CHAPTER 4: RESULTS AND DISCUSSION

SECTION I: PROOF OF CONCEPT

Preamble and rationale

This section of the dissertation focused on the treatment of OFMSW and LW is studied through the concatenation of three processes:

1. **Thermal hydrolysis** pretreatment followed by a separation of the liquid and solid fractions

2. **Anaerobic digestion** of the solid fraction

3. **Photo-biological conversion** of the liquid fraction with mixed cultures of PPB.

In this section, we have discussed the rate of hydrolysis of organic matter to the liquid fraction during pretreatment; analyzed the methanogenic potential of the remaining solid fraction, and settle whether a mixed culture of PPB can grow and transform the complex liquid fraction into high value-added products such as proteins, H2 and PHA. This approach has been tested on a laboratory scale in batch tests. Energy requirements and the yield of the different products was conducted by means of preliminary mass and energy balances in order to validate the proof of concept.

Experimental design

OFMSW (Pre-sorted and selective) was used in section I.1 and LW in section I.2. Prior to thermal hydrolysis pretreatment, the amount of water was adjusted to 20% TS in order to be able to compare results. In section I.1 the pretreatment at 180ºC is studied, since it is the optimum temperature determined by Cano and three reaction times: 5, 15 and 30 min. Seeing that the reaction time does not affect the results too much, in section I.2 three temperatures (120ºC, 150ºC and 180ºC) were studied at a reaction time of 5 min. After thermal pretreatment the effluent is centrifuged to separate the solid and liquid fractions and stored at 4ºC until use. The solid fraction is used in BMP tests and the liquid fraction in SPA tests. Together with the SPA tests of

the liquid fraction, a control with MOM medium is studied. Table I.1 shows a summary of the experimental design.

Tabla I.1 Summary of the experimental design used in section I.

Variable / Process	Section I.1	Section I.2
Waste	Pre-Sorted and Selective OFMSW	LW
Thermal hydrolysis	Batch reactions at 180 °C Reaction times of: 5, 15 and 30 min	Batch reactions at 120 °C, 150°C and 180°C and a reaction time of 5 min
Anaerobic digestion	BMP batch tests of the untreated waste and the 3 solid fractions	BMP batch tests of the untreated waste, the 3 treated wastes (liquid + solid) and the 3 solid fractions.
Photoheterotrophic process	SPA batch tests of the 3 liquid fractions and control with the same inoculum and optimized media growth.	SPA batch tests of the 3 liquid fraction and control with the same inoculum and optimized media growth.

The results of this section have been published in the following papers:

Allegue, L. D., Puyol, D., and Melero, J. A., (2020). Novel approach for the treatment of the organic fraction of municipal solid waste: Coupling thermal hydrolysis with anaerobic digestion and photofermentation . Science of the Total Environment (2020), 714, 136845.

Allegue, L.D., Ventura, M., Melero, J.A. and Puyol, D., (2021). Integrated sustainable process for polyhydroxyalkanoates production from lignocellulosic waste by purple phototrophic bacteria. GCB Bioenergy, 13, 862-875.

Allegue, L. D., Puyol, D., and Melero, J. A, (2020). Food waste valorization by purple phototrophic bacteria and anaerobic digestion after thermal hydrolysis. Biomass and Bioenergy, 142, 105803.

SECTION I.1

4.I.1. OFMSW Proof of Concept

In this part of the dissertation, we have analyzed the treatment of the OFMSW with different origins: a fraction pre-sorted by the citizens (denoted as pre-sorted) and the other one sorted by the waste treatment plant (denoted as selective). The temperature selection was based on previous works (Cano *et al.*, 2014), while reaction time was set for 5, 15 and 30 min. Table 3.1 shows the main macroscopic properties of both feedstocks. Surprisingly, there is no significant difference between both raw wastes, at least in macroscopic terms. Nevertheless, we have decided to carry out the study with both wastes to elucidate if some other compounds could affect the performance of the coupled process and if there is any benefit of the pre-sorting in origin by the citizens.

4.I.2. Thermal hydrolysis

Table I.2 summarizes the macroscopic characteristics of the liquid and solid phases after thermal treatment at different times and at 180 ^{o}C for pre-sorted and selective OFMSW. VS destruction efficiencies ranged from 27-30% and 37-38% for pre-sorted and selective OFMSW, respectively. On the other hand, COD, N, and P solubilization averaged 40% and 38%, 11% and 12%, and 27% and 25%, respectively, for pre-sorted and selective OFMSW, respectively. These values led to a very high COD/N/P mass ratios (100/0.62/0.72 on average), within the liquid phase, which theoretically benefits the accumulation of carbon in the form of PHA by PPB (Fradinho *et al.*, 2019).

No statistically relevant differences were found in regards to the three different hydrolysis reaction times. High COD and low nutrient release is achieved independent of the reaction time. Nevertheless, a more comprehensive characterization of the hydrolysate, in terms of proteins, carbohydrates, lipids, volatile fatty acids, sugars, etc. composition is necessary for a better understanding of the reaction mechanism. Although this subject is encouraged, it is out of the scope of this proof-of-concept study.

4.I.3. Anaerobic Digestion

The potential for product recovery from the solid fraction upon thermal hydrolysis is performed by analyzing their BMP and the metals and nutrients composition of the digestate. Figure I.1 depicts the time course of the methane potential of different remaining solids after pretreatment and the fresh waste untreated (raw). The methanogenic potential is referenced to the initial concentration of VS before

Table I.2. Macroscopic characteristics of raw OFMSW samples and solid and liquid fractions after the hydrothermal pretreatment under different temperatures.

	Pre-sorted				Selective			
	Raw	5'	15'	30'	Raw	5'	15'	30'
TS ($g\ kg^{-1}$)	367 ± 51	245 ± 26	243 ± 82	263 ± 62	343 ± 42	201 ± 8	240 ± 24	207 ± 33
VS ($g\ kg^{-1}$)	298 ± 46	214 ± 23	211 ± 83	218 ± 33	297 ± 19	187 ± 28	182 ± 26	181 ± 22
COD ($g\ kgTS^{-1}$)	112 ± 12	89.2±8.5	95.3 ± 3.5	94.1 ± 3.3	135 ± 7	85.7 ± 4.5	87.9 ± 2.5	89.1 ± 4.4
TP ($gP\ kgTS^{-1}$)	1.2 ± 0.1	0.9 ± 0.5	1.0 ± 0.7	1.0 ± 0.6	1.1 ± 0.6	0.9 ± 0.5	1.0 ± 0.4	0.7 ± 0.4
TKN ($gN\ kgTS^{-1}$)	3.7 ± 1.7	3.2 ± 0.1	3.4 ± 0.2	3.2 ± 0.1	3.5 ± 0.8	3.2 ± 0.1	3.1 ± 0.1	3.0 ± 0.2

	Liquid fraction					
TSS (mg L^{-1})	0.95 ± 0.21	1.10 ± 0.18	1.05 ± 0.11	1.21 ± 0.08	1.14 ± 0.11	1.16 ± 0.13
VSS (mg L^{-1})	0.94 ± 0.1	1.07 ± 0.12	1.02 ± 0.17	1.18 ± 0.05	1.11 ± 0.09	1.13 ± 0.08
TKN (mgN L^{-1})	446 ± 5	351 ± 2	423 ± 5	495 ± 4	483 ± 5	377 ± 3
TP (mgP L^{-1})	675 ± 40	684 ± 34	694 ± 41	545 ± 35	512 ± 21	578 ± 41
TCOD (g L^{-1})	54.2 ± 1.5	54.5 ± 3.2	61.4 ± 3.5	51.7 ± 1.5	51.6 ± 1.5	51.8 ± 1.3
SCOD (g L^{-1})	53.9 ± 0.4	53.3 ± 0.8	61.9 ± 0.8	50.2 ± 0.3	50.7 ± 0.4	51.9 ± 1.4
NH_4+ (mgN L^{-1})	269 ± 8	253 ± 9	227 ± 10	227 ± 6	281 ± 18	294 ± 36
PO_4+ (mgP L^{-1})	338 ± 178	514 ± 170	567 ± 214	365 ± 98	307 ± 73	373 ± 66

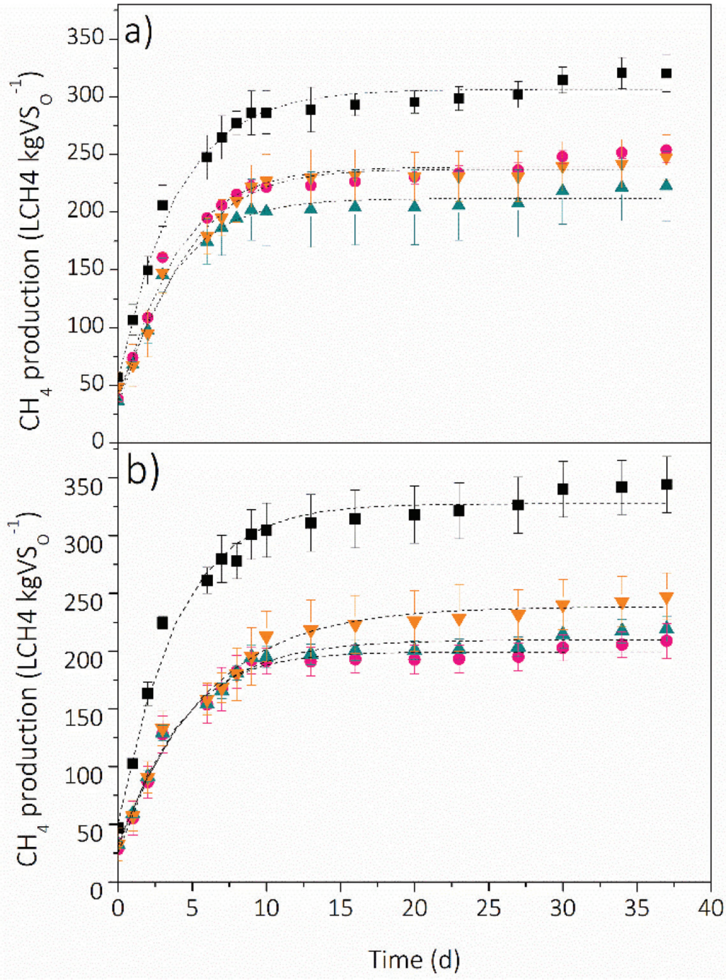
the hydrolysis in the raw waste (VS_o), thus including into the story line the effect of the removal of the liquid fraction. A maximum of 253 ± 11 and 246 ± 20 LCH_4 gVS_o^{-1} were obtained for pre-sorted and selective biowastes respectively after pretreatment. The results are compared with a control test where anaerobic digestion was performed to the untreated OFMSW and where all the mass of the residue sum up for the methane production potential. As expected, methane production values of the treated samples are lower than those from the fresh solids that yielded 320 ± 15 and 342 ± 22 LCH_4 gVS^{-1} respectively. As seen in the previous section, the removal of the liquid fraction upon hydrolysis led to 30-45% of solids destruction, probably the most biodegradable part, and consequently affecting the reduction of the BMP. Nevertheless, these results are within the range of similar studies reported in the literature: ranging from 180 to 570 LCH_4 gVS^{-1} (Bala *et al.*, 2019; Cabbai *et al.*, 2013; Campuzano and González-Martínez, 2016; Cano *et al.*, 2014). Hence, the effect of liquid removal upon the hydrolysis did not imply a significant reduction of the methane potential of the remaining solids.

Figure I.2 reports the hydrolysis constant (K_h) and the biodegradability extent (B_o), with 95% confidence regions for the conditions of the different tests. Although overall methane production is lower after the pretreatment (Figure I.1), kinetic parameters are quite similar to that obtained from the fresh OFMSW.

This demonstrates that thermal hydrolysis pretreatment is increasing the anaerobic biodegradability of the less biodegradable fraction, thus it increases the total profitability of the proposed treatment. Results show no statistically relevant differences between thermal hydrolysis times for OFMSW except for 30 min reactions on the selective OFMSW, which was slightly improved probably due to a small difference in the homogeneity of the raw samples. It must be stated that the main goal of thermal hydrolysis is to obtain an organic liquid feedstock with a high COD/N/P ratio. The benefit obtained with the treatment of the liquid fraction is key to check on the viability of the proposed approach, as will be furtherly discussed.

In addition to the energy potential of biogas, anaerobic digestion also produces a digestate that must be studied for its possible applications. The use of digestate from stabilized OFMSW has been shown to have benefits to soil, crops, and the environment (Prabpai *et al.*, 2009) due to the low organic content of the residual solid after digestion. In our results, a minimum of 40% VS and 70% COD removal was achieved after anaerobic digestion. However, the digestate must comply with some requirements before it is used as a marketable product. First, the digestate must be free of pathogens before land application. The pathogens reduction has not been checked in this work. However, it has been demonstrated that a 180 ^{0}C thermal hydrolysis is very effective for the pathogen's reduction (Ruiz-Espinoza *et al.*, 2012). Second, urban waste may contain high levels of heavy metals, which is of concern for a fertilizer due

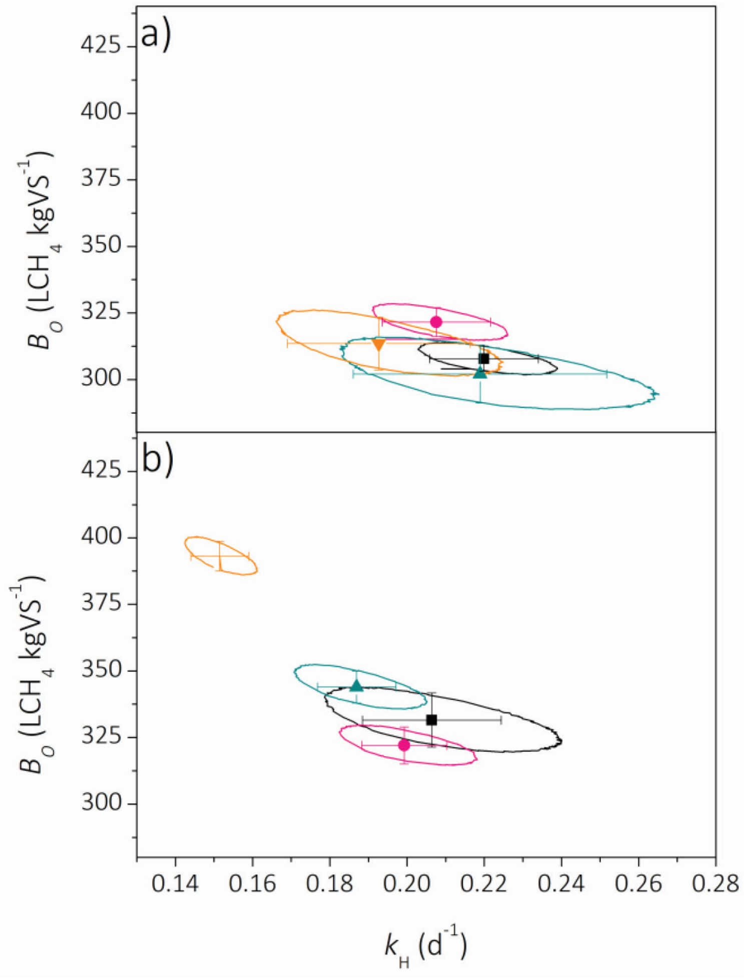
Figure I.1 BMP tests during the anaerobic digestion of the different solid fractions. Presorted OFMSW (a) and Selective OFMSW (b). Initial OFMSW without thermal pretreatment (■), 5' (●), 15' (▲) and 30' (▼) min of reaction time.



to potential soil fertility deterioration, ground-water quality damage, and food chain contamination (McBride, 1995). Metal content in the solid digestate was measured and the results were compared to the Spanish law (Real Decreto 506/2013) that regulates organic fertilizers (see detail in Table I.3).

Considering the metals composition of the final digestate, it may be registered as a Class B fertilizer, which is not subject to limitations on its use. It seems that the pre-sorted OFMSW has better properties than the selective OFMSW regarding metal composition and its values are close to limit

Figure I.2: Results from BMP tests (added feedstock basis). 95% confidence regions for the first-order kinetic parameters during the BMP tests of the solid fraction pre-sorted OFMSW (a) and Selective OFMSW (b). Initial OFMSW without thermal pretreatment (■), 5' (●), 15' (▲) and 30' (▼) min of reaction time.



values to be considered as Class A fertilizer. Thereby, an improvement of the pre-sorting efficiency (through e.g. better awareness campaigns) can decrease the metal contamination of the residue and may benefit the quality of the produced fertilizer. Finally, selective OFMSW had a higher nitrogen content on dry mass, just above the 2% necessary to be considered organic fertilizer type N, as specified in the Spanish law aforementioned (Real Decreto 506/2013). Thereby, depending on the initial composition of the OFMSW the digestate can be potentially considered as an organic fertilizer or as an organic amendment, closing the carbon and nutrient cycle.

Table I.3 Metals and nutrient composition of the digestate samples upon hydrolysis and centrifugation compared to the Spanish legislative limits for organic fertilizers.

	Measured data (mg kg $^{-1}$)		Limit values RD 506/2013 (mg kg $^{-1}$)		
	Pre-sorted	Selective	Class A	Class B	Class C
Cd	0.9	1.6	0,7	2	3
Cu	134	277	70	300	400
Ni	20	21	25	90	100
Pb	58	39	45	150	200
Zn	104	466	200	500	1000
Hg	0.5	1.1	0.4	1.5	2.5
Total N (%, d.b.)	1.9 ± 0.5	2.4 ± 0.4			
P_2O_5 (%, d.b.)	0.28 ± 0.02	0.27 ± 0.03			

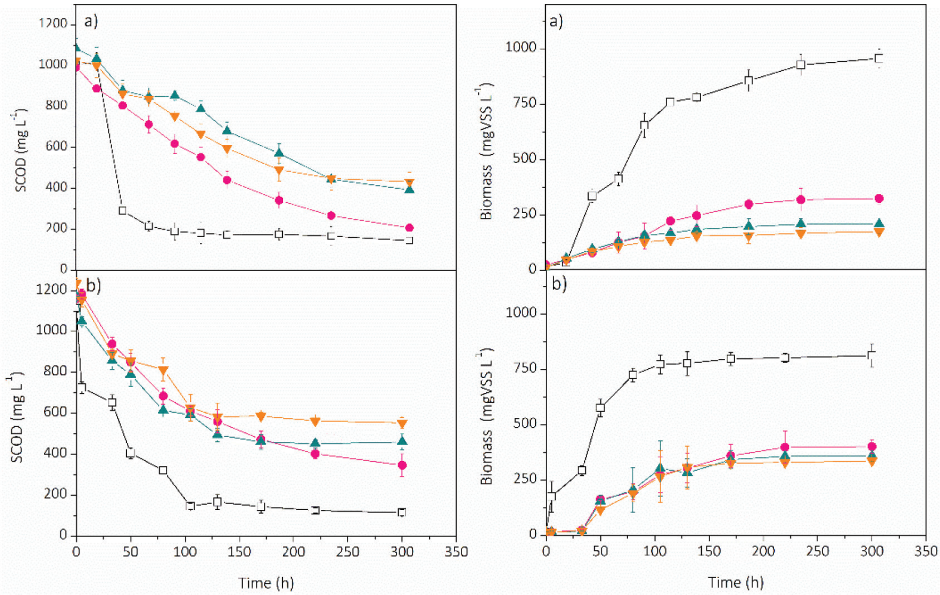
4.I.4. Specific phoheterotrophic activity tests (SPA)

Growth process

Standard SPA tests were performed on the liquid fraction upon thermal hydrolysis. Figure I.3 shows the results about COD consumption and biomass growth in terms of mgVSS^{-1}. The control test that used the same inoculum but grown in the MOM served as a reference for the maximum theoretical specific growth rate. Active biomass assimilated between 59-82% of the SCOD in the experimental tests. The experiments lasted 300 h for both sources of OFMSW with more than 80% of the consumed SCOD assimilated in the first 200 h and a biomass growth up to 400 mgVSS L^{-1}.

Near to 100% of main nutrients (N and P) were consumed, likely due to their low concentration compared to the organic carbon concentration (see Figure I.4). The low nutrient content is therefore limiting biomass development, especially ammonium. High P removal even upon ammonium depletion, suggests the appearance of accumulative processes (e.g. polyphosphate accumulation), which improves the potential of nutrients recovery and increases the quality of the phototrophic biomass as a product. The pH at the end of all the assays was on average 8.5. As the initial pH was set at 6.5 the pH increase was likely due to consumption of volatile fatty acids by PPB. The liquid

Figure I.3 Comparison of SCOD consumption and biomass growth in the SPA tests using the liquid fraction. Results tests for pre-sorted OFMSW (a) and selective OFMSW (b) at 5' (●), 15' (▲) and 30' (▼) min are compared with a control experiment (□) with the same inoculum.

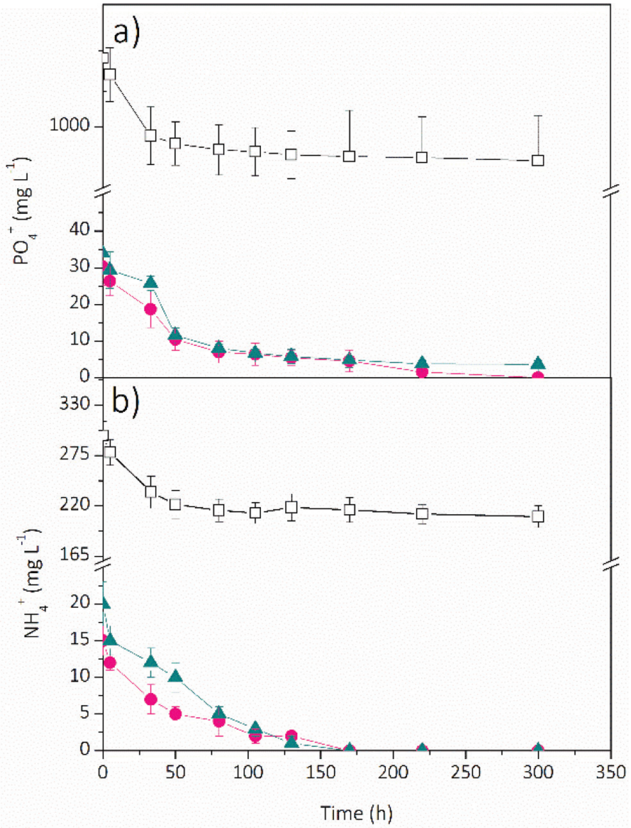


fraction after 5 min pretreatment has proven to be the most biodegradable in all cases, whereas there were no relevant statistical differences between 15 and 30 min of pretreatment. The release of poorly biodegradable organics due to long hydrolysis times may be the main reason for the lower biodegradability of the soluble organics. This fact has been previously related to Maillard reactions, which form recalcitrant melanoidins at temperatures higher than 160 °C (Dwyer *et al.*, 2008).

In the SPA tests, initial and final biomass samples showed prominent VIS/NIR absorption peaks at 590, 805, and 865 nm in all cases, corresponding to the typical absorption spectra of bacteriochlorophyll a, thus confirming the dominance of the PPB within the MMC culture under IR light, which concurs with previous studies (Hülsen *et al.*, 2014).

The quantification of the photoheterotrophic metabolism was assessed by calculating the specific phototrophic activity (k_M) and the biomass yield ($Y_{x/s}$), respectively (Figure I.5). The k_M of control tests yielded 0.035 ± 0.005 g SCOD $gVSS^{-1}$ h^{-1}, and the tests carried out with the two types of OFMSW yielded on average 0.022 ± 0.003 g SCOD $gVSS^{-1}$ h^{-1}. These results are in line with

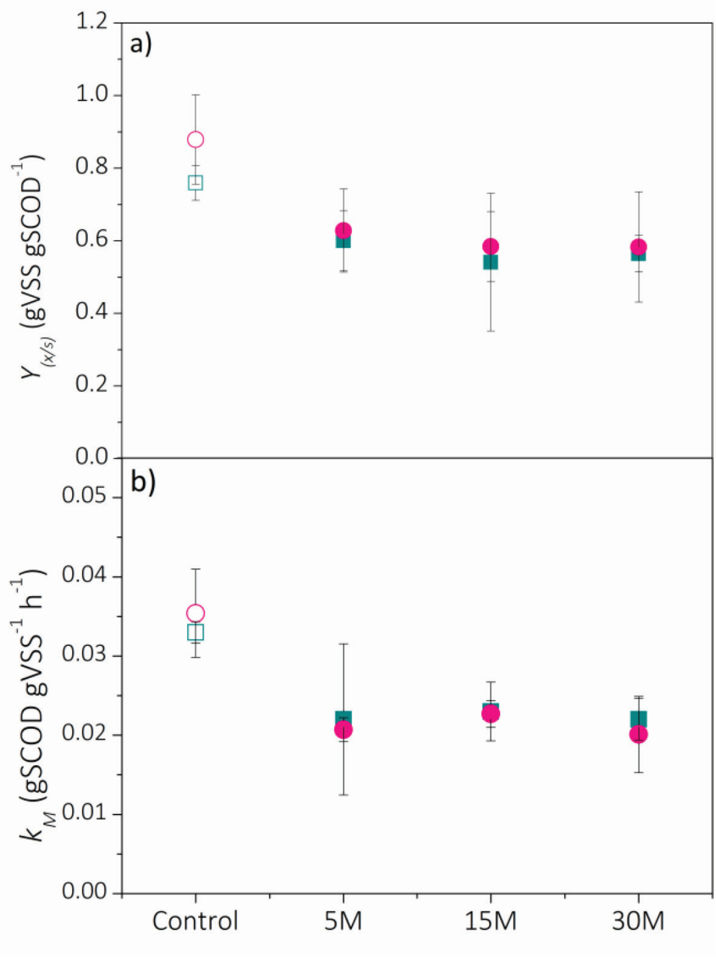
Figure I.4: Comparison of PO_4^{3-} (a) and NH_4^+ (b) consumption in the SPA tests using the liquid fraction (Average of the 3 different thermal hydrolysis reactions). Results tests for pre-sorted OFMSW (●) and selective OFMSW (▲) are compared with a control experiment (□) with the same inoculum.



previous studies (Puyol *et al.*, 2017) that reported a k_M of 0.036 g SCOD g VSS^{-1} h^{-1} on acetate for a PPB mixed culture.

On the other hand, the $Y_{x/s}$ was 0.87 ± 0.07 and 0.75 ± 0.08 gVSS gSCOD^{-1} for the control tests while averages for OFMSW are 0.60 and 0.57 gVSS gSCOD^{-1} for selective and pre-treated OFMSW, respectively. Although $Y_{x/s}$ is lower compared to the control, the biomass yield obtained in these tests were higher than the values reported in the literature (up to 0.56 gVSS gCOD^{-1}) for the treatment of domestic wastewater (Dalaei *et al.*, 2019) and poultry processing wastewater (Hülsen *et al.*, 2018) with PPB. Biomass yield values lower than the controls suggest the presence of non-phototrophic organic transformations (e.g. fermentation or anaerobic oxidation processes), as in

Figure I.5: Comparison of biomass yield ($Y_{x/s}$) (a) and specific phototrophic activity (k_M) (b) in SPA tests using the liquid fraction of every waste upon the thermal hydrolysis at different reactions times. Results tests for Pre-Sorted OFMSW (■) and Selective OFMSW (●) are compared with a control experiment (open symbols) with the same inoculum.



essence photoheterotrophy implies almost a complete conversion of organics into biomass (Hülsen *et al*, 2016).

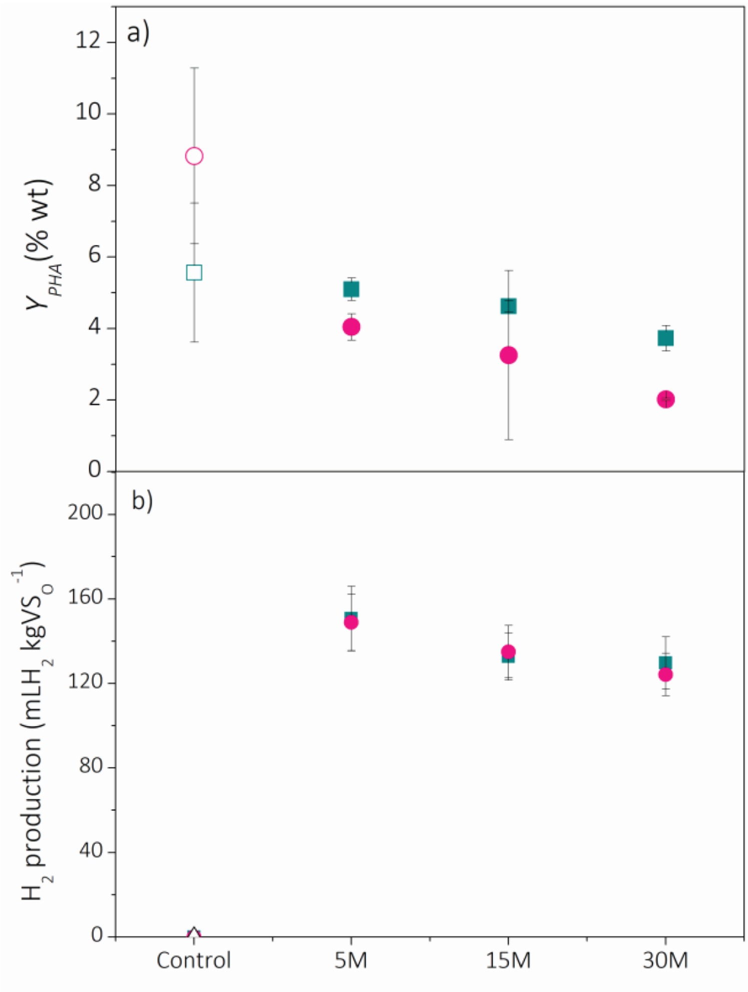
Moreover, the hydrolysis reaction times caused non-significant differences over the two metabolic parameters, which means that the lower biodegradability due to the extended hydrolysis time did not cause toxicity over the PPB metabolism. It must be noteworthy that the inoculum was not adapted to the feedstock.

Thus, the ability of non-adapted PPB to grow on these organic sources demonstrates the feasibility of the proposed concept.

Tests Products

During SPA tests, PHA and biohydrogen productions were measured, and SCP production was estimated based on biomass production. Figure I.6 (a) shows a maximum of 5.1 ± 0.3% (wt.) of PHA production yield (Y_{PHA}) for the

Figure I.6: Comparison of PHA production yield (Y_{PHA}) (a) and H_2 production (b) in SPA tests using the liquid fraction of every. Results tests for pre-sorted OFMSW (■) and selective OFMSW (●) are compared with a control experiment (open symbols) with the same inoculum.



presorted OFMSW upon 5 min of thermal hydrolysis and a minimum of 2.0 ± 0.1% (wt.) for the selective one upon 30 min thermal hydrolysis. PHA production in control assays was slightly higher, averaging 5.8 ± 1.9% (wt.). Data shows that PHA production is not depending on the thermal hydrolysis times, as there are no clear trends of statistical relevance. Low PHA content (even in controls) is due to the sampling at the end of each essay, instead of at the time of the peak of maximum PHA production. In any case, the PHA yields obtained are considerable, stating that the inoculum is a PPB mixed microbial culture, and are within the lower range found in the literature (3 to 30%), even in works where an adapted phototrophic consortium was used (Fradinho *et al.*, 2016, 2019). Therefore, **these results are promising and encourage future research to maximize the production of PHA in a continuous process using the OFMSW as an efficient and low-cost feedstock.**

Figure I.6 (b) shows the H_2 production along with the experiment. The maximum production was achieved upon 5 min of thermal hydrolysis (150 ± 15 mLH_2 kgVS_0^{-1}), whereas the minimum production was achieved upon 30 min of thermal hydrolysis (124 ± 10 mLH_2 kgVS_0^{-1}). These data mean an average production of 0.071 LH_2 L^{-1} on the hydrolysate. The thermal hydrolysis times caused non-significant differences. The controls had no hydrogen evolution due to the high ammonium content, which inhibits the hydrogen production in the nitrogenase complex (Sasikala *et al.*, 1993). H_2 production is lower than recently published results, where values ranging from 0.4 to 8.6 L H_2 L^{-1} of waste have been reported (Ghosh, *et al.*, 2017). Low H_2 production responds to the presence of ammonia at the beginning of the assay and the rise of pH above 8, which is also a key factor along with temperature in H_2 production with phototrophic bacteria (Bolatkhan *et al.*, 2019). In any case, these results, although modest, may open the opportunity for future research in the optimization of H_2 production using this feedstock. A combination between dark fermentation and photo-fermentation may be a promising option as it has been described previously (Argun and Kargi, 2011). In addition, H_2 is not generated directly as a result of a metabolic pathway but occurs as a mechanism for controlling the reducing power through the nitrogenase enzyme complex (Kotay and Das, 2008).

Finally, SCP estimation content was performed following the literature (Eding *et al.*, 2006). PPB uses IR light as a catabolic driver and enables a nondestructive, assimilative uptake of organics, nitrogen, and phosphorous (Hülsen, *et al.*, 2016). Recent studies show that in a medium with high salinity or high concentrations of ammonium, PPB develops specific proteins as a method of defense against chemical stress (Delamare-Deboutteville *et al.*, 2019). On one hand, content in control assays was high, up to 777 ± 31 mgSCP gVSS^{-1}, likely due to excess ammonium in the media. These results are in line with a recent study in which a maximum of 720 mgSCP gVSS^{-1} was obtained using pork flush as a substrate, which also has a high nitrogen content (Hülsen *et al.*, 2018). The

PPB mixed cultures from the OFMSW assays show a lower protein content yield of up to 482 ± 21 and 421 ± 19 mgSCP $gVSS^{-1}$ for presorted and selective OFMSW, respectively. These results are in agreement with the low biomass growth and ammonium content of these tests. Nitrogen is a key factor in protein synthesis, and its deficiency in the media causes a low protein content. However, a recent study showed protein content in PPB averaging 45%, but a production rate up to 1.7 g dry weight L^{-1} d^{-1} of SCP was possible by using a wise combination of synthetic VFAs (Alloul *et al.*, 2019). **This makes PPB an economically viable alternative source of microbial protein in contrast with the current conventional agricultural-based supply route for nutritive animal proteins which has a high environmental and water impact.** This alternative route is a more environmentally friendly way of protein production that can be optimized using the OFMSW as a feedstock.

