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## Implications of light attenuation for the upscaling of mixed purple phototrophic bacteria processes

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**Abstract:** Light attenuation in purple phototrophic bacteria (PPB) has been studied. The results show that increased biomass concentrations lead to higher light attenuation, and that PPB absorb both visible and near-infrared wavelengths. Both spectrum fractions were equally absorbed at PPB concentrations above  $1 \text{ g COD} \cdot \text{L}^{-1}$ . A flat plate configuration showed less attenuation than top-illuminated cylindrical reactors, representative of open ponds. Neither a complex wastewater matrix nor the presence of polyhydroxyalkanoates affected light attenuation. The concentration of pigments (*e.g.* carotenoids and bacteriochlorophyll) had a strong effect, with much higher attenuation in the presence of pigments compared to non-pigmented biomass. In dense outdoor PPB cultures ( $\geq 1 \text{ g COD} \cdot \text{L}^{-1}$ ), effective light penetration is only 5 cm. This, together with the increased oxygen diffusion in large open ponds, biases design away from horizontal lagoons, and towards multi-panel systems such as flat plate reactors.

**Keywords:** nutrient recovery; photoheterotrophy; purple non-sulfur bacteria

### Introduction

Purple phototrophic bacteria (PPB) have been proposed as a potential mediator for resource recovery from wastewater, with a focus on the production of value-added products, such as polyhydroxyalkanoates (PHA), carotenoids, or biomass (to be used as single-cell protein or fertilizer), to balance the overall costs (Capson-Tojo et al., 2020).

As any phototrophic process, the performances of PPB reactors are limited by light availability. To achieve efficient light distribution, studying light attenuation is critical, especially for scaled systems, as attenuation will directly affect the culture strategy and the reactor design. Light attenuation in PPB cultures has not been studied, and extrapolating behaviors observed with other phototrophs (*e.g.* microalgae) is not recommended, as PPB behave differently, absorbing mostly near-infrared (NIR) wavelengths for harvesting light energy, via bacteriochlorophylls (BChls).

This work aimed at studying the effect of different biomass concentrations, reactor configurations, wastewater matrices, and growth conditions, on the attenuation extent of NIR and ultraviolet-visible (UV-VIS) light spectra, in enriched PPB cultures.

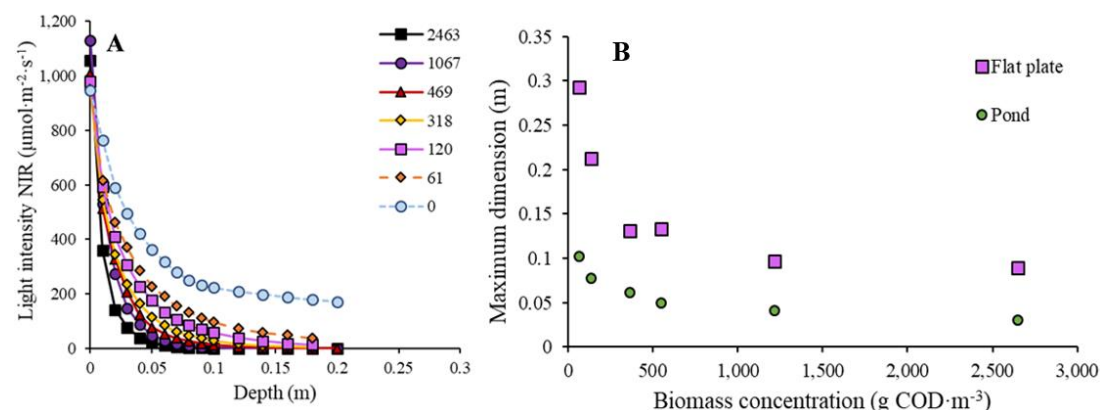
### Materials and methods

Light attenuation (both NIR and UV-VIS wavelengths separately) was evaluated at different biomass concentrations ( $0\text{--}2,463 \text{ mg COD} \cdot \text{L}^{-1}$ ) in three different reactors (one flat plate reactor, and two cylindrical reactors with different diameters illuminated from the top, representative of open ponds).

Furthermore, the impact on light attenuation of the presence of PHA granules within cells, of the concentrations of pigments (*i.e.* BChl and carotenoids), and of the wastewater matrix (*i.e.* synthetic medium *vs.* effluent from a pilot-scale PPB reactor treating real poultry-processing wastewater) was also evaluated.

## Results and conclusions

Increased biomass concentrations lead to a more pronounced light attenuation (Figure 1A), mostly caused by light absorption by PPB biomass. At low PPB concentrations, the preferential absorption of NIR light by water resulted in a more pronounced loss of NIR light with depth compared to UV-VIS light. Nevertheless, at biomass concentrations above 1 g COD·L<sup>-1</sup>, both spectrum fractions were absorbed equally, confirming that PPB utilize both NIR and VIS light for energy generation. This means that, for practical implementation, both NIR and VIS light will be equally attenuated.



**Figure 1.** (A) NIR-light attenuation curves and different biomass concentrations (g COD·m<sup>-3</sup>) for a cylindrical reactor, and (B) maximum dimensions of different reactor configurations allowing a light intensity of 15 W·m<sup>-2</sup> throughout the whole reactor volume (assuming an incident light intensity of 400 W·m<sup>-2</sup>).

Amongst the different reactor configurations tested, a flat plate configuration showed less attenuation than the cylindrical reactors (proven by the corresponding attenuation coefficients from fitted Lambert-Beer equations). The presence of PHA granules or a more complex wastewater matrix did not have any effect on light attenuation through the cultures, with similar attenuation curves and coefficients. The concentration of pigments (*e.g.* carotenoids and bacteriochlorophyll) increased considerably the attenuation effect, which was tested comparing a pigmented culture with non-pigmented biomass (grown under aeration according to Capson-Tojo *et al.* (2021)).

Overall, the effect of light attenuation in dense outdoor PPB cultures ( $\geq 1$  g COD·L<sup>-1</sup>) resulted in an effective light penetration of only 5 cm. If PPB are to keep their main competitive advantage (photoheterotrophic growth), reactors/ponds must be designed considering that any “dark”, non-illuminated, area will result in PPB outcompetition. Figure 1B shows that to avoid dark areas caused by attenuation, flat plate widths and pond depths should not exceed 10 and 5 cm, respectively. In the case of ponds, this will result in extremely shallow systems, with large surface areas. This will lead to a promotion of the oxygen diffusion into the culture, which might result in photoheterotrophy inhibition due to the concomitant oxidative conditions. Although further research is needed, these findings bias design away from horizontal lagoons, towards multi-panel systems such as flat plate reactors.

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