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# Modelling H<sub>2</sub> conversion by purple bacteria enriched cultures: evaluating kinetics of gas-fed processes to assess feasibility

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## Abstract

Purple phototrophic bacteria (PPB) can be used to convert gaseous streams (e.g., H<sub>2</sub> and CO<sub>2</sub>) into single-cell protein. This work aimed to determine the specific uptake rates of enriched/mixed PPB cultures under different environmental conditions (i.e., temperatures, pH and light intensities), using a mechanistic model considering relevant biological and physico-chemical processes. The model accurately represented results from batch tests with different gas-transfer kinetics (i.e.,  $k_{L,a}$  values), providing similar biological uptakes rates. Optimal rates of 1.9-2.0 g COD·g COD<sup>-1</sup>·d<sup>-1</sup> were reached at pH 7, 25°C and light intensities over 30 W·m<sup>-2</sup>, with biomass yields of ~1 g COD·g<sup>-1</sup> COD. The influence of light and temperature on the uptake rates was modelled using the Steele's equation and the cardinal temperature model with inflection. The obtained rates are similar to those for pure PPB cultures and those achieved via photoheterotrophy.

## Keywords

Autotrophy; gas valorisation; modelling; purple phototrophic bacteria; resource recovery; single-cell protein

## INTRODUCTION

Processes based on purple phototrophic bacteria (PPB) are a promising option for resource recovery due to the high biomass yields that their phototrophic metabolism allows. PPB can efficiently generate a wide range of value-added products (e.g., single-cell protein (SCP), fertilisers, or polyhydroxyalkanoates) at biomass yields up to 1 g COD·g COD<sup>-1</sup> (Capson-Tojo et al., 2020).

While their photoheterotrophic capabilities have been considerably studied in the past few years for resource recovery from wastewater (Hülßen et al., 2022a, 2022b, 2022c), their photoautotrophic capabilities have barely been researched for recovery. PPB grown autotrophically could allow the efficient conversion of H<sub>2</sub> and CO<sub>2</sub> into SCP, generating a pathogen-free high value-added product usable as feed/food (Delamare-Deboutteville et al., 2019). If the H<sub>2</sub> and nutrient sources are sustainable (e.g., from bioH<sub>2</sub> and anaerobic digestate extracts), this approach could be a game changer for valorising gaseous effluents in a biorefinery, while recovering remaining nutrients. The proof of concept of H<sub>2</sub> conversion by PPB has been recently realised using pure cultures (Spanoghe et al., 2021). Results from our group have shown that enriched PPB cultures can valorise H<sub>2</sub> and CO<sub>2</sub> at higher yields than pure cultures (unpublished). However, the parameters influencing specific rates (crucial for implementation at large scale) are still to be determined using dedicated models.

The mechanistic models available to represent resource recovery by PPB are the Photo-anaerobic model (PANM) (Puyol et al., 2017) and its extension, the ePANM (Capson-Tojo et al., 2023), which have been successfully applied for wastewater treatment. Photoautotrophic uptake is included in both models, but they do not consider detailed gas-transfer kinetics. This is essential for modelling gas-fed processes, as models must account for rate limitation by mass transfer due to the low solubility of gaseous substrates.

Results from dedicated batch tests have been used here to calibrate a mechanistic model considering PPB as biomass component. Once it was confirmed that the model could account for both physical and biological rate limitations, the model was used to determine the specific uptake rates of enriched PPB cultures under different environmental conditions (e.g., pH, temperature (T) and light intensities). The impact of these variables on the rates was studied and modelled.

## MATERIALS AND METHODS

Photoautotrophic enrichments were grown in Schott flasks of 500 mL using the mineral synthetic medium (MSM) proposed by Ormerod et al. (1961) but modified to provide CO<sub>2</sub> as C source (as carbonate) and providing H<sub>2</sub> in the headspace. The flasks were filled with 190 mL of MSM and 10 mL of PPB enrichment as inoculum. After, they were closed and the headspace was flushed with N<sub>2</sub> prior to H<sub>2</sub> addition. The initial headspace pressure was adjusted to 1.30-1.35 bar. The flasks were covered with UV/VIS filters (Hülse et al., 2022c) and incubated under continuous illumination using infrared LED lights (850 nm). Two tests at different mixing intensities (and thus gas transfer rates) were performed, at 150 and 600 rpm. Assays at initial pH values of 6, 7 and 8.5, T of 15, 25, 38 and 50°C, and light intensities of 0, 5, 15, 30 and 50 W·m<sup>-2</sup> were carried out at 600 rpm (in triplicate). Gas composition and pressure were monitored 4-5 times per day. Liquid samples were drawn to determine biomass yields, crude protein contents and microbial communities.