4.I.5. Mass and energy balances

Energy balance

The energy balance of the thermal hydrolysis pretreatment was assessed extrapolating the BMP results from laboratory tests. Thermal hydrolysis requires a large amount of thermal energy which is one of the main limitations of the process to be economically feasible (Passos and Ferrer, 2015). A simplified energy balance (see Figure 3.5) has been carried out by simulating a CHP system following the scenarios of energetic integration proposed in Cano *et al*. (2014) and Murphy and McKeogh, (2004). Table I.4 summarizes the energy balances for the different scenarios in terms of electrical and thermal balance (that means energy produced in the CHP system minus energy requirements in the overall process). Test controls, which are the biowastes directly undergoing anaerobic digestion, yielded the highest results in thermal (341 and 367 kWh $tonne^{-1}$ for pre-sorted and selective OFMSW, respectively) and electrical balances (190 and 206 kWh $tonne^{-1}$ for pre-Sorted and selective OFMSW, respectively). The value of electrical and thermal outputs for the assays with pretreatment ranged from 128 to 103 kWh $tonne^{-1}$ and 70 to 23 kWh $tonne^{-1}$, respectively. These values are lower than that shown by the control sample due to the thermal energy requirements necessary in the hydrolysis step. But all the scenarios independent of the raw material and the hydrolysis time show a positive energetic balance.

These findings are analogous to the results reported in the literature (Cano *et al.*, 2014; Dasgupta and Chandel, 2019). Further optimization of different pretreatment conditions with respect to energy integration (e.g. temperature, pressure, reaction time, or moisture) is mandatory in order to determine the scalability of hydrothermal pretreatment of OFMSW prior to anaerobic digestion and photo-fermentation.

Table I.4 Energy integration balance. Results were simulated for CHP system for electricity and thermal energy production. Data referenced to a tonne (t) of FORSU.

Substrate	Total Energy biogas kWh t^{-1}	Electrical Output kWh t^{-1}	Thermal Output kWh t^{-1}	Electrical balance kWh t^{-1}	Thermal energy balance kWh t^{-1}	Electric output Euro t^{-1}
Pre-sorted Control[a]	619	205	341	190	341	29
Pre-sorted 5'	491	163	270	128	70	19
Pre-sorted 15'	431	143	237	108	26	16
Pre-sorted 30'	479	159	263	124	43	19
Selective Control[a]	666	221	367	206	367	31
Selective 5'	417	138	230	103	30	15
Selective 15'	424	141	233	106	23	16
Selective 30'	478	159	263	124	43	19

[a] *Direct anaerobic digestion (AD)*

[b] *Electrical energy produced minus that required for the centrifugation and mixing for AD.*

[c] *Thermal energy produced minus that required for pretreatment and AD.*

Mass balance

This section shows the preliminary analysis of the global mass balance of the process on initial total solids in the raw OFMSW. Figure I.7 shows the total mass balance of the proposed approach. As a general finding, a maximum of 15% of the initial TS become PHA, H_2, and biomass jointly to microbial protein upon thermal treatment with a 59-61% volume reduction of the waste to be disposed of (5-11% more volume reduction as compared with the traditional treatment of an anaerobic digestion process). Figure I.7 clearly shows the diversity of potential products that can be derived from this new concept. Complete and efficient utilization of resources is important for circular economies as a zero-waste policy is the aim of bioprocesses.

In order to preliminary analyze the economic viability of this new process, we have compared the selling prices of each individual product. Current benefits are not included and would require a complete social and economic life cycle

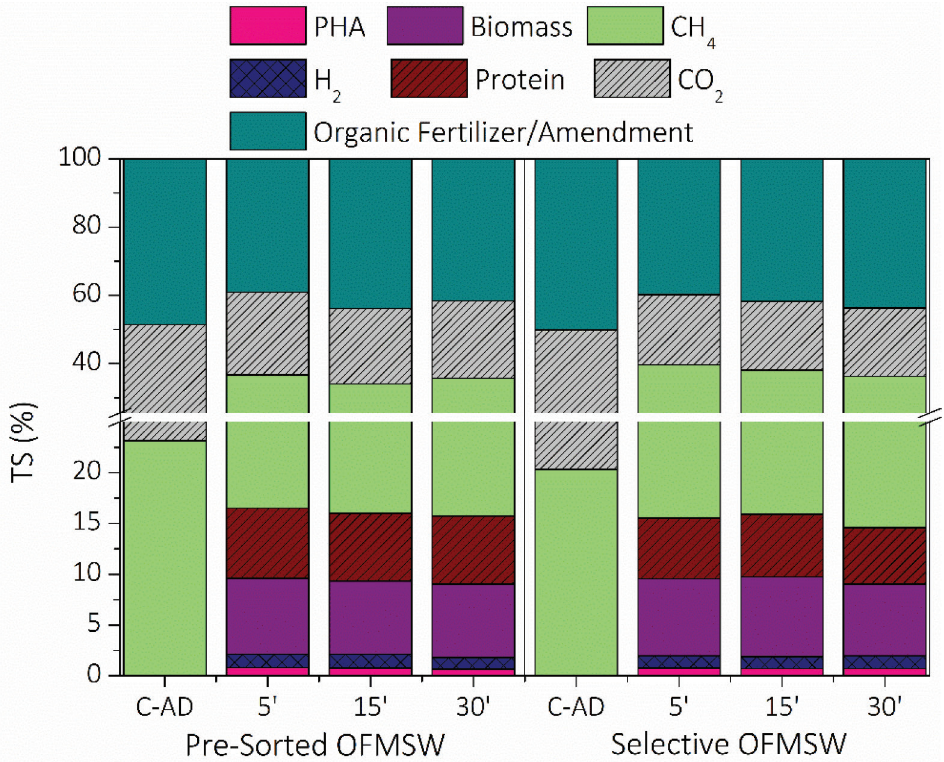


Figure I.7 Mass balance of the overall process at different pretreatment times (5, 15, and 30 min), compared to the traditional direct anaerobic digestion of the wastes (AD).

assessment once the process is conveniently optimized. The biogas produced has been partially used for the energy integration of the process, and only its excess in the production and sale of electricity is considered in this analysis. The incomes expected from electricity are on average 30 € tonne^{-1} for the control tests, and 18 € tonne^{-1} for the assays undergoing the new process. Despite the mass percentage of organic fertilizer being the most important (more than 40% in all cases), its selling price is very low. An average selling price of 4 € tonne^{-1} for certified (RAL) bio-waste fertilizer has been reported in the EU (Meyer-Kohlstock *et al.*, 2015). On the other hand, although PHA constitutes an average of 5.1 g PHA kgTS$_0^{-1}$ based on the mass of the residue, its market price is relatively high: 4.5-5.5 € kg^{-1} (Castilho *et al.*, 2009). The final costs of PHA mainly depend on the price of substrates added as a carbon source for microbial growth. Furthermore, the PHA yield on carbon source, PHA productivity, and downstream costs determine their introduction into the global market. The cost of carbon sources was reported to constitute about 50% of the final production cost (Choi and Lee, 1999). Taking this into account, the process proposed uses a virtually cost zero-carbon substrate, adding more interest to the process. Microbial protein constitutes the largest fraction of possible income, due to the large amount of biomass growth produced in photo-fermentation tests. Up to 68 gProtein kgTS$_0^{-1}$ was produced. Microbial protein has a market price of 1.1 € kg^{-1} in (Matassa *et al.*, 2016). H_2 costs via PPB are considered 10 € GJ^{-1} (Basak and Das, 2007). The purification cost exceeds this figure if we consider that the purity of H_2 issued in these batch tests are very low (about 9%). Considering those, H_2 is the least economically interesting product of this platform. In any case, the reduction costs derived from the lowering of the waste to be disposed of in landfills, as well as the reduction of the C-footprint, should be put front into an economic life cycle analysis once the concept is verified. Thereby**, the proposed platform may potentially enlarge the economic benefit by targeting PHA and microbial protein production, and future trends should focus on process optimization with these targets in mind.**

4.I.6. Conclusions

This preliminary results confirm the proof of concept for future optimization of an integrated photo-biorefinery process using OFMSW as feedstock. Thermal hydrolysis achieves up to 40% solubilization of the COD depending on the used waste, where the reaction time (5 to 30 min) seems to have low significance over the overall process. The photo-process using PPB mixed cultures has proven to be a resilient technology capable of assimilating organic matter from the OFMSW after pretreatment of thermal hydrolysis, yielding PHA, SCP, and H_2. Different metabolic pathways can be favored to produce these products in specific cases, so the proposed treatment concept is highly versatile depending on factors such as market needs, feedstock composition, and environmental conditions. This new process also has interesting economic prospects based on the expected income from the production of high added-value products. Finally, the anaerobic digestion process closes the carbon cycle, reduces the amount of waste and decreases the C footprint and enables an energetically sustainable process, thus embracing this alternative concept within the circular economy framework

SECTION I.2

4.I.7. Lignocellulosic waste: proof of concept

In this part of the discussion, we have extended the proof of concept using as feedstock LW (in particular gardening and pruning wastes). It must be noted that this type of LW accounts for 20% of the OFMSW. In this way, in this section, we have performed a parallel work to that described in section I.1. As seen in the previous section, pretreatment reaction time is not an especially relevant parameter, therefore, it has been decided to change the reaction temperatures to 120 °C, 150 °C and 180 °C and keeping reaction time of 5 min.

4.I.8. Hydrothermal pretreatment

Table I.5 shows the main macroscopic characteristics of the raw waste, and the slurry and liquid fractions upon the pretreatment. Thermal conditions were 120 °C, 150 °C, and 180 °C including the temperature gradient which corresponds to a severity factor of 2.1, 3.1, and 3.9, respectively. Previous studies have shown that the optimal severity for maximizing sugar yield is 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 suffer the highest solubilization on a severity factor of 4 (Carvalheiro *et al.*, 2009). The elemental analysis carried out shows that there are almost no changes in the percentage of carbon, nitrogen, or hydrogen. This means that the pretreatment process did not entail the loss of matter by means of 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 pretreatment. The crystallinity indexes may indirectly indicate the amorphous phase signal of the biomass such as hemicellulose, cellulose, and lignin domains (Table I.5). The cellulose in lignocellulosic biomass is composed of crystalline and amorphous structures, which have a great influence on 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).

Table I.5 Macroscopic characteristics of raw LW samples and solid and liquid fractions after the hydrothermal pretreatment under different temperatures.

Parameters*	Raw	120 °C	150 °C	180 °C
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 ± 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 (%)[a]	45.9	45.3	45.1	43.5
Liquid fraction				
SCOD (g L^{-1})		19.6 ± 0.9	22 ± 1	45 ± 2
Monosaccharids (%)[b]		2.96	2.74	1.23
Other CA (%)[b]		1.15	3.04	3.41
Oligosaccharides, and proteins (%)[b]		95.90	94.22	95.35
TKN (gN 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
NH_4+ (mgN L^{-1})		610 ± 10	710 ± 10	930 ± 20
PO_4^{3-}(mgP L^{-1})		100 ± 10	122 ± 8	140 ± 10
pH		5.8	5.2	4.8

[a]*% 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.*

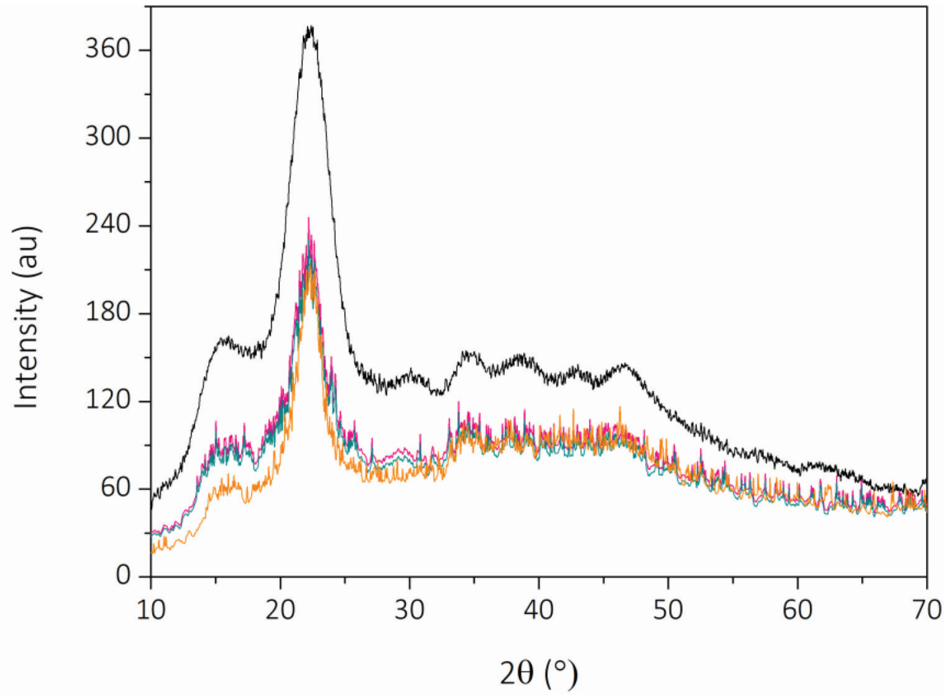
[b]*Percentage calculated based on SCOD. The %CA was calculated as SCOD - (SCCA + monosaccharides).*

Figure I.8 shows the CrI XRD spectra comparing raw and pretreated samples. The composition of the biomass influences the CrI since hemicellulose and lignin are amorphous while cellulose is crystalline (Jeoh *et al.*, 2007). Raw lignocellulosic waste had a CrI of 68.6%, which increased up to 73.5, 76.8, and 76.7% upon the 120°C, 150°C, and 180°C, respectively. The increase in CrI was caused by the removal of amorphous substances in the biomass, mostly

Table I.5 2θ theoretical values

Cellulose type	Amorphous structure	Crystalline structure
I	15-17^{0}	22-23^{o}
II	12-13^{o}	20-22^{o}

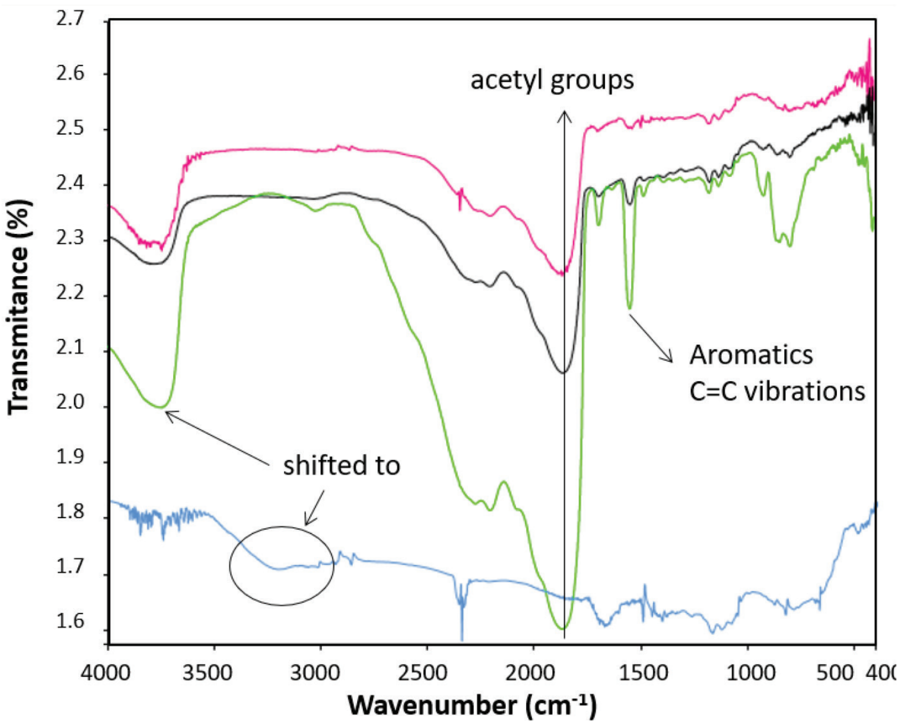
Figure I.8 Comparison of DRX spectra of the raw waste (Black) and the hydrothermal pretreatments solid phases were 120 °C (Pink), 150 (Cyan) and 180 °C (Orange).



hemicellulose, which exposed the crystalline cellulose core and increased the glucan content in the pretreated solid fraction. 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 measurements were also used to characterize the solid fraction after pretreatment. The structural changes of hemicelluloses, cellulose, and lignin after the thermal treatment can be observed in the FTIR spectra. Figure I.9 shows the effect of pretreatment on the change of functional groups of feedstock. The wavenumber at 3252 cm^{-1} shifted to 3759 cm^{-1} with the pretreatment, 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. The aromatic ring vibrations of C=C (between 1600-1545 cm^{-1}) changed as temperature increased, which is indicative that the lignin of the biomass was affected. The results observed in the FTIR measurements show clear changes in the crystallinity of cellulose, which agrees with the XRD analyses, partial solubilization of

Figure I.9 FTIR spectra of the untreated waste (blue) and the three hydrothermal pretreatments, 120 °C (Pink), 150 (black) and 180 °C (green).



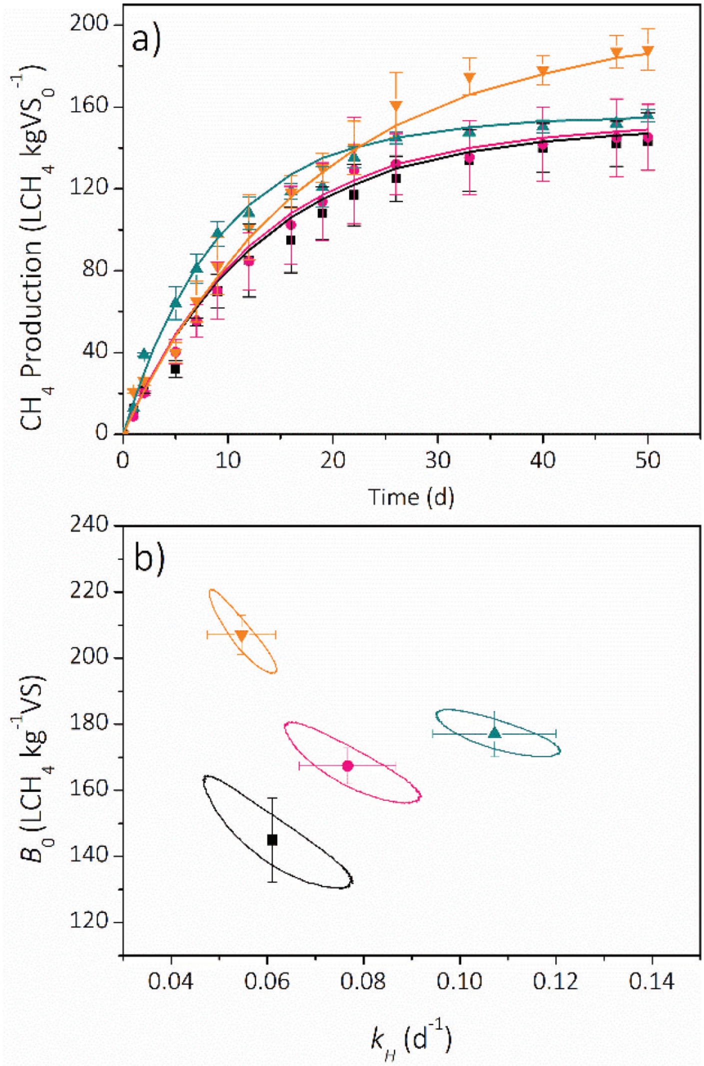
hemicellulose, and slight changes in the structure of lignin. Besides, a clear peak at 1877 cm^{-1} was found in the treated biomass, being more intense as temperature increases. This is related to the acetyl groups of hemicelluloses or the ester linkages of ferulic and p-coumaric acids with lignin (Li and Jin, 2015). Hence, the greater the severity factor the more changes in the crystallinity, solubilization of hemicellulose, and changes in lignin structure.

The increase of the severity factor also affected the composition and characteristics of the liquid hydrolysate. The solubilization of COD accounts for 9, 14, and 24% for 120 °C, 150 °C, and 180 °C respectively. This means a 69% increment in COD solubilization for 180 °C compared to 120 °C. A similar trend happens with the destruction of solids, yielding 13, 19, and 29% destruction efficiencies at 120 °C, 150 °C, and 180 °C, respectively. As shown in Table I.4, the pH of the hydrolysate decreased significantly with increasing temperature, due to the presence of SCCA such as acetic and propionic acids that are usually formed during pretreatment of wood biomass (Wang *et al.*, 2018). Values strongly suggest the predominance (about 95% of SCOD) of oligosaccharides and proteins in the liquid phase. Indeed, the liquid fraction obtained after centrifugation had variation in color according to the pretreatment temperature and turned darker at high temperatures likely due to the solubilization of sugars and their subsequent caramelization. Previous works agree with these results and consistently showed that the liquid fraction resulting from the pretreatment of LW like the feedstock used in the current research is mainly composed of oligosaccharides (higher than 95% on a mass basis). Xylose and glucose, followed by galactose, were found as the major components for severity factors similar to the reported in this work, at a temperature of 180 °C. In these conditions, the resulting SCCA concentrations were very low, with values under 1 gCOD L^{-1}, which agrees with our results (Ballesteros *et al.*, 2011; Cara *et al.*, 2006, 2008). Nitrogen solubilization was also dependent on temperature, with up to 1.4 gN L^{-1} at 180 °C, and a 52% increase in inorganic nitrogen content ($NH4^{+}$) compared to the 120 °C. At first glance, 180 °C seems to be the most interesting pretreatment due to the higher solubilization of organic compounds.

4.I.9. Biochemical methane potential tests

Figure I.10 shows the results from BMP tests on the raw waste and the solid fractions after pretreatment. An increase in the cumulative methane yield as pretreatment temperature increases is clearly observed. 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 closer to the industrial scale. For that reason, the effect

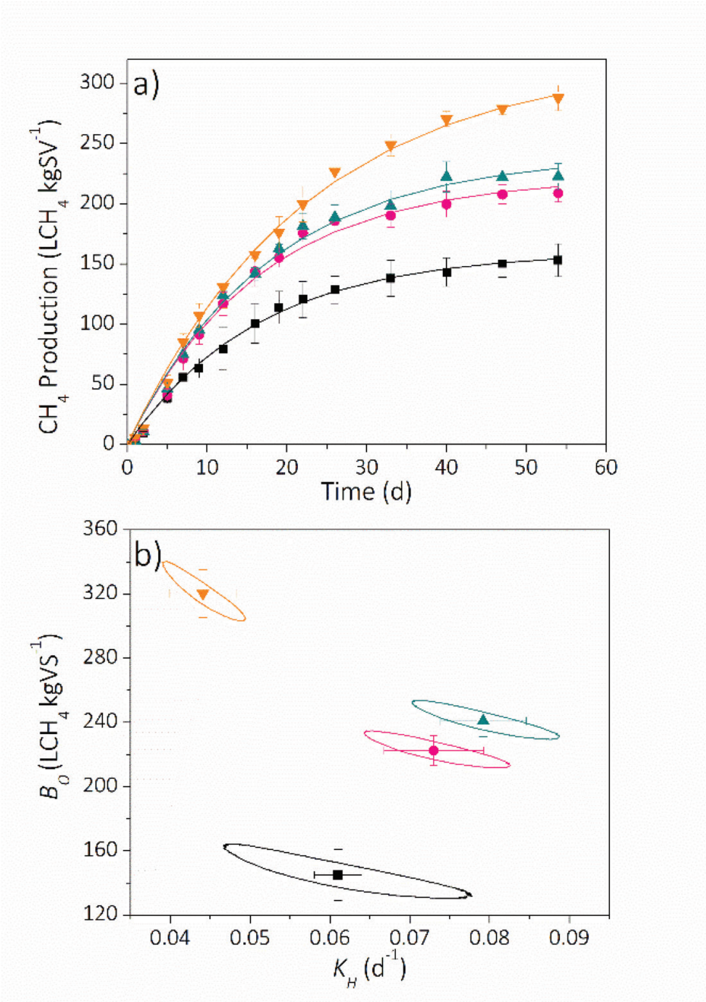
Figure I.10 BMP tests (a) and 95% confidence regions for the first-order kinetic parameters (b) during the anaerobic digestion of the raw waste (■) and the solid fraction after 120 °C (●), 150 °C (▲) and 180 °C (▼) pretreatments.



of the pretreatment over the biodegradability of the solid phase alone has been ignored in this work. The raw untreated waste produces an important amount of methane (145 ± 14 LCH_4 gVS_0^{-1}). 180 °C pretreatment resulted in the highest cumulative methane yield, representing an increase in methane production of 27% compared to the raw sample.

The increment is noteworthy despite the removal of liquid fraction, which may reduce the methane potential of the sample significantly. Figure I.11 shows that the anaerobic digestion of raw slurry yielded up to 53% higher methane potential than the solid fraction alone, indicating that the removal of the liquid fraction decreased significantly the methane potential. Thereby, the increase of the BMP due to the pretreatment 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.

Figure I.11 BMP tests (a) and 95% confidence regions for the first-order kinetic parameters (b) during the anaerobic digestion of the raw waste (■) and the slurry after 120 °C (●), 150 °C (▲) and 180 °C (▼) pretreatments.



The experimental results from BMP tests have been fitted to a simple first-order model following Batstone *etal.* (2003). The 95% confidence surfaces for the hydrolysis constant (k_H) and the biodegradability extent (B_O) were calculated (Figure I.10 and I.11, respectively). Results are presented by using the real feedstock added as a basis (kg VS added), thereby the direct (nonrelative) kinetic parameters are representative of the anaerobic digestion process. Estimated values generally predicted accurately the experimental data and confirms the positive relation of the pretreatment temperature with the increase of the biogas production. As an example, the B_O of the experiment using the solid fraction upon 180 °C is 210 ± 10 mLCH4 kgVS^{-1}, which represents an improvement of 31% with respect to the value estimated for the raw waste (146 ± 10 mLCH$_4$ kgVS ').

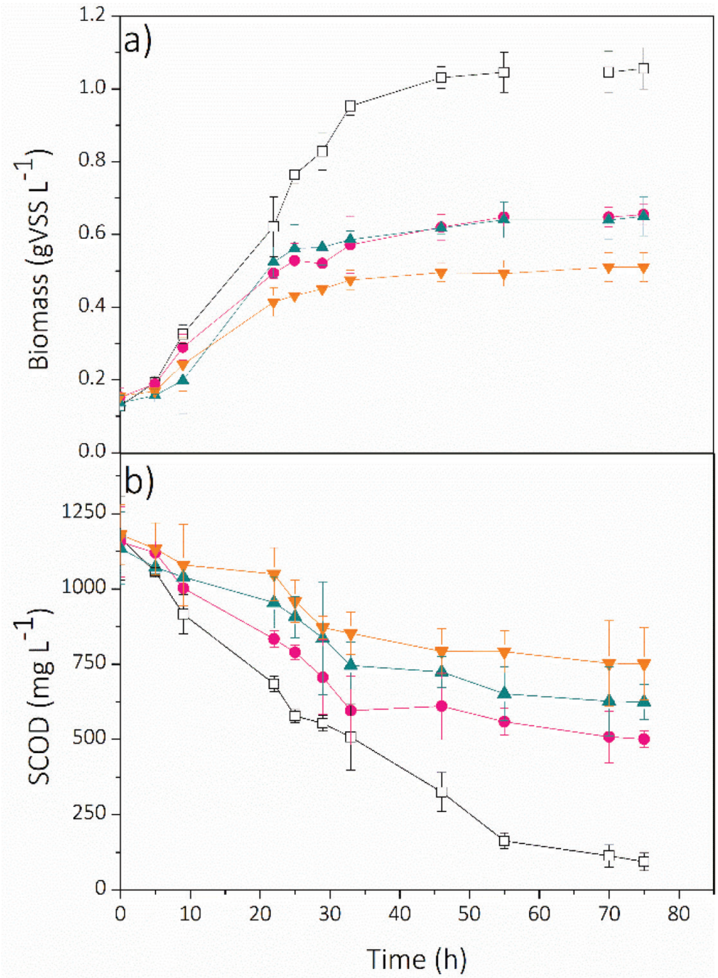
The estimated value for the k_H of the untreated sample was 0.060 ± 0.003 d^{-1}, while treated samples reached 0.078 ± 0.002, 0.113 ± 0.004 and 0.057 ± 0.005 d^{-1} for 120 °C, 150 °C and 180 °C, respectively. This means that the pretreatment increased the process rate at 150 °C but maintained at 180 °C despite the increment of the BMP. This trend is even more clear observing the results when using both solid and liquid fraction as feedstock (Figure I.11): in the 180 °C experiment, the B_O increased by 128% (320 ± 10 mLCH$_4$ kgVS^{-1}) but k_H decreased by 33% (0.045 ± 0.002 d^{-1}) with respect to the untreated biowaste. This reduction of the hydrolytic activity for high temperatures would be linked with the formation of inhibitory furan components, which have been reported for temperatures close to 180 °C (severity factor around 4) (Steinbach *et al.*, 2019). Indeed, after removing the liquid fraction, B_O decreases but k_H increases, which clearly points towards the solubilization of a large portion of the organic fraction, but also the presence of potentially inhibitory organics. Hence, the increase of the severity may amplify the formation of these compounds, making the organic matter recalcitrant and less biodegradable. Thereby, temperatures higher than 180 °C are not recommended for this biorefinery platform.

4.1.10. Specific phototrophic activity tests

Growth process

Figure I.12 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 tests, respectively. This caused an increase of the biomass concentration at the end of the test up to 1046 ± 30 mgVSS L^{-1} in the control test and 649 ± 20 and 640 ± 30 mgVSS L^{-1} in the 120 and 150 °C. A considerable reduction occurred for the 180 °C,

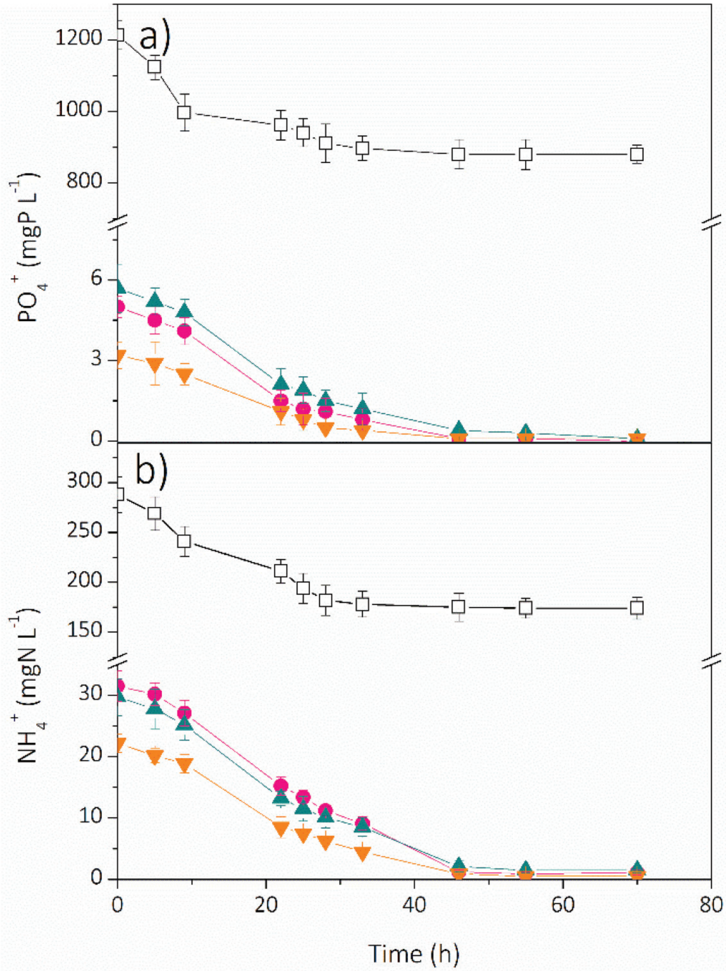
Figure I.12 Comparison of biomass growth (a) and SCOD consumption (b) in the SPA tests using the liquid fraction. Results tests for hydrolyzed at 120 °C (●), 150 °C (▲), and 180 °C (▼) are compared with a control experiment (□) with the same inoculum.



where just 37% of SCOD was consumed and biomass concentration reached 490 ± 30 mgVSS L^{-1}. In parallel, Figure I.13 shows that close to 100% of the main nutrients (N and P) were consumed, likely due to their low concentration compared to the organic carbon concentration.

Therefore, the limitation of nutrients that leads to the accumulation of PHA can be due to both N and P. Likewise, some H_2 was produced on the experimental tests, which accounted for 8, 10, and 17 mgCOD L^{-1} at the end of the

Figure I.13 Comparison of PO_4^+ (a) and NH_4^+ (b) consumption in the SPA tests using the liquid fraction. Results tests for hydrolyzed at 120 °C (●), 150 °C (▲), and 180 °C (▼) are compared with a control experiment (□) with the same inoculum.



120, 150, and 180 °C tests, respectively. H_2 production was coincident with ammonium depletion, and the control experiment did not produce H_2 due to an excess of nutrients. The highest COD/N ratio found at the 180 °C test was coincident with the lowest SCOD consumption and the highest biohydrogen production. This indicates that the nutrient limitation was the main cause of the lower COD consumption efficiency during the phototrophic tests, which was furtherly analyzed through model-based analysis.

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 and Buitrón, 2020). As shown in Table I.4, the raw lignocellulosic waste contains a low N proportion, resulting in a COD/N ratio of 100/0.71. Upon pretreatment, 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 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 pretreatment, but while the temperature is rising, more carbohydrates are being solubilized, resulting in lower COD/N ratios. As it has been been shown, this had strong effects on 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 COD to nutrient ratios entails a preferential usage of COD for PHA accumulation, as the medium lacks key nutrients to allow for bacterial growth.

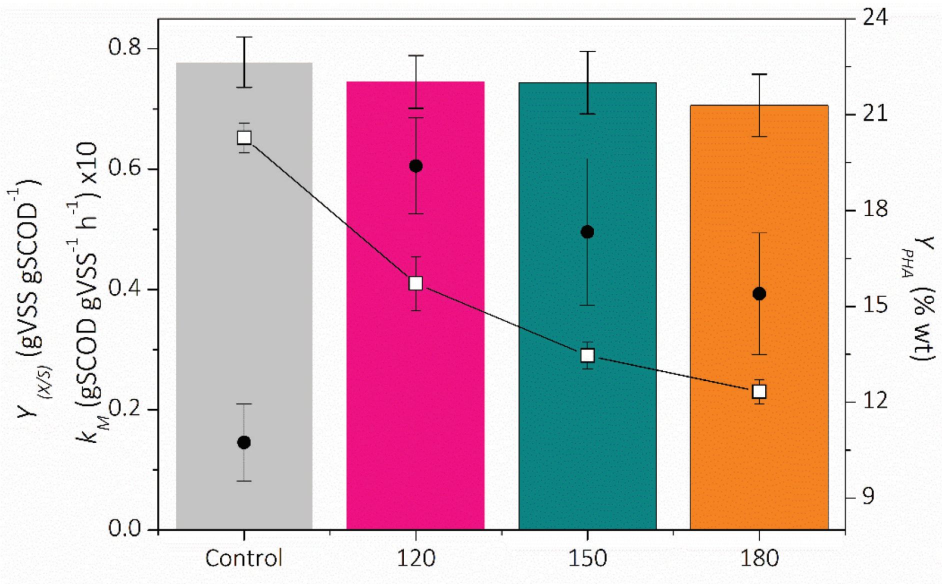
Figure I.14 depicts the kinetic parameters of the tests ($Y_{x/s}$ and k_M). The temperature caused a negligible effect on $Y_{x/s}$ while the k_M of the control was significantly higher than found for the treated samples, but the values for the experimental tests were not significantly different from each other. This is likely due to the higher biodegradability of the synthetic media in comparison with the complex hydrolysate. In any case, k_M values for the experimental control tests averaged 0.78 gSCOD gVSS^{-1} d^{-1}, which are in the same range as the values reported in the literature for simple organics (Puyol *et al.*, 2017).

