

# Lessons learnt from the first outdoors demonstrations-scale purple phototrophic bacteria flat-plate anaerobic photobioreactor

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**Abstract:** If purple phototrophic bacteria (PPB)-based systems are to become a reality for resource recovery, research must be performed outdoors, using scaled reactors. Here we present the results from an outdoors 10 m long PPB-enriched flat plate photobioreactor (PBR), with a volume of 0.95 m<sup>3</sup>. Results show that, over a long period (192 days), the PBR was able to effectively remove volatile fatty acids (VFAs), N, and P, with average removal efficiencies of >90%, 34-77%, and 28-45% (despite varying environmental conditions and a changing influent). Nutrient removal was limited by the availability of COD (*i.e.*, VFAs). Operating anaerobically under a semicontinuous/day-only feeding regime, estimated biomass productivities of 6-24 g VS·m<sup>-2</sup>·d<sup>-1</sup> were achieved, with soluble COD removal rates up to 1.0 g·L<sup>-1</sup>·d<sup>-1</sup>. Optimal conditions led to a relative PPB abundance of 0.56, at a minimum HRT of 2.1-2.4 d (1.0-1.2 d during day-only feeding). Biomass was harvested at ~90% VS/TS, with a crude protein content of 58% and an amino acid profile suitable for feeding purposes.

**Keywords:** wastewater treatment; anoxygenic photosynthesis; resource recovery; photobioreactor

## Introduction

Purple phototrophic bacteria (PPB) have lately reappeared as mediators for resource recovery from wastewater, thanks to their photoheterotrophic metabolism, using light as energy source and organics as source of C and electrons. This allows the simultaneous assimilation of C and nutrients, producing biomass (*e.g.*, microbial protein) at yields up to 1 g COD·g COD<sup>-1</sup>. Median removal efficiencies by PPB-based treatment systems of 76%, 53% and 58% for COD, N and P have been reported, depending on the COD:N:P ratios of the wastewater (Capson-Tojo et al., 2020).

Due to economic constraints caused by artificial illumination, PPB systems for resource recovery will have to be run outdoors, using sunlight (Capson-Tojo et al., 2020). To date, only a handful of articles have dealt with this topic, and most did not focus on wastewater treatment or nutrient recovery, but on generation of H<sub>2</sub> and PHA from artificial media (Adessi et al., 2012; Carlozzi et al., 2006; Carlozzi and Sacchi, 2001). In addition, they are not relevant for wastewater treatment applications, as artificial media and pure cultures were used.

This study presents the results of a demonstration scale PPB photobioreactor (PBR) operated outdoors (950 L). Various design and operational parameters were modified during a period of 192 days, aiming at obtaining design and performance data, including wastewater treatment capacities and biomass productivities.

## Material and Methods

The demonstration plant consisted of a flat plate PBR of 1 m<sup>3</sup> (10 m long), covered with UV-VIS absorbing foil (Figure 1). To the knowledge of the authors, this is the first demonstration-scale PPB PBR in operation. The wastewater fed, the feeding regime, the growth strategy (suspended vs. attached) and the HRT were varied over the operational period. Accordingly, six operational phases were defined during a period of 192 days, aiming at optimising the plant performance (see Table 1). Samples were taken twice a week, measuring concentrations of soluble and total COD, total phosphorus (TP) and total Kjeldahl nitrogen (TKN), NH<sub>4</sub><sup>+</sup>-N, PO<sub>4</sub><sup>3-</sup>-P, VFAs, and total and suspended solids. The harvested biomass (via centrifugation) was also studied, measuring the metal contents, the elemental composition, and the aminoacid profile. The microbial community was also studied via 16S sequencing.

## Results

Overall data from the PBR plant show that, despite the varying environmental conditions (*i.e.*, day-night cycles, peaks daily temperatures ranging between 14-42 °C, and daily irradiances ranging between 3-32 MJ·m<sup>-2</sup>), the PBR provided effective VFA, N, and P removal, with average removal efficiencies of >90% (in most phases), 34-77%, and 21-45%, respectively; see Figure 2. Results show that PPB were able to photoheterotrophically assimilate the VFAs in the wastewater, at rates over 0.5 g SCOD·L<sup>-1</sup>·d<sup>-1</sup>, with peaks up to 1.0 g SCOD·L<sup>-1</sup>·d<sup>-1</sup>. The removal of N and P was limited by the availability of biodegradable COD (*i.e.*, VFAs), as the wastewater had a COD:N:P far from the uptake ratio for PPB (due to the presence of non-biodegradable SCOD in the wastewater (Figure 2).

