Polyhydroxyalkanoates production from agricultural wastes using phototrophic purple bacteria operated in non-aerated systems

J.C. Fradinho[1], M. Palhas[1], J. Almeida[1], A. Oehmen[1,†], M.A.M. Reis[1]

[1]UCIBIO-REQUIMTE, Department of Chemistry, Faculty of Sciences and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal
[†] Present address: School of Chemical Engineering, University of Queensland, Brisbane, QLD, 4072, Australia

Keywords: Polyhydroxyalkanoates (PHA), Phototrophic Purple Bacteria (PPB), Agricultural wastes valorisation
Presenting author email: palhasmiguel@gmail.com

Polyhydroxyalkanoates (PHA) are biodegradable biopolymers with physical-chemical properties similar to conventional plastics. These biopolymers are precursors for bioplastics production which have reduced carbon footprint and improved biodegradability, representing a sustainable alternative to synthetic plastics. In an effort to decrease PHA production costs, mixed microbial cultures fed with cheap agro-industrials wastes have been studied in the last years, and recently, a new PHA producing system has been proposed. It consists in operating phototrophic mixed cultures (PMC) and enriching them in PHA producing phototrophic purple bacteria (PPB). These phototrophic bacteria can obtain energy from light and therefore, do not require aeration, preferring to grow in anaerobic systems. This is a clear advantage in relation to current PHA producing systems where aeration is continuously or partially present during the system operation, strongly contributing to high operational costs.

Thus far, studies with PHA producing PPB occurred mostly at laboratory scale with very promising results. Indeed, when PMC were operated in anaerobic conditions using a permanent carbon feast selection strategy specifically designed for PPB, PHA accumulation levels up to 60% (Fradinho *et al*. 2016) were achieved. Currently, studies are occurring under the Horizon 2020 European project NoAW. These studies aim to determine, at laboratory scale, what are the optimal operational conditions for valorising fermented agricultural wastes (mixture of manure and waste maize silage) through PMC's PHA production. Also, data is being collected to develop a model that describes the photosynthetic PHA producing metabolism. Finally, project results will allow to design photobioreactors specifically for PHA producing PMCs from industrial waste and wastewater streams that best suit their operational requirements for a maximized PHA production.

Methods

A 4 L photosynthetic bioreactor operated in a sequential mode (SBR) was inoculated with sludge from a wastewater treatment plant and fed with synthetic effluent simulating the organic acids and nutrients present in the fermented stream of manure and maize silage mixture. The synthetic effluent contained acetic, propionic, butyric and valeric acids with a total VFA concentration of 12372 mg COD/L (Table 1). The photo bioreactor was operated under a permanent carbon feast strategy at 30ºC and transient illumination (12h dark/12h light) in order to simulate a 24h diurnal cycle. The reactor was internally illuminated by a halogen lamp (200W) providing a volumetric light intensity of 1.8 W/L of culture broth during the 12h illuminated phase.

To further increase the culture PHA content, sludge from the SBR was collected at the end of the cycle, placed in separate accumulator reactors and subjected to higher illumination conditions of 7.5 W/L using two halogen lamps (60W). The culture was regularly pulse fed with synthetic medium to prevent acids depletion and maintain the culture in the permanent presence of carbon.

Table 1 – Composition of the synthetic influent fed to the NoAW PBR

Acetic	**Propionic**	**Butyric**	**Valeric**	**Total VFAs**	**$N-NH_4$**	**$P-PO_4$**
4838 mgCOD/L	2224 mgCOD/L	3828 mgCOD/L	1483 mgCOD/L	12372 mgCOD/L	350 mgN/L	316 mgP/L

Results

Starting with wastewater sludge and synthetic feed, the PBR quickly became enriched with a PMC dominated by phototrophic purple bacteria (Fig 1).

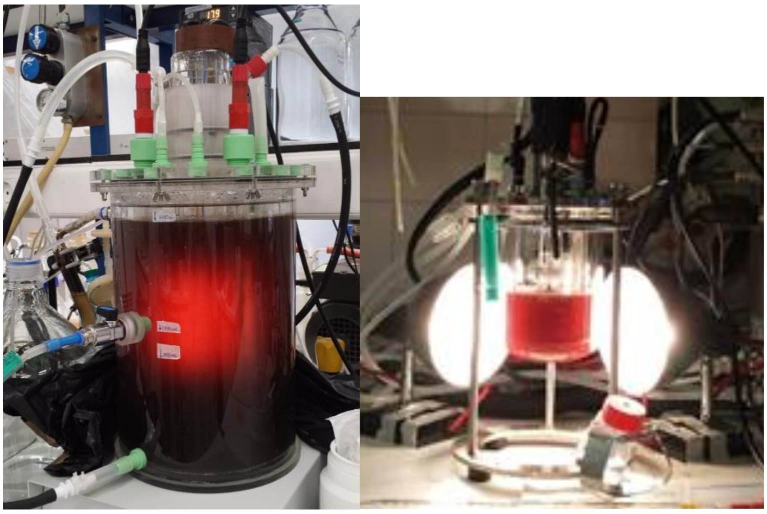

Figure 1 – Left: Selector PBR enriched in PHA accumulating purple bacteria showing the characteristic red colour of these bacteria. Right: Accumulator reactor where the culture PHA content was further increased.

The PMC was capable of maintaining a stable PHA content around 20 % PHA/VSS in the selector reactor, a value that could increase up to 35% in the accumulator reactor. From Figure 2 it is clear that most of the phototrophic population was capable of accumulating PHA.

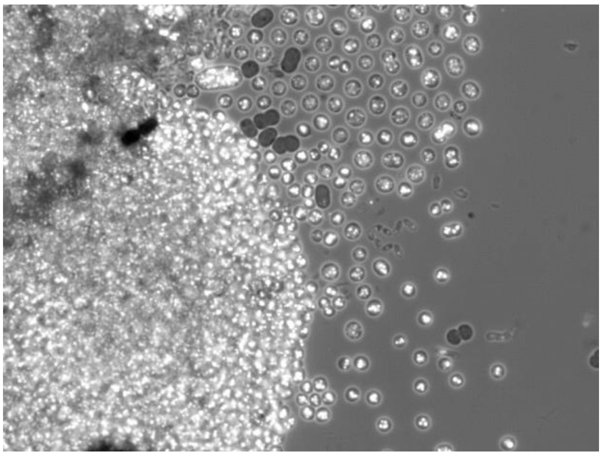

Figure 2 - Fluorescence microscopic picture of Nile blue staining showing cocci shaped purple bacteria accumulating PHA.

Seeing that these results were obtained in non-aerated PBR in completely anaerobic conditions, we prospect that phototrophic mixed cultures can lead to more cost-effective and environmentally sustainable PHA production processes. Currently, operating conditions are being adjusted to introduce the real fermented agriculture waste as influent and improve the culture PHA production capacity.

Acknowledgements

This work was supported by the Applied Molecular Biosciences Unit- UCIBIO which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019). NoAW project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement n° 688338).

References

Fradinho, J.C, Oehmen, A., Reis, M.A.M., 2016. Beyond feast and famine: Selecting a PHA accumulating photosynthetic mixed culture in a permanent feast regime. Water Research, 105, 421-428