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## ► To cite this version:

Gabriel Capson-Tojo, Damien J Batstone, Tim Hülsen. Implications of fundamental aspects of purple phototrophic bacteria for process upscaling. IWA World Water Congress & Exhibition, Sep 2022, Copenhagen, Denmark. hal-03782809

**HAL Id: hal-03782809**

**<https://hal.inrae.fr/hal-03782809>**

Submitted on 21 Sep 2022

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## Implications of fundamental aspects of purple phototrophic bacteria for process upscaling

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**Abstract:** Purple phototrophic bacteria (PPB) have a great potential for resource recovery from wastewater thanks to their photoheterotrophic capabilities. This work puts together available information on PPB biochemical fundamentals and the latest results from pilot-scale PPB cultivation, aiming to point research towards realising PPB full-scale implementation, with a focus on reactor design. PPB have two main requirements for an efficient phototrophic growth in enriched cultures: (i) a sufficient supply of near-infrared light and (ii) anaerobic conditions. Recent results show that effective light penetration in PPB cultures does not exceed 5-10 cm, implying that PPB open ponds (OPs) will be shallow (increasing O<sub>2</sub> diffusion), and photobioreactors (PBRs) will be thin (increasing capital costs). Results suggest that PPB OPs operation might be more challenging than for algae, tipping the balance towards the application of PBRs. Cost analyses must be performed using reliable data.

**Keywords:** anoxygenic photosynthesis; biological wastewater treatment; carbon and nutrient recovery

### INTRODUCTION

Purple phototrophic bacteria (PPB) are receiving increasing attention as mediators for resource recovery from wastewaters. PPB can use light as energy source and organics as C and electron donor, via photoheterotrophic growth. This allows the simultaneous recovery of COD and nutrients as value-added products contained within the biomass (*e.g.* single-cell protein), obtained at high yields (Capson-Tojo et al., 2021b).

The main limitation for the implementation of phototrophic processes is their higher costs compared to traditional waste treatment approaches (Ación Fernández et al., 2019). If PPB processes are to become a reality, profits must be maximised, and costs must be minimised. Profit maximisation entails and optimal treatment performance and product value. Regarding cost minimisation, enriched PPB cultures must be used to avoid sterilisation costs, and sunlight must be the energy source, as artificial light sources are prohibitive. An optimal reactor design is also crucial for cost minimisation and efficient operation. PPB reactors must provide cheap/efficient mixing, efficient light distribution and must allow maximal loads, minimising reactor volumes.

Decades of research have been dedicated to optimise microalgal reactor design (Posten, 2009). The two most common cultivation technologies are photobioreactors (PBRs) and open ponds (OPs). PBRs lead to improved performances and productivities, but are more expensive, making OPs the most commonly applied configuration for growing phototrophic microorganisms (IEA Bioenergy, 2017).

While previous research on microalgae can serve as starting point for PPB, conclusions cannot be directly extrapolated, as the underlying biochemical processes in algae and PPB systems are completely different. This work aims to use the current available knowledge on PPB biochemistry to point research towards realising a feasible full-scale implementation of PPB processes. We address different crucial challenges that the technology faces, such as efficient reactor design or biomass harvesting. The latest data on pilot-scale PPB cultivation using both PBRs and OPs has been used to validate our statements.

## PPB REACTOR DESIGN: CONSIDERING PPB FUNDAMENTALS

Opposed to oxygenic photosynthetic by algae, PPB perform anoxygenic photosynthesis, which has two main implications: (i) PPB use mostly near-infrared (NIR) light to grow phototrophically, and (ii) anoxic/anaerobic conditions are required. NIR-light uptake implies a more significant light attenuation, due to the higher selective absorption of NIR-light by water compared to visible-light (used by algae). Moreover, PPB reactors are operated at higher biomass concentrations than algal OPs ( $>1.0$  vs.  $<0.5$  gvs·L<sup>-1</sup>), further amplifying light attenuation in PPB reactors (Alloul et al., 2021; Robles et al., 2020). Anaerobic conditions are required in PPB systems because oxidative conditions (resulting from the presence of O<sub>2</sub>) inhibit the expression of most genes required for chromatophore synthesis (Gregor and Klug, 1999). This results in the suppression of photoheterotrophic growth, colour loss, and PPB outcompetition in mixed cultures in a matter of days (Capson-Tojo et al., 2021b).

The fundamental differences pointed out above have two main implications for reactor design: (i) light-path lengths (*i.e.* width for PBRs and depth for OPs) need to be shorter in PPB systems compared to algae (*e.g.* 5-10 cm vs. 30 cm deep OPs, see Figure 1), and (ii) O<sub>2</sub> diffusion must be minimised. The latter will be incredibly challenging in open systems with high surface exchange areas, such as OPs, particularly considering that shallow ponds are needed, thus increasing the surface for gas diffusion. All considered, PPB OPs operation might be much more challenging than for algae, which might favour PBR implementation (Figure 2 shows a qualitative comparison of PBRs and OPs).