The differences between the $Y_{x/s}$ calculated for the experiments at the three temperatures were also not statistically relevant, which indicates the solubilization of organic compounds does not affect the $Y_{x/s}$. 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, this data strongly supports the viability of the proposed photobiorefinery. It must be noted that the $Y_{x/s}$ values obtained in this work are generally higher than others 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 ($[C_4H_6O_2]$n) is more oxidized than PPB biomass ($CH_{1.8}O_{0.38}N_{0.18}$, (Puyol *et al.*, 2017)) (1.62 vs 1.75 gCOD gVSS^{-1}, respectively). Therefore, a detailed analysis of the PHA accumulation has been conducted.

Tests Products

Figure I.14 shows the PHA production yield (Y_{PHA}) that was measured midtrial, 30 h after the experiment began when the SCOD consumption in the

Figure I.14 Biomass yield ($Y_{X/S}$) (columns), specific phototrophic activity (k_M x 10) (□) and PHA production yield (Y_{PHA}) (●) in SPA tests using the liquid fraction at different temperatures. Results tests are compared with a control experiment using the same inoculum.



experiments was around 80%, and 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 gCOD L^{-1} was negligible. Data shows 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, respectively. 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 *et al.*, 2020). Lower Y_{PHA} on the control tests were 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). The PHA yields are significantly higher than those found in the literature (5-6.3%) when using PPB MMCs with real waste substrates (Monroy and 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 PPB MMC 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). **Nevertheless, these results are, to the best of our knowledge, the first instance to report the production of PHA in waste-lignocellulosic hydrolysates using PPB.**

Most of the studies published on MMC agree that acetate and other SCCA are the predominant carbon source for PHA production (Lee *et al.*, 2014; Reis *et al.*, 2003). But the presence of these volatile fatty acids in our liquid fractions were low (1.66% of the SCOD or up to 570 mg L^{-1} of acetic acid and 43 mg L^{-1} of propionic acid for 180 °C). 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 and Jagietto-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 have 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 PPB. 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 photo-heterotrophic process by PPB and PHA accumulation are encouraged but are beyond the scope of this study.

4.I.11. Energy balance

A theoretical energy balance of a full-scale CHP plant was estimated using the experimental data from the batch pretreatment and BMP tests and other parameters from the literature. 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 and Glasser, 1998). As shown in Table I.6, positive thermal and electrical balances were achieved on all temperatures, confirming the energetic viability of the proposed process.

Table I.6 Energy integration balance. Results were simulated for a CHP system for electricity and thermal energy production. Data referenced to a tonne (t) of LW.

Substrate	Total biogas energy kWh t^{-1}	Thermal Output kWh t^{-1}	Electrical Output kWh t^{-1}	Electrical balance kWh t^{-1}	Thermal energy balance kWh t^{-1}	Electric output Euro t^{-1}
Raw[a]	1343	739	443	428	739	64
120°C	1343	739	443	408	92	61
150°C	1398	769	461	426	26	64
180°C	1693	931	559	524	49	79

[a] *Direct anaerobic digestion (AD)*
[b] *Electrical energy produced minus that required for the centrifugation and mixing for AD.* [c] *Thermal energy produced minus that required for pretreatment and AD.*

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.I.12. Prospects and industrial implications

PHAs have gained much attention both in research and industry, but their production cost is still their greatest disadvantage. But the approach of the European Commission towards 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, opens the door to new investments and research in this field, especially in Europe.

To improve the PHA production process, industrial producers are currently working towards 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 PPB have several advantages over aerobic fermentation microorganisms, which are currently been used in commercial PHA. The use of PPB 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 PPB is 0.9 molPHA $molAcetate^{-1}$ (Fradinho *et al.*, 2019) vastly higher than any aerobic process (Bengtsson *et al.,* 2010), which could potentially increase the volumetric production of PHA.

The illumination requirement might be one of the biggest drawbacks when employing a photoheterotrophic process using PPB. 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. On the other hand, if artificial illumination is used, MPBR with cellretention systems could circumvent the problem of low biomass production. MPBR have been extensively applied in wastewater treatment with PPB (Hülsen *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} d^{-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 any case, it is key to increase the amount of SCCA in the liquid effluent, in order to make the organic matter more readily available for accumulation in PHA. **Accordingly, in the following sections of this Thesis, the acidogenic fermentation of the hydrolysate after thermal pretreatment will be studied, with the objective of increasing the percentage of SCCA present in the liquid fraction.**

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 **OFMSW**) 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 re-use of the remnant COD caused by its partial consumption during photo- heterotrophic 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 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 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.

4.I.13. 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 has been achieved). Thermal pretreatment improves the organic matter solubilization as well as digestibility of the remaining solids but may also limit PPB growth due to low nutrient release. These nutrients 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 future research.

4.I.14. References

Alloul, A., Wuyts, S., Lebeer, S., and Vlaeminck, S. E. (2019). Volatile fatty acids impacting phototrophic growth kinetics of purple bacteria: Paving the way for protein production on fermented wastewater. Water Research, 152(2019), 138-147. https://doi.org/10.1016Zj.watres.2018.12.025

Argun, H., and Kargi, F. (2011). Bio-hydrogen production by different operational modes of dark and photo-fermentation: An overview. International Journal of Hydrogen Energy, 36, 74437459. https://doi.org/10.1016/j.ijhydene.2011.03.116

Avellar, B. K., and Glasser, W. G. (1998). Steam-assisted biomass fractionation. I. Process considerations and economic evaluation. Biomass and Bioenergy, 14(3), 205-218. https://doi.org/10.1016/S0961-9534(97)10043-5

Azman, S., Khadem, A. F., Lier, J. B. Van, Zeeman, G., and Caroline, M. (2015). Critical Reviews in Environmental Science and Technology Presence and role of anaerobic hydrolytic microbes in conversion of lignocellulosic biomass for biogas production. 45(23), 2523-2564. https://doi.org/10.1080/10643389.2015.1053727

Bala, R., Gautam, V., and Mondal, M. K. (2019). Improved biogas yield from organic fraction of municipal solid waste as preliminary step for fuel cell technology and hydrogen generation. International Journal of Hydrogen Energy, 44(1), 164-173. https://doi.org/10.1016/j.ijhydene.2018.02.072

Ballesteros, I., Ballesteros, M., Cara, C., Sáez, F., Castro, E., Manzanares, P., Negro, M. J., and Oliva, J. M. (2011). Effect of water extraction on sugars recovery from steam exploded olive tree pruning. Bioresource Technology, 102(11), 6611-6616. https://doi.org/10.1016/j.biortech.2011.03.077

Basak, N., and Das, D. (2007). The prospect of purple non-sulfur (PNS) photosynthetic bacteria for hydrogen production: The present state of the art. World Journal of Microbiology and Biotechnology, 23(1), 31-42. https://doi.org/10.1007/s11274-006-9190-9

Batstone, D. J., Pind, P. F., and Angelidaki, I. (2003). Kinetics of thermophilic, anaerobic oxidation of straight and branched chain butyrate and valerate. Biotechnology and Bioengineering, 84(2), 195-204. https://doi.org/10.1002/bit.10753

Bayon-vicente, G., Wattiez, R., and Leroy, B. (2020). Global Proteomic Analysis Reveals High Light Intensity Adaptation Strategies and Polyhydroxyalkanoate Production in Rhodospirillum rubrum Cultivated With Acetate as Carbon Source. Frontiers in Microbiology, 11(March), 1-17. https://doi.org/10.3389/fmicb.2020.00464

Bengtsson, S., Pisco, A. R., Reis, M. A. M., and Lemos, P. C. (2010). Production of polyhydroxyalkanoates from fermented sugar cane molasses by a mixed culture enriched in glycogen accumulating organisms. Journal of Biotechnology, 145(3), 253-263. https://doi.org/https://doi.org/10.1016/j.jbiotec.2009.11.016

Bolatkhan, K., Kossalbayev, B. D., Zayadan, B. K., Tomo, T., Veziroglu, T. N., and Allakhverdiev, S. I. (2019). Hydrogen production from phototrophic microorganisms: Reality and perspectives. International Journal of Hydrogen Energy, 44(12), 5799-5811. https://doi.org/10.1016/j.ijhydene.2019.01.092

Brandl, H., Gross, R. A., Lenz, R. W., Lloyd, R., and Fuller, R. C. (1991). The accumulation of poly(3-hydroxyalkanoates) in Rhodobacter sphaeroides. Archives of Microbiology, 155, 337-340.

Buitrón, G., Hernández-Juárez, A., Hernández-Ramírez, M. D., and Sánchez, A. (2019). Biochemical methane potential from lignocellulosic wastes hydrothermally pretreated. Industrial Crops and Products, 139, 111555. https://doi.org/https://doi.org/10.1016/j.indcrop.2019.111555

Cabbai, V., Ballico, M., Aneggi, E., and Goi, D. (2013). BMP tests of source selected OFMSW to evaluate anaerobic codigestion with sewage sludge.

Waste Management, 33(7), 1626-1632. https://doi.org/10.1016/j.wasman.2013.03.020

Campuzano, R., and González-Martínez, S. (2016). Characteristics of the organic fraction of municipal solid waste and methane production: A review. Waste Management, 54, 3-12. https://doi.org/10.1016/j.wasman.2016.05.016

Cano, R., Nielfa, A., and Fdz-Polanco, M. (2014). Thermal hydrolysis integration in the anaerobic digestion process of different solid wastes: Energy and economic feasibility study. Bioresource Technology, 168, 14-22. https://doi.org/10.1016/j.biortech.2014.02.007

Capson-tojo, G., Batstone, D. J., Grassino, M., Vlaeminck, S. E., Puyol, D., Verstraete, W., Kleerebezem, R., Oehmen, A., Ghimire, A., Pikaar, I., Lema, J. M., and Hülsen, T. (2020). Purple phototrophic bacteria for resource recovery: Challenges and opportunities. Biotechnology Advances, 43(May), 107567. https://doi.org/10.1016/j.biotechadv.2020.107567

Cara, C., Ruiz, E., Ballesteros, I., Negro, M. J., and Castro, E. (2006). Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline peroxide delignification. Process Biochemistry, 41(2), 423-429. https://doi.org/10.1016/j.procbio.2005.07.007

Cara, C., Ruiz, E., Ballesteros, M., Manzanares, P., Negro, M. J., and Castro, E. (2008). Production of fuel ethanol from steam-explosion pretreated olive tree pruning. Fuel, 87(6), 692700. https://doi.org/10.1016/j.fuel.2007.05.008

Carvalheiro, F., Silva-Fernandes, T., Duarte, L. C., and Gírio, F. M. (2009). Wheat straw autohydrolysis: Process optimization and products characterization. Applied Biochemistry and Biotechnology, 153(1-3), 84-93. https://doi.org/10.1007/s12010-008-8448-0

Castilho, L. R., Mitchell, D. A., and Freire, D. M. G. (2009). Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solidstate fermentation. Bioresource Technology, 100(23), 5996-6009. https://doi.org/10.1016/j.biortech.2009.03.088

Choi, J., and Lee, S. Y. (1999). Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation. Applied Microbiology and Biotechnology, 51(1), 13-21. https://doi.org/10.1007/s002530051357

Dalaei, P., Bahreini, G., Nakhla, G., Santoro, D., Batstone, D., and Hülsen, T. (2020). Municipal wastewater treatment by purple phototropic bacteria at low infrared irradiances using a photo- anaerobic membrane bioreactor. Water Research, 173, 115535. https://doi.org/10.1016/j.watres.2020.115535

Dalaei, P., Ho, D., Nakhla, G., and Santoro, D. (2019). Low temperature nutrient removal from municipal wastewater by purple phototrophic bacteria (PPB). Bioresource Technology, 288. https://doi.org/10.1016/j.biortech.2019.121566

Dasgupta, A., and Chandel, M. K. (2019). Enhancement of biogas production from organic fraction of municipal solid waste using hydrothermal pretreatment. Bioresource Technology Reports, 7. https://doi.org/10.1016/j.biteb.2019.100281

Delamare-Deboutteville, J., Batstone, D. J., Kawasaki, M., Stegman, S., Salini, M., Tabrett, S., Smullen, R., Barnes, A. C., and Hülsen, T. (2019). Mixed culture purple phototrophic bacteria is an effective fishmeal replacement in aquaculture. Water Research X, 4. https://doi.org/10.1016/j.wroa.2019.100031

Duque, A., Manzanares, P., Ballesteros, I., and Ballesteros, M. (2016). Steam Explosion as Lignocellulosic Biomass Pretreatment. In Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery. Elsevier Inc. https://doi.org/10.1016/B978-0-12-802323-5.00015-3

Dwyer, J., Starrenburg, D., Tait, S., Barr, K., Batstone, D. J., and Lant, P. (2008). Decreasing activated sludge thermal hydrolysis temperature reduces product colour, without decreasing degradability. Water Research, 42(18), 4699-4709. https://doi.org/10.1016/j.watres.2008.08.019

Dziga, D., and Jagietto-Flasinska, D. (2015). Wheat straw degradation and production of alternative substrates for nitrogenase of Rhodobacter sphaeroides. Acta Biochimica Polonica, 62(3), 395-400. https://doi.org/10.18388/abp.2015_995

Eding, E. H., Kamstra, A., Verreth, J. A. J., Huisman, E. A., and Klapwijk, A. (2006). Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. Aquacultural Engineering, 34(3), 234-260. https://doi.org/10.1016/j.aquaeng.2005.09.007

Evans, R., Wallis, A. F. A., Newman, R. H., Roick, U. C., and Suckling, I. D. (1995). Changes in Cellulose Crystallinity During Kraft Pulping: Comparison of Infrared, X-ray Diffraction and Solid State NMR Results. Holzforschung, 49(6), 498-504. https://doi.org/10.1515/hfsg.1995.49.6.498

Fradinho, J. C., Domingos, J. M. B., Carvalho, G., Oehmen, A., and Reis, M. A. M. (2013). Polyhydroxyalkanoates production by a mixed photosynthetic consortium of bacteria and algae. Bioresource Technology, 132, 146-153. https://doi.org/10.1016/j.biortech.2013.01.050

Fradinho, J. C., Oehmen, A., and Reis, M. A. M. (2019). Improving polyhydroxyalkanoates production in phototrophic mixed cultures by optimizing accumulator reactor operating conditions. International Journal of Biological Macromolecules, 126, 1085-1092. https://doi.org/10.1016/j.ijbiomac.2018.12.270

Fradinho, J. C., Reis, M. A. M., and Oehmen, A. (2016). Beyond feast and famine: Selecting a PHA accumulating photosynthetic mixed culture in a permanent feast regime. Water Research, 105, 421-428. https://doi.org/10.1016/j.watres.2016.09.022

Ghosh, S., Chowdhury, R., and Bhattacharya, P. (2017). A review on single stage integrated dark-photo fermentative biohydrogen production : Insight into salient strategies and scopes. International Journal of Hydrogen Energy, 43(4), 2091-2107. https://doi.org/10.1016/j.ijhydene.2017.12.018

Hongyuan, L., Chen, J., Jia, Y., Cai, M., and Lee, P. K. H. (2016). Transcriptomic responses of the interactions between Clostridium Cellulovorans 743b and Rhodopseudomonas Palustris CGA009 in a cellulose-grown coculture for enhanced hydrogen production. Applied and Environmental Microbiology, 82(15), 4546-4559. https://doi.org/10.1128/AEM.00789-16

Hülsen, T., Barry, E. M., Lu, Y., Puyol, D., and Batstone, D. J. (2016). Low temperature treatment of domestic wastewater by purple phototrophic bacteria: Performance, activity, and community. Water Research, 100, 537-545. https://doi.org/10.1016/j.watres.2016.05.054

Hülsen, T., Barry, E. M., Lu, Y., Puyol, D., Keller, J., and Batstone, D. J. (2016). Domestic wastewater treatment with purple phototrophic bacteria using a novel continuous photo anaerobic membrane bioreactor. Water Research, 100, 486-495. https://doi.org/10.1016/j.watres.2016.04.061

Hülsen, T., Batstone, D. J., and Keller, J. (2014). Phototrophic bacteria for nutrient recovery from domestic wastewater. Water Research, 50, 18-26. https://doi.org/10.1016/j.watres.2013.10.051

Hülsen, T., Hsieh, K., Tait, S., Barry, E. M., Puyol, D., and Batstone, D. J. (2018). White and infrared light continuous photobioreactors for resource recovery from poultry processing wastewater - A comparison. Water Research, 144, 665-676. https://doi.org/10.1016/j.watres.2018.07.040

Jeffries, T. W. (1983). Utilization of xylose by bacteria, yeasts, and fungi. In Pentoses and Lignin. Advances in Biochemical Engineering/Biotechnology (pp. 1-32). Springer. https://doi.org/https://doi.org/10.1007/BFb0009101

Jeoh, T., Ishizawa, C. I., Davis, M. F., Himmel, M. E., Adney, W. S., and Johnson, D. K. (2007). Cellulase Digestibility of Pretreated BiomassIs Limited by Cellulose Accessibility. Biotechnology and Bioengineering, 98, 112-122. https://doi.org/https://doi.org/10.1002/bit.21408

Lee, D. Y., Xu, K. Q., Kobayashi, T., Li, Y. Y., and Inamori, Y. (2014). Effect of organic loading rate on continuous hydrogen production from food waste in submerged anaerobic membrane bioreactor. International Journal of Hydrogen Energy, 39(30), 16863-16871. https://doi.org/10.1016Zj.ijhydene.2014.08.022

Li, Y., and Jin, Y. (2015). Effects of thermal pretreatment on acidification phase during two- phase batch anaerobic digestion of kitchen waste. Renewable Energy, 77, 550-557. https://doi.org/10.1016Zj.renene.2014.12.056

Matassa, S., Boon, N., Pikaar, I., and Verstraete, W. (2016). Microbial protein: future sustainable food supply route with low environmental footprint. Microbial Biotechnology, 9(5), 568-575. https://doi.org/10.1111/1751-7915.12369

McBride, M. B. (1995). Toxic Metal Accumulation from Agricultural Use of Sludge: Are USEPA Regulations Protective? Journal of Environment Quality, 24(1), 5. https://doi.org/10.2134/jeq1995.00472425002400010002x

Meher Kotay, S., and Das, D. (2008). Biohydrogen as a renewable energy resource-Prospects and potentials. International Journal of Hydrogen Energy, 33(1), 258-263. https://doi.org/10.1016/j.ijhydene.2007.07.031

Meyer-Kohlstock, D., Schmitz, T., and Kraft, E. (2015). Organicwaste for compost and biochar in the Eu: Mobilizing the potential. Resources, 4, 457-475. https://doi.org/10.3390/resources4030457

Monroy, I., and Buitrón, G. (2020). Production of polyhydroxybutyrate by pure and mixed cultures of purple non-sulfur bacteria : A review. Journal of Biotechnology, 317(May), 39-47. https://doi.org/10.1016/j.jbiotec.2020.04.012

Murphy, J. D., and McKeogh, E. (2004). Technical, economic and environmental analysis of energy production from municipal solid waste. Renewable Energy, 29(7), 1043-1057. https://doi.org/10.1016/j.renene.2003.12.002

O'Dwyer, J. P., Zhu, L., Granda, C. B., and Holtzapple, M. T. (2007). Enzymatic hydrolysis of lime-pretreated corn stover and investigation of the HCH-1 Model: Inhibition pattern, degree of inhibition, validity of simplified HCH-1 Model. Bioresource Technology, 98(16), 2969-2977. https://doi.org/10.1016/j.biortech.2006.10.014

Passos, F., and Ferrer, I. (2015). Influence of hydrothermal pretreatment on microalgal biomass anaerobic digestion and bioenergy production. Water Research, 68, 364-373. https://doi.org/10.1016/j.watres.2014.10.015

Pattanamanee, W., Choorit, W., Deesan, C., Sirisansaneeyakul, S., and Chisti, Y. (2012). Photofermentive production of biohydrogen from oil palm waste hydrolysate. International Journal of Hydrogen Energy, 37(5), 4077-4087. https://doi.org/10.1016/j.ijhydene.2011.12.002

Prabpai, S., Charerntanyarak, L., Siri, B., Moore, M. R., and Noller, B. N. (2009). Effects of residues from municipal solid waste landfill on corn yield and heavy metal content. Waste Management, 29(8), 2316-2320. https://doi.org/10.1016/j.wasman.2009.02.009

Puyol, D., Barry, E. M., Hülsen, T., and Batstone, D. J. (2017). A mechanistic model for anaerobic phototrophs in domestic wastewater applications: Photo-anaerobic model (PAnM). Water Research, 116, 241-253. https://doi.org/10.1016/j.watres.2017.03.022

Real Decreto 506/2013, sobre productos fertilizantes, Boletín Oficial del Estado (BOE) 59 (2013). https://www.boe.es/boe/dias/2013/07/10/pdfs/B0EA-2013-7540.pdf

Reis, M. A. M., Serafim, L. S., Lemos, P. C., Ramos, A. M., Aguiar, F. R., and Van Loosdrecht, M. C. M. (2003). Production of polyhydroxyalkanoates by mixed microbial cultures. Bioprocess and Biosystems Engineering, 25(6), 377-385. https://doi.org/10.1007/s00449-003-0322-4

Ruiz-Espinoza, J. E., Méndez-Contreras, J. M., Alvarado-Lassman, A., and Martinez- Delgadillo, S. A. (2012). Effect of low temperature thermal pre-treatment on the solubilization of organic matter, pathogen inactivation and mesophilic anaerobic digestion of poultry sludge.

Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering, 47(12), 1795-1802. https://doi.org/10.1080/10934529.2012.689237

Sasikala, K., Ramana, C. V., Rao, P. R., and Kovacs, K. L. (1993). Anoxygenic Phototrophic Bacteria: Physiology and Advances in Hydrogen Production Technology. Advances in Applied Microbiology, 38(C), 211-295. https://doi.org/10.1016/S0065-2164(08)70217-X

Silva-Fernandes, T., Duarte, L. C., Carvalheiro, F., Loureiro-Dias, M. C., Fonseca, C., and Gírio, F. (2015). Hydrothermal pretreatment of several lignocellulosic mixtures containing wheat straw and two hardwood residues available in Southern Europe. Bioresource Technology, 183, 213220. https://doi.org/10.1016Zj.biortech.2015.01.059

Steinbach, D., Wüst, D., Zielonka, S., Krümpel, J., Munder, S., Pagel, M., and Kruse, A. (2019). Steam explosion conditions highly influence the biogas yield of rice straw. Molecules, 24(19), 114. https://doi.org/10.3390/molecules24193492

Wang, D., Shen, F., Yang, G., Zhang, Y., Deng, S., Zhang, J., Zeng, Y., Luo, T., and Mei, Z. (2018). Can hydrothermal pretreatment improve anaerobic digestion for biogas from lignocellulosic biomass? Bioresource Technology. https://doi.org/10.1016/j.biortech.2017.09.197

SECTION II: ACIDOGENIC COFERMENTATION

Preamble and rationale

The LW is the least biodegradable part of the OFMSW. According to the latest published data, in Spain, 42% of the MSW is the organic fraction (OFMSW), 20% of which is lignocellulosic pruning and gardening waste. This section aims to settle the possible synergistic effect when co-fermented FW and LW wastes produce SCCA and H_2. For the first time, the work also analyses the effect of a steam explosion pretreatment on the co-fermentation performance. Finally, this work explores several strategies for producing high- value-added products from SCCA within the framework of the EU bioeconomy.

Experimental design

The experiments have been conducted using as substrate the FW and LW described in Chapter 3. The steam explosion pretreatment was performed at 150 °C and 40 min of reaction, since these are the conditions optimized by our research group in the context of the DEEP PURPLE project and the results are currently in preparation. The acidogenic fermentation of FW and LW with mixtures of 20%, 50% and 80% LW by volume of VS, both without and after pretreatment, is studied. The experimental desing conditions are summarized in Table II.1.

The results of this section have been published or is in preparation in the following papers:

Allegue, L. D., Puyol, D., and Melero, J. A., (2021). Synergistic thermophilic co-fermentation of food and lignocellulosic urban waste with steam explosion pretreatment for efficient hydrogen and carboxylic acid production. Biofpr, 16, 499-509.

Tabla II.1 Summary of the experimental design used in section II.

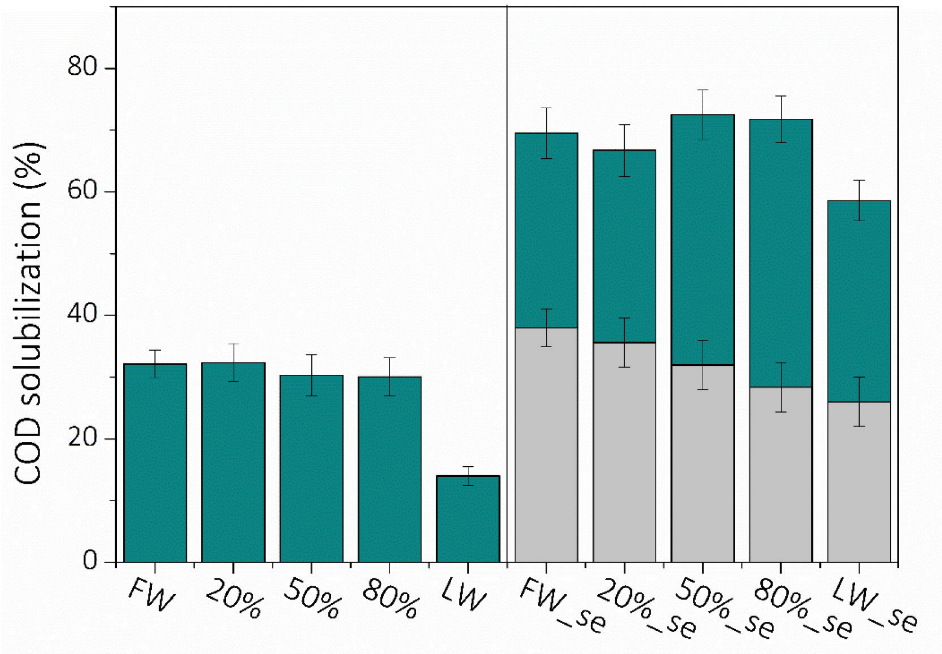
Variable / Process	Section II
Waste	FW and LW
Thermal hydrolysis	Steam explosion reactions 150 °C and 40 min
Acidogenic fermentation	Thermophilic batch tests of the untreated and pretreated wastes. Mixtures with 20%, 50% and 80% of LW in volume.

Villamil, J. A., Allegue, L. D., Pérez-Elvira, S., Martinez, F., Melero, J. A., and Puyol, D. (2022). New concept for valorization of OFMSW : coupling steam explosion pre-treatment with PHA production by purple phototrophic bacteria, and anaerobic digestion . *In preparation*

4.Ii.1. COD solubilization and steam explosion pretreatment

Figure II.1 reports the COD solubilization in the fermentation process. Usually, thermophilic temperatures favor the solubilization of organic matter compared to mesophilic temperatures, as previously observed (Soomro *et al.*, 2020). The solubilization of COD for FW reached 32%. However, the hydrolytic performance in the LW case was significantly lower (14%). The proliferation of hydrolytic microorganisms inherent to food waste and the difference in compositional characteristics of LW and FW may have impacted the hydrolytic microbial populations in batch tests, resulting in reduced hydrolysis of LW (Arras *et al.*, 2019). The co-fermentation processes offer a positive synergy

Figure II.1 Effect of steam explosion pretreatment (grey bars) and acidogenic fermentation (dark cyan bars) on COD solubilization for different mixtures of FW and LW.



since COD solubilization of approximately 30% is achieved, even for a high percentage of LW (80%), probably due to these different communities.

Soluble COD increases coupling steam explosion pretreatment and acidogenic fermentation. Table II.2 shows the main characteristics of the pretreated substrates as FW_se and LW_se. The decrease in VS mainly indicates that hemicellulose and cellulose were decomposed to lower molecular organics, mostly oligo- and monosaccharides and some organic acids (Bundhoo *et al.*, 2015). Lipids are hydrolyzed into fatty acids through beta oxidation (Yin *et al.*, 2014). On the other hand, protein is hardly decomposed during hydrothermal pretreatment (Pavlovic *et al.*, 2013), which agrees with our results. We obtained a 42% TS destruction and 38% COD solubilization of the FW, and a 30% TS destruction and 26% COD solubilization of the LW. However, the total C: N ratio of the FW after pretreatment was 100:3.1, whereas it was 100:1.4 in the liquid phase. This data indicates preferential dissolution of organic carbon instead of nitrogen. A similar pattern occurs after the LW pretreatment, where a total C/N ratio was 100:1.9, but the liquid fraction was 100:1.0. Also, a small amount of SCCA was released, being acetic acid the most abundant, accounting for 72% of the obtained SCCA.

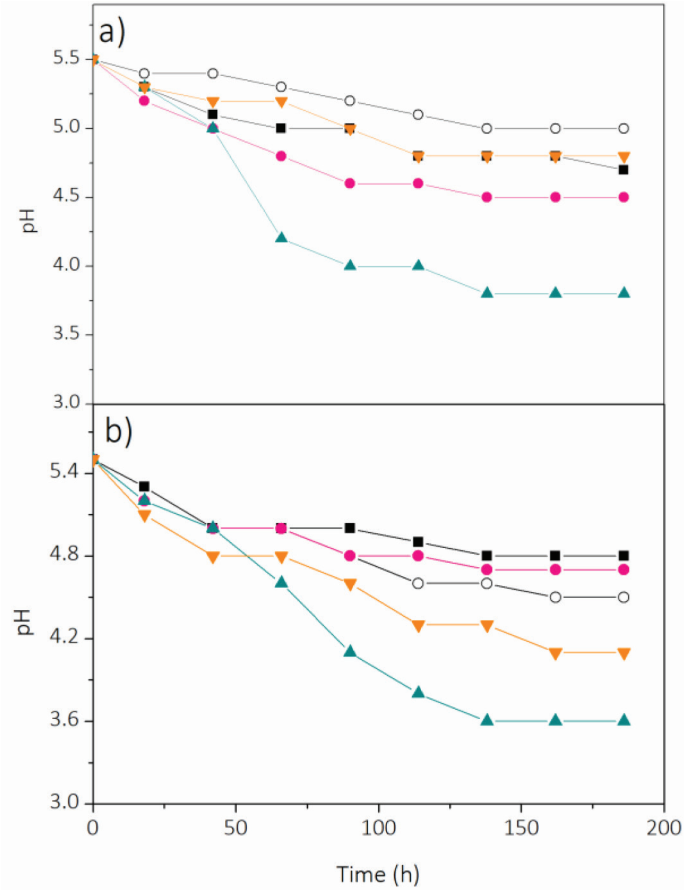
The pretreatment also improved the solubilization of COD in the fermentation process, with similar solubilizations of around 44% for FW_se and 20%_se and LW_se. For 50%_se and 80%_se, solubilizations of 50% and 53% occurred, respectively, with maximum solubilization through both processes close to

Table II.2 Average and 95% confidence intervals of the macroscopic characteristics of the organic solid waste used in this studio: FW (Food Waste), LW (Lignocellulosic waste), FW_se (Food waste after the steam explosion), LW_se (Lignocellulosic waste after the steam explosion).

	FW	LW	FW_se	LW_se
TS (g kg^{-1})	115 ± 12	950 ± 12	55 ± 14	66 ± 16
vs (g kg^{-1})	99 ± 6	911 ± 11	48 ± 9	75 ± 6
TKN (gN $kgTS^{-1}$)	3.2 ± 0.5	2.5 ± 0.8	3.6 ± 0.9	2.6 ± 1.2
TKN (mgN L^{-1}) [a]	150 ± 15		644 ± 22	389 ± 33
TCOD (g L^{-1})	122 ± 5	1075 ±8**	115 ± 10	136 ± 13
SCOD (g L^{-1}) [a]	9.5 ± 0.6		46.2 ± 5.8	38.5 ± 7.5
HAc (% SCOD) [a]			0.5 ± 0.1	2.2 ± 0.3

[a] *In the liquid phase **g gTS^{-1}*

Figure II.2 Change in pH pattern during the batch fermentation using non-pretreated waste (a) and pretreated waste (b). Wastes used were 100% FW (•), 100% LW (o), and mixtures of 20:80% (●), 50:50% (▲) and 80:20% (▼) of FW and LW, respectively.



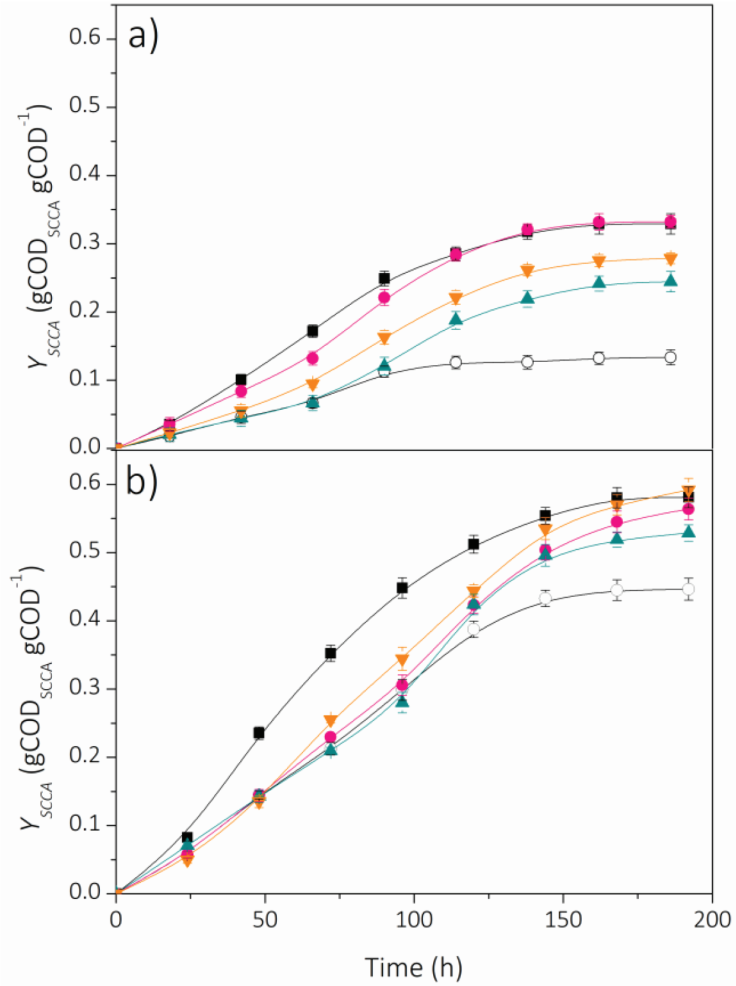
80% of the COD. These results showed a highly efficient combined treatment in COD solubilization, with an intimate link to solids destruction and process waste reduction (Yin *et al.*, 2014).