Changing the raw feed for prefermented wastewater and feeding only during daytime hours (to increase COD availability during daytime hours) allowed to maximise biomass productivities and PPB relative abundances (up to 0.56), while maintaining the treatment performance. Under these conditions, optimal retention times of 2.1-2.4 d in Phases V-VI (values to be halved considering daytime-only feeding) lead to estimated biomass productivities of up to 24 g VS·m<sup>-2</sup>·d<sup>-1</sup> (conservative; see Figure 3), at organic loading rates around 1.5 g COD·L<sup>-1</sup>·d<sup>-1</sup>. Different daily cycle studies showed that, even with day-only feeding, VFAs accumulated in the reactor during the night, outlining the importance of feeding only during daytime during fully anaerobic operation to avoid the discharge of untreated wastewater.

The produced biomass was harvested at 90±1% VS/TS ratio, with a crude protein content of 58±14% and an amino acid profile suitable for feeding purposes. The only heavy metal whose concentration could be worrisome was Al (2,470 g·kg<sup>-1</sup>). Its origin is uncertain.

## Conclusions

The demo-PBR showed a relatively constant performance despite varying environmental conditions and influent characteristics. The removal of N and P was limited by the availability of biodegradable COD, a property of the feed wastewater, an issue that can be solve via stream co-treatment. The harvested biomass had low inert contents and proteins contents of 58%, with an aminoacid profile suitable for animal feeding. Semicontinuous day-only feeding is recommended, as well as a minimum overall HRT of 1.6-2.4 d (to avoid PPB washout). These results show the technical feasibility of outdoors PPB-based treatment systems, and are the first step towards determining their economic viability.

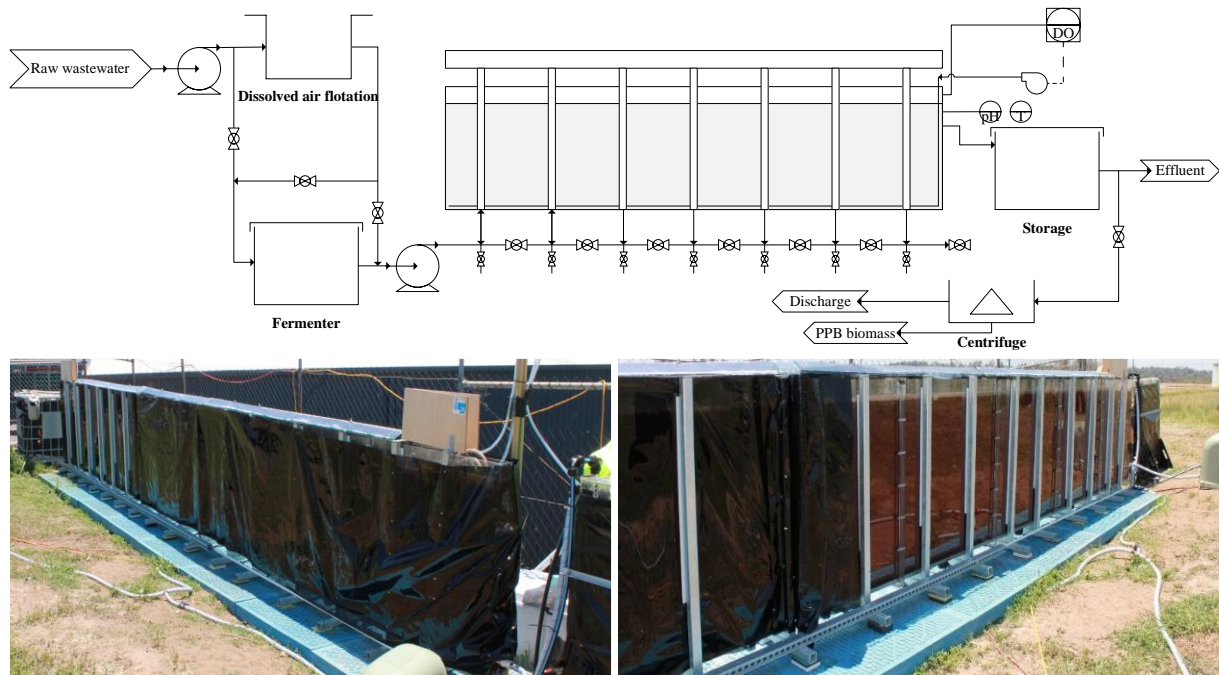
**Table 1.** Operational conditions of the PBR during the different periods.