The model considered PPB as single biomass component and growth on H<sub>2</sub> and CO<sub>2</sub> and biomass death as biological processes. Table 1 shows the corresponding Petersen matrix. Mass transfer processes and chemical equilibria were modelled as in the ADM1 (Batstone et al., 2002), modifying the model to account for gas diffusivities and consumption and for relevant chemical species for pH calculation. The model followed IWA standards. Stoichiometric parameters were either determined experimentally (yields) or gathered from the literature. Matlab was used to perform the simulations. Estimation of parameters related to H<sub>2</sub> uptake ( $k_m$  and  $K_{H_2}$ ) was performed as in Puyol et al. (2017). The effect of T and light on uptake rates was modelled using the cardinal temperature model with inflexion (CTMI) (Ruiz-Martínez et al., 2016) and The Steele's equation (Wágner et al., 2018).

**Table 1.** Petersen matrix of the proposed model. The nomenclature is the same as in Capson-Tojo et al. (2023). Parameter values are not shown due to lack of space.

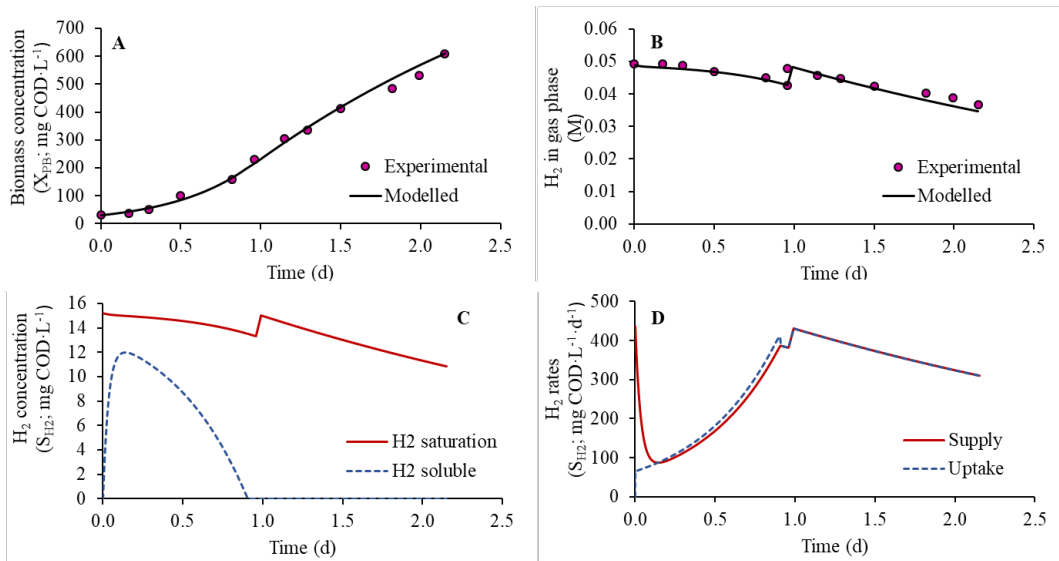
Component →		i	1	2	3	4	5	6	Rate (mg COD·L <sup>-1</sup> ·d <sup>-1</sup> )
j	Process ↓		$S_{IC}$	$S_{H_2}$	$S_{IN}$	$S_{IP}$	$X_{PB}$	$X_C$	
1	Autotrophic uptake of H <sub>2</sub> by PPB	$-f_{IC,au}$	1	$-\sum_{i=1,2,4-6} N_i \cdot v_{i,1}$	$-\sum_{i=1,3,5-6} P_i \cdot v_{i,1}$	$Y_{PB,au}$			$k_m \cdot S_{IC} / (K_{IC} + S_{IC}) \cdot S_{H_2} / (K_{H_2} + S_{H_2}) \cdot X_{PB} \cdot I_{IN} \cdot I_{IP}$
2	Decay of $X_{PB}$						-1	1	$k_{dec,PB} \cdot X_{PB}$
Units		mol C·L <sup>-1</sup>	mg COD·L <sup>-1</sup>	mg N·L <sup>-1</sup>	mg P·L <sup>-1</sup>	mg COD·L <sup>-1</sup>	mg COD·L <sup>-1</sup>	mg COD·L <sup>-1</sup>	