Another critical factor in the hydrolytic process is the pH, as a decrease of the pH values below 4.5 - 5.0 strongly inhibits the hydrolytic and fermentative bacteria (Moretto *et al.*, 2019). Figure II.2 shows the pH evolution and in almost all cases the pH drop occurs down to 5. We encourage further studies to focus on thermophilic operations under strict pH control to sustain high hydrolysis rates and reduce the fermentation time.

4.II.2. Short Chain Carboxylic Acids production

Figure II.3 depicts the time course of the released SSCA on a total COD added basis during the acidogenic fermentation. The endogenous production of the inoculum is negligible, but it is still subtracted from the production. Figure II.2a summarizes the results of the SCCA production in acidogenic fermentation when using non-pretreated substrates. Y_{SCCA} obtained with FW is 0.33 ± 0.01 $gCOD_{SCCA}$ $gCOD_{added}^{-1}$, and that of the LW is significantly lower with 0.13 ± 0.01

Figure II.3 SCCA production during batch tests on non-pretreated (a) and pretreated wastes (b) at different FW-LW ratios: 100% FW (■), 100% LW (o), and mixtures of 20:80% (●), 50:50% (▲) and 80:20% (▼) of FW and LW, respectively.



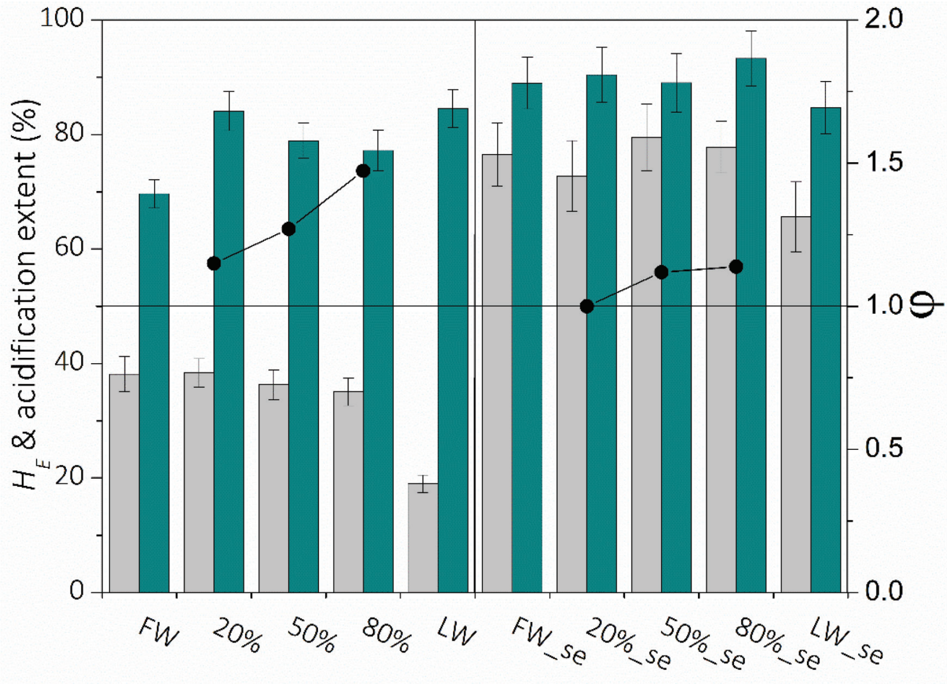
$gCOD_{SCCA}$ $gCOD_{added}^{-1}$. Data on SCCA production has not been as extensively reported as on hydrogen production. An overview of the available literature shows that the range of transformation into SCCA for wastes of these characteristics, including studies on continuous reactors, is 0.06 to 0.61 $gCOD_{SCCA}$ $gCOD_{added}^{-1}$ (Garcia-Aguirre *et al.*, 2017; Moretto *et al.*, 2019; Soomro *et al.*, 2020; Yin *et al.*, 2014). Considering that this range involves production with substrates that have undergone pretreatment or continuous reactors, the FW results are substantial. Concerning the different co-fermentations, in this case, a strong positive synergistic effect is observed. The results were 0.33 ± 0.01, 0.24 ± 0.02 and 0.28 ± 0.01 $gCOD_{SCCA}$ $gCOD_{added}^{-1}$ for the 20% 50% and 80% mixtures, respectively. With these data, we can calculate a Y_{SCCA} positive synergistic effect, with an increase in yield with respect to the theoretical of 15%, 5%, and 61%, respectively.

Figure II.2b shows the results obtained on SCCA production using pretreated substrates. The Y_{SCCA} obtained for FW_se was 0.56 $gCOD_{SCCA}$ $gCOD_{added}^{-1}$, representing a 70% increase compared to the untreated sample. This result is among the highest in the literature range, and considering that these are batch tests, there is room for improvement in continuous mode as previously demonstrated, for example, in Yin *et al.*, (2016). In the article by Yin *et al.* (2014), the increase was 42.5% in batch-type tests. Although the result obtained for LW_se is the lowest (Y_{SCCA}: 0.44 $gCOD_{SCCA}$ $gCOD_{added}^{-1}$), this represents an increase of 215% compared to the same waste without pretreatment. Again, minimal research has been done on SCCA production with this substrate when combining steam explosion pretreatment with fermentation. For example, in the article by Perimenis *et al.* (2016), they reported very low productions on substrates with high lignin and cellulose content such as wheat bran (0.17 $gCOD_{SCCA}$ $gCOD_{added}^{-1}$) or on miscanthus (0.11 $gCOD_{SCCA}$ g COD_{added}^{-1}), but even worse results (0.17 vs. 0.22 $gCOD_{SCCA}$ $gCOD_{added}^{-1}$) on wheat bran. The co-fermentation achieved the highest yields in our work, with 0.58 $gCOD_{SCCA}$ $gCOD_{added}^{-1}$ for the 80%_se mixture and 0.56 and 0.57 $gCOD_{SCCA}$ $gCOD_{added}^{-1}$ for the 20%_se and 50%_se mixtures, respectively. This result translates to a positive synergistic effect and an increase in Y_{SCCA} of 5%, 6%, and 8%, respectively. Although the synergistic effect was clear without pretreatment, the overall performance in SCCA production was significantly superior after pretreatment by steam explosion.

4.II.3. Process efficiency: Hydrolysis extent, acidification, and synergy effect

Figure II.4 shows the variations in the acidification extent, the hydrolysis extent (H_E), and the synergy effect (φ). The H_E is assimilable to the efficiency of the process. It represents the particulate COD solubilized and hydrolyzed during the fermentation by the hydrolytic bacteria and the hydrogen produced

Figure II.4 Overall productivities of the fermentation process. Grey bars represent hydrolysis extent (H_E), and dark cyan bars show acidification degrees (AD). Black dots show the synergy index (*φ*).



from that SCOD. COD solubilization is far more relevant in establishing this parameter than hydrogen production since the $CODH_2$ in these fermentation trials ranged from 0.9 to 2.1% of the initial total COD. Without pretreatment, the maximum H_E achieved was 38%, observed with FW. This result fairly agrees with others reported in the literature, ranging from 31 to 40% (Soomro *et al.*, 2020). Furthermore, the LW got the lowest H_E with 19%, which concurs with the more serious difficulty of hydrolyzing lignocellulosic wastes.

Co-fermentations yielded very positive synergistic effects (*φ*), the highest being 1.53 for the 80:20 FW/LW mix ratio and 1.12 and 1.27 for the 20:80 and 50:50 mix ratios, respectively. These results confirm the possibility of using this technology with the usual mixtures obtained from municipal solid waste. The use of lignocellulosic wastes as co-substrate considerably improved the efficiency of the process compared to the treatment of single substrates. This type of synergy was also observed, for example, in the co-fermentation of MSW and LW for bioethanol production, where synergistic effects of between 1.10 and 1.49 were achieved (Zhang *et al.*, 2020). Also, Soomro *et al.* (2020) reported a synergistic effect between synthetic food and paper waste, with synergies ranging between 1.08 and 1.66. Considering that the substrates used in this study are urban wastes, pruning LW is much less biodegradable than paper waste, and the batch nature of our tests, the synergistic effect of our results is highly remarkable.

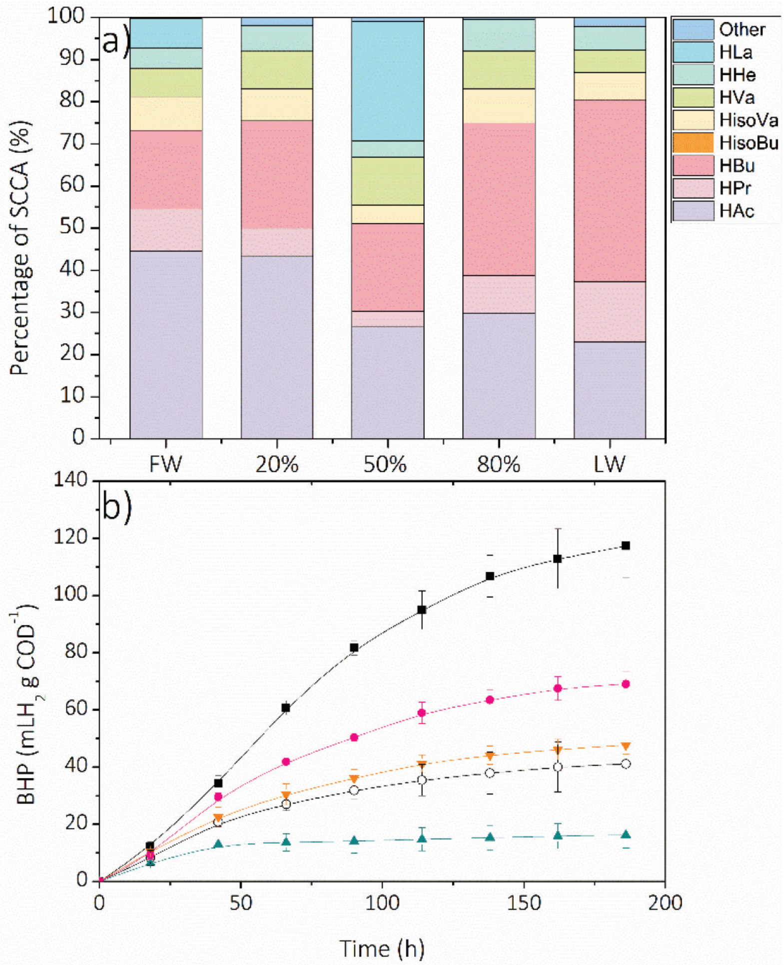
On the other hand, as shown previously, the steam explosion pretreatment considerably improved the fermentation efficiency, using both single and mixtures of urban waste. The H_E reached up to 72% and 65% on single FW and LW fermentation, respectively, representing a 100% and 245% increase compared to the non-pretreated process. But co-fermentations reached the highest H_E, achieving the following results: 72, 79, and 77% for 20%_se, 50%_Se, and 80%_se, respectively. In this case, the synergies obtained were slightly lower than those found in the non-pretreated experiments, with φ values of 1, 1.12, and 1.15, respectively. Again, these results confirm the suitability of mixing these wastes within an acidogenic fermentation process.

The acidification obtained without pretreatment are in the range of 7085%. In this case, the highest acidification range was for the LW, undoubtedly due to the soluble protein and carbohydrates still present in the FW. For this parameter, we found a wide range of results in the literature, from 48% to 94% (Moretto *et al.*, 2019; Perimenis *et al.*, 2016). This study also showed an increase in the FW_se from 69% to 89%. However, in the literature, we found that thermal pretreatments can be detrimental to this parameter. Yin *et al.* (2014), reported that the acidification range decreases from 80% to 70% after a thermal pretreatment at 140ºC. On the other hand, in the LW_se, acidification does not vary, keeping it at 85%. Previous studies with lignocellulosic residues showed a slight increase when miscanthus was used (from 79 to 83%) or even a decrease with wheat bran (from 65 to 55%) (Perimenis *et al.*, 2016)v. In this case, the different co-fermentation mixtures obtained the highest acidification range values with 90, 89, and 93% for 20, 50, and 80%, respectively, again demonstrating the improvement of the process caused by mixing these wastes.

4.II.4. SCCA Composition and Hydrogen Production

Figure II.5a shows the distribution of SCC for the different non thermally pretreated wastes. The features of the waste stream can change the microbial community and change the dominant species in the anaerobic digestion process (Garcia-Aguirre *et al.*, 2017). As shown in Figure 5.4a, when the substrate is mainly composed of FW, acetic acid is the primary fermentation product (up to 44%), and lactic acid production is close to 6%. Indeed, acetogenic fermentation is the most common pathway during FW fermentation due to the equilibrated composition in carbohydrates, lipids, and proteins (Yin *et al.*, 2014). On the other hand, the LW drifted to butyric fermentation

Figure II.5 Distribution of SCCA at the end of batch tests using non-pretreated waste sources (a) and associated H2 production (b) at different FW-LW mix ratios. FW (●), LW (O), and mixtures of 20% (●), 50% (▲) and 80% (▼). HAc: Acetic acid, HPr: Propanoic Acid, HBu: Butiric acid, HisoVa: Isovaleric acid, HVa: Valeric acid, HHe, Hexanoic acid and HLa: Lactic acid.



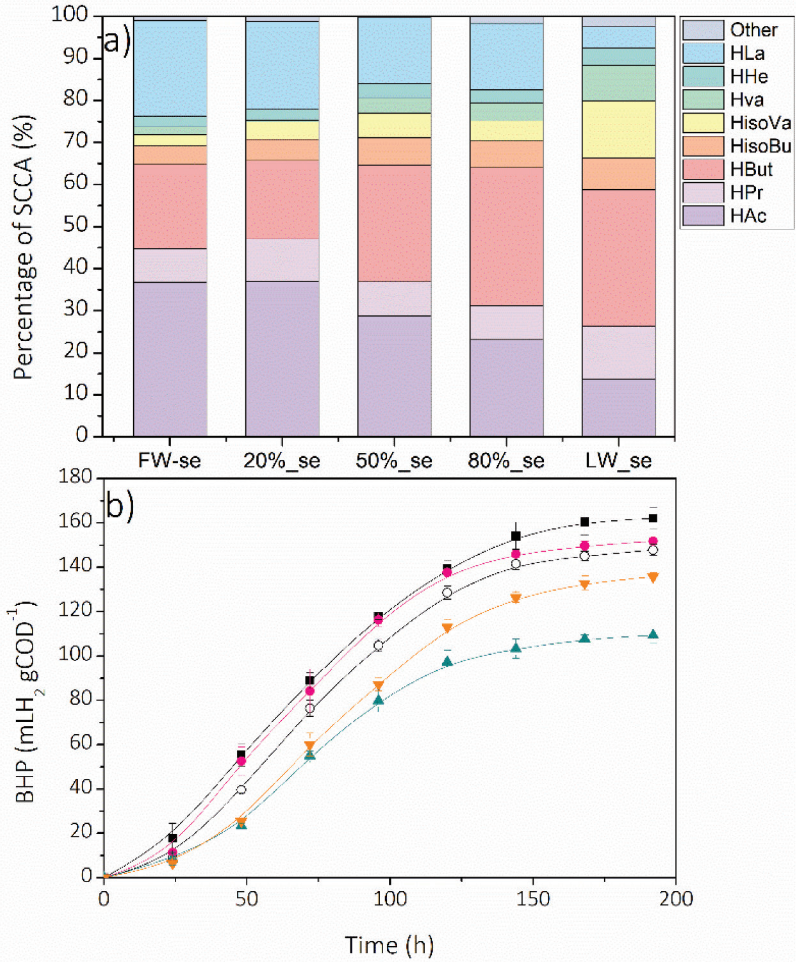
(up to 43% butyric acid), and no lactic acid appeared. Butyric fermentation is also typical when dealing with LW residues, mainly composed of sugars and oligomers (Perimenis *et al.*, 2016). Also, LW causes an increase in propionic acid production (14% compared to 10%) but a slight reduction in valeric and isovaleric acid compared to FW. Both urban wastes yielded up to 5% hexanoic acid, and oddly, no iso-butyric acid production was observed in almost all conditions. Other carboxylic acids found in small traces were caproic, oxal-acetic, succinic, or fumaric acid. The maximum observed was 2% caproic acid obtained with LW. However, the prominent exception occurred in the 50:50 mix condition, where a high generation of lactic acid happened. This lactic acid production is consistent in triplicates and reaches up to 28% prominence, which required further analysis that was possible after checking the hydrogen production data.

Figure II.5b shows hydrogen production from the batch tests with nonpretreated single and mixed substrates. Hydrogen was produced successfully with both substrates, although production is much higher with FW than with LW. Hydrogen yields were 117 ± 11 and 41 ± 8 mlH_2 $gCOD^{-1}$ added for the FW and LW experiments, respectively. The mixtures produced 69 ± 4, 16 ± 4 and 47 ± 3 mlH_2 $gCOD^{-1}$ for 20:80, 50:50 and 80:20 FW/LW mix ratios, respectively. The literature reported highly variable values of hydrogen production from the urban wastes, ranging from 12 - 248 mlH_2 $gCOD^{-1}$ (Basak *et al.,* 2020; Bundhoo *et al.*, 2015; Kuang *et al.*, 2020). This variability is mainly due to the heterogeneity of the substrate and the different technologies and pretreatments used. Our results fall in the middle of this range. However, these are significant results considering that neither the substrates nor the inoculums were pretreated.

Figure II.6a shows the analysis of the fermentation products coming from the pretreated mixtures. The fermentation of FW is still driven to acetic acid (36%) but at a lower percentage than without pretreatment. In addition, the fermentation of FW promoted the production of lactic acid (23%). Again, the fermentation of LW fostered butyric acid production, although in lower values (up to 33%) than without pretreatment. LW_se also produces lactic acid, up to 5%, which was much lower than in FW. Indeed, the mixtures promoted the production of lactic acid when the proportion of FW increased. In contrast, when the ratio of LW increased, the fermentation products trend towards propionic acid (8% -12%), valeric acid (2% - 8%), and isovaleric acid (2.7 - 13.5%), to clear detriment of the lactic acid production. Besides, isobutyric acid appeared in all conditions, which differed from the tests without pretreatment.

Figure II.6 shows the time course of the hydrogen production during these experiments. Hydrogen production on the pretreated substrates increased considerably. A 28% increase in hydrogen production after the steam explosion was achieved for FW. This increase is slightly higher than typical values in the literature where hydrogen yield improved by 5-20 times compared to the control (Bundhoo *et al.*, 2015). For the pretreated lignocellulosic residue LW_se, an increase of 260% was obtained. The highest increase found in the literature is with an ultrasonic pretreatment with dilute HCl, where a 311% increase in hydrogen production is obtained (Datar *et al.*, 2007). As for the cofermentation mixtures, the results obtained were as follows: 152 ± 2, 109 ± 4, and 136 ± 2 mlH_2 $gCOD^{-1}$ for 20%_se, 50%_se, and 80%_se, respectively, which indicates a reduction in hydrogen production in the co-fermentations,

Figure II.6 Distribution of SCCA at the end of batch tests using pretreated waste sources (a) and associated H2 production (b) at different FW-LW mix ratios. FW (●), LW (O), and mixtures of 20% (●), 50% (▲) and 80% (▼).HAc: Acetic acid, HPr: Propanoic Acid, HBu: Butiric acid, HisoBut: Isobutiric acid, HisoVa: Isovaleric acid, HVa: Valeric acid, HHe, Hexanoic acid and HLa: Lactic acid.



except for 20%_se. Overall, hydrogen yields after pretreatment are higher than without pretreatment, and differences between substrates are reduced.

Based on our results, the predominance of acetic acid formation links with a high hydrogen yield, which agrees with the available literature (Hoelzle *et al.*, 2014). Likewise, the butyric acid pathway can produce an excess of acetate as the main metabolites, yielding high hydrogen production. The third most- produced metabolite in almost all cases is lactic acid, but its production tends to increase when there is more presence of LW. On the other hand, when the substrate is LW, the production of valeric, iso-valeric, and propionic acid is increased. This is to be expected, since each substrate enters the fermentation metabolism at a different point, and therefore has a unique set of pathways and yields available to it. The SCCA production profiles and the higher concentration of lactic acid in FW may be due to its higher starch concentration or to the naturally-occurrent presence of lactic acid-producing microorganisms in FW and is similar to the profile obtained in previous studies (Vidal-Antich *et al.*, 2021).

Low hydrogen yields are associated with propionic acid and reduced end-products such as alcohols and lactate (Ochoa *et al.*, 2021). In our experiments on substrates without pretreatment, the production of lactic acid results in a sudden drop in pH on the 50% ratio test as can be seen in Figure II.2, which appears to be inhibiting hydrogen production since an excess of electrons that may be preventing the acetogenesis process. This mixture of FW and LW contains a high concentration of sugars but also nutrients, and therefore the excess of electrons due to the consumption of sugars leads to lactic acid production, which in turn leads to a decrease in hydrogen production, as explained in Hoelzle *et al.*, (2014).

In the acidogenic fermentation with mixed microbial cultures, lactate and propionate accumulation are viewed as evidence of inhibition within the methanogenic process and are often associated with shock loads of substrate (Hoelzle *et al.*, 2021). Our results point towards that explanation, where the steam explosion pretreatment makes the organic matter much more accessible, thus increasing lactic and propionic acid production. The hydrolysis step is not quite as constraining, and the bacteria are overloaded. They can divert some of the excess electrons to the production of lactic acid, propionic acid, butyric acid, and to a lesser extent other higher molecular weight SCCA, limiting hydrogen production.

4.II.5. Implications and perspectives

A total SCCA concentration up to 0.58 $gCOD_{SCCA}$ $gTCOD^{-1}$ with a high acidification rate as herein obtained is quite promising. In this study, we have shown that, depending on the percentage of lignocellulosic residue that we have in the urban waste, we can anticipate the type of carboxylic acids produced. This fact is essential because, depending on the molecular weight and the chemical structure of the acids, their price varies considerably. For example, caproic and valeric acids have the highest market prices (3800 € $tonne^{-1}$) (Ramos-Suarez *et al.*, 2021), followed by propionic acid (2000-2500 € $tonne^{-1}$), butyric (1500-1650 € $tonne^{-1}$) (Atasoy *et al.*, 2018), lactic acid (5501950 € $tonne^{-1}$, depending on the lactic acid grade) (Manandhar and

Shah, 2020), and acetic acid (400-800 € tonne^{-1}) (Atasoy *et al.*, 2018). SCCA are fundamental building blocks of chemicals such as esters, ketones, aldehydes, alcohols, and alkanes (Greses *et al.*, 2020). But for this purpose, the different acids must be separated and purified, which may not be cost-effective. The work of Bonk and Schmidt, (2015) indicated that SCCA obtained from dark fermentation would be cost-effective if the operating costs of the separation/purification did not exceed 15 \$ m^{-3} effluent. To bypass this bottleneck, direct usage of the fermentation products in other bioprocesses is a promising alternative, wherein in any case, some physical processes like centrifugation allow the separation of solid and liquid streams. These bioprocesses entail the production of hydrogen, high added-value products like PHA and biodiesel, chain elongation products, or the removal of nitrogen and phosphorus from wastewater (Ramos-Suarez *et al.*, 2021). Biogas production from the fermentation products is not desirable as recent studies indicated that the generation of SCCA is more profitable than the more traditional AD with biogas upgrading (Bastidas-Oyanedel and Schmidt, 2018). In any case, the AD is useful to produce energy from the degradation of the solid streams upon physical separation of the fermentate, thus making the overall biorefinery process energetically more sustainable (Righetti *et al.*, 2020).

Generally, steam reforming produces massive amounts of hydrogen from non-renewable hydrocarbons, causing high greenhouse gas emissions. In the last years, research efforts focused on developing biotechnological routes to produce hydrogen in an environmentally friendly manner through acidogenic fermentation and photochemical fermentation, even in a single stage (Shiladitya *et al.*, 2017). In this platform, the synergy of both biological processes is the crucial point, where phototrophic bacteria can produce hydrogen with a conversion efficiency of up to 0.98 $gCOD_{H2}$ $gCOD_{fed}^{-1}$ (Puyol *et al.*, 2019). This platform is sui*table* for low nitrogen-bearing feedstock, like lignocellulosic hydrolysates.

A promising bioprocess is chain elongation, which appeared around ten years ago. It mainly consists of converting SCCA into medium-chain carboxylic acids such as caproic acid, enanthic acid, caprylic acid, or pelargonic acid, which, as mentioned before, have a higher market value. This process occurs by p-reverse oxidation, where an electron donor (mainly acetyl-CoA and ethanol) is oxidized to reduce an electron acceptor, e.g., acetic acid (Magdalena *et al.*, 2020). Although most studies on this technology have focused on synthetic media, upscaling chain elongation technologies are underway (Candry and Ganigué, 2021) and could be an important biotechnological pillar for the production of high value-added products from waste.

Another common application of SCCA is the biological nutrient (nitrogen and phosphorous) removal from wastewaters. Even removing P seems to be more effective by using SCCA from acidogenic fermentation than the traditional use of synthetic acetic acid. In this respect, Tong and Chen (2007) observed that the phosphorus removal efficiency was around 98% with the fermentative SCCA and about 71% using pure acetic acid. Also, several studies have demonstrated that waste-derived SCCA outperforms commercial chemicals in terms of nitrate removal efficiency and denitrification rate when used as a carbon source (Tong and Chen, 2007). Therefore, integrating SCCA production in wastewater treatment plants offers a bioeconomic alternative to removing nutrients.

One of the most promising applications for the SCCA obtained from acidogenic fermentation is PHA production, and the most common process used is sequential enrichment and accumulation steps using aerobic mixed cultures. In this sense, Bengtsson *etal.* (2017) reported an accumulation of up to 49% PHA of volatile suspended solids using SCCA from fermented organic residues as substrates in a pilot-scale demonstration (Bengtsson *et al.*, 2017). However, this technology is still looking for its breakthrough to be economically viable since PHA is not yet competitive compared to equivalent petrochemical plastics such as polyethylene terephthalate (Ramos-Suarez *et al.*, 2021).

Finally, PPB are perfect candidates for PHA production since they can obtain energy from IR light instead of oxygen, reducing operating costs. **In this Thesis, the feasibility of co-producing PHA and hydrogen from the organic fraction of municipal solid waste and lignocellulosic waste has already been demonstrated. However, several challenges remain and will be addressed in the next section of this Thesis.**

4.II.6. Conclusions

A significant synergy was found between the two most common substrates found in municipal solid waste (FW and LW) in the production of SCCA but not in the production of H_2. Also, the steam explosion pretreatment significantly increases the process yield with a maximum of 0.58 $gCOD_{SCCA}$ $gCOD^{-1}$ and a 93% acidification rate. The results obtained in this study indicate the robustness of the acidogenic fermentation technology. They can contribute to the valorization of municipal solid as the first step of a biorefinery platform dedicated to recovering added-value bioproducts (such as PHA) through a combined anaerobic multi-step process.

4.II.7. References

Arras, W., Hussain, A., Hausler, R., and Guiot, S. R. (2019). Mesophilic, thermophilic and hyperthermophilic acidogenic fermentation of food waste in batch: Effect of inoculum source. Waste Management, 87, 279-287. https://doi.org/10.1016Zj.wasman.2019.02.011

Atasoy, M., Owusu-Agyeman, I., Plaza, E., and Cetecioglu, Z. (2018). Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. Bioresource Technology, 268(July), 773-786. https://doi.org/10.1016Zj.biortech.2018.07.042

Basak, B., Jeon, B., Hyun, T., Lee, J., Kumar, P., and Lim, H. (2020). Dark fermentative hydrogen production from pretreated lignocellulosic biomass : Effects of inhibitory byproducts and recent trends in mitigation strategies. Renewable and Sustainable Energy Reviews, 133(August), 110338. https://doi.org/10.1016/j.rser.2020.110338

Bastidas-Oyanedel, J.-R., and Schmidt, J. E. (2018). Increasing Profits in Food Waste Biorefinery - A Techno-Economic Analysis. Energies, 11(6), 1511. https://doi.org/10.3390/en11061551

Bengtsson, S., Karlsson, A., Alexandersson, T., Quadri, L., Hjort, M., Johansson, P., Morgan- sagastume, F., Anterrieu, S., Arcos-hernandez, M., Karabegovic, L., Magnusson, P., and Werker, A. (2017). A process for polyhydroxyalkanoate (PHA) production from municipal wastewater treatment with biological carbon and nitrogen removal demonstrated at pilot-scale. New Biotechnology, 35, 42-53. https://doi.org/10.1016/j.nbt.2016.11.005

Bonk, F., and Schmidt, J. E. (2015). Converting the organic fraction of solid waste from the city of Abu Dhabi to valuable products via dark fermentation: Economic and energy assessment. Waste Management, 40, 82-91. https://doi.org/10.1016/j.wasman.2015.03.008

Bundhoo, M. A. Z., Mohee, R., and Hassan, M. A. (2015). Biohydrogen production from food waste Current status, limitations,.pdf. In Journal of Environmental Management (Vol. 157, pp. 20-48). Academic Press. https://doi.org/10.1016/j.jenvman.2015.04.006

Candry, P., and Ganigué, R. (2021). Chain elongators, friends, and foes. Current Opinion in Biotechnology, 67, 99-110. https://doi.org/10.1016/j.copbio.2021.01.005

Datar, R., Huang, J., Maness, P. C., Mohagheghi, A., Czernik, S., and Chornet, E. (2007). Hydrogen production from the fermentation of corn stover biomass pretreated with a steamexplosion process. International Journal of Hydrogen Energy, 32(8), 932-939. https://doi.org/10.1016/j.ijhydene.2006.09.027

Garcia-Aguirre, J., Aymerich, E., González-Mtnez. de Goñi, J., and Esteban-Gutiérrez, M. (2017). Selective VFA production potential from organic waste streams: Assessing temperature and pH influence. Bioresource Technology, 244(July), 1081-1088. https://doi.org/10.1016/j.biortech.2017.07.187

Ghosh, S., Chowdhury, R., and Bhattacharya, P. (2017). A review on single stage integrated dark-photo fermentative biohydrogen production : Insight into salient strategies and scopes. International Journal of Hydrogen Energy, 43(4), 2091-2107. https://doi.org/10.1016/j.ijhydene.2017.12.018

Greses, S., Tomás-Pejó, E., and Gónzalez-Fernández, C. (2020). Agroindustrial waste as a resource for volatile fatty acids production via anaerobic fermentation. Bioresource Technology, 297, 122486. https://doi.org/10.1016/j.biortech.2019.122486

Hoelzle, R. D., Puyol, D., Virdis, B., and Batstone, D. (2021). Substrate availability drives mixed culture fermentation of glucose to lactate at steady state. Biotechnology and Bioengineering, 118(4), 1617-1629. https://doi.org/10.1002Zbit.27678

Hoelzle, R. D., Virdis, B., and Batstone, D. J. (2014). Regulation mechanisms in mixed and pure culture microbial fermentation. Biotechnology and Bioengineering, 111(11), 2139-2154. https://doi.org/10.1002/bit.25321

Kuang, Y., Zhao, J., Gao, Y., Lu, C., Luo, S., Sun, Y., and Zhang, D. (2020). Enhanced hydrogen production from food waste dark fermentation by potassium ferrate pretreatment. Environmental Science and Pollution Research, 27, 8145-1815. https://doi.org/10.1007/s11356- 020-08207-3

Magdalena, J. A., Ballesteros, M., and González-Fernández, C. (2020). Chapter 17 - Acidogenesis and chain elongation for bioproduct development. In J. A. Olivares, D. Puyol, J. A. Melero, and J. Dufour (Eds.), Wastewater Treatment Residues as Resources for Biorefinery Products and Biofuels (pp. 391-414). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-816204-0.00017-5

Manandhar, A., and Shah, A. (2020). Techno-Economic Analysis of Bio-Based Lactic Acid. Processes, 8(2), 199.

Moretto, G., Valentino, F., Pavan, P., Majone, M., and Bolzonella, D. (2019). Optimization of urban waste fermentation for volatile fatty acids production. Waste Management, 92, 21-29. https://doi.org/10.1016Zj.wasman.2019.05.010

Ochoa, C., Hernández, M. A., Bayona, O. L., Cabeza, I. O., and Candela, A. M. (2021). Value- Added By-Products During Dark Fermentation of Agro-Industrial Residual Biomass: Metabolic Pathway Analysis. Waste and Biomass Valorization, 0123456789. https://doi.org/10.1007/s12649-021-01421-1

Pavlovič, I., Eljko Knez, *Ž*., and Kerget, M. S.[v]. (2013). Hydrothermal Reactions of Agricultural and Food Processing Wastes in Sub-and Supercritical Water:

A Review of Fundamentals, Mechanisms, and State of Research. J. Agric. Food Chem, 61(34), 8003-8025. https://doi.org/10.1021/jf401008a

Perimenis, A., Aarle, I. M. Van, Nicolay, T., Jacquet, N., Meyer, L., Richel, A., Gerin, P. A., van Aarle, I. M., Nicolay, T., Jacquet, N., Meyer, L., Richel, A., and Gerin, P. A. (2016). Metabolic profile of mixed culture acidogenic fermentation of lignocellulosic residues and the effect of upstream substrate fractionation by steam explosion. Biomass Conversion and Biorefinery, 6(1), 25-37. https://doi.org/10.1007/s13399-015-0164-8

Puyol, D., Monsalvo, V. M., Marin, E., Rogalla, F., Melero, J. A., Martínez, F., Hülsen, T., and Batstone, D. J. (2019). Purple phototrophic bacteria as a platform to create the next generation of wastewater treatment plants: Energy and resource recovery. In Wastewater Treatment Residues as Resources for Biorefinery Products and Biofuels (pp. 255-280). https://doi.org/10.1016/B978-0-12-816204-0.00012-6

Ramos-Suarez, M., Zhang, Y., and Outram, V. (2021). Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste. In Reviews in Environmental Science and Biotechnology (Vol. 20, Issue 2). Springer Netherlands. https://doi.org/10.1007/s11157-021- 09566-0

Righetti, E., Nortilli, S., Fatone, F., Frison, N., Bolzonella, D., and Nortilli, S. (2020). A Multiproduct Biorefinery Approach for the Production of Hydrogen, Methane and Volatile Fatty Acids from Agricultural Waste Statement of Novelty. Waste and Biomass Valorization, 11, 52395246. https://doi.org/10.1007/s12649-020-01023-3

Soomro, A. F., Ahmed, I., Ni, Z., Ying, L., Liu, J., Abbasi, I. A., Ni, Z., Ying, L., and Liu, J. (2020). Influence of temperature on enhancement of volatile fatty acids fermentation from organic fraction of municipal solid waste: Synergism between food and paper components. Bioresource Technology, 304, 122980. https://doi.org/10.1016Zj.biortech.2020.122980

Tong, J., and Chen, Y. (2007). Enhanced Biological Phosphorus Removal Driven by ShortChain Fatty Acids Produced from Waste Activated Sludge Alkaline Fermentation. Environmental Science and Technology, 41(20), 7126-7130. https://doi.org/10.1021/es071002n

Vidal-Antich, C., Perez-Esteban, N., Astals, S., Peces, M., Mata-Alvarez, J., and Dosta, J. (2021). Assessing the potential of waste activated sludge and food waste co-fermentation for carboxylic acids production. Science of the Total Environment, 757, 143763. https://doi.org/10.1016/j.scitotenv.2020.143763

Yin, J., Wang, K., Yang, Y., Shen, D., Wang, M., and Mo, H. (2014). Improving production of volatile fatty acids from food waste fermentation by hydrothermal pretreatment. Bioresource Technology, 171, 323-329. https://doi.org/10.1016/j.biortech.2014.08.062

Yin, J., Yu, X., Wang, K., and Shen, D. (2016). Acidogenic fermentation of the main substrates of food waste to produce volatile fatty acids. International Journal of Hydrogen Energy, 41(46), 21713-21720. https://doi.org/10.1016/j.ijhydene.2016.07.094

Zhang, L., Loh, K. C., Dai, Y., and Tong, Y. W. (2020). Acidogenic fermentation of food waste for production of volatile fatty acids: Bacterial community analysis and semi-continuous operation. Waste Management, 109, 75-84. https://doi.org/10.1016/j.wasman.2020.04.052

SECTION III: INTEGRATED PHOTOBIOREFINERY

Preamble and rationale

Having tested the feasibility of growing PHA-accumulating PPB from OFMSW hydrolysates and optimizing both the thermal pretreatment and the acidogenic fermentation process, this Section aims to optimize PHA accumulation in a PPB enriched mixed microbial culture and explore how its production is determined via organic overloading. Furthermore, this study has been carried out in a **continuous novel membrane photobioreactor (MPBR)** using for the first time an OFMSW eluent pre-treated by steam explosion and fermentation as substrate. Finally, the holistic valorization of organic waste was studied in the integrated photobiorefinery proposed to understand the overall impact of this multistrategy approach.