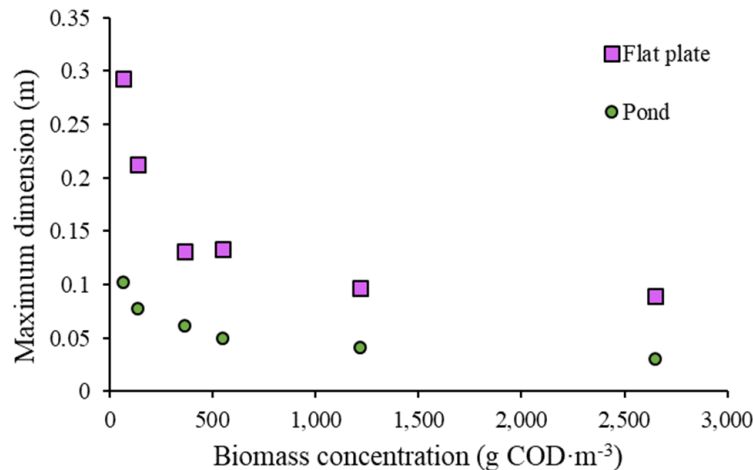
## LINK WITH LATEST PILOT-SCALE PPB DATA

The light-path limits shown above agree with recent data. Results from a 8 cm thick demonstration-scale flat plate PBR (of 1 m<sup>3</sup>, the 1<sup>st</sup> in operation; recently presented by the authors (Hülßen et al., 2021)) showed that there was no effective light limitation. Recent results from OPs (100 L) reported light limitation at depths of 10-20 cm (Alloul et al., 2021). Similar results were obtained with 15 cm deep OPs (Sepúlveda-Muñoz et al., 2020). Light limitation at 15 cm deep OPs was confirmed by other studies, where much longer retention times (4-11 d) than those commonly applied were needed (García et al., 2019; López-Serna et al., 2019).


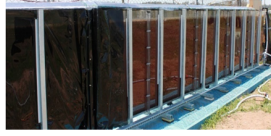
Regarding anaerobic conditions, while this was easy to achieve in the 8 cm PBR (even when open (Hülßen et al., 2021)), this was challenging for OPs. A recent study by Alloul et al. (2021) recognised this issue, observing that dissolved oxygen (DO) minimisation in 10-20 cm deep OPs by mixing only during daytime (using a common paddlewheel) increased the PPB proportions from 14 to 56%. In addition, DO was further reduced by partial O<sub>2</sub> stripping caused by the CO<sub>2</sub> sparging system used for pH control. This need to minimise DO concentrations by low mixing intensities and small surface/volume ratios (S/Vs) to reduce O<sub>2</sub> diffusion (opposed to the high S/V needed for efficient light supply), will be a key factor when designing PPB OPs.

## CONCLUSIONS

Although more research using demonstration-scale PBRs and OPs must be done, preliminary results and fundamental differences between PPB and microalgae suggest that PPB OP operation might be more challenging than for algae. This might tip the balance towards the application of PBRs, although the development of unexpensive options will be required. Cost analysis must be performed using experimental data.



**Figure 1.** Maximum dimensions of different reactor configurations allowing a light intensity of  $15 \text{ W} \cdot \text{m}^{-2}$  (minimum for effective phototrophic uptake rates) throughout the whole reactor volume (assuming an incident light intensity of  $400 \text{ W} \cdot \text{m}^{-2}$ ) (extracted from recent work of the authors, see Capson-Tojo et al. (2021a)).

	Light	DO	Cost	Harvesting method
<b>Open ponds</b> 	Efficient light distribution at surface  Light limitation at depths over 10 cm	Increase in DO due to diffusion and mixing  Potential competition with aerobes	Low capital costs  Low operational costs	Flocculation → Cheap; potential product contamination  Membranes → More expensive; higher recovery; biomass retention
<b>Photobioreactors</b> 	Efficient light distribution over the whole volume  Light limitation at widths over 10-20 cm	Minimal DO  Negligible presence of aerobes	Higher capital costs  Low operational costs	Flocculation → Cheap; potential product contamination Membranes → More expensive; higher recovery; biomass retention Granulation → Enhanced recovery; light-limited Biofilm → Pure product; light-limited; lower recovery

Pictures from: (up) Phototrophic Purple Bacteria Ponds © AQUALIA and (down) flat plate PBR from the University of Queensland

**Figure 2.** Qualitative comparison between open ponds and photobioreactors. Their main characteristics regarding light distribution, dissolved oxygen (DO) presence, cost, and applicable harvesting methods are given. Harvesting is not discussed in the text due to space limitations.

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