CHAPTER 5: CONCLUSIONS AND PERSPECTIVE

5.1. General conclusions

Although specific conclusions have been included in each Section of the results and discussion Chapter, the following is answers to the questions stated Chapter 2 of this present Doctoral Thesis.

1) Is it possible to grow a mixed culture of PPB and accumulate PHA using OFMSW hydrolysates?

A mixed culture of PPB has been successfully cultivated from the liquid fraction of OFMSW hydrolysate, both pre-sorted by citizens and selectively sorted at the waste plant. PPB growth has been limited by the availability of nutrients, not by the biodegradability of organic matter. However, this nutrient limitation stimulates the accumulation of PHA from the PPBs, achieving a result of 5% wt. Furthermore, even with low nitrogen concentrations in the substrate, the PPBs contained 48% wt of protein which can also be seen as a high value- added product to be considered. The strategy of concatenating thermal hydrolysis and anaerobic digestion with a photoheterotrophic process results in high value-added products, but also reduces the volume of remaining solids by 5-11% compared with the traditional treatment of an anaerobic digestion process.

2) How does using a carbohydrate-based residue such as lignocellulosic waste affect the PHA accumulation by PPB?

Using the same strategy, mixed cultures of PPB were successfully cultivated from liquid LW hydrolysates. Although these hydrolysates are mainly composed of sugars, PPB managed to assimilate them and also to accumulate PHA (21% wt.). Likewise, as with OFMSW, PPB grows from this organic matter without inhibition, and growth is limited by nutrient consumption. This Thesis is the first time that PHA accumulation is studied with LW, which allows to open this technology to agri-food sectors and to study synergies within classical biorefineries.

3) How do high percentages of lignocellulosic residue affect the acidogenic co-fermentation of the OFMSW?

Given that PPB has a higher capacity to accumulate PHA using SCCA as substrate, this Thesis optimized the acidogenic fermentation process, evaluating whether there is synergy in the co-fermentation of FW and LW wastes. First, the combination of steam explosion and acidogenic fermentation achieves COD solubilizations of up to 80%, even with high percentages of LW.

Under the best-tested conditions, co-fermentation produced up to 162±5mlH$_2$ gCOD^{-1}. H$_2$ production decreased notably with the increase of LW, the effect being stronger in the absence of thermal treatment. However, the fraction of COD derived to H$_2$ is very low (below 4%), and co-fermentation increases SCCA production which translates into a positive synergy effect in the overall efficiency. Values up to 0.58 gCOD of SCCA per gCOD added were achieved with coupled steam explosion and acidogenic fermentation of mixed urban waste, resulting in 93% acidification. Overall, the co-fermentation of FW and LW produces positive synergies, enabling this technology to be used on OFMSW with high LW content or even wastes from agricultural sectors. The results of this Thesis demonstrate the robustness of the acidogenic fermentation technology, which is not only key to optimize the photobiorefinery proposed in this Thesis but can also be used within the context of the carboxylate platform for a wide range of applications such as the biological removal of nitrogen and phosphorous from wastewater, biodiesel production or chain-elongation technology, increasing its versatility.

4) Is it possible, and which parameters are key to maintain a good PHA productivity from OFMSW fermentate?

This Thesis studied the continuous accumulation of PHA in a novel MPBR and obtained the best results of PHA accumulation with mixed PPB cultures and residual substrate in literature (42% wt), achieving a mass balance of the overall process of 0.15 gCOD$_{PHA}$ gCOD which is in the same order of magnitude as the aerobic chemotrophic feast and famine processes, which are much more optimized and researched processes. Besides, the quantification of PHH, in addition to the usual PHB and PHV, represented up to 30% of the total PHA at certain stages, which increased the cumulative PHA identified. On the other hand, increasing the organic load of the reactor resulted in a decrease in PHA but an increase in glycogen, EPS and H$_2$ which shows, for the first time, alternatives to PHA accumulation in PPB mixed cultures: the storage of carbon in both glycogen and extracellular polymers (EPS) while deriving the excess electrons into hydrogen even in the presence of organic ammonium. Furthermore, this Thesis showed the need to study in-depth the functionalities of each bacterial community within mixed cultures of PPB. This

Thesis demonstrated the importance of acquiring a thorough understanding of the carbon accumulation and electron allocation strategies of PPB under stressful environmental conditions and shows promising results for a larger scale implementation of a PPB-based photo-biorefinery.

5) Is the anaerobic digestion process suitable to close the biorefinery's carbon cycle and energy balance?