Experimental design

Table III.1 summarizes the most important experimental design conditions. The waste used as substrate was OFMSW, described in Chapter 3. The steam explosion pretreatment was carried out at 150 ºC and a reaction time of 40 min. The conditions chosen for the steam explosion were based on previously performed optimization experiments carried out in the context of the DEEP PURPLE project as explain in the previous Section.

The hydrolysate obtained after the steam explosion is used directly as feed for an acidogenic CSTR fermenter reactor. An OLR of 4 gCOD L^{-1} d^{-1} and a HRT of 5 d were fixed and kept for 98 d. These operating conditions were

Tabla III.1 Summary of the experimental design used in section III.

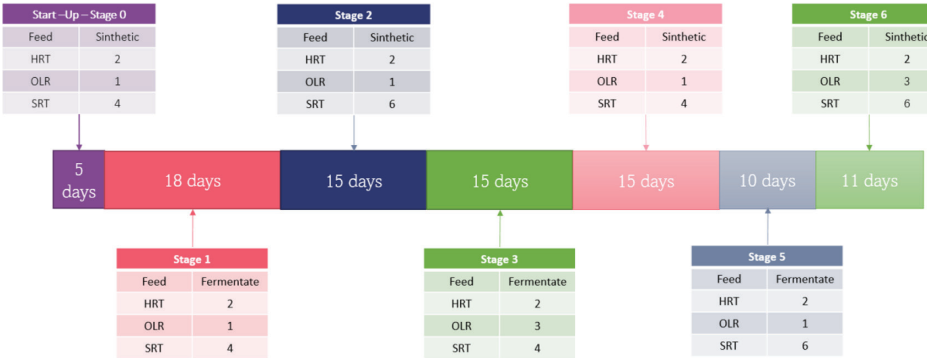
Variable / Process	Section III
Waste	OFMSW
Thermal hydrolysis	Steam explosion at 150 °C and 40 min.
Acidogenic fermentation	Continuous treatment of the pretreated waste on a CSTR at thermophilic temperature and pH 5.5
Photoheterotrophic process	Continuous treatment of the liquid fraction of the fermentate MPBR.
Anaerobic digestion	BMP batch tests of the solid fraction accumulated of the fermentate

chosen to give operational continuity to the adapted inoculum fed with AFM over a two year period, as explained in Chapter 3. The fermentate was centrifuged to separate the solids from the liquid phase. Next, the solids are fed to an BMP tests to determine its the methanogenic potential, and the liquid phase is fed to the continuous photoheterotrophic process in a MPBR.

The MPBR operation conditions (OLR and SRT) were changed to increase PHA productivity. During the first five days of operation, the MPBR was fed with MOM for acclimatization (described in Chapter 3). Afterward, the liquid fraction of the fermenter effluent was fed, and the operating conditions were varied in 7 Stages as can be seen in Figure III.1, which can be classified as Startup (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 reactor sampling was not performed from day 54 to 60. Representative samples were chosen from the MPBR twice a week for the analysis of 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.

A version of this Section is provisionally accepted or in preparation in the following papers: Allegue, L. D., Ventura, M., Melero, J. A., and Puyol, D., (2022).Unravelling PHA production from urban organic waste with purple phototrophic bacteria via organic overload. Renewable and Sustainable Energy Reviews. *Provisionally Accepted*. Gamboa, M., Allegue, L. D., Puyol, D., Dugour, J., and Melero, J. A. (2022). Environmental life cycle assessment of polyhydroxyalkanoates production by purple phototrophic bacteria. *In preparation*.

Figure III.1 Schematic timeline of changes in the operating conditions of the MPBR.

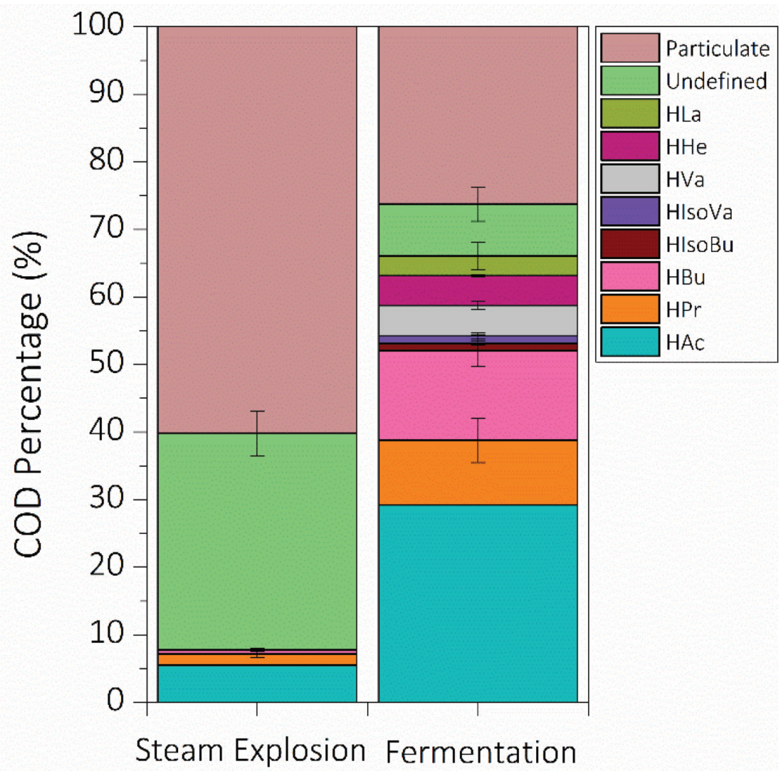


4.III.1. Steam explosion and acidogenic fermentation pretreatments

The steam explosion disruption apparatus and experimental conditions provided reproducible conditions between the different batches performed. Figure III.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, but also 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 enhance the hydrolysis rate, a limiting factor in most biological processes, including acidogenic fermentation.

A thermophilic acidogenic fermentation was carried out for nearly 100 d as a complementary pretreatment. This process significantly increases the soluble COD, especially the percentage of SCCA produced (Figure III.1). Soluble COD

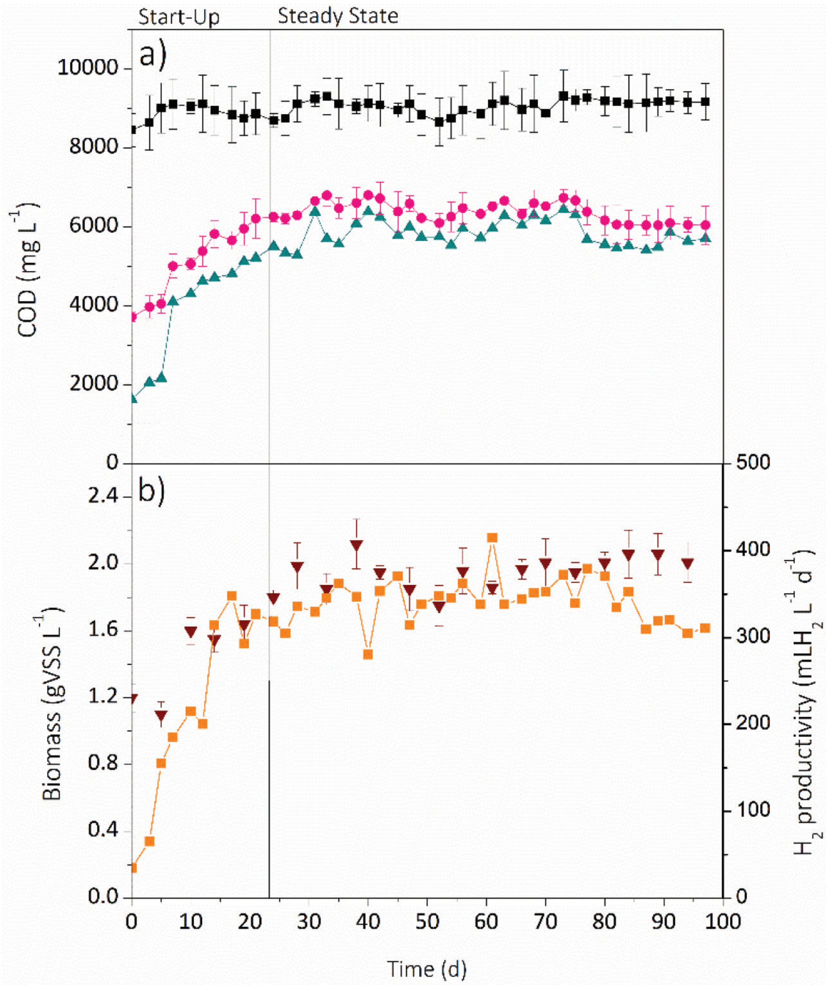
Figure III.2 Steam explosion (4 batches) and subsequent acidogenic fermentation (average of 70 d, after reaching steady-state) in terms of COD mass balance.



increased up to 74% of the total COD. A total of 65.6% of all COD was transformed into SCCA, which represents acidification of the process of 90%.

HAc and HBu are the two major acids produced, accounting for 63% of the COD equivalent of all SCCA. If we compare these results with the batch results of Section II, we can observe that we obtain a similar acidification (close to 90%) but a 13% higher SCCA production, as expected for a continuous operation. Figure III.3a shows a stabilized yield of about 0.66 gCODSCCA $gCODfeed^{-1}$. Combining both pretreatments on the OFMSW to produce SCCA

Figure III.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 in the reactor (b).



achieves high yields and high stability over time. In addition, H2 is co-produced at high yields during the acidogenic fermentation process.

Figure III.3b shows the hydrogen production and VSS amount present in the fermenter. The stabilization of both parameters is similar to the COD and H_2, which could serve as an online indicator of fermentation performance. Average H_2 productivity was 345 mLH_2 L^{-1} d^{-1}, with a hydrogen percentage of 57% in the outlet gas (43% CO2), and stabilized biomass close to 1.8 gVSS L^{-1}. In any case, based on the total COD balance, H_2 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.

4.III.2. The photoheterotrophic treatment of the liquid fraction with PPB

Start-up (S0)

Figure III.4 shows the evolution of biomass, COD removal, PHA (Y_{PHA}), glycogen (Y_{GLY}) and EPS (Y_{EPS}) accumulation and H_2 production for the different scenarios. The culture underwent an adaptation period during the first 5 d (Stage 0) to promote purple bacteria'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%. The correct acclimatization is corroborated through bacterial community analysis with a sample from day 4 (D4).

Figure III.5 shows a culture highly enriched in PPB (>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 d 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, NHT consumption is complete (Figure III.6), but no H_2 production is detected. The acclimatization Stage was considered finished on day 5 when COD removal efficiency stabilized, and hydrogen production started. Then, the liquid fraction of the fermentate was fed as a substrate.

Operation under stable biomass growth (S1, S2)

Stable biomass growth and high PHA 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

Figure III.4. MPBR operation profile for 88 d. a) shows biomass (■) and COD removal (●). b) shows PHB/PHV percentage (■) and total percentage of PHA (PHB/PHV/PHH) (●), represent glycogen percentage (▲) and represent EPS percentage (●). c) Shows hydrogen productivity (■).

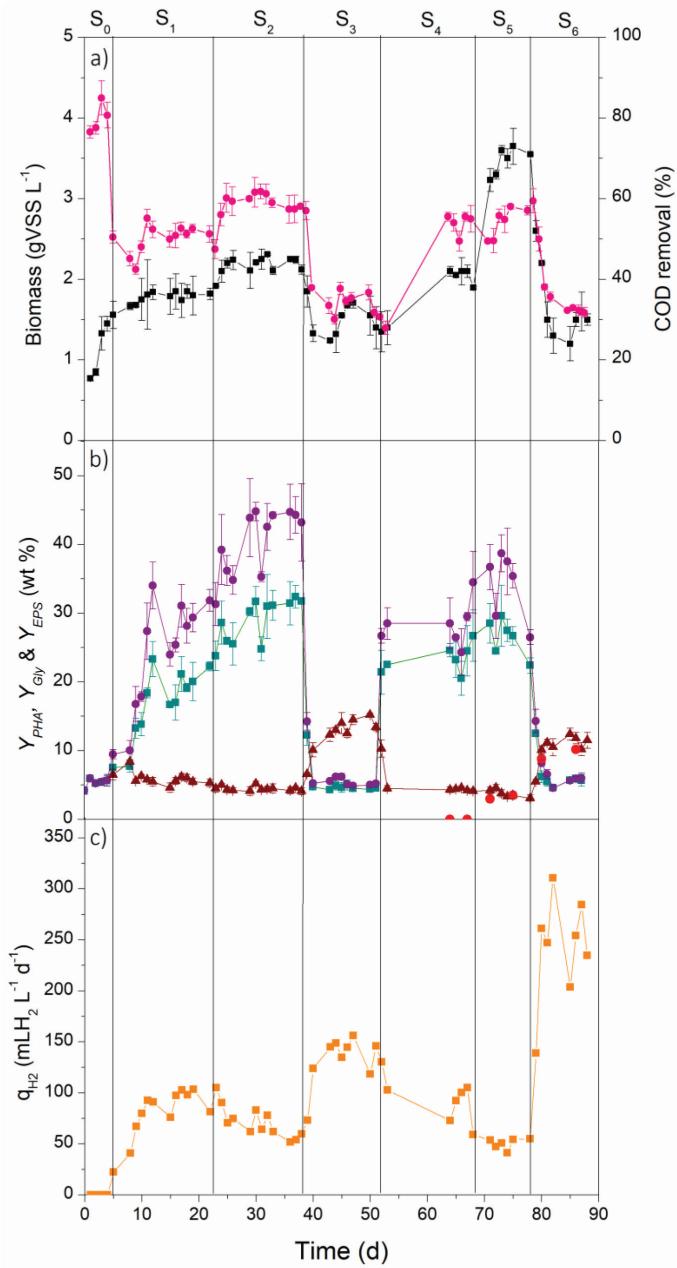
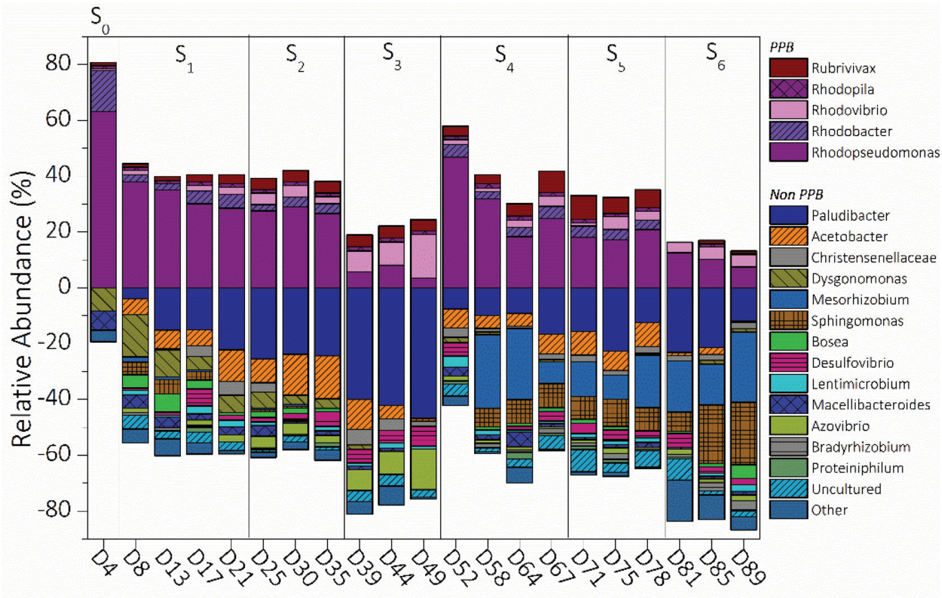


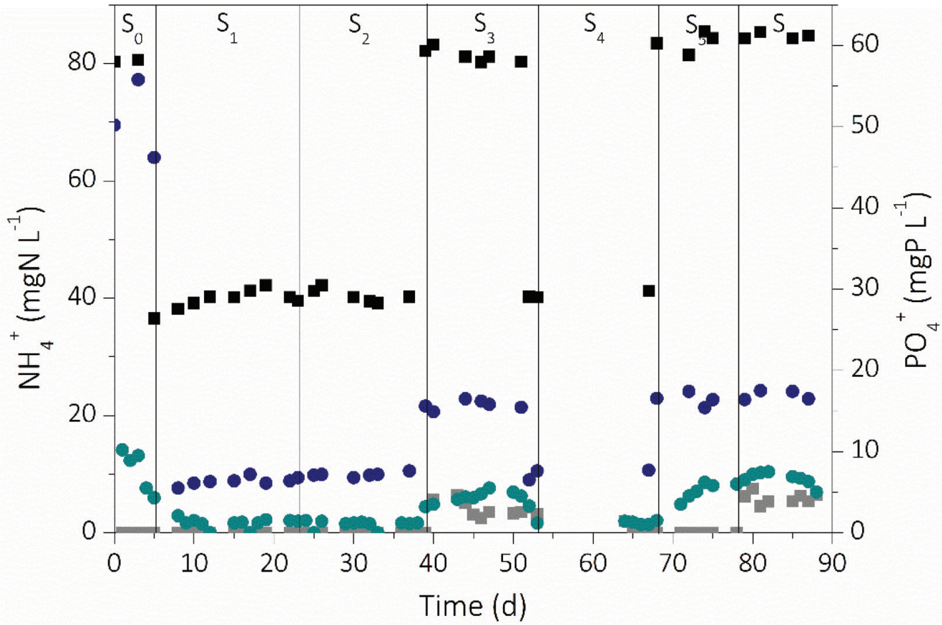
Figure III.5 Microbial community structure at genus level MPBR at different phases.



shown in Figure III.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 (Figure III.6). PHA accumulation increased significantly, and then it stabilized at around 31% dry weight during the following 12 d. This PHA comprised PHB, PHV (20% and 5% by dry weight), and PHH that was 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 d 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 (Figure III.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 possible alterations in the metabolic pathways of PHA accumulation.

Figure III.6 Nutrient profile during the MBPr operation. NH_4^+ (■) and PO_4^+ (●) in the reactor feed. NH_4^+ (■) and PO_4^+ (●) at the reactor outlet.



First carbon overload (S3)

In Stage 3, the OLR was increased up 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 Figure III.4, biomass collapsed, reducing to 1.3 gVSS L^{-1}, although after 5 d, 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 III.1. The most drastic reduction was PHA accumulation, reduced to 5%. This minimal accumulation remained stable and did not increase during the 14-d phase. As PHA accumulation dropped, glycogen increased to 12%, and hydrogen production doubled. In addition, a decrease in PPB to less than 20% was observed, with *Rhodovibrio sp.* unusually increasing to 15% (Figure III.5).

The next step would be to recover the biomass and a more PPB-enriched culture, thereby studying whether PHA accumulation is regained backing to the conditions of Stage 1.

Recovery of the biomass (S4, S5)

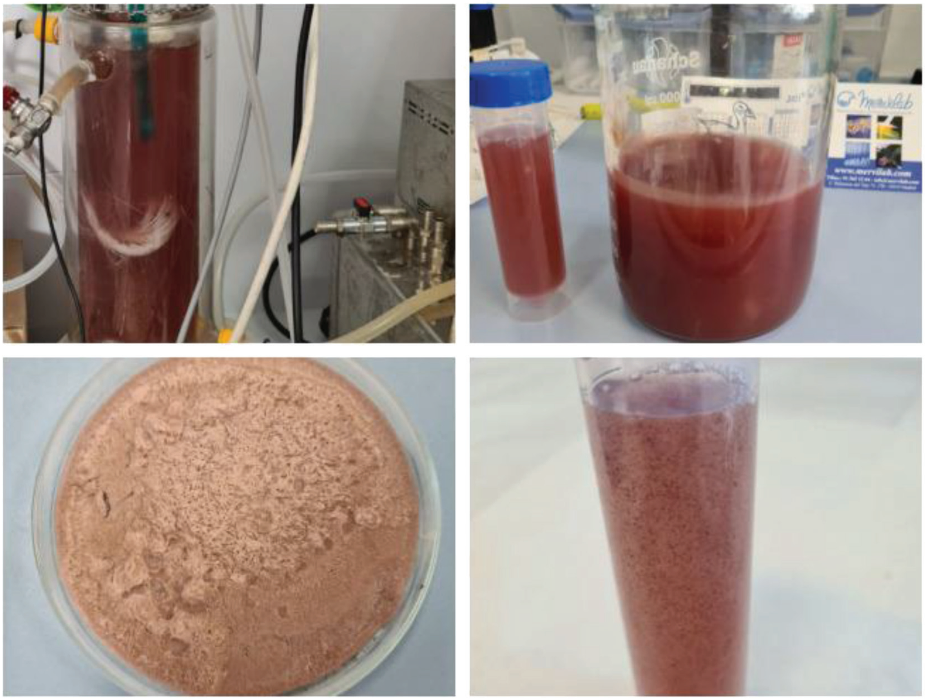
Under the operation conditions of Stage 1, the culture gradually recovered its performance, demonstrating the resilience of this technology. In Stage 4, biomass stabilizes at 1.8 gVSS L^{-1}, and COD consumption is up to 55%. The PHA accumulation was recovered, achieving 28% dry weight (5% PHH), and glycogen again lowered to 5%. The hydrogen production trend was also negative, decreasing its productivity. In Stage 5, the SRT was increased and the same trend as Stage 2 was observed, 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 (Figure III.7), EPS were measured to see if the culture 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% abundance of PPB was recovered in Stage 4 (Figure III.5) but stabilized at around 40% in Stage 5, with the only difference being 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 in the last Stage.

Second carbon overload (S6)

As it occurred in 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 H2 productivity at 310 mLH_2 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 Figure III.4 are also corroborated when we observe the performance parameters in Table III.1. The highest PHA productivities occurred at Stages 2 and 5, while higher glycogen and hydrogen productivities occurred at Stages 3 and 6, matching with 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

Figure III.7 Photos showing MPBR (top left), biomass collected at S2 (top right), lyophylized biomass (bottom left), and granulated biomass collected at P3 (bottom right).



when the biomass yield drops, the non-consumed COD is being diverted to H_2 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 in Stage 5, but, interestingly, this parameter was not significantly reduced in the organic-overloaded Stages. As discussed previously, 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.

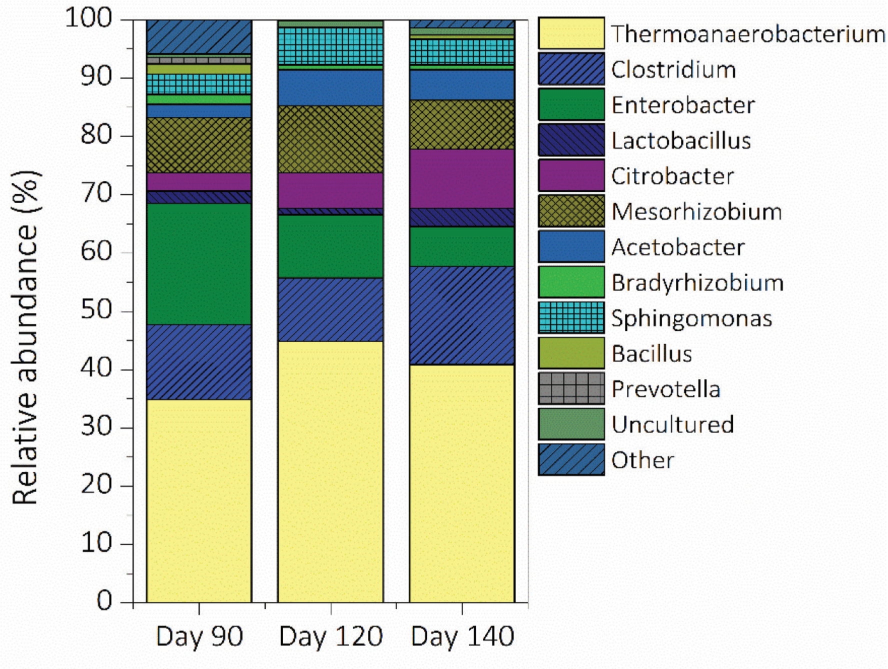
4.III.3. Statistical analysis of the evolution of microbial community profiles

Figure III.9 shows the development of the microbial communities evaluated by PCA ordination based on Hellinger transformed rarefied abundance. This

Table III.1 MBPR performance parameters on each Stage. Average ± standard deviation.

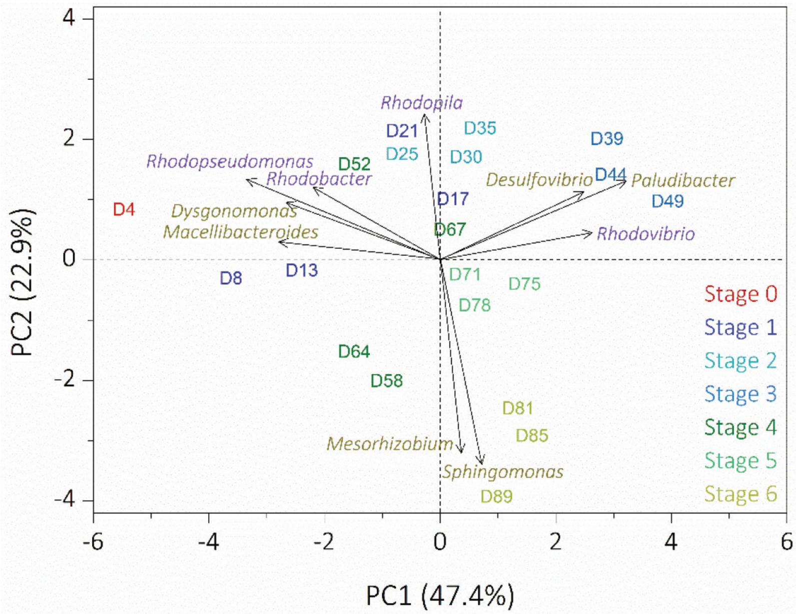
Parameters	S1	S2	S3	S4	S5	S6
$Y_{X/S}$ (gCOD gCOD-[1])	0.83 ±	0.97 ±	0.62	0.86	0.94 ±	0.62 ±
	0.07	0.05	±0.08	±0.25	0.02	0.07
$-q_S$ (mgCOD L [1] d 1)	541 ± 45	638 ± 32	677 ± 62	688 ± 28	1055 ± 66	665 ± 49
q_{PHA} (gPHA L-[1] d-[1])	0.22 ±	0.45 ±	0.05 ±	0.28 ±	0.61 ±	0.06 ±
	0.05	0.05	0.02	0.02	0.06	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 ±	0.09 ±	0.19 ±	0.09 ±	0.13 ±	0.17 ±
	0.03	0.01	0.04	0.00	0.01	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

Figure III.8 Microbial community structure at genus level of the thermophilic fermentation reactor at 3 different days after stabilization.



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. In Stage 1, the start-up with the synthetic feed caused a high enrichment 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 *Desufovibrio 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. which are some of the species present in the acidogenic fermenter (Figure III.8). which are less specialized in thermophilic temperatures and can therefore survive more efficiently in the MPBR. The clustering of the Stages and how most PPB genera were negatively related to organic overloads was confirmed. To further analyze these results, an RDA was carried out to assess the contributions of environmental variables to variances in the microbial communities.

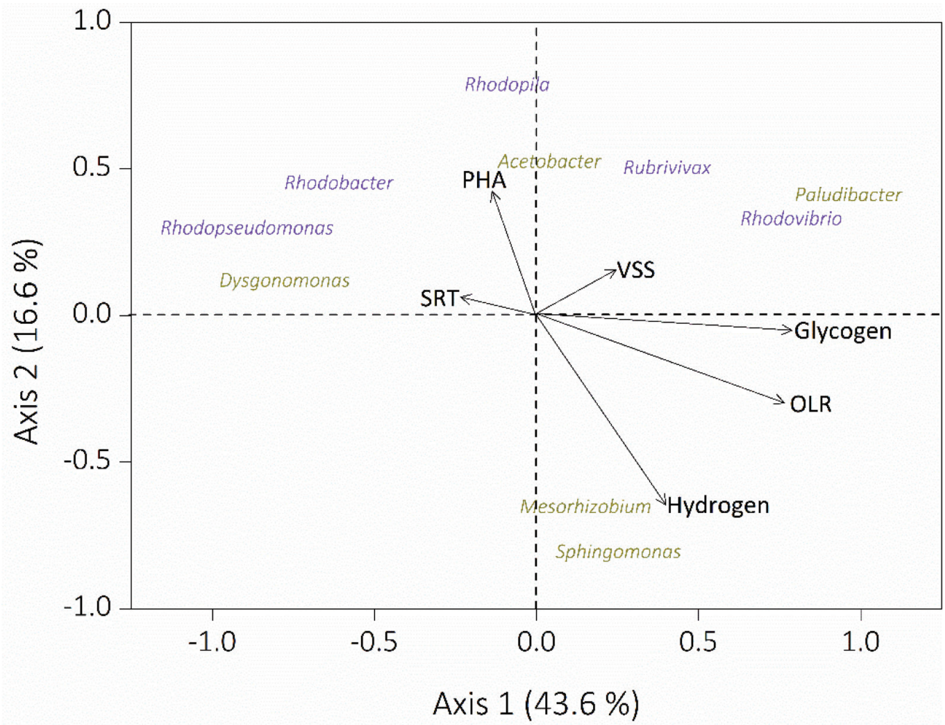
Figure III.9 PCA plot of the microbial communities. The samples are shown on day code (D4: day 4) and colored according to the Stage. Names indicate the 10 genera that contribute the most weight to PC1 and PC2, colored according to PPB (purple) or another genus (yellow). The arrows indicate the weight of each genus in each PC.



RDA analysis, shown in Figure III.10, 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 correlated 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 (Figure III.11). 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 PPB that are gaining ground with time are *Rubrivivax sp.* and *Rhodovibrio sp.*, but also *Paludibactersp.*, not only associated with time

Figure III.10 RDA of bacteria related to environmental variables: PHA (gVSSpHA L^{-1}), SRT (d), VSS (gVSS L^{-1}), Glycogen (gVSSoLY L^{-1}) OLR (d) and H2 (mLH2 L^{-1}). Nodes show the 10 genera with the most weight in both axis (PPB in purple, other genera in yellow), and the arrows indicate the weight of each environmental variable.



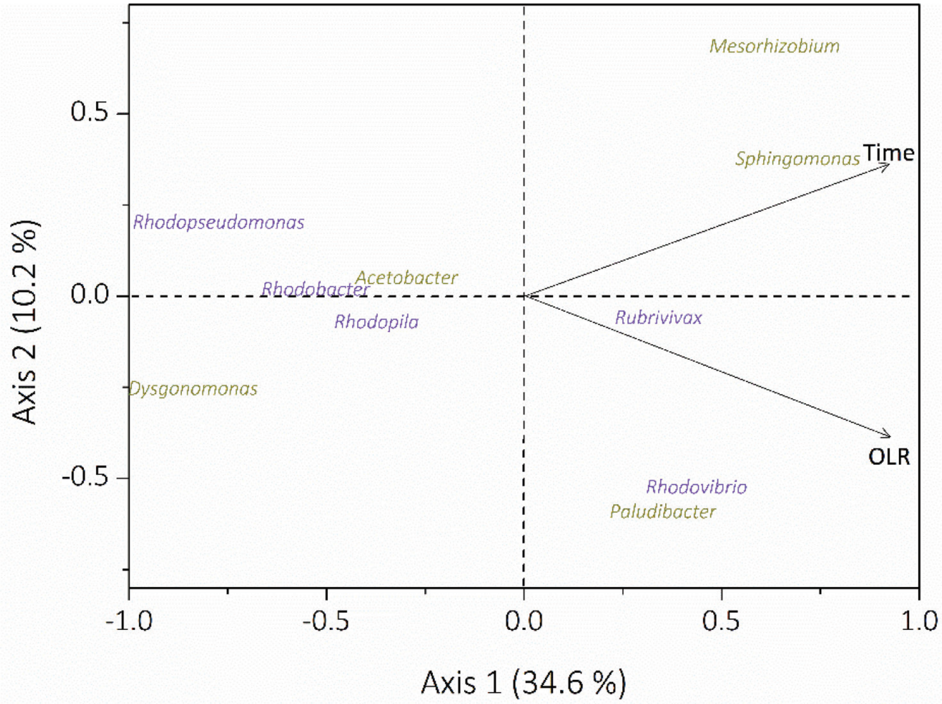
but also with higher organic loads. Finally, fermentative genera had a great weight on hydrogen production.

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 factor.

4.III.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,

Figure III.11 RDA diagram of bacteria related to environmental variables: Time (d) and OLR (d). The nodes show the 10 genera with the most weight in both axis (PPB in purple, other genera in yellow), and the arrows indicate the weight of each environmental variable.