Parameter	Phase I	Phase II	Phase III	Phase IV	Phase V	Phase VI
Duration (d)	1-32	35-60	63-95	93-118	120-127	130-192
Substrate	FWW	FDAF	FDAF	FDAF	FDAF	FDAF
Feeding strategy	Cont.	Cont.	Cont.	Cont.	Daytime	Daytime
HRT (d)*	4.4-5.7	2	2	1	2.4	2.1
Growth strategy	Susp.	Att.	Susp.	Susp.	Susp.	Susp.
Average temperature inside reactor (°C)**	26 (2.8)	25 (3.5)	25 (3.1)	24 (3.0)	18 (1.5)	16 (1.3)
Daily average irradiance (MJ·m <sup>-2</sup> )	26 (5.7)	20 (7.9)	19 (7.1)	18 (4.1)	15 (2.3)	13 (1.7)

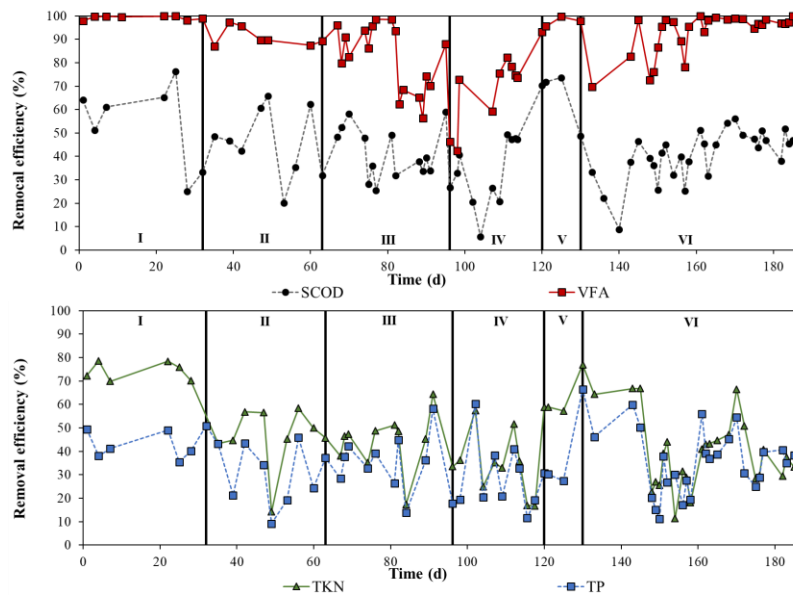
FWW stands for fermented wastewater, FDAF for fermented dissolved air flotation effluent, HRT for hydraulic retention time, cont. for continuous, susp. for suspended, and att. for attached.

\* Note that when feeding only during daytime, the daytime (effective) HRT is half of the given value.

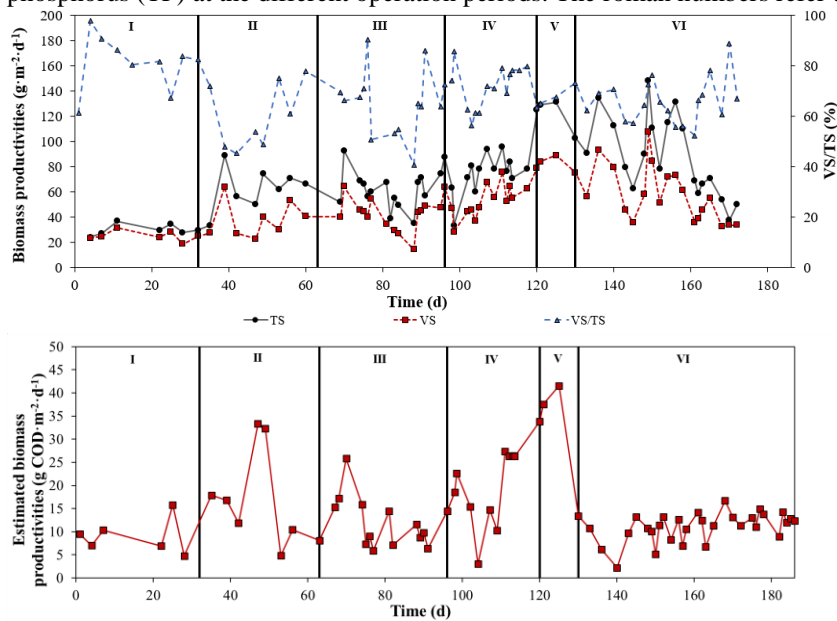
\*\* These values correspond to the moments when the samples were taken (10-12 am).



**Figure 1.** Schematic representation of the treatment plant, including the PBR.



**Figure 2.** Removal efficiencies of (up) SCOD and VFAs and (down) total Kjeldahl nitrogen (TKN) and total phosphorus (TP) at the different operation periods. The roman numbers refer to the phases described in Table 1.



**Figure 3.** (up) Measured biomass productivities and VS/TS ratios in the reactor, and (down) estimated biomass productivities from the soluble COD removals. The roman numbers refer to the phases described in Table 1.

## References

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