## RESULTS

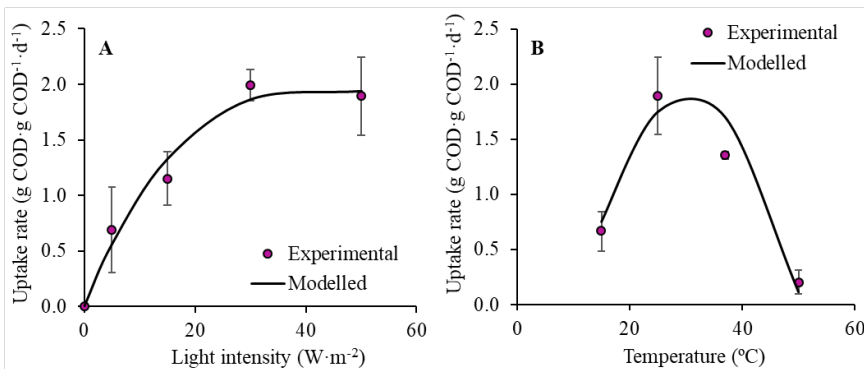
Figure 1 shows, as example, the results for one of the batch tests at 150 rpm. Despite the change in limiting rates, the model was able to represent the whole process, predicting accurately biomass and hydrogen concentrations. Before 0.9 d, H<sub>2</sub> was sufficient, and consumption rates were limited by biological uptake (typical exponential curve, soluble H<sub>2</sub> concentrations higher than saturation constant for H<sub>2</sub> ( $K_{H_2}$ ; always below 0.05 mg COD·L<sup>-1</sup>) and uptake rates increased with supply). After 0.9 d, the H<sub>2</sub> transfer rate became limiting due to the high biomass concentration and the low  $k_L a$  (28.7 d<sup>-1</sup>; determined experimentally), a situation that did not change despite the injection of extra H<sub>2</sub> in the headspace. After this point, the concentration of H<sub>2</sub> in the liquid was always close to zero, the growth curve became linear, and the uptake rate was limited by the supply rate. The model predicted accurately this behaviour, confirming its applicability for rate determination.

The tested environmental conditions affected considerably the H<sub>2</sub> uptake rates. Optimal rates of 1.9-2.0 g COD·g COD<sup>-1</sup>·d<sup>-1</sup> were achieved at initial pH values of 7, 25°C, and light intensities over 30 W·m<sup>-2</sup> (Table 2). Lower or higher pH values and T resulted in decreased rates (Table 2, Figure 2). No photoinhibition was observed at 50 W·m<sup>-2</sup>. With R<sup>2</sup> values of 0.97 and 0.91, the Steele's equation and the CTMI when able to represent these impacts accurately (Figure 2). The biomass

yields close to  $1 \text{ g COD} \cdot \text{g}^{-1} \text{ COD}$  confirmed effective phototrophic growth. The optimal conditions are similar to those reported for photoheterotrophic PPB growth, confirming these values and suggesting that the growth mode does not affect optimal conditions (Capson-Tojo et al., 2022, 2020). Optimal specific uptake rates are close to those for photoheterotrophic processes ( $2.3\text{-}2.7 \text{ g COD} \cdot \text{g COD}^{-1} \cdot \text{d}^{-1}$ ; (Capson-Tojo et al., 2023; Puyol et al., 2017)) and similar to those reported for pure PPB cultures grown autotrophically (below  $2.3 \text{ g COD} \cdot \text{g COD}^{-1} \cdot \text{d}^{-1}$ ; (Spanoghe et al., 2021)).



**Figure 1.** Results for the batch test at 150 rpm, showing (A) biomass concentrations, (B) H<sub>2</sub> gas concentrations, (C) H<sub>2</sub> liquid concentrations, and (D) H<sub>2</sub> supply and uptakes rates.



**Figure 2.** Influence of the (A) light intensity and (B) temperature on the specific H<sub>2</sub> uptake rates. Average and confidence intervals are shown (95%; n=3). The modelled results correspond to the Steele's equation (light intensity) and the cardinal temperature model with inflection (temperature).

**Table 2.** Main biomass yields and uptake rates from the batch tests.

	Mixing (rpm)		pH		Temperature (°C)		Intensity (W·m <sup>-2</sup> )		
	150 <sup>1</sup>	600 <sup>1</sup>	6	8.5	15	38	15	30	50 <sup>2</sup>
<b>Biomass yield</b> (g COD <sub>biomass</sub> · g COD <sub>consumed</sub> <sup>-1</sup> )	1.01±0.03	1.01±0.01	1.00±0.07	0.93±0.01	0.85±0.07	0.99±0.07	1.02±0.06	1.05±0.01	1.01±0.01
<b>Specific uptake rates</b> (g COD·g COD <sup>-1</sup> ·L <sup>-1</sup> )	2.01±0.50	1.89±0.35	1.50±0.05	0.83±0.02	0.66±0.18	1.35±0.04	1.15±0.24	2.00±0.14	1.89±0.35

1.  $k_{LaH_2}$  of 28.7 d<sup>-1</sup> at 150 rpm and 70.4 d<sup>-1</sup> at 600 rpm.
2. These are also the results for the tests at pH 7 and 25°C.