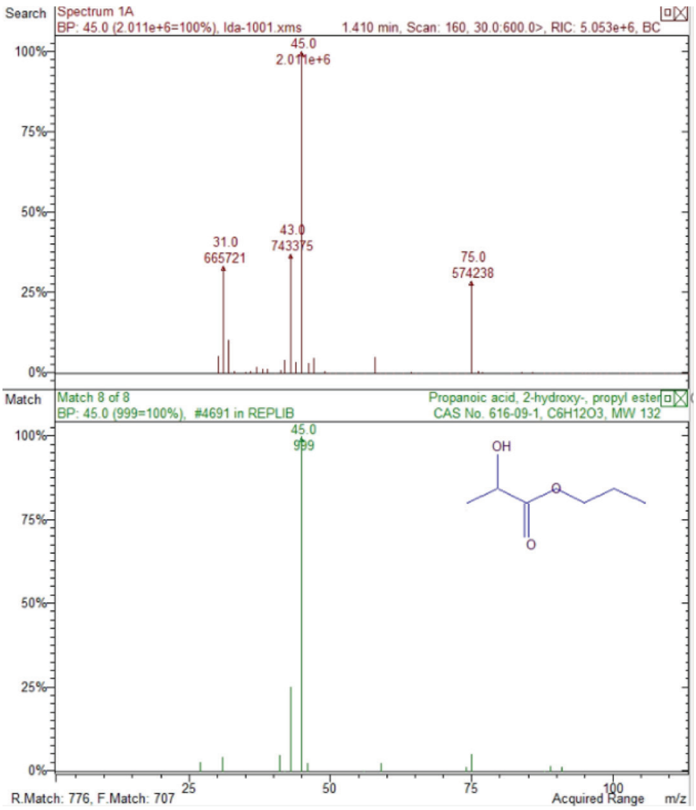
where a yield of 0.74 $gCOD_{SCCA}$ $gCOD_{feed}^{-1}$ was achieved (Matos *et al.*, 2021). Another work showed fermentation of OFMSW to obtain an overall yield of 0.65 ± 0.04 $gCOD_{VFA}$ $gVS_{(0)}^{-1}$ and acidification of 86% (Moretto *et al.*, 2020). In our work, we obtained slightly higher acidification (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 (Almeida *et al.*, 2021). The remainder of the undefined COD could be oligo- and monosaccharides derived from hemicellulose, lipids, proteins, complex carbohydrates, and amino acids (Romero-Cedillo *et al.*, 2017). Within the SCCA 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 (Fradinho *et al.*, 2014). The second SSCA was butyric acid, corresponding to 25% of the COD-equivalent of SCCA. 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 acid favors the accumulation of PHV (Fradinho *et al.*, 2019), and 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* (Fradinho *et al.*, 2019). If these results are kept in the process scale-up, the SCCA productivity and the fast reaching of a steadystate, reproducibility, and process stability highlight the practicality and high suitability of these pretreatments for PHA production with PPB.

The PPB-enriched mixed culture accumulated high percentages of PHA by assimilating fermented effluent from OFMSW feed. The maximum percentage of PHA accumulation achieved was 42%, which is the maximum achieved with mixed cultures of PPB using residual substrates (Sali and Mackey, 2021), the closest result being the treatment of fermented domestic wastewater, where 30.8% was achieved (Almeida *et al.*, 2021). Figure III.9 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 (de las Heras *et al.*, 2020). In other studies, the bacterial communities fluctuate in their percentage of PPB, for example, from the dominance of *Rhodopseudomonas* to *Rhodobacter* (Hülsen *et al.*, 2016), and 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 85% by weight in pure cultures (Sali and Mackey, 2021), which 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* sp., for example, can oxidize some short-chain acids such as lactate and butiryc acid and ethanol to acetic acid (Nakano and Fukaya, 2008), and PPB can assimilate this substrate to accumulate PHA faster. A more comprehensive metaproteomic 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 is accumulated when hexanoic acid is present in the medium, assimilated via p-oxidation, and incorporated into PHA chains (Silva *et al.*, 2022). 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 (Cabecas Segura *et al.*, 2022). 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 PHH production by phototrophic bacteria, one where C6 and C7 monomers were detected in *Rhodospirillum rubrum* (Brandl *et al.*, 1989), and another where PHH was detected in a mixed PPB culture (Liebergesell *et al.*, 1993). 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, and Figure III.12 depicts the results. Both PHH and PHP, which are generally not accounted for, indicate that PHA accumulation is usually un-

Figure III.12 GC/MS mass spectra and fractionation pattern. The pattern above is the analyzed sample, and the pattern below is the theoretical polyhydroxypropionate compound in its esterified form.



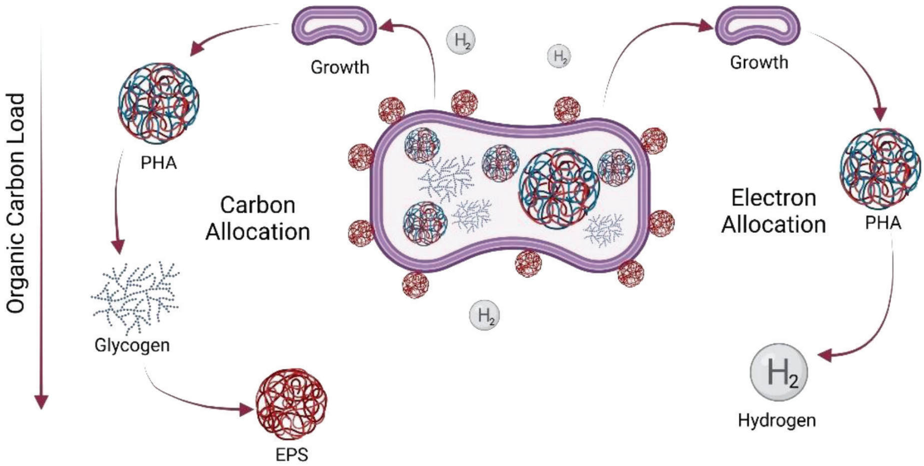
derestimated with mixed PPB cultures, which further increases the interest in the technology.

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} (Fradinho *et al.*, 2019), 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} (Almeida *et al.*, 2021). 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 gPHA L^{-1} d^{-1} (Capson-tojo *et al.*, 2020). The primary constraint is biomass growth, which is limited by nutrients in this work. 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} (Fradinho *et al.*, 2021). 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} (Capson-tojo *et al.,* 2020). 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 increase of organic load in the MPBR led to the destabilization of the culture and the collapse of PHA accumulation (Stages 3 and 6, Figure III.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 (Figure III.13). Glycogen accumulation by PPB has been known for a long time. Still, only one study has examined the relationship between PHB and glycogen production (Philippis *et al.*, 1992), 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: (i) the maintenance of photosynthetic efficiency in light and (ii) of viability in periods of starvation, such as in darkness or macronutrient depletion, and (iii) the acclimation to macronutrients deficiency (Mas and Van Gemerden, 1995). It is noteworthy that after Stage 3 and the lowering of OLR, there was a rapid

Figure III.13 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.



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 (Hustede *et al.*, 1993). The conversion of PHB accumulated in *Rhodovalum sufidophilum* to hydrogen in the absence of other substrates was demonstrated (Maeda *et al.*, 1997). In our experiment, this inversely proportional relationship is evident in Figure III.9. 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 H2 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, PPB lower their cellular efficiency and divert excess carbon and electrons to EPS and H_2, respectively. Another hypothesis for the increased hydrogen production is that EPS, which are exopolymers composed mainly of carbohydrates, may be optimal substrates for the growth of fermentative bacteria. These fermentatives can come from cross-contamination between bioreactors as explained in previous sections, increasing their biomass over time and producing more

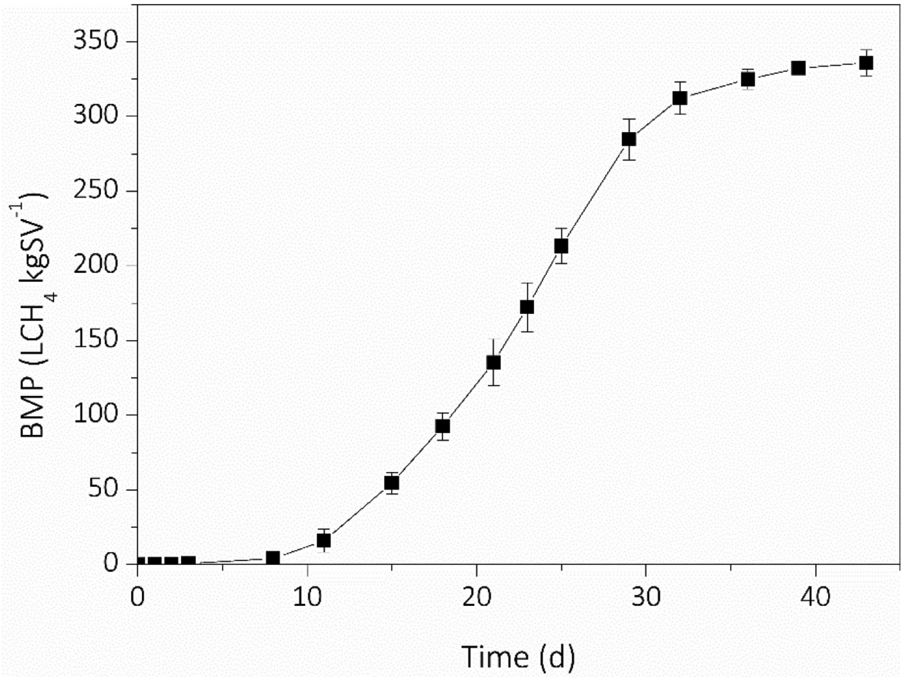
H_2. In addition, the COD of the biomass decreases considerably, as well as the cell yield, which is linked to fermentative processes. Fermentative bacteria produce more oxidized compounds for PPB, which can assimilate them for growth, causing a symbiotic relationship already evidenced in Figures III.9 and III.10. 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 (Tavares Ferreira *et al.*, 2021).

The granulation capacity of PPB has been studied recently, and EPS accumulations of up to 35% in dry mass were observed (Stegman *et al.*, 2021). 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, and are widely being employed at industrial scale in cosmetic, pharmaceutical and petroleum industries (Sreejita Ghosh *et al.*, 2021). In the work where PPB granulation is analyzed, the relative abundance obtained is between 40 to 70% (Stegman *et al.*, 2021), 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 (Zhao *et al.*, 2021), as shown in Figure III.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 that the settling cohesion was enhanced and stabilized by PPB (Larson *et al.*, 2009), 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 (Fernández-Dacosta *et al.*, 2015), **the granulation of mixed PPB cultures and the simultaneous production of PHA and EPS can reduce costs in the scale-up of the technology** (Kopperi *et al.*, 2021) **and opens up exciting research opportunities for the future.**

4.III.5. Implications for an integrated 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 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

Figure III.14 Biochemical potential test (BMP) of the solid phase obtained after the acidogenic fermentation pretreatment. Error bars are 95% confidence intervals.



of the solid fraction obtained after fermentation, yielding 336 LCH_4 $kgVS^{-1}$ (Figure III.14). This additional energy source would close the carbon and energy cycle of the biorefinery.

As for the COD removed, on average more than 40% of the COD is not assimilated, mainly due to nutrients limitation. 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 (Alloul *et al.*, 2019; Delamare-Deboutteville *et al.*, 2019). Nonetheless, the biggest challenge of the technology shown in this Thesis is the industrial scalability of the MBPR.

4.III.6. Mass and energy balance of a conceptual photobiorefinery

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 being studied at a pilot plant scale (Matos *et al.*, 2021; Moretto, Lorini, *et al.*, 2020). This technology's best performance achieved so far is 0.45 gCODPHA $gCODFeed^{-1}$ (Matos *et al.*, 2021). A conceptual photobiorefinery has been proposed (Figure III.15). Based on the data obtained in this section a preliminary mass balance of the overall novel photobiorefinery proposed here shows a total yield of **109 kg-PHA $tonneTS^{-1}$(0.15 $gCOD_{PHA}$ $gCOD_{Feed}$)**, which is two orders of magnitude above that achieved in the proof of concept in Section I. However, it is 3 times less than the aerobic process, but anyway we have to take into account that it is a far more mature technology that has been studied for several years. 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 PPB and artificial illumination is considered to be economically unfeasible (Capson-tojo *et al.*, 2020), the most logical solution being the scaling of the technology using the irradiance of the sun. Another important consideration of this balance is that we obtain a combined production between the acidogenic and photoheterotrophic fermentation process of **347 kg H_2 $tonneTS^{-1}$** treated. However, 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, as discussed in Section IV. Operational strategies adaptable to the different seasons of the year and the feedstock arriving in the biorefinery is a crucial point for its development.**

Several streams should be accounted for and recirculated/recycled within the proposed integrated photobiorefinery, as illustrated in Figure III.8. For example, the photoheterotrophic process requires an 11-fold dilution, thus requiring a significant amount of water. The aqueous stream remaining after the PHA extraction process could be used to save water and costs. Also, the membrane output stream has a high organic content, basically composed of SCCA. This stream would have several potential applications, such as the purification of the SCCA or their use in another photoheterotrophic PPB reactor in combination with a nutrient source for the production of microbial protein, for instance. Furthermore, in the interest of completely closing the carbon cycle, we have proposed that the share of biomass (TS) remaining after PHA extraction can be can be recirculated to the anaerobic digestion process, increasing biomass production. To calculate the methanogenic potential we take as reference the value of 210 mLCH4 gVS 1 determined by Hülsen *et al*. (2020).

If we only estimate the anaerobic digestion process based on the ST from the acidogenic fermentation, another major point to note is the reduction of the solid residue by up **to 90%, with the production of 91 kg of digestate per tonneTS**. Nonetheless, since we add the biomass remaining from the PHA extraction, the digestate increases up to 141 kgTS, but would also increase the nutrient content of this digestate, improving its potential applicability as an organic fertilizer.In any case, this digestate could be used as an organic soil amendment, or even the soluble fraction could be recycled together with the high-organic content stream in order to produce proteins, as mentioned above.

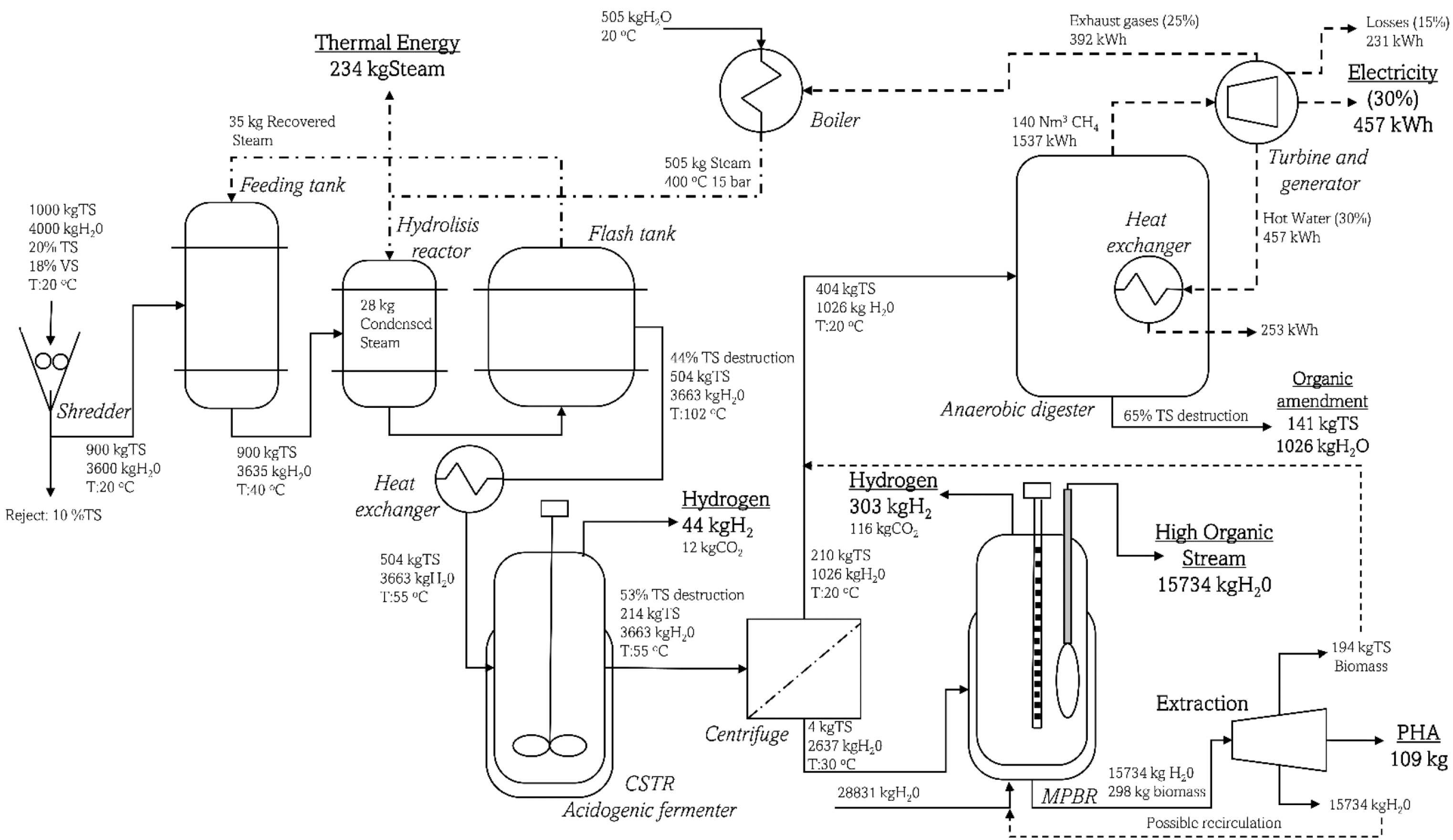
We can conclude from the preliminary balances that the anaerobic digestion and steam explosion pretreatment would be energetically autarkic. If we use the biogas produced in the anerobic digestion as proposed by Cano *et al*. (2014) and as described in Chaper 3, we would produce hot water with enough thermal energy to heat the anerobic digester. We would also produce up to **457 kWh of electricity per TonneTS**, which can be used for plant self-consumption or sold to the grid. And finally, the exhausted gases would be used to generate steam. This steam produced would be sufficient to provide the steam needed for the steam explosion pretreatment, leaving up to **234 kgSteam TonneTS^{-1}.**

The possibility of producing different products seasonally (H_2, glycogen, EPS), in addition to 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 H2 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 (Pereira *et al.*, 2019). 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 constant. All this Information gives us tools for optimizing and controlling this technology and paves the way for future large-scale feasibility analyses.

4.III.7. Conclusions

This study demonstrates the possibility of increasing PHA production by combining different operating strategies. The association of steam explosion pretreatment and acidogenic fermentation leads to stable production of SCCA

Figure III.15 Preliminary mass and energy balance of the proposed photobiorefinery



and H2 from OFMSW. Understanding the different metabolic pathways of carbon assimilation and consequent electron allocation is critical to enhancing PHA production. This production is shown to be stable and resistant to severe organic stress, and although the community within the PPB mixed culture may change, its functionality remains consistent. Finally, this comprehensive overview helps design a PPB-based pho-biorefinery with seasonal PHA, EPS, and H2 production, depending on the culture feed, contributing to urban solid waste management and taking a step forward in the direction of a circular economy society.

4.III.8. References

Alloul, A., Wuyts, S., Lebeer, S., and Vlaeminck, S. E. (2019). Volatile fatty acids impacting phototrophic growth kinetics of purple bacteria: Paving the way for protein production on fermented wastewater. Water Research, 152(2019), 138-147. https://doi.org/10.1016Zj.watres.2018.12.025

Almeida, J. R., Serrano, E., Fernandez, M., Fradinho, J. C., Oehmen, A., and Reis, M. A. M. (2021). Polyhydroxyalkanoates production from fermented domestic wastewater using phototrophic mixed cultures. Water Research, 197, 117101. https://doi.org/10.1016/j.watres.2021.117101

Brandl, H., Knee, E. J., Fuller, R. C., Gross, R. A., and Lenz, R. W. (1989). Ability of the phototrophic bacterium Rhodospirillum rubrum to produce various poly (P-hydroxyalkanoates): Potential sources for biodegradable polyesters. International Journal of Biological Macromolecules, 11(1), 49-55. https://doi.org/10.1016/0141-8130(89)90040-8

Cabecas Segura, P., Onderwater, R., Deutschbauer, A., Dewasme, L., Wattiez, R., and Leroy, B. (2022). Study of the production of poly(hydroxybutyrate-co-hydroxyhexanoate) and poly(hydroxybutyrate- co -hydroxyvalerate-co-hydroxy hexanoate) in Rhodospirillum rubrum. Applied and Environmental Microbiology, 6. https://doi.org/10.1128/aem.01586-21

Cano, R., Nielfa, A., and Fdz-Polanco, M. (2014). Thermal hydrolysis integration in the anaerobic digestion process of different solid wastes: Energy and economic feasibility study. Bioresource Technology, 168, 14-22. https://doi.org/10.1016/j.biortech.2014.02.007

Capson-tojo, G., Batstone, D. J., Grassino, M., Vlaeminck, S. E., Puyol, D., Verstraete, W., Kleerebezem, R., Oehmen, A., Ghimire, A., Pikaar, I., Lema, J. M., and Hülsen, T. (2020). Purple phototrophic bacteria for resource recovery: Challenges and opportunities. Biotechnology Advances, 43(May), 107567. https://doi.org/10.1016/j.biotechadv.2020.107567 de las Heras, I., Molina, R., Segura, Y., Hülsen, T., Molina, M. C., Gonzalez-Benítez, N., Melero, J. A., Mohedano, A. F., Martínez, F., and Puyol, D. (2020). Contamination of N-poor wastewater with emerging pollutants does not affect the performance of purple phototrophic bacteria and the subsequent resource recovery potential. Journal of Hazardous Materials, 385, 121617. https://doi.org/10.1016Zj.jhazmat.2019.121617

Delamare-Deboutteville, J., Batstone, D. J., Kawasaki, M., Stegman, S., Salini, M., Tabrett, S., Smullen, R., Barnes, A. C., and Hülsen, T. (2019). Mixed culture purple phototrophic bacteria is an effective fishmeal replacement in aquaculture. Water Research X, 4. https://doi.org/10.1016/j.wroa.2019.100031

Fernández-Dacosta, C., Posada, J. A., Kleerebezem, R., Cuellar, M. C., and Ramirez, A. (2015). Microbial community-based polyhydroxyalkanoates (PHAs) production from wastewater: Techno-economic analysis and ex-ante environmental assessment. Bioresource Technology. https://doi.org/10.1016/j.biortech.2015.03.025

Fradinho, J., Allegue, L. D., Ventura, M., Melero, J. A., Reis, M. A. M., and Puyol, D. (2021). Up-scale challenges on biopolymer production from waste streams by Purple Phototrophic Bacteria mixed cultures: A critical review. Bioresource Technology, 327. https://doi.org/10.1016/j.biortech.2021.124820

Fradinho, J. C., Oehmen, A., and Reis, M. A. M. (2014). Photosynthetic mixed culture polyhydroxyalkanoate (PHA) production from individual and mixed volatile fatty acids (VFAs): Substrate preferences and co-substrate uptake. Journal of Biotechnology, 185, 19-27. https://doi.org/10.1016/jjbiotec.2014.05.035

Fradinho, J. C., Oehmen, A., and Reis, M. A. M. (2019). Improving polyhydroxyalkanoates production in phototrophic mixed cultures by optimizing accumulator reactor operating

conditions. International Journal of Biological Macromolecules, 126, 1085-1092. https://doi.Org/10.1016/j.ijbiomac.2018.12.270

Ghosh, S., Lahiri, D., Nag, M., Dey, A., Sarkar, T., Pathak, S. K., Edinur, H. A., Pati, S., and Ray, R. R. (2021). Bacterial biopolymer: Its role in pathogenesis to effective biomaterials. Polymers, 13(8), 1-28. https://doi.org/10.3390/polym13081242

Hülsen, T., Barry, E. M., Lu, Y., Puyol, D., Keller, J., and Batstone, D. J. (2016). Domestic wastewater treatment with purple phototrophic bacteria using a novel continuous photo anaerobic membrane bioreactor. Water Research, 100, 486-495. https://doi.org/10.1016/j.watres.2016.04.061

Hülsen, T., Lu, Y., Rodríguez, I., Segura, Y., Martínez, F., Puyol, D., and Batstone, D. J. (2020). Anaerobic digestion of purple phototrophic bacteria - The release step of the partition-release- recover concept. Bioresource Technology, 306, 123125. https://doi.org/10.1016/j.biortech.2020.123125

Hustede, E., Steinbüchel, A., and Schlegel, H. G. (1993). Relationship between the photoproduction of hydrogen and the accumulation of PHB in non-sulphur purple bacteria. Applied Microbiology and Biotechnology, 39(1), 87-93. https://doi.org/10.1007/BF00166854

Kopperi, H., Amulya, K., and Venkata Mohan, S. (2021). Simultaneous biosynthesis of bacterial polyhydroxybutyrate (PHB) and extracellular polymeric substances (EPS): Process optimization and Scale-up. Bioresource Technology, 341(August), 125735. https://doi.org/10.1016/j.biortech.2021.125735

Larson, F., Lubarsky, H., Gerbersdorf, S. U., and Paterson, D. M. (2009). Surface adhesion measurements in aquatic biofilms using magnetic particle induction: MagPI. Limnology and Oceanography: Methods, 7(JUL.), 490-497. https://doi.org/10.4319/lom.2009.7.490

Liebergesell, M., Mayer, F., and Steinbüchel, A. (1993). Anaylsis of polyhydroxyalkanoic acidbiosynthesis genes of anoxygenic phototrophic bacteria reveals synthesis of a polyester exhibiting an unusal composition. Applied Microbiology and Biotechnology, 40(2-3), 292-300. https://doi.org/10.1007/BF00170383

Maeda, I., Idehara, K., Okayama, N., Miura, Y., Yagi, K., and Mizoguchi, T. (1997). Poly(3- hydroxybutyrate) as an endogeneous substrate for H2 evolution in Rhodovulum sulfidophilum. Biotechnology Letters, 19(12), 1209-1212. https://doi.org/10.1023/A:1018437921006

Mas, J., and Van Gemerden, H. (1995). Storage Products in Purple and Green Sulfur Bacteria. In R. E. Blankenship, M. T. Madigan, and C. E. Bauer (Eds.), Anoxygenic Photosynthetic Bacteria (pp. 973-990). Springer Netherlands. https://doi.org/10.1007/0-306-47954-0_45

Matos, M., Cruz, R. A. P., Cardoso, P., Silva, F., Freitas, E. B., Carvalho, G., and Reis, M. A. M. (2021). Combined Strategies to Boost Polyhydroxyalkanoate Production from Fruit Waste in a Three-Stage Pilot Plant. ACS Sustainable Chemistry and Engineering, 9(24), 8270-8279. https://doi.org/10.1021/acssuschemeng.1c02432

Moretto, G., Lorini, L., Pavan, P., Crognale, S., Tonanzi, B., Rossetti, S., Majone, M., and Valentino, F. (2020). Biopolymers from urban organic waste: Influence of the solid retention time to cycle length ratio in the enrichment of a Mixed

Microbial Culture (MMC). ACS Sustainable Chemistry and Engineering, 8(38). https://doi.org/10.1021/acssuschemeng.0c04980

Moretto, G., Russo, I., Bolzonella, D., Pavan, P., Majone, M., and Valentino, F. (2020). An urban biorefinery for food waste and biological sludge conversion into polyhydroxyalkanoates and biogas. Water Research, 170, 115371. https://doi.org/10.1016/j.watres.2019.115371

Nakano, S., and Fukaya, M. (2008). Analysis of proteins responsive to acetic acid in Acetobacter: Molecular mechanisms conferring acetic acid resistance in acetic acid bacteria. International Journal of Food Microbiology, 125(1), 54-59. https://doi.org/10.1016/j.ijfoodmicro.2007.05.015

Pereira, J. R., Araújo, D., Marques, A. C., Neves, L. A., Grandfils, C., Sevrin, C., Alves, V. D., Fortunato, E., Reis, M. A. M., and Freitas, F. (2019). Demonstration of the adhesive properties of the medium-chain-length polyhydroxyalkanoate produced by Pseudomonas chlororaphis subsp. aurantiaca from glycerol. International Journal of Biological Macromolecules, 122, 1144-1151. https://doi.org/10.1016/j.ijbiomac.2018.09.064

Philippis, R. De, Ena, A., Guastini, M., Sili, C., and Vincenzini, M. (1992). Factors affecting poly- / 3-hydroxybutyrate accumulation in cyanobacteria and in purple non-sulfur bacteria. 103, 187-194.

Romero-Cedillo, L., Poggi-Varaldo, H. M., Ponce-Noyola, T., Ríos-Leal, E., Ramos-Valdivia, A. C., Cerda-García Rojas, C. M., and Tapia-Ramírez, J. (2017). A review of the potential of pretreated solids to improve gas biofuels production in the context of an OFMSW biorefinery. Journal of Chemical Technology and Biotechnology, 92(5), 937-958. https://doi.org/10.1002/jctb.5116

Sali, S., and Mackey, H. R. (2021). The application of purple non-sulfur bacteria for microbial mixed culture polyhydroxyalkanoates production. Reviews in Environmental Science and Biotechnology, 7(4), 959-983. https://doi.org/10.1007/s11157-021-09597-7

Silva, F., Matos, M., Pereira, B., Ralo, C., Pequito, D., Marques, N., Carvalho, G., and Reis, M. A. M. (2022). An integrated process for mixed culture production of 3-hydroxyhexanoate-rich polyhydroxyalkanoates from fruit waste. Chemical Engineering Journal, 427(June 2021), 131908. https://doi.org/10.1016Zj.cej.2021.131908

Stegman, S., Batstone, D. J., Rozendal, R., Jensen, P. D., and Hülsen, T. (2021). Purple phototrophic bacteria granules under high and low upflow velocities. Water Research, 190, 116760. https://doi.org/10.1016/j.watres.2020.116760

Tavares Ferreira, T. J., Luiz de Sousa Rollemberg, S., Nascimento de Barros, A., Machado de Lima, J. P., and Bezerra dos Santos, A. (2021). Integrated review of resource recovery on aerobic granular sludge systems: Possibilities and challenges for the application of the biorefinery concept. Journal of Environmental Management, 291(April). https://doi.org/10.1016/j.jenvman.2021.112718

Zhao, L., Bao, M., Zhao, D., and Li, F. (2021). Correlation between polyhydroxyalkanoates and extracellular polymeric substances in the activated sludge biosystems with different carbon to nitrogen ratio. Biochemical Engineering Journal, 176(August), 108204. https://doi.org/10.1016/j.bej.2021.108204

SECTION IV: SCALING-UP CHALLENGES OF PHOTOHETEROTROPHIC PROCESS

Preamble and Rationale

As discussed in the previous section, the scale-up of the photoheterotrophic process is key to achieving a cost-effective process. The successful scale-up for biopolymer production on an industrial scale depends on several factors, such as the cost of precursor substrates, yield over substrate rate, volumetric productivity, and the cost of downstream processing, among others. While bioengineering aims to improve upstream processes (low-cost substrates and increased productivity), bioprocess optimization of upstream and downstream processes is necessary for scalable and cost-effective manufacturing. In this last section of the discussion Chapter, we have considered reviewing and performing a critical analysis of the current state of the art in scaling up these PPB-based biopolymer production technologies.

4.IV.1. PPB Storage Compounds

Storage compounds are produced by living organisms and/or synthesized by processive enzymes that link building blocks to yield high molecular weight molecules. Figure IV.1 shows the main environmental conditions for the accumulation of storage compounds and their industrial applications are schematically shown in Figure IV.1. Depending on the conditions in which PPB are growing, five major storage compounds are produced: zero-valence sulfur, glycogen, PHA, and polyphosphate (poly-P). These compounds are stored intracellularly (and therefore are inclusions) except for sulfur and EPS, which can also be stored extracellularly.

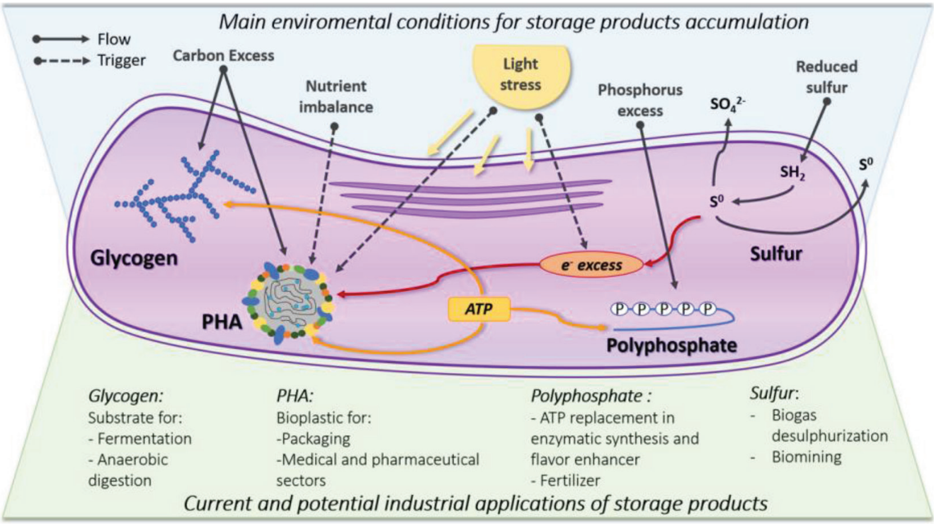
Poly-phosphate

Inorganic poly-P is the only polyanhydride found in all living cells forming linear polymers with variable chain length, which are constructed from repeating $PO4^{-3}$ and connected by high-energy anhydride bonds (Liang *et al.*, 2010). It has been shown that poly-P affects numerous aspects of bacterial physiology, such as survival during the stationary growth phase, response to stress, motility, quorum sensing, biofilm formation or pathogenicity. The enzyme responsible for poly-P biosynthesis is the highly conserved poly-P

The results of this section have been published in the following paper:

J. Fradinho, L.D. Allegue, M. Ventura, J.A. Melero, M.A.M. Reis, D. Puyol, (2021). Up-scale challenges on biopolymer production from waste streams by Purple Phototrophic Bacteria mixed cultures: A critical review. Bioresource Technology, 327, 124820.

