

# Converting HRAP into phototrophic purple bacteria ponds for polyhydroxyalkanoates production from wastewater

J.C. Fradinho<sup>1</sup>, J. Almeida<sup>1</sup>, E. Serrano<sup>2</sup>, A. Oehmen<sup>1,†</sup>, E. Lara<sup>2</sup>, M.A.M. Reis<sup>1</sup>

<sup>1</sup>UCIBIO-REQUIMTE, Department of Chemistry, Faculty of Sciences and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

<sup>2</sup>FCC Servicios Ciudadanos, Av. del Camino de Santiago, 40, edificio 3, 4<sup>a</sup> planta, 28050 Madrid, Spain

<sup>†</sup> Present address: School of Chemical Engineering, University of Queensland, Brisbane, QLD, 4072, Australia

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Presenting author email: [jro.almeida@campus.fct.unl.pt](mailto:jro.almeida@campus.fct.unl.pt)

## Introduction

In the last decades, polyhydroxyalkanoates (PHAs) production using aerobic mixed microbial cultures (MMC) has been thoroughly studied. Several systems have been optimized in order to increase mixed culture's PHA accumulation capacity, while decreasing operational costs. With the goal of further decreasing PHA production costs, a new PHA producing system has been recently 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. Since aeration is the parameter that most strongly contributes to operational costs in MMCs, its abatement/elimination from PMCs operation can lead to a more cost-effective PHA production system.

Thus far, studies with PHA producing PPB occurred mostly at laboratory scale with very promising results. Indeed, whether by applying the typical selection strategy of carbon feast and famine used in aerobic MMCs or by applying a permanent carbon feast strategy specifically designed for PMCs, PHA accumulation levels up to 30% (Fradinho *et al.* 2013) and 60 % (Fradinho *et al.* 2016), have been respectively achieved. Currently, studies with PHA producing PMCs are occurring under the Horizon 2020 European project INCOVER. This project is run at demonstration scale and aims to do a retrofitting of already existing high rate algae pond (HRAP) facilities used for domestic wastewater treatment, adapting their operation for PHA production using PPB, under local outdoor conditions. This conversion of HRAP into Phototrophic Purple Bacteria ponds (PPBponds) will allow determination of the advantages of using already installed facilities and replacing the currently used algae communities by PHA producing phototrophic bacterial communities.

## Materials and Methods

Domestic wastewater from El Torno Wastewater treatment plant in Chiclana (pre-treated with sand screening, oil/grease removal and sieved in 1 mm rotary filter) was mixed with molasses residues (0.5% molasses in wastewater) and fermented in demonstration scale anaerobic reactors (20 m<sup>3</sup> UASB) to obtain a volatile fatty acid (VFA) rich stream. This effluent was fed to two High Rate Algae Ponds (HRAP) operated at El Torno retrofitted for PHA production (total 64m<sup>2</sup> of illuminated surface and working volume of 19m<sup>3</sup>). Both ponds were operated under a feast and famine strategy with the feast phase occurring during the day time and the famine phase occurring during the night. During the day the ponds were fed with the UASB effluent and were illuminated with natural sunlight (anaerobic light phase). During the night the ponds were aerated (aerobic dark phase). The ponds were subjected to the typical variability in illumination and temperature conditions experienced at Chiclana.

## Results

The fermentation in the UASB of the 0.5% molasses in wastewater led to an effluent mostly composed of valeric acid (46%), butyric acid (25%), ethanol (10%), acetic acid (11%) and propionic acid (8%). By feeding this VFA rich stream to the ponds it was possible to convert the initial algae culture into PMCs enriched in PHA accumulating purple bacteria (Fig. 1).



Figure 1. Incover demonstration ponds. On the left side of the pictures it is shown the initial stage of HRAP retrofitting (green color from algae). On the right side of the pictures it is shown an already retrofitted HRAP converted into a PPBpond and enriched in PHA-accumulating phototrophic purple bacteria (red-brown color).

During the 2018 summer campaign, the PPBponds achieved a stable operation being attained a PHA content of 30% PHA/VSS (Fig. 2).

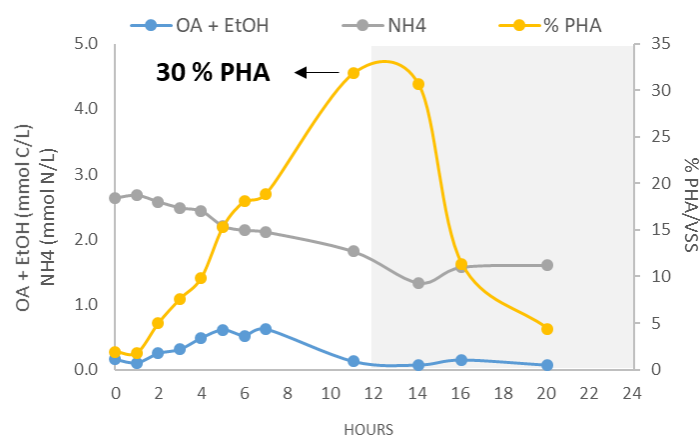


Figure 2. Ammonia, organic acids plus ethanol (OA + EtOH) and PHA content profile of the PMC enriched in phototrophic purple bacteria during a 24h cycle operation in the INCOVER demonstration PPBpond. White zone represents the day time (from 9 AM to 9 PM) and the grey zone represents the night (from 9 PM to 9 AM).

Figure 2 indicates that PHA production occurs during the day time when the sunlight availability enables PPB to take up organic acids and accumulate them as PHA. At the end of this stage part of the biomass is harvested and directed for polymer extraction. Afterwards, in the night, the pond is aerated which allows the consumption of PHA by the remaining bacteria, establishing a feast and famine profile that promotes the continuous selection of PHA storing bacteria. In this new phototrophic process, the high costs with aeration that typically occur in aerobic mixed cultures during the feast phase are eliminated and only minimal aeration is required during the famine/night phase. These results indicate that phototrophic mixed cultures can lead to a more cost-effective and environmentally sustainable PHA production process.

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