## CONCLUSIONS

These results show, for the first time, that enriched PPB cultures can be grown autotrophically from H<sub>2</sub> at similar rates than pure cultures, proving that axenic conditions are not needed for effective growth. Optimal values of 1.9-2.0 g COD·g COD<sup>-1</sup>·d<sup>-1</sup> were reached at pH 7, 25°C and light intensities higher than 30 W·m<sup>-2</sup>. The developed model was able to represent the overall kinetics of the process, accurately predicting rate limitations. The Steele's equation and the CTMI represented accurately the variations in the specific uptake rates due to light and T. The high yields and rates achieved confirm the great potential of enriched PPB cultures for H<sub>2</sub> valorisation. Volumetric production rates must be assessed and the model must be validated using continuous bioreactors.

## REFERENCES

- Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhny, S. V, Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin, V.A., 2002. Anaerobic digestion model no. 1 (ADM1). IWA Publishing.
- Capson-Tojo, G., Batstone, D.J., Grassino, M., Vlaeminck, S.E., Puyol, D., Verstraete, W., Kleerebezem, R., Oehmen, A., Ghimire, A., Pikaar, I., Lema, J.M., Hülsen, T., 2020. Purple phototrophic bacteria for resource recovery: Challenges and opportunities. *Biotechnol. Adv.* **43**, 107567.
- Capson-Tojo, G., Batstone, D.J., Grassino, M., Hülsen, T., 2022. Light attenuation in enriched purple phototrophic bacteria cultures: implications for modelling and reactor design. *Water Res.* **219**, 118572.
- Capson-Tojo, G., Batstone, D.J., Hülsen, T., 2023. Expanding mechanistic models to represent purple phototrophic bacteria enriched cultures growing outdoors. *Water Res.* **229**, 119401.
- Delamare-Deboutteville, J., Batstone, D.J., Kawasaki, M., Stegman, S., Salini, M., Tabrett, S., Smullen, R., Barnes, A.C., Hülsen, T., 2019. Mixed culture purple phototrophic bacteria is an effective fishmeal replacement in aquaculture. *Water Res. X* **4**, 100031.
- Hülsen, T., Barnes, A.C., Batstone, D.J., Capson-Tojo, G., 2022a. Creating value from purple phototrophic bacteria via single-cell protein production. *Curr. Opin. Biotechnol.* **76**, 102726.
- Hülsen, T., Stegman, S., Batstone, D.J., Capson-Tojo, G., 2022b. Naturally illuminated photobioreactors for resource recovery from piggery and chicken-processing wastewaters utilising purple phototrophic bacteria. *Water Res.* **214**, 118194.
- Hülsen, T., Züger, C., Batstone, D.J., Solley, D., Ochre, P., Porter, B., Capson-Tojo, G., 2022c. Outdoor demonstration-scale flat plate photobioreactor for resource recovery with purple phototrophic bacteria. *Water Res.* **216**, 118327.
- Ormerod, J.G., Ormerod, K.S., Gest, H., 1961. Light-Dependent Utilization of Organic Compounds and Photoproduction of Molecular bHydrogen by Photosynthetic Bacteria; Relationships with Nitrogen Metabolism. *Arch. Biochem. Biophys.* **94**, 449–463.
- Puyol, D., Barry, E.M., Hülsen, T., Batstone, D.J., 2017. A mechanistic model for anaerobic phototrophs in domestic wastewater applications: Photo-anaerobic model (PAnM). *Water Res.* **116**, 241–253.
- Ruiz-Martínez, A., Serralta, J., Seco, A., Ferrer, J., 2016. Modeling light and temperature influence on ammonium removal by *Scenedesmus* sp. under outdoor conditions. *Water Sci. Technol.* **74**, 1964–1970.
- Spanoghe, J., Vermeir, P., Vlaeminck, S.E., 2021. Microbial Food From Light, Carbon Dioxide And Hydrogen Gas: Kinetic, Stoichiometric And Nutritional Potential Of Three Purple Bacteria. *Bioresour. Technol.* **337**, 125364.
- Wágner, D.S., Valverde-Pérez, B., Plósz, B.G., 2018. Light attenuation in photobioreactors and algal pigmentation under different growth conditions – Model identification and complexity assessment. *Algal Res.* **35**, 488–499.