Figure IV.1 Schematic representation of PPB anaerobic phototrophic metabolism that support storage products accumulation and their industrial applications.



kinase (PPK), which helps to store energy excess from light through phototrophic growth when low C / P ratios are present (Lai *et al.*, 2017). Poly-P forms intracellular storage particles but may also form a membrane-anchored complex with low molecular weight polyhydroxybutyrate (PHB), facilitating the uptake of DNA and various ions (Reusch and Sadoff, 1988). This polymer can be accumulated as P reserve in response to periods of P starvation, but some organisms can accumulate Poly-P as energy storage (ATP), without requiring a previous P starvation phase in environments with excess P. This capacity has been observed in phototrophs like microalgae (Solovchenko *et al.*, 2016), PPB (Lai *et al.*, 2017, Liang *et al.*, 2010) and heterotrophs like the poly-P accumulating organisms (PAOs) (Desmidt *et al.*, 2015). Some PPB species can accumulate internal $PO4^{-3}$ into the form of poly-P up to 13%-15% of its cell dry weight (Liang *et al.*, 2010), nevertheless, there is still a great gap of knowledge regarding its accumulation patterns on PPB. Unlike poly-P accumulating chemoheterotrophic organisms, the driver for poly-P accumulation in PPB is related to the presence of high light availability, similarly to other photosynthetic microorganisms (Carvalho *et al.*, 2019). Therefore, the process design is simplified as there is no need to have a succession of aerobic/anoxic stages in the photobioreactors, considerably decreasing the operation costs.

Phosphorus presents vast applications in the chemistry and food industry, but particularly, in the agriculture field where P is used as fertilizer (Solovchenko *et al.*, 2016). Commercial production of bacterial poly-P for industrial applications is not currently economically feasible due to a much more efficient production by chemical synthesis (Iliescu *et al.*, 2006). However, controlling the phosphorus propagation in nature is a highly important matter in environmental biotechnology, and the biological capture and recovery of P in waste is key for the protection of the aquatic environment. PPB systems are still under study for P recovery, which can boost the use of PPB biomass as C/N/P organic fertilizers (Sakarika *et al.*, 2020).

Glycogen

Glycogen, a polysaccharide made up by glucose units, is another intracellular stored resource common in evolutionarily divergent species. It is a widespread form of carbon and energy storage that promotes survival during starvation (Sekar *et al.*, 2020). Its role in bacteria is highly diverse, for example, it contributes as an energy source when there is a lack of other energy sources, or as a temporary resource used during the physiological transitions required by dynamic environmental conditions. It can be accumulated in either stationary phase or under excess carbon and/or limited-growth conditions, and contributes to survival or maintenance in bacterial environments in which nutrient availability frequently fluctuates (Sekar *et al.*, 2020). The glycogen biosynthesis capacity of PPB has been already measured (Igarashi and Meyer, 2000). However, there is still a gap of knowledge in the relation between PHA, and glycogen accumulation in PPB, as addressed in this Thesis. A previous study indicated that *Rhodopseudomonas palustris* accumulate glycogen in higher percentage in almost all conditions (5-15% d.w.), synthesizing PHA when the environment has reducing equivalents in excess (0.3-7% d.w.) and the PPB are in static mode (De Philippis *et al.*, 1992). However, glycogen accumulation remains almost constant, with decreasing values related to starvation conditions. The possible competition between glycogen and PHA accumulation in photoheterotrophic conditions, as well as the genetic regulation for this process, is still unclear. We may hypothesize that glycogen acts always as a carbon and energy storage during growth, whereas PHA regulates the electron balance, acting also as carbon storage during static mode. The energy required for glycogen is higher than that for PHB accumulation (starting from acetate, 1 mol of glycogen requires 4 ATP, 2 CO2 and 4 reducing equivalents, whereas 1 mol PHB only requires 2 ATP and 1 reducing equivalent (Fradinho *et al.*, 2014)). However, glycogen is on the polysaccharides biosynthesis pathway, and therefore it is the natural carbon storage process during growing conditions. PHA accumulation is more feasible in static mode, requiring less energy. Also, the lack of CO2 is a trigger for PHA accumulation in detriment of glycogen, as Acetyl-CoA needs CO2 for initializing any biosynthetic pathway (Bayon-Vicente *et al.*, 2020).

The use of PPB-based glycogen as a carbon source for a variety of applications remains untapped. Recently, it has been studied the accumulation of glycogen in cyanobacteria to use the bacterial biomass as a feedstock for octanoic acid through dark fermentation (Comer *et al.*, 2020), which may be a feasible alternative for PPB. In addition, the anaerobic digestion of PPB-based biomass has been recently studied (Hülsen *et al.*, 2020). The role of glycogen in this process should be stated as it can potentially boost the biochemical methane potential of PPB biomass rejection coming from the extraction of higher value-added products. In this sense, glycogen may be a byproduct of biorefinery platforms. In any case, it interferes with most of the metabolic pathways of PPB and must be considered in any biopolymers' application of PPB.

Sulfur

PPB have the ability to grow using reduced sulfur compounds (mainly H2S) as electron donors for their biosynthesis (Pokorna and Zabranska, 2015). Sulfide is oxidized to sulfate and, as an intermediate, elemental So is produced and accumulated in the form of globules inside or outside the cells. In principle, So can serve as both an electron acceptor (when it is being accumulated) and as a donor (Trüper, 1984), therefore sulfur compounds, and So in particular, is expected to have a considerable influence on the production and/or consumption of H2 by PPB (Laurinavichene *et al.*, 2007). An excess of sulfur in the environment can induce sulfur accumulation of over 30% of the dry weight of the cell (Pedrós-Alió *et al.*, 1985).

Although there is no industrial scale of sulfur removal by PPB so far, this is a promising technology that can be used, for example, for biogas desulphurization. A previous study performed in the laboratory and in a pilot plant, achieved a complete oxidation of H2S of the biogas using the purple sulfur bacterium Ectofhiorhodospira shaposhnikovii (Vainshtein *et al.*, 1994). A recent paper have shown potential capacity of mixed cultures of PPB for photoautotrophic sulfide removal where the purple sulfur bacterium *Allochromatium* sp. predominated in the consortium (Egger *et al.*, 2020). In this sense, mixed cultures of PPB may allow a potential technology for simultaneous wastewater treatment and biogas upgrading, including both biogas desulphurization, removal of carbon dioxide, and organic matter and nutrients removal (Marín *et al.*, 2019).

Biomining is another option to use the ability of PPB to transform sulfide into sulfur globules. These organisms may be useful as intermediates in bio leaching of metals after metal recovery by sulfate-reducing microorganisms in a two-stage biological approach. Though this has been never tested with PPB, there are evidences indicating that sulfide-oxidizing microorganisms are suitable for these purposes (Suzuki, 2001).

Polyhydroxyalkanoates (PHA)

This Ph.D. Thesis is mainly focused on the production of PHA, which are linear polyesters, formed by the accumulation of carbon as reserve material in response to substrate excess when growth is limited owing to starvation of some nutrient, usually nitrogen, phosphorus or sulfur (Monroy and Buitron, 2020). PHA is deposited as spherical intracellular inclusion with an amorphous and hydrophobic PHA core that is mainly surrounded by proteins involved in such PHA metabolism (Jendrossek, 2009). PHA accumulation occurs in high-carbon and nutrient-limited (N, P, S) environments, but it is also enhanced in case of redox imbalance, to act as an electron sink (Bayon-Vicente *et al.*, 2020, De Philippis *et al.*, 1992), therefore sudden light increase could be a way to induce redox stress in the culture, thus forcing higher PHA accumulation. However, long-term cultivation could lead to metabolic adaptation and eventual decrease of PHA accumulation.

The most investigated PHA is the homopolymer poly-3-hydroxybutyrate (P3HB). Compared to the P3HB, PHA copolymers, composed of a mixture of hydroxybutyrate and hydroxyvalerate (PHBV), show better mechanical properties with decreased stiffness and brittleness, increased flexibility and a decreased melting and glass transition temperatures, which allows for a wider temperature processing window and thus increase its processability (Albuquerque *et al.*, 2011). A thorough review of PHB (homo- and copolymers) production with PPB mixed cultures has been recently published (Monroy and Buitron, 2020), where it has been shown the copolymer production capacity of PPB when using heterogeneous substrates (propionate among others), with up to 51% fraction of PHV moiety (Fradinho *et al.*, 2014).

As discussed in Chapter 1, among bioplastics, PHA has the best biodegradability even in marine environments, but this feature has been barely considered when the production costs are calculated in cradle-to-grave approaches (e.g. life cycle assessment). An important aspect to consider in this context is the production by PPB of hydrogen as a by-product in the accumulation of PHA. This process have been widely studied in different bioreactors (Basak *et al.*, 2014), and can be a possible strategy to improve the profitability of the overall process as has also been demonstrated in Section III. A solar-powered photo-biorefinery could be as well a promising way for sustainable biodegradable PHA production, as will be discussed in subsequent sections.

4.IV.2. Enriching phototrophic mixed cultures in PHA accumulating PPB

In order to develop strategies that promote PHA production in PPB mixed cultures, it is essential to recall that in PPB, PHA can be stored as a carbon reserve and as a sink for reducing power. Therefore, strategies can be designed by specifically targeting each function. Up to now, studies have been mainly conducted with synthetic feedstock and indoor artificial illumination, creating a knowledge base for higher complex system operation with real wastes in outdoor conditions.

The two most important strategies used for the production of PHA are feast-famine and permanent feast and their key features are summarized as follows:

The **feast and famine strategy (FF)** is widely identified as an prominent method for PHA accumulation by mixed microbial culture within aerobic systems. This processes are usually based on the alternance of periods in which substrate (energy/C source) is available, named feast phase, and periods in which substrate is no longer available, named famine phase. The aim of these processes is pushing towards the survival of the of the most resistant strains (strains that under this cultivation condition accumulate intracellular energy and reserve compounds like PHA in the feast phase, and then use them as energy substrate in the famine phase) in place of the survival of the faster growing strains (Montiel-Corona and Buitrón, 2021), otherwise obtained with culture medium fully replete in every nutrient. This type of strategy is typically used in SBR reactors or two continuous CSTR reactors (Di Caprio, 2021). There are two main ways of operation:

1) Feast and famine with coupled C/energy and N supply: In this strategy the organic substrate (C and energy source) is supplied together to the N substrate, at the beginning of the cultivation (feast phase). The C/energy source is quickly consumed. When the C/energy source is depleted, cells enter in the famine phase, in which they can survive depending on its duration and on the amount of the compounds previously storage.

2) Feast-famine with uncoupled C/energy and N supply: In this strategy there is a feast phase corresponding to the supply of C/energy source in N-starvation, followed by a famine phase corresponding to the supply of N in absence of the external energy source. In the feast phase, in N-starvation, the cell duplication is arrested, and the biomass concentration increases because of the sole accumulation of organic compounds. For phototrophic cultivation of PPB this uncoupling could be attained easier because the energy source used is light, that was just turned off at night.

The other main strategy would be to maintaining the culture in a carbon feast regime, with permanent presence of external carbon. The selection principle of this **permanent feast** (PF) relies on the distinctive properties of anoxygenic photosynthetic bacteria, where no oxygen is released during photosynthesis. In illuminated environments, photosynthetic bacteria can use the ATP produced by photosynthesis to uptake external carbon. If no electron acceptors are present, the cells have to activate internal mechanisms to oxidise the reduced molecules. One of them is the accumulation of PHA that requires the reduction of its precursors during polymer formation. Thus, PPB are able to grow exponentially and accumulate PHA simultaneously (Montiel-Corona and Buitrón, 2021). In addition, the selection and accumulation process can be carried out in a single reactor, since in this reactor would be simultaneously a selector and a PHA accumulator reactor, where organisms would be permanently selected while accumulating PHA, which can potentially reduce production costs (Fradinho *et al.*, 2016).

Nevertheless, in order to attain high PHA accumulations in PPB cultures, it is essential to enrich the culture in PHA-accumulating bacteria. There are different advantages and disadvantages to the aforementioned strategies when specifically addressing mixed PPB cultures, which will be discussed below.

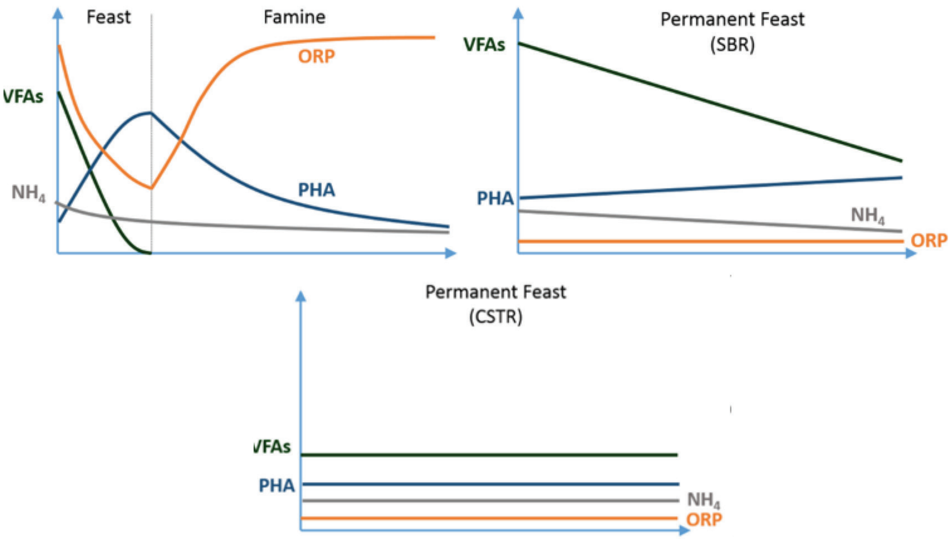
4.IV.3. Feast and Famine (FF) strategy

To select for PPB that stores PHA as carbon reserves, a FF strategy can be applied. Repeated FF cycles create a selection pressure that enriches the culture in organisms with high PHA storing capacity. Since this strategy requires the presence of an electron acceptor for the PHA consumption in the famine phase, Fradinho *et al.* (2013) proposed the operation of mixed cultures comprised of PPB and microalgae, with the latest being the oxygen providers. When the consortium is operated in a FF regime, the system is not completely anaerobic due to the oxygen production by microalgae, but it is under sub-oxic conditions. During the Feast phase, the oxidation and reduction potential (ORP) can drop to -300 mV, and PPB use the photosynthetically generated ATP to anaerobically take up external carbon and store it as PHA (Figure IV.2). In this situation, high carbon conversion efficiencies can be expected. During the famine phase, the cells' oxygen demand decreases, and the oxygen that is continuously produced by microalgae increases the ORP up to +0/+50 mV. Oxygen values are never detected, minimizing an eventual inhibition of PPB pigments expression.

Studies under these FF conditions and continuous illumination have shown the mixed cultures capability of accumulating up to 20% gPHA $gVSS^{-1}$, producing a PHA copolymer (HB:HV molar fraction of 84:16) when fed with VFA mixtures (Fradinho *et al.*, 2014). When the cultures were operated under dark/light cycles, microalgae levels could be decreased relatively to PPB, further enriching the culture in PPB and enabling PHB contents up to 30% (Fradinho *et al.*, 2013). In this case, the PHB storage yield reached 0.90 ± 0.09 $Cmol_{PHB}$ $Cmol_{Substrate}^{-1}$ (acetate + internal glycogen), confirming that high carbon recovery and conversion to PHA are possible during the feast phase.

Overall, studies under the FF strategy indicate that it is possible to efficiently enrich the mixed cultures in PHA accumulating PPB by promoting PHA storage as carbon reserves. The cultures not only can produce PHA copolymers when fed with SCCA mixtures (compounds commonly found in fermented organic wastes) but are also robust to transient illumination, an important feature for outdoor operation.

Figure IV.2 General profile of phototrophic mixed cultures during PHA production under different selection strategies and reactor operation. A: Feast and famine selection in an SBR; B: Permanent feast selection in an SBR; C: Permanent feast selection in a CSTR.



4.IV.4. Permanent Feast (PF) strategy

Another strategy that can be used to select for PHA accumulating PPB, explores the PHA storage as a sink for reduced power. Firstly, the PPB mixed culture must be cultured in an illuminated anaerobic environment under the permanent presence of SCCA (so-called permanent carbon feast, PF) (Fig. IV.2). In these conditions, PPB does not have access to the typical electron acceptors (oxygen, nitrate), and when they take up the organic carbon, PPB must activate metabolic pathways that allow electrons dissipation, thus balancing the cell's internal redox state. PHA accumulation, CO_2 fixation via Calvin-Benson-Bassam (CBB) cycle, and N_2 fixation/H_2 production via nitrogenase enzyme are some metabolic processes that PPB can use to dissipate electrons (Alsiyabi *et al.*, 2019; McKinlay and Harwood, 2010). The key is to provide operating conditions that select for PPB that favor the PHA accumulation pathway. As such, if the PPB mixed culture is grown on feedstocks that contain NH_4^+ (which is usually the case in municipal wastewaters and some fermented organic wastes), nitrogenase expression is repressed, and electrons cannot be dissipated through N2 fixation/H2 production (Koku *et al.*, 2002). Regarding CO2 fixation via the CBB cycle, this is an important pathway used by PPB to achieve redox homeostasis, especially when the cells are growing on organic substrates more reduced than the biomass (Koku *et al.*, 2002; McKinlay and Harwood, 2010). However, the CBB cycle is an ATP-dependent and high-energy-demanding pathway (Alsiyabi *et al.*, 2019). On the contrary, no ATP is required if reduced cofactors are re-oxidized via PHA production (Laycock *et al.*, 2014). With both pathways at hand, the organisms that favor electron dissipation via PHA production have more energy available, benefiting their growth and enriching the PPB mixed culture in PHA storing bacteria.

It is essential to point out that PHA production can occur side-by-side with cell growth with this PF selection strategy, with no detriment to the culture selection (Fradinho *et al.*, 2016, 2019). However, it is natural that more PHA can be stored if the carbon precursors are not being used for growth. This can be achieved by limiting growth (nutrient limitation) and/or increasing the light availability, allowing cells to take up more VFA than necessary to grow. This was observed in a study by Fradinho *et al.* (2016), where a PPB mixed culture selected under permanent acetate feast, P limitation, and low light availability could accumulate up to 60% PHB when exposed to higher light availability in accumulator reactors. Also, high PHB storage yields of 0.83 ± 0.07 $Cmol_{PHB}$ $Cmol_{Acetate}^{-1}$ and global carbon yields (∑Y accounting biomass + PHB + glycogen production) of 1.03 ± 0.05 Cmol $Cmol_{Acetate}^{-1}$ could be achieved (Fradinho *et al.*, 2019), further reinforcing the potential of PPB systems for high carbon recovery during PHA production.

The results show that targeting PHA storage as an electron sink is an attractive strategy that allows PPB mixed cultures enrichment in PHA storing PPB. Furthermore, in anaerobic conditions and PF the culture becomes fully enriched in PPB (algae are outcompeted). High PHA content and carbon recovery can then be achieved with light energy demands supported by natural sunlight illumination (Fradinho *et al.*, 2019).

4.IV.5. Potentials for Poly-P production as P recovery

In the last decades, the world requirement for more pastures and crops for food production has increased the phosphorous demand, and since P is mostly obtained from finite phosphate rock mines, there are concerns that P rock reserves may become depleted (Solovchenko *et al.*, 2016). Furthermore, phosphate's widespread use and uncontrolled leakage into waterways can cause detrimental environmental problems, such as eutrophication, with profound negative effects on the ecosystems (Solovchenko *et al.*, 2016). Therefore, phosphorus recovery methods like the EBPR systems, have been implemented and widely used in wastewater treatment plants (WWTP) to recover phosphate from waste streams. In these systems, Poly-P storage requires intensive aeration (with associated costs), and part of the organic carbon removed from the wastewater is dissipated during PAOs' aerobic respiration. Implementation of photosynthetic processes can overcome these drawbacks, exploring, for example, the PPB capacity to store Poly-P, and designing specific systems to recover P anaerobically from waste liquid streams. Studies with PPB strains from *Rhodobacter sphaeroides* (Hirais *et al.*, 1991) and *Rhodopseudomonas palustris* (Liang *et al.*, 2010) showed that under illuminated anaerobic conditions and upon entering the stationary growth phase, these organisms accumulated substantial amounts of Poly-P. Unlike the chemoheterotrophic bacteria in EBPR systems that obtain energy by consuming organic carbon sources, ATP production in PPB is independent of carbon uptake. Therefore, with ATP constantly available from light, PPB could continue to take up phosphate, and because growth was limited, cells were no longer assimilating phosphate into biomass but instead storing it as Poly-P. These findings are even more interesting considering that *R. sphaeroides* could accumulate 2 - 4% of their cell dry weight as phosphorous, and *R. palustris* could achieve P contents of 4 - 10%. These values are in the range of P contents observed in PAOs, 5 -15% (Liang *et al.*, 2010), demonstrating the high storage potential of PPB.

In PPB mixed cultures, polyphosphate storage has also been reported, for instance, in cultures grown under P limiting conditions in a system aimed at PHA production (Fradinho *et al.*, 2016, 2019). In these studies, the mixed cultures were operated under low illumination conditions in the selector SBR. When exposed to high light availability, the phosphate uptake increased up to 95% of the removed phosphate accumulated and not assimilated into the biomass (Fradinho *et al.*, 2016). Despite the culture's previous conditioning to P starvation periods, the higher light supply in this study demonstrates the importance of an excess of ATP availability to boost phosphate uptake and PolyP storage.

The common finding between all these studies is that a surplus of ATP seems to be the critical factor that triggers high P storage. As such, a PPB system for P removal from wastewater can be devised by including periods with an excess of ATP availability. These conditions can be achieved by controlling several parameters like the light supply, which directly impacts ATP production, and by regulating the activity of some metabolic pathways that compete for ATP (e.g., ensuring the presence of ammonia to inhibit H2 production). However, the hypothesis of combining poly-P storage with PHA production cannot be excluded if the operating conditions allow an excess of ATP capable of sustaining both phosphate and high carbon uptake. Indeed, simultaneous Poly-P formation and PHA storage were observed in (Fradinho *et al.*, 2016, 2019) with mixed and single strains of PPB, respectively. The prospect of implementing such a PPB system to waste streams processing would be very economically attractive and environmentally beneficial due to the concurrent P and C recovery and discharge of a better-quality effluent.

From the studies on P storage in PPB, Table IV.1 proposes a simple description of the different possibilities of producing Poly-P in combination (or not)

Table IV.1 Proposed combinations of Poly-P production with other compounds by PPB under different C, N, S cultivation conditions, assuming external P and ATP in excess for growth. TCA: Tricarboxylic acid cycle.

	High Carbon		Low Carbon or Low S
	VFAs	Organic acids (from TCA)	
NH4 present	Poly-P + PHA (Fradinho *et al.*,	Poly-P (Hirais *et al.*,	Poly-P (Kitamura and Hiraishi, 1985)
	2016)	1991)	
NH4 limited	Poly-P + PHA (Lai *et al.*, 2017)[1]	No Poly-P; likely H2 (Kitamura and Hiraishi, 1985)	Likely Poly-P (no references)

[1] *glutamate was used as N source*

with other compounds, assuming that cells have external P and ATP in excess for growth.

Mostly all the situations lead to Poly-P production, except for PPB growth in NH4 limited conditions and the presence of TCA organic acids, a condition known for promoting H2 production that competes with Poly-P for energy. A small note must be given regarding the Poly-P production with NH4 presence and low carbon because, in this situation, it is fundamental to filtrate the visible light to prevent microalgae growth in such a nutrient-rich environment. With this filtration assured, PPB systems also become a potential technology for wastewater treatment and P recovery from streams with a low COD/P ratio.

4.IV.6. Upscaling challenges and research needs

At the current moment, a large amount of knowledge has been harnessed on PPB technologies, and these are starting to be implemented in outdoor pilot/demonstration conditions for a complete evaluation of their potential. However, this technology transfer from lower laboratory scales to larger facilities is usually associated with upscaling challenges that must be identified and tackled.

Regional limitations and challenges

As a light-dependent technology, large-scale operation of PPB systems must consider the conditions of the implementation site in terms of local climate (sunlight availability) and land requirement (enough space to accommodate the facilities). Insolation is not uniform around the globe, with some regions receiving higher irradiance levels than others, thus being more likely for implementing the photosynthetic process. Also, due to the Earth's tilt, the day length changes along the year, with the variations between day/night time becoming more intense with increasing latitudes. These seasonal variations will strongly impact the system operation during the winter periods and imply process adjustments (e.g., OLR, SRT) if an all-year operation is planned.

Local temperature is also an essential factor when selecting the operating site. The very high temperatures generally found in regions with the highest insolation may be excessive for PPB activity, preferably growing in the range of 25 to 35°C (Chen *etal.*, 2020). On the other hand, Hülsen *etal.* (2016a) reported that PPB cultures could adapt to lower operating temperatures (10°C) with performances comparable to temperate temperatures (22°C), which enlarges the regional applicability of PPB systems.

The implementation of PPB systems is very dependent on space availability. Like other photosynthetic processes, a high illuminated surface-to-volume ratio is fundamental to increasing productivity. While different reactor configurations have been proposed to increase this ratio (e.g., tubular reactors, flat panels), their high investments and maintenance costs are prohibitive for large waste streams applications (Gupta *et al.*, 2019). Open raceway ponds are a cheaper and simple alternative that can support the anaerobic growth of PPB mixed cultures, providing that organic carbon is present, which maintains the system under zero oxygen concentration levels. However, open ponds present liquid heights between 15 -30 cm, thus requiring a high ground surface area in order to process high volume loads. As such, their implementation is suited for the outskirts of small towns or nearby waste-producing industrial areas. This last case would be ideal for PPB systems since there would be (i) easy access to the waste stream, (ii) access to qualified technicians for the system operation, (iii) the possibility to integrate the PPB polymer production system with downstream and polymer processing units within the industrial zone, establishing a grid that follows the biorefinery concept. It is important to mention that the utilization of products recovered from waste streams is subject to national regulation. Therefore, effective communication between technology developers, companies, and political institutions is required to develop standards that ensure the quality and safety of the PPB technology and its products.

Bottlenecks in upscaling artificial light systems

Unlike sunlight, which has daily/seasonal irradiation variations and is affected by weather conditions, artificial light can be fully controlled in terms of irradiation time, intensity, wavelength, and spatial distribution. In the case of light wavelength, the utilization of LED lamps that emit in the near-infrared region (NIR) can even be used to selectively grow purple bacteria over microalgae (Puyol *et al.*, 2017). From a process point of view, artificial illumination would be the optimal light source for increased productivities of biomass and associated biopolymers (Fradinho *et al.*, 2019). However, it brings extra capital and operating costs from the economic side. Capson-Tojo *et al.* (2020) estimated an illumination cost of 1.9 $ per kg of PPB biomass grown under artificial LED illumination (based on 2019 US energy costs and assuming a maximum empirical value of 59 g COD kWh^{-1} for the biomass energy yield). In a biopolymer production process, which must also account for biomass downstream processing costs, these illumination costs would be an additional weight that likely could not be compensated from the incomes of PPB biopolymers produced from waste streams. Similar observations have been made over the years concerning microalgae bioprocesses in which the utilization of artificial illumination is only advised for high-value molecules production (Blanken *et al.*, 2013). As such, free sunlight illumination is fundamental for the economic viability of PPB systems dedicated to the production of commodities, like PHA or fertilizers, which have lower commercial prices. Hence, PPB technologies should be designed in accordance with the constraints of solar irradiance and set up for outdoor operation.

4.IV.7. Limitations of light irradiation based on rector design and potential measures to solve these limitations

As stressed above, light irradiance is a key factor in developing the PPB bioprocess. These organisms can be cultivated via open or closed systems. Open raceways systems made of closed-loop recirculation channels are typically shallow (<0.3 m deep), unlined, and have a moderate surface-to- volume ratio of 3-10/m (Gupta *et al.*, 2019). These systems are also prone to contamination by pathogens and predators, experience water loss via evaporation, and low illumination efficiency. Nevertheless, they account for 95% of worldwide algal production (Mendoza *et al.*, 2013) due to the low capital and operation costs and easy scalability and operation. Closed systems or PBRs were designed to overcome the problems associated with open-pond systems, and culture growth in them is much more controlled. PBRs can be tilted at different angles using diffuse and reflected light that can play an important role in productivity. Although there are many examples of different PBR designs, two main types are commercially applied nowadays: tubular reactors and flat panel reactors. Both have already been heavily studied on PPB (Lu *et al.*, 2019), although mainly as indoor reactors. Regardless of the type of reactor used, sunlight is still the key limiting factor due to low light intensity, uneven distribution, shifts during the day/night cycle, changing weather conditions, seasonal changes, or direct exposure to UV irradiation. This light limitation does not allow fully exploring the growth potential of PPB and the production of associated polymers. Fradinho *et al.* (2016) reported specific growth rates values around 0.5-0.8 d^{-1} in PHA producing PPB mixed cultures selected under low light conditions. However, specific growth rates can range from 0.6 up to 12 d^{-1} in axenic cultures (Madigan and Gest, 1979). This variation naturally results from the diversity of PPB organisms tested and from the culturing conditions where temperature, carbon source, and light irradiation needs are known to impact the growth rates. For comparison purposes, in PHA producing aerobic mixed cultures, specific growth rates have been reported in the range of 0.96 to 2.16 d^{-1} (Oliveira *et al.*, 2017). However, in these systems, culture selection occurs in the constant presence of oxygen (minimal oxygen saturation levels ~10%), supporting the culture's oxygen needs for ATP production and consequent growth. In photosynthetic systems, the difficulty in providing the ideal light input will limit ATP production and the capability to reach higher specific growth rates. This can explain why, in comparison to aerobic chemotrophic organisms, PPB systems typically present lower biomass production rates. As such, it is fundamental to develop new strategies to enhance solar illumination efficiency, exploring different solar collection and distribution systems, thermochromic materials or solar filtration technologies.

Solar collection and spatial light dilution systems

Spatial light dilution is a method to decrease photon flux density and distribute it in a larger surface area. This method requires diffusers and collectors such as optical fibers, parabolic discs, green solar collectors, or luminescent solar concentrator panels. Optical fibers are glass or plastic transparent fibers that can transport light from one place to another (for example, from a parabolic disc to inside the reactor). They have shown significantly increased productivities in microalgae cultivation and have already been studied on PPB, with a 1.38-fold increase in hydrogen production (Chen *et al.*, 2008). A breakthrough in new materials for the cost-effective production of optical fibers must occur since their excessive prices still make them unsuitable for large-scale applications. An exciting option is the use of poly- methyl-methacrylate as the primary material. This material has been proved efficient in increasing the phototrophic growth of the green algae *Haematococcus pluvialis* by around 95% (Wondraczek *et al.*, 2019). Interestingly, multi-wavelength light penetration through these optical fibers seems to be better in the near-infrared range, so it is a promising material for being used with PPB. However, biofilm formation over the optical fiber is a major drawback for continuous operation if the process is focused on biopolymers production, which considerably limits the volumetric productivity. A potential solution for this is applying a smart low-voltage electric field to avoid bacterial adhesion over the illuminated surface. This has been successfully applied to avoid microbial fouling problems in MBR reactors (J. Zhang *et al.*, 2015).

The most promising spatial dilution method is luminescent solar concentration panels, built by luminescent particles like organic dyes or quantum dots that absorb and re-emit light at longer wavelengths (Hill *et al.*, 2019). The use of luminescent solar concentrations has already been tested on raceway reactors for microalgae and cyanobacteria cultivation, with Raeisossadati *et al.* (2019) finding a 26% increase in biomass productivity for *Arthrospira platensis*. Luminescent solar concentrations are easy to construct, cost-effective, and feasible to use in outdoor open pond systems, but they must be tested on PPB since the wavelengths needed to improve productivity differ from microalgae cyanobacteria.

Solar filtration technologies

The development of wavelength filtration systems has allowed the specialized culture of PPB using only IR or NIR wavelengths. The more typically used method in laboratory-scale PPB cultivation is UV-VIS absorbing foil, but even though it is a cheap material, its use in outdoors large-scale reactors does not seem suitable since it is an easily tear-off material. Another option is colored filter glass, a relatively inexpensive filter type that attenuates light but sacrifices absorption. The design of PBR with this material has already been tested, improving productivity in microalgae (Sun *et al.*, 2018). Temperature upsurge due to the absorbed wavelengths is still the biggest drawback, thus making it less adequate under intense illumination conditions. Finally, thin-film coatings destructively interfere with unwanted wavelengths instead of absorbing them, solving at the same time the overheating problems of glass coatings. These materials can easily be adapted for a PBR design, and they were recently tested on a cascade photobioreactor to cultivate high-density microalgal cultures for biodiesel production (Tan *et al.*, 2020). They have not been proven for PPB growth yet, but it has the potential to be a cost-efficient technology.

Thermochromic materials

One of the biggest problems in outdoor reactors is seasonal temperature changes. Specifically, in winter, low temperatures can cause a decrease in metabolic efficiency and even freeze the reactor. Thermochromics materials can change their transmittance and reflectance properties when subjected to temperature variations. They are usually employed as glazing materials in architecture, thus regulating the amount of light transmitted or reflected depending on atmospheric temperature (Kamalisarvestani *et al.*, 2013). They act as specific wavelength filters and transmit more heat associated with IR and near-IR radiations at low temperatures (Granqvist and Niklasson, 2017). On the other hand, they reflect IR wavelengths at high temperatures, so they should be adapted to the reactor and easily removed during hot days. These properties can be perfectly adapted to the needs of PPB cultivation through effective sunlight wavelength management and temperature regulation, but it needs an exhaustive experimental study to determine its suitability. No work has been published so far applying this technology.

4.IV.8. PPB retention systems for biopolymers production and potential measures to solve bottlenecks

PPB can be cultivated in reactors/tanks with varied configurations, where cells can freely circulate in the system or be retained through the presence of carriers, support materials, or membranes (Chen *et al.*, 2020). Biomass retention systems have been proposed to reduce the system volume by concentrating cells, improving nutrient removal from the liquid stream, and facilitating liquid separation from the biomass (Chen *et al.*, 2020). Cells can be retained, for example, by using photo-anaerobic membrane bioreactors (PAnMBR) as proposed by Hülsen *etal.* (2016b) for wastewater treatment with PPB. However, when PPB are operated for polymer production, the goal is quite the opposite of biomass retention. Instead, the aim is to remove as much biomass as possible for stable polymer extraction. Therefore, it is even more critical for up-scaled systems that simple and direct access to the biomass is implemented with the lowest possible manual labor requirement. At the current technology development, suspended cells systems may be a better option, and while some settling issues may be pointed out, they may be minimized by the capacity of PPB to attach to particles present in the waste stream, forming granules. This can improve sludge settling and subsequent biomass recovery in PPB mixed culture systems.