Anaerobic digestion has proven to be an adaptable and mature technology for the treatment of the non-hydrolyzed organic matter. Hydrothermal pre-treatment of LW affects the overall biodegradability more positively than that of OFMSW, since the methanogenic potential is improved after pretreatment even when the liquid fraction is removed (contrary to OFMSW). The methanogenic potential of the organic fraction of the acidogenic fermentation effluent obtained high results (336 LCH_4 $kgVS^{-1}$), reducing the volume of solids of the integrated photobiorefinery by up to 90% in total. The energy produced in a CHP fed with biogas from anaerobic digestion is sufficient to satisfy the energy requirements of the steam explosion pretreatment and the heat required in the biodigester. This energy autarky is key to achieving a cost-effective integrated photobiorefinery.

6) What are the major challenges in scaling up this technology?

The major challenge of scaling up the photoheterotrophic process with mixed PPB cultures is the design of a bioreactor that can maximize the volumetric irradiance received, without being critically reduced by increasing the biomass concentration. In the short term, the most viable solutions are to place these reactors in regions with high solar irradiance throughout the year, as well as to install solar collectors that increase the irradiance fed back into the reactor. Closed reactors would be ideal to avoid contamination of other communities (microalgae) and to ensure an anaerobic environment, but open raceway reactors would be more economical and IR light filters could drastically reduce contamination. Scaling up the system to a permanent feast operation would also streamline the process to a single reactor, thereby reducing costs. Research is in the sweet spot for higher TRL PPB-based integrated photobiorefineries, from which rawn conclusions to eventually achieve industrial development.

5.2. Recommendations for the future

Based on the results of this Thesis, and on research currently being carried out by other research groups, future directions in PPB culture research, are discussed below:

- **Upscale research**. Some guidelines for future research on PHA accumulation with PPB crops are already discussed in Section IV. Upscaling this technology is the hotspot of the research that is taking place now and probably in the years to come. This upscale will be performed most likely in raceway bioreactors with either solar or solar plus artificial lighting. The strategy most likely to succeed is the permanent feast, which has much higher carbon recovery, but raises the yet unresearched question of whether culture selection and high PHA production can be maintained simultaneously. Recent research indicates that the overall PHA production may decrease due to the acclimatization of the culture after light stress (Bayon-Vicente *et al.*, 2020). But more research is needed in this respect.

- **Nutrient decoupling.** In this Thesis, primarily substrates with a very high COD/nutrient ratio have been used. This nutrient limitation is critical for the PPB culture to accumulate PHA but restricts growth and therefore limits the volumetric productivities of the reactor. Therefore, decoupling nutrient limitation could be achieved by first allowing a high nutrient content to maximize growth and then limiting the nutrient content to maximize PHA accumulation. This is reasonable, as waste such as OFMSW or lignocellulosic waste can be mixed with, e.g., domestic wastewater, which has a high nutrient content.

- **Modeling the metabolism of PPB towards PHA production.** One key challenge is the integration of the complex metabolism of these organisms to allow control and optimization, which needs a dedicated modeling approach with novel components, including energy, electrons, and light harvesting complex. Due to the extraordinarily complex metabolism of PPB, the quest for a comprehensive and usable model is challenging as it should be stoichiometrically correct (thereby should be based on metabolic mechanisms) and easy to be implemented in real plants. Unfortunately, most of the models published so far address the identification of metabolic mechanisms of PPB from a systems biology perspective (including energy, carbon, and redox flows) and are not suited to analyze the optimal process conditions to produce biopolymers without major simplifications.

- **Granulation of PPB cultures.** In recent years, some research has been done on this aspect (Stegman *et al.*, 2021), but mainly focused on the recovery of wastewater resources and not on the production of high value-added products. As discussed in chapters 3 and 4, fractionation can reduce costs in the downstream process. Furthermore, optimization and versatility based on biopolymer accumulation (PHA, glycogen, and EPS) should be studied. Furthermore, H2 production with this type of technology has not yet been studied.

- **Combination of catalytic and biotechnological pretreatments**. Especially considering lignocellulosic waste, a large part of the organic carbon is not being fully exploited, catalytic treatments either in situ or ex situ can be explored. For example, thermo-catalytic treatment prior to fermentation can reduce the necessity of hydrolysis and therefore improve the overall efficiency of the process (Ventura *et al.*, 2021).

- **Microbial protein production**. Microbial protein is also a high added value product, and thus many studies on the valorization of organic waste by biotechnological methods, including PPB, are turning towards this strategy (Alloul *et al.*, 2019; Hülsen ***et al.***, 2020). It is important to mention that PHA increases the value of the microbial protein as it increases its probiotic quality. In any case, a photobiorefinery capable of adapting to produce different products according to seasonality or the type of substrate received in the plant would be an immense advantage. To this end, studies on process optimization and synergies in the downstream are still pending.

5.3. References

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