Settling

One of the highest operational costs in polymer extraction is the dewatering of biomass after its cultivation (Alloul *et al.*, 2018). Settling is one of the most established methods, but PPB collection is challenging due to their small size (0.2-4 μm), high electronegativity, and stable suspension state in the culture medium (Chen *et al.*, 2020). The easiest and cheapest would be gravitational settling, but if not possible, membrane filtration can be performed followed by centrifugation (Alloul *et al.*, 2018), although it will increase operating costs. Recently, Cerruti *et al.* (2020) developed an enriched, concentrated and well-settling PPB mixed-culture operated under the SBR regime. This mixed-culture forms bio-aggregates with good settling properties. Settled biomass accounted for a 3-fold higher relative abundance of PPB (80% as the sum of *Rhodobacter*, *Rhodopseudomonas*, and *Blastochloris* sp.) than the non-settled biomass (25%) with sedimentation G-flux of solids up to 4.7 kg $h^{-1}m^{-2}$. This should lead to an efficient solid to liquid separation, lowering costs for downstream processing by potentially reducing the need for ultrafiltration and centrifugation to concentrate the biomass.

Flocculation

Generally, flocculation has some advantages for industrial processes because of the simplicity of liquid/solid separation and the ease of mass cell retention in the reactor. However, flocculating photosynthetic bacteria have not yet been practically applied for industrial use due to their high cost associated. Some studies demonstrate how sodium, pH, and light intensity affect flocculation for *Rhodobacter sphaeroides* (Lu *et al.*, 2019). Watanabe *et al.* (1998) studied the growth and flocculation of *Rhodovulum* sp and tried to improve it by adding metal cations. The only study found about flocculation on PPB mixed cultures was published in 1983 and uses aluminum sulfate as a flocculant (Freedman *et al.*, 1983). So, this is an open field of research in this process with PPB mixed cultures.

Photo-Granulation

From a bioprocess perspective, the high potential of granules to settle facilitates the recovery and reuse of the active microbial biomass, and in the wastewater treatment context, the development of oxygenic photo-granules is a good novelty (Abouhend *et al.*, 2018). Also, granules can give way to IR light photons better than suspended biomass. Following the Beer-Lambert law, light absorbance will be enhanced with concentrated biomass, making deeper bioreactors possible, improving volumetric productivity and reducing economic costs. Most studies have been performed on microalgae, but the research on other microorganisms is attracting increased interest, and PPB's ability to form granules has been proven (Stegman *et al.*, 2021; Wilbanks *et al.*, 2014). In Section III, we have already shown that the formation of EPS granules directly affects PHA accumulation. Controlling the formation of photo granules is an open invitation to interdisciplinary collaborations between ecologists, bioprocess engineers, and environmental scientists.

4.IV.9. Homogeneity of the operation

Mixing is a crucial feature in the cultivation of any photosynthetic microorganism. Homogeneous mixing can reduce the gradient of nutrients, pH, temperature, and substrate in the reactor, preventing biomass settling, stagnant or dead zones, and cell aggregation (Kumar *et al.*, 2015). Besides, homogeneity ensures that all cells are equally exposed to the light and promotes mass transfer between phases. However, mixing accounts for approximately 69%

of the total utility cost, in the range of 1.5-8.4 W m^{-3} (Mendoza *et al.*, 2013). Therefore, unnecessarily mixing should be avoided, reducing the mixing velocity during the night and even in the winter season to minimize the operational cost. On the positive side, power consumption can be minimized under lower volume depths, which agrees well with PPB cultivation, and deteriorates its growth over 20 cm of depth due to restricted illumination.

Several modifications in PBR have been proposed to tackle mixing costs and demonstrated to enhance biomass productivity, mixing efficiency, and light penetration, such as closed ponds, hybrid raceways, different flows through modular stack raceways, or unlevelled baffled raceways (Kumar *et al.*, 2015). Another vital point to consider is the inlets and outlet points on the reactor. A single inlet point can create poor mixing and substrate gradient throughout the reactor (Enfors *et al.*, 2001). Thus, multiple inputs to the PBR should be considered, even more so in larger reactors.

4.IV.10. Automatization of the system

Control systems play an essential role in the development of bioreactors. In general, the main functions of a bioreactor control system include process control, monitoring, data gathering, and processing. The current trends in bioreactor control systems can be grouped into two aspects: some studies are focused on control strategies and algorithms adapted to bioreactor control systems (Steinwandter *et al.*, 2019), while others mainly investigate the physical structure of the instrumental organization of bioreactor control systems (Wang *et al.*, 2020).

PPB activity can be assessed by key performance indicators such as pH, absorbance, temperature, illumination, nutrient and substrate recovery rates, and biomass productivity, for which suspended solids and nutrient concentrations must be measured. Although online sensors can monitor ammonium, nitrate, and suspended solids concentrations, they usually have higher capital and maintenance costs but are not always as reliable as expected. Therefore, they are often measured by time-consuming and expensive laboratory analyses (Foladori *et al.*, 2018). The same happens with the production of biopolymers since their intracellular concentration can only be measured in the laboratory, which amplifies the need for online monitoring strategies based on dynamic modeling dependent on indirect parameters such as pH, absorbances at 805 and 8065 nm, illumination, temperature, and the hydraulic retention time. But the complexity, inherent nonlinearity, and high kinetic uncertainty can make this approach very complicated. There are recent examples to control microalgae activity, such as the works published by DeLuca *et al.* (2018), where a growth optimization based on uncertain weather forecasts is shown, or Robles *et al.* (2020), which demonstrated a community stabilization based on pH and dissolved oxygen. However, no work on the control of PPB activity has been published yet. Control systems should be implemented as part of a SCADA (Supervisory, Control, and Data Acquisition) program for supervision, data acquisition, and equipment control. Reactor level control, feeding times, mixing, among others, can be output signals that can allow for better system optimization upon the dynamic model algorithm implementation.

4.IV.11. Future directions on PHA production with PPB mixed cultures

In prospecting the transfer of the PHA producing technology with PPB mixed cultures to larger-scale outdoor facilities, it is essential to mention that the two selection strategies currently applied to enrich the mixed culture in PHA-producing PPB, FF and PF, have very distinct modes of operation and requirements. Both strategies present solid points but also aspects that can be further improved. Table IV.2 compares, side by side, the main characteristics of the two strategies.

Looking at the characteristics of the FF strategy, the major factor that can hinder the technology transfer to large outdoor operations is the system dependence on microalgae to produce the oxygen that enables the PHA consumption during the famine phase. There is, however, margin to develop the FF technology by making the system independent of the microalgae presence. The microalgae elimination could further enrich the mixed culture in PPB while the advantageous features of the FF operation could be maintained. As previously mentioned, the system requires an electron acceptor during the famine phase, which can be provided through external aeration. Although it brings additional energy costs to the famine phase, the oxygen needs during the famine phase are substantially lower than the feast phase (2 - 5 times lower) (Third *et al.*, 2003), and thus minimal aeration would be required.

Therefore, future studies should evaluate if the costs incurred with aeration in the famine phase could be overcome by the following advantages: (i) the famine aerated phase could be schedule to the night time, and thus, the night would no longer be an idle period, but instead, could actively contribute to the FF process; (ii) with the famine phase occurring in the night, the feast phase could be extended up to the night time increasing the system PHA productivity and eventually eliminate the need for a secondary accumulator tank reactor (iii) aeration during the night famine phase allows the consumption of residual organic compounds that are not assimilated by PPB during the day anaerobic feast phase, permitting the discharge of a better quality effluent, (iv) aeration rates can be easily tuned to address the culture oxygen requirements at each night famine phase, maintaining the culture under very low oxygen concentrations (0.0-1.0 $mgO_2\ L^{-1}$) which decreases aeration energy costs and minimizes inhibition of PPB pigments expression, (v) the

Table IV.2 Characteristics and specific requirements of the feast and famine and the permanent feast strategies used for the selection of PHA producing PPB mixed cultures.

	Feast and Famine (PPB and microalgae)	**Permanent Feast (PPB enriched culture)**
Aeration	Aeration is eliminated since the microalgae provide autooxygenation; FF cycle must occur during the daytime.	Anaerobic operation, no aeration requirements.
Carbon recovery	High carbon recovery and conversion to PHA is achieved during the feast phase; PHA is partly dissipated as CO_2 during the famine phase.	Up to 100% carbon recovery (approaching the system to CO2 neutrality) with high conversion to PHA.
Nutrient limitation	Carbon is the limiting nutrient that triggers PHA storage; No other nutrient limitation is required.	High PHA content is achieved under nutrient limitation, requiring feedstock with high carbon to nutrient ratios (e.g., high C:P or C:S ratios)
Electron balance	Oxygen availability during the famine phase reduces PPB internal redox stress allowing the uptake of more reduced substrates during the feast phase (e.g., butyric and valeric acids).	Anaerobic conditions induce consumption of less reduced substrates (e.g., acetate, propionate) to facilitate redox balance, leading to the accumulation of less preferable compounds
OLR	Strict control in order to maintain a stable feast to famine ratio.	No famine phases; Organic carbon is continuously present, simplifying system operation

Table IV.2 Characteristics and specific requirements of the feast and famine and the permanent feast strategies used for the selection of PHA producing PPB mixed cultures. (*continued*)

	Feast and Famine (PPB and microalgae)	Permanent Feast (PPB enriched culture)
Sugar presence in the feedstock	Oxygen presence inhibits fermentative metabolism.	Full fermentation of the feedstock must be assured to prevent fermentation processes that conflict with PHA production
Presence of Non-PHA producers	Although essential for oxygenation, microalgae decrease the overall PHA content in the biomass and may compete for carbon (e.g., acetate);	Microalgae are outcompeted; Fermenting organisms may grow if fermentable compounds are present in the feedstock (e.g., sugars, lactate)

operational conditions lead to a microbial selection that favours the mixed culture enrichment in PPB that are more oxygen tolerant, which further overcomes eventual oxygen inhibitions due to the night aerated time. With these adaptations, the FF strategy would be ideal for operation with a feedstock with highly reduced carbon compounds and high nutrients since it does not rely upon nutrient limitation for improved PHA accumulation. However, carbon dissipation will always occur in the famine phase during PHA consumption.

Concerning the PF strategy, its major challenge is related precisely to the feedstock composition. Since this strategy relies on the observance of anaerobic conditions, the presence of sugars and high sulfate concentrations in the feedstock can lead to the growth of a side-population composed of fermentative organisms or even of sulfate-reducing bacteria (the latest supported by the highly reductive conditions promoted by the sugars). Therefore, it is essential to control the sugar present in the feedstock by implementing, for example, upstream fermentation processes. Furthermore, high nutrient concentrations in the feedstock can also challenge the production of high PHA content. In this situation, to favor the PHA storage over the growth, additional metabolic constraints may be needed in parallel to the selection under the permanent feast strategy. For example, adjusting the ratio of food to microorganisms (F/M) may be a possible solution since F/M has been suggested to impact the dynamics of internal carbon flow during PHA storage in PPB systems (Fradinho *et al.*, 2016). Also, eventual growth decouplers (aromatics, metals) in the feedstock may help limit growth but not the PHA production activity (Puyol *et al.*, 2019). Therefore, the PF selection strategy is suited for operation with a feedstock that contains less reduced carbon sources (e.g., acetate, propionate) and low nutrient concentration (P, S) that allow the PHA storage over the growth. Compared to the FF strategy, it has the advantage of high carbon recovery that approaches PF systems to CO2 emission neutrality.

For the outdoor operation of PPB mixed cultures under the PF selection strategy, the current knowledge proposes the operation of two reactors/tanks: one for culture selection and growth with low light availability and a second for an external accumulation step under higher light availability. Decreasing the implementation area through one single reactor operation would be a more economically attractive alternative. This raises the interesting and not yet addressed question of whether simultaneous culture selection and high PHA production are possible. The culture would permanently present high intrinsic levels of PHA, but this would imply the system operation with higher light availability. A possible conflict with the culture selection could occur (as previously discussed). However, a decrease in the culture's overall photosynthetic efficiency could be observed (reduction of light-harvesting complex levels due to acclimation to high light operation) (Muzziotti *et al.*, 2017). Further research can bring more insight to this challenging question.

4.IV.12. Conclusions

The sustainable production of biopolymers from waste sources by mixed cultures of PPB is an emerging field facing several challenges. Using current knowledge on mixed outdoor reactors based on PPB mixed cultures systems is crucial for achieving full-scale deployment of the technology. In addition, the need to advance on upscaling challenges like improved sun illumination, biomass retention and collection, and feed control has promoted the development of specific projects focused on these challenges.

4.IV.13. References

Abouhend, A. S., McNair, A., Kuo-Dahab, W. C., Watt, C., Butler, C. S., Milferstedt, K., Hamelin, J., Seo, J., Gikonyo, G. J., El-Moselhy, K. M., and Park, C. (2018). The Oxygenic Photogranule Process for Aeration-Free Wastewater Treatment. Environmental Science andamp; Technology, 52(6), 3503-3511. https://doi.org/10.1021/acs.est.8b00403

Albuquerque, M. G. E., Martino, V., Pollet, E., Avérous, L., and Reis, M. A. M. (2011). Mixed culture polyhydroxyalkanoate (PHA) production from volatile fatty acid (VFA)-rich streams: Effect of substrate composition and feeding regime on PHA productivity, composition and properties. Journal of Biotechnology, 151(1), 66-76. https://doi.org/10.1016/j.jbiotec.2010.10.070

Alloul, A., Ganigué, R., Spiller, M., Meerburg, F., Cagnetta, C., Rabaey, K., and Vlaeminck, S. E. (2018). Capture-Ferment-Upgrade: A Three-Step Approach for the Valorization of Sewage Organics as Commodities. Environmental Science and Technology, 52(12), 6729-6742. https://doi.org/10.1021/acs.est.7b05712

Alsiyabi, A., Immethun, C. M., and Saha, R. (2019). Modeling the Interplay between Photosynthesis, CO2 Fixation, and the Quinone Pool in a Purple Non-Sulfur Bacterium. Scientific Reports, 9(1), 1-9. https://doi.org/10.1038/s41598-019-49079-z

Basak, N., Jana, A. K., Das, D., and Saikia, D. (2014). Photofermentative molecular biohydrogen production by purple-non-sulfur (PNS) bacteria in various modes: The present progress and future perspective. International Journal of Hydrogen Energy, 39(13), 6853-6871. https://doi.org/10.1016/j.ijhydene.2014.02.093

Bayon-Vicente, G., Wattiez, R., and Leroy, B. (2020). Global Proteomic Analysis Reveals High Light Intensity Adaptation Strategies and Polyhydroxyalkanoate Production in Rhodospirillum rubrum Cultivated With Acetate as Carbon Source. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.00464

Blanken, W., Cuaresma, M., Wijffels, R. H., and Janssen, M. (2013). Cultivation of microalgae on artificial light comes at a cost. Algal Research, 2(4), 333-340. https://doi.org/10.1016Zj.algal.2013.09.004

Capson-tojo, G., Batstone, D. J., Grassino, M., Vlaeminck, S. E., Puyol, D., Verstraete, W., Kleerebezem, R., Oehmen, A., Ghimire, A., Pikaar, I., Lema, J. M., and Hülsen, T. (2020). Purple phototrophic bacteria for resource recovery: Challenges and opportunities. Biotechnology Advances, 43(May), 107567. https://doi.org/10.1016/j.biotechadv.2020.107567

Carvalho, V. C. F., Freitas, E. B., Fradinho, J. C., Reis, M. A. M., and Oehmen, A. (2019). The effect of seed sludge on the selection of a photo-EBPR system. New Biotechnology, 49, 112-119. https://doi.org/10.1016/j.nbt.2018.10.003

Cerruti, M., Stevens, B., Ebrahimi, S., Alloul, A., Vlaeminck, S. E., and Weissbrodt, D. G. (2020). Enriching and aggregating purple non-sulfur bacteria in an anaerobic sequencing-batch photobioreactor for nutrient capture from wastewater. Frontiers in Bioengineering and Biotechnology, 8. https://doi.org/10.3389/fbioe.2020.557234

Chen, J., Wei, J., Ma, C., Yang, Z., Li, Z., and Yang, X. (2020). Photosynthetic bacteria-based technology is a potential alternative to meet sustainable wastewater treatment requirement. Environment International, 137, 105417. https://doi.org/10.1016/j.envint.2019.105417

Comer, A. D., Abraham, J. P., Steiner, A. J., Korosh, T. C., Markley, A. L., and Pfleger, B. F. (2020). Enhancing Photosynthetic Production of Glycogen-Rich Biomass for Use as a Fermentation Feedstock. Frontiers in Energy Research, 8. https://doi.org/10.3389/fenrg.2020.00093

De-Luca, R., Trabuio, M., Barolo, M., and Bezzo, F. (2018). Microalgae growth optimization in open ponds with uncertain weather data. Computers and Chemical Engineering, 117, 410419. https://doi.org/https://doi.org/10.1016/j.compchemeng.2018.07.005

Di Caprio, F. (2021). Cultivation processes to select microorganisms with high accumulation ability. Biotechnology Advances, 49, 107740. https://doi.org/10.10167j.biotechadv.2021.107740

Philippis, R. de, Ena, A., Guastini, M., Sili, C., and Vincenzini, M. (1992). Factors affecting poly- / 3-hydroxybutyrate accumulation in cyanobacteria and in purple non-sulfur bacteria. 103, 187194.

Desmidt, E., Ghyselbrecht, K., Zhang, Y., Pinoy, L., van der Bruggen, B., Verstraete, W., Rabaey, K., and Meesschaert, B. (2015). Global Phosphorus Scarcity and Full-Scale P-Recovery Techniques: A Review. Critical Reviews in Environmental Science and Technology, 45(4), 336384.

Egger, F., Hülsen, T., Tait, S., and Batstone, D. J. (2020). Autotrophic sulfide removal by mixed culture purple phototrophic bacteria. Water Research, 182, 115896. https://doi.org/10.1016Zj.watres.2020.115896

Enfors, S.-O., Jahic, M., Rozkov, A., Xu, B., Hecker, M., Jürgen, B., Krüger, E., Schweder, T., Hamer, G., O'Beirne, D., Noisommit-Rizzi, N., Reuss, M., Boone, L., Hewitt, C., McFarlane, C., Nienow, A., Kovacs, T., Tragardh, C., Fuchs, L., ... Manelius, A. (2001). Physiological responses to mixing in large scale bioreactors. Journal of Biotechnology, 85(2), 175-185. https://doi.org/https://doi.org/10.1016/S0168-1656(00)00365-5

Foladori, P., Petrini, S., and Andreottola, G. (2018). Evolution of real municipal wastewater treatment in photobioreactors and microalgae-bacteria consortia using real-time parameters. Chemical Engineering Journal, 345, 507-516. https://doi.org/https://doi.org/10.1016/j.cej.2018.03.178

Fradinho, J. C., Domingos, J. M. B., Carvalho, G., Oehmen, A., and Reis, M. A. M. (2013). Polyhydroxyalkanoates production by a mixed photosynthetic consortium of bacteria and algae. Bioresource Technology, 132, 146-153. https://doi.org/10.1016/j.biortech.2013.01.050

Fradinho, J. C., Oehmen, A., and Reis, M. A. M. (2013). Effect of dark/light periods on the polyhydroxyalkanoate production of a photosynthetic mixed culture. Bioresource Technology, 148, 474-479. https://doi.org/10.1016/j.biortech.2013.09.010

Fradinho, J. C., Oehmen, A., and Reis, M. A. M. (2014). Photosynthetic mixed culture polyhydroxyalkanoate (PHA) production from individual and mixed volatile fatty acids (VFAs): Substrate preferences and co-substrate uptake. Journal of Biotechnology, 185, 19-27. https://doi.org/10.1016/j.jbiotec.2014.05.035

Fradinho, J. C., Oehmen, A., and Reis, M. A. M. (2019). Improving polyhydroxyalkanoates production in phototrophic mixed cultures by optimizing accumulator reactor operating conditions. International Journal of Biological Macromolecules, 126, 1085-1092. https://doi.org/10.1016/j.ijbiomac.2018.12.270

Fradinho, J. C., Reis, M. A. M., and Oehmen, A. (2016). Beyond feast and famine: Selecting a PHA accumulating photosynthetic mixed culture in a permanent feast regime. Water Research, 105, 421-428. https://doi.org/10.1016/j.watres.2016.09.022

Freedman, D., Koopman, B., and Lincoln, E. P. (1983). Chemical and biological flocculation of purple sulphur bacteria in anaerobic lagoon effluent. Journal of Agricultural Engineering Research, 28(2), 115-125. https://doi.org/https://doi.org/10.1016/0021-8634(83)90081-1

Granqvist, C. G., and Niklasson, G. A. (2017). Thermochromic oxide-based thin films and nanoparticle composites for energy-efficient glazings. Buildings, 7(1). https://doi.org/10.3390/buildings7010003

Gupta, S., Pawar, S. B., and Pandey, R. A. (2019). Current practices and challenges in using microalgae for treatment of nutrient rich wastewater from agro-based industries. Science of the Total Environment, 687, 1107-1126. https://doi.org/10.1016Zj.scitotenv.2019.06.115

Hill, S. K. E., Connell, R., Peterson, C., Hollinger, J., Hillmyer, M. A., Kortshagen, U., and Ferry, V. E. (2019). Silicon Quantum Dot-Poly(methyl methacrylate) Nanocomposites with Reduced Light Scattering for Luminescent Solar Concentrators. ACS Photonics, 6(1), 170-180. https://doi.org/10.1021/acsphotonics.8b01346

Hirais, A., Yanase, A., and Kitamura, H. (1991). Polyphosphate Grown Accumulation under Different by Rhodobacter Environmental on the sphaeroides

Conditions with Special Emphasis Phosphate Effect of External Concentrations AKIRA HIRAISHI , 1 * AKINOIIU YANASE , 2 and HIROSHI KITAMURA2 2Department of Ind. Bulletin of Japanese Society of Microbial Ecology, 6(1), 25-32.

Hülsen, T., Barry, E. M., Lu, Y., Puyol, D., and Batstone, D. J. (2016). Low temperature treatment of domestic wastewater by purple phototrophic bacteria: Performance, activity, and community. Water Research, 100, 537-545. https://doi.org/10.1016/j.watres.2016.05.054

Hülsen, T., Barry, E. M., Lu, Y., Puyol, D., Keller, J., and Batstone, D. J. (2016). Domestic wastewater treatment with purple phototrophic bacteria using a novel continuous photo anaerobic membrane bioreactor. Water Research, 100, 486-495. https://doi.org/10.10167j.watres.2016.04.061

Igarashi, R. Y., and Meyer, C. R. (2000). Cloning and sequencing of glycogen metabolism genes from Rhodobacter sphaeroides 2.4.1. expression and characterization of recombinant ADP-glucose pyrophosphorylase. Archives of Biochemistry and Biophysics, 376(1), 47-58. https://doi.org/10.1006/abbi.1999.1689

Iliescu, S., Ilia, G., Plesu, N., Popa, A., and Pascariu, A. (2006). Solvent and catalyst-free synthesis of polyphosphates. Green Chemistry, 8(8), 727. https://doi.org/10.1039/b602462a

Jendrossek, D. (2009). Polyhydroxyalkanoate Granules Are Complex Subcellular Organelles (Carbonosomes). Journal of Bacteriology, 191(10), 3195-3202. https://doi.org/10.1128/JB.01723-08

Kamalisarvestani, M., Saidur, R., Mekhilef, S., and Javadi, F. S. (2013). Performance, materials and coating technologies of thermochromic thin films on smart windows. Renewable and Sustainable Energy Reviews, 26(2013), 353-364. https://doi.org/10.1016/j.rser.2013.05.038

Kitamura, H., and Hiraishi, A. (1985). Changes in the Polyphosphate Content of Photosynthetically Grown Rhodobacter sphaeroides Due to Nutrient Limitation. Agricultural and Biological Chemistry, 49(11), 3343-3345. https://doi.org/10.1271/bbb1961.49.3343

Koku, H., Erolu, I., Gündüz, U., Yücel, M., and Türker, L. (2002). Aspects of the metabolism of hydrogen production by Rhodobacter sphaeroides. International Journal of Hydrogen Energy, 27(11-12), 1315-1329. https://doi.org/10.1016/S0360-3199(02)00127-1

Kumar, K., Mishra, S. K., Shrivastav, A., Park, M. S., and Yang, J.-W. (2015). Recent trends in the mass cultivation of algae in raceway ponds. Renewable and Sustainable Energy Reviews, 51, 875-885. https://doi.org/https://doi.org/10.1016/j.rser.2015.06.033

Lai, Y. C., Liang, C. M., Hsu, S. C., Hsieh, P. H., and Hung, C. H. (2017). Polyphosphate metabolism by purple non-sulfur bacteria and its possible application on photo-microbial fuel

cell. Journal of Bioscience and Bioengineering, 123(6), 722-730. https://doi.org/10.1016/jjbiosc.2017.01.012

Laurinavichene, T. v., Rákhely, G., Kovács, K. L., and Tsygankov, A. A. (2007). The effect of sulfur compounds on H2 evolution/consumption reactions, mediated by various hydrogenases, in the purple sulfur bacterium, Thiocapsa roseopersicina. Archives of Microbiology, 188(4), 403410. https://doi.org/10.1007/s00203-007-0260-7

Laycock, B., Halley, P., Pratt, S., Werker, A., and Lant, P. (2014). The chemomechanical properties of microbial polyhydroxyalkanoates. Progress in Polymer Science, 39(2), 397-442. https://doi.org/10.1016/j.progpolymsci.2013.06.008

Liang, C. M., Hung, C. H., Hsu, S. C., and Yeh, I. C. (2010). Purple nonsulfur bacteria diversity in activated sludge and its potential phosphorus-accumulating ability under different cultivation conditions. Applied Microbiology and Biotechnology, 86(2), 709-719. https://doi.org/10.1007/s00253-009-2348-2

Lu, H., Dong, S., Zhang, G., Han, T., Zhang, Y., and Li, B. (2019). Enhancing the autoflocculation of photosynthetic bacteria to realize biomass recovery in brewery wastewater treatment. Environmental Technology, 40(16), 2147-2156. https://doi.org/10.1080/09593330.2018.1439107

Madigan, M. T., and Gest, H. (1979). Growth of the photosynthetic bacterium Rhodopseudomonas capsulata chemoautotrophically in darkness with H2 as the energy source. Journal of Bacteriology, 137(1), 524-530. https://doi.org/10.1128/jb.137.1.524-530.1979

Marín, D., Posadas, E., García, D., Puyol, D., Lebrero, R., and Muñoz, R. (2019). Assessing the potential of purple phototrophic bacteria for the simultaneous treatment of piggery wastewater and upgrading of biogas. Bioresource Technology, 281, 10-17. https://doi.org/10.1016/j.biortech.2019.02.073

McKinlay, J. B., and Harwood, C. S. (2010). Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria. Proceedings of the National Academy of Sciences of the United States of America, 107(26), 11669-11675. https://doi.org/10.1073/pnas.1006175107

Mendoza, J. L., Granados, M. R., de Godos, I., Acién, F. G., Molina, E., Heaven, S., and Banks, C. J. (2013). Oxygen transfer and evolution in microalgal culture in open raceways. Bioresource Technology, 137, 188-195. https://doi.org/10.1016/j.biortech.2013.03.127

Monroy, I., and Buitrón, G. (2020). Production of polyhydroxybutyrate by pure and mixed cultures of purple non-sulfur bacteria: A review. Journal of Biotechnology, 317, 39-47. https://doi.org/10.1016/j.jbiotec.2020.04.012

Montiel-Corona, V., and Buitrón, G. (2021). Polyhydroxyalkanoates from organic waste streams using purple non-sulfur bacteria. Bioresource Technology, 323, 124610. https://doi.org/10.1016/j.biortech.2020.124610

Muzziotti, D., Adessi, A., Faraloni, C., Torzillo, G., and De Philippis, R. (2017). Acclimation strategy of Rhodopseudomonas palustris to high light irradiance. Microbiological Research, 197, 49-55. https://doi.org/https://doi.org/10.1016/j.micres.2017.01.007

Reusch, N., and Sadoff, H. L. (1988). Putative structure and functions of a poly-,B- hydroxybutyrate/ calcium polyphosphate channel in bacterial plasma membranes (pore/transport/DNA). PNAS, 85, 4176-4180.

Oliveira, C. S. S., Silva, C. E., Carvalho, G., and Reis, M. A. (2017). Strategies for efficiently selecting PHA producing mixed microbial cultures using complex feedstocks: Feast and famine regime and uncoupled carbon and nitrogen availabilities. New Biotechnology, 37, 69-79. https://doi.org/10.1016/j.nbt.2016.10.008

Pedrós-Alió, C., Mas, J., and Guerrero, R. (1985). The influence of poly-hydroxybutyrate accumulation on cell volume and buoyant density in alcaligenes eutrophus. Archives of Microbiology, 143(2), 178-184. https://doi.org/10.1007/BF00411044

Pokorna, D., and Zabranska, J. (2015). Sulfur-oxidizing bacteria in environmental technology. Biotechnology Advances, 33(6), 1246-1259. https://doi.org/10.1016/j.biotechadv.2015.02.007

Puyol, D., Monsalvo, V. M., Marin, E., Rogalla, F., Melero, J. A., Martínez, F., Hülsen, T., and Batstone, D. J. (2019). Purple phototrophic bacteria as a platform to create the next generation of wastewater treatment plants: Energy and resource recovery. In Wastewater Treatment Residues as Resources for Biorefinery Products and Biofuels (255-280). https://doi.org/10.1016/B978-0-12-816204-0.00012-6

Puyol, D., Batstone, D. J., Hülsen, T., Astals, S., Peces, M., and Kromer, J. O. (2017). Resource Recovery from Wastewater by Biological Technologies:

Opportunities, Challenges, and Prospects . In Frontiers in Microbiology (Vol. 7). https://www.frontiersin.org/article/10.3389/fmicb.2016.02106

Raeisossadati, M., Moheimani, N. R., and Parlevliet, D. (2019). Red and blue luminescent solar concentrators for increasing Arthrospira platensis biomass and phycocyanin productivity in outdoor raceway ponds. Bioresource Technology, 291, 121801. https://doi.org/10.1016Zj.biortech.2019.121801

Robles, Á., Capson-Tojo, G., Gales, A., Ruano, M. V., Sialve, B., Ferrer, J., and Steyer, J.-P. (2020). Microalgae-bacteria consortia in high-rate ponds for treating urban wastewater: Elucidating the key state indicators under dynamic conditions. Journal of Environmental Management, 261, 110244. https://doi.org/https://doi.org/10.1016/j.jenvman.2020.110244

Sakarika, M., Spanoghe, J., Sui, Y., Wambacq, E., Grunert, O., Haesaert, G., Spiller, M., and Vlaeminck, S. E. (2020). Purple non-sulphur bacteria and plant production: benefits for fertilization, stress resistance and the environment. Microbial Biotechnology, 13(5), 1336-1365. https://doi.org/10.1111/1751-7915.13474

Sekar, K., Linker, S. M., Nguyen, J., Grünhagen, A., Stocker, R., and Sauer, U. (2020). Bacterial Glycogen Provides Short-Term Benefits in Changing Environments. Applied and Environmental Microbiology, 86(9). https://doi.org/10.1128/AEM.00049-20

Solovchenko, A., Verschoor, A. M., Jablonowski, N. D., and Nedbal, L. (2016). Phosphorus from wastewater to crops: An alternative path involving microalgae. Biotechnology Advances, 34(5), 550-564. https://doi.org/10.1016/j.biotechadv.2016.01.002

Stegman, S., Batstone, D. J., Rozendal, R., Jensen, P. D., and Hülsen, T. (2021). Purple phototrophic bacteria granules under high and low upflow velocities. Water Research, 190, 116760. https://doi.org/10.1016/j.watres.2020.116760

Steinwandter, V., Borchert, D., and Herwig, C. (2019). Data science tools and applications on the way to Pharma 4.0. Drug Discovery Today, 24(9), 1795-1805. https://doi.org/https://doi.org/10.1016/j.drudis.2019.06.005

Sun, H., Zhao, W., Mao, X., Li, Y., Wu, T., and Chen, F. (2018). High-value biomass from microalgae production platforms: Strategies and progress based on carbon metabolism and energy conversion. Biotechnology for Biofuels, 11(1), 1-23. https://doi.org/10.1186/s13068- 018-1225-6

Suzuki, I. (2001). Microbial leaching of metals from sulfide minerals. Biotechnology Advances 19, 119-13. https://doi.org/10.1016/S0734-9750(01)00053-2

Tan, C. H., Tan, X., Ho, S. H., Lam, S. S., Show, P. L., and Nguyen, T. H. P. (2020). Conceptual design of a hybrid thin layer cascade photobioreactor for microalgal biodiesel synthesis. International Journal of Energy Research, 44(12), 9757-9771. https://doi.org/10.1002/er.5699

Third, K. A., Newland, M., and Cord-Ruwisch, R. (2003). The effect of dissolved oxygen on PHB accumulation in activated sludge cultures. Biotechnology and Bioengineering, 82(2), 238250. https://doi.org/https://doi.org/10.1002/bit.10564

Trüper, H. G. (1984). Phototrophic Bacteria and their Sulfur Metabolism (pp. 367-382). https://doi.org/10.1016/B978-0-444-42355-9.50023-2

Vainshtein, M. B., Gogotova, G. I., and Heinritz, N.-J. (1994). Removal of H2S by the purple sulphur bacterium Ectothiorhodospira shaposhnikovii. World Journal of Microbiology and Biotechnology, 10(1), 110-111. https://doi.org/10.1007/BF00357574

Wang, B., Wang, Z., Chen, T., and Zhao, X. (2020). Development of Novel Bioreactor Control Systems Based on Smart Sensors and Actuators . In Frontiers in Bioengineering and Biotechnology (Vol. 8). https://www.frontiersin.org/article/10.3389/fbioe.2020.00007

Watanabe, M., Sasaki, K., Nakashimada, Y., Kakizono, T., Noparatnaraporn, N., and Nishio, N. (1998). Growth and flocculation of a marine photosynthetic bacterium Rhodovulum sp. Applied Microbiology and Biotechnology, 50(6), 682-691. https://doi.org/10.1007/s002530051351

Wilbanks, E. G., Jaekel, U., Salman, V., Humphrey, P. T., Eisen, J. A., Facciotti, M. T., Buckley, D. H., Zinder, S. H., Druschel, G. K., Fike, D. A., and Orphan, V. J. (2014). Microscale sulfur cycling in the phototrophic pink berry consortia of the Sippewissett Salt Marsh. Environmental Microbiology, 16(11), 3398-3415. https://doi.org/10.1111/1462-2920.12388

Wondraczek, L., Gründler, A., Reupert, A., Wondraczek, K., Schmidt, M. A., Pohnert, G., and Nolte, S. (2019). Biomimetic light dilution using side-emitting optical fiber for enhancing the productivity of microalgae reactors. Scientific Reports, 9(1), 1-10. https://doi.org/10.1038/s41598-019-45955-w

Zhang, J., Satti, A., Chen, X., Xiao, K., Sun, J., Yan, X., Liang, P., Zhang, X., and Huang, X. (2015). Low-voltage electric field applied into MBR for fouling suppression: Performance and mechanisms. Chemical Engineering Journal, 273, 223-230. https://doi.org/10.1016Zj.cej.2015.03.044