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Abstract
Purple phototrophic bacteria (PPB) can be used to convert gaseous streams (e.g., H_2 and CO_2) 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_La values), providing similar biological uptakes rates. Optimal rates of 1.9-2.0 g COD·g COD^{-1}·d^{-1} were reached at pH 7, 25ºC and light intensities over 30 W·m^{-2}, with biomass yields of ~1 g COD·g^{-1} 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^{-1} (Capson-Tojo et al., 2020).

While their photoheterotrophic capabilities have been considerably studied in the past few years for resource recovery from wastewater (Hülsen et al., 2022a, 2022b, 2022c), their photoautotrophic capabilities have barely been researched for recovery. PPB grown autotrophically could allow the efficient conversion of H_2 and CO_2 into SCP, generating a pathogen-free high value-added product usable as feed/food (Delamare-Debouteville et al., 2019). If the H_2 and nutrient sources are sustainable (e.g., from bioH_2 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_2 conversion by PPB has been recently realised using pure cultures (Spanoghe et al., 2021). Results from our group have shown than enriched PPB cultures can valorise H_2 and CO_2 at higher yields than pure cultures (unpublished).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_2 as C source (as carbonate) and providing H_2 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_2 prior to H_2 addition. The initial headspace pressure was adjusted to 1.30-1.35 bar. The flasks were covered with UV/VIS filters (Hülsen 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 $W \cdot m^{-2}$ 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_2 and CO_2 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_2 uptake (k_m and K_{H2}) 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^{-1}·d^{-1})**
j	**Process ↓**	S_{IC}	S_{H2}	S_{IN}	S_{IP}	X_{PB}	X_C	
1	Autotrophic uptake of H_2 by PPB	$-f_{IC,au}$	1	$-\Sigma_{i=1-2,4-6} N_i \cdot \nu_{i,1}$	$-\Sigma_{i=1-3,5-6} P_i \cdot \nu_{i,1}$	$Y_{PB,au}$		$k_m \cdot S_{IC}/(K_{IC}+S_{IC}) \cdot S_{H2}/(K_{H2}+S_{H2}) \cdot X_{PB} \cdot I_{IN} \cdot I_{IP}$
2	Decay of X_{PB}					-1	1	$k_{dec,PB} \cdot X_{PB}$
Units		mol C·L^{-1}	mg COD·L^{-1}	mg N·L^{-1}	mg P·L^{-1}	mg COD·L^{-1}	mg COD·L^{-1}	

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_2 was sufficient, and consumption rates were limited by biological uptake (typical exponential curve, soluble H_2 concentrations higher than saturation constant for H_2 (K_{H2}; always below 0.05 mg COD·L^{-1}) and uptake rates increased with supply). After 0.9 d, the H_2 transfer rate became limiting due to the high biomass concentration and the low k_La (28.7 d^{-1}; determined experimentally), a situation that did not change despite the injection of extra H_2 in the headspace. After this point, the concentration of H_2 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_2 uptake rates. Optimal rates of 1.9-2.0 g COD·g COD^{-1}·d^{-1} were achieved at initial pH values of 7, 25ºC, and light intensities over 30 $W \cdot m^{-2}$ (Table 2). Lower or higher pH values and T resulted in decreased rates (Table 2, Figure 2). No photoinhibition was observed at 50 $W \cdot m^{-2}$. With R^2 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 g COD·g^{-1} 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 (2.3-2.7 g COD·g COD^{-1}·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 g COD·g COD^{-1}·d^{-1}; (Spanoghe et al., 2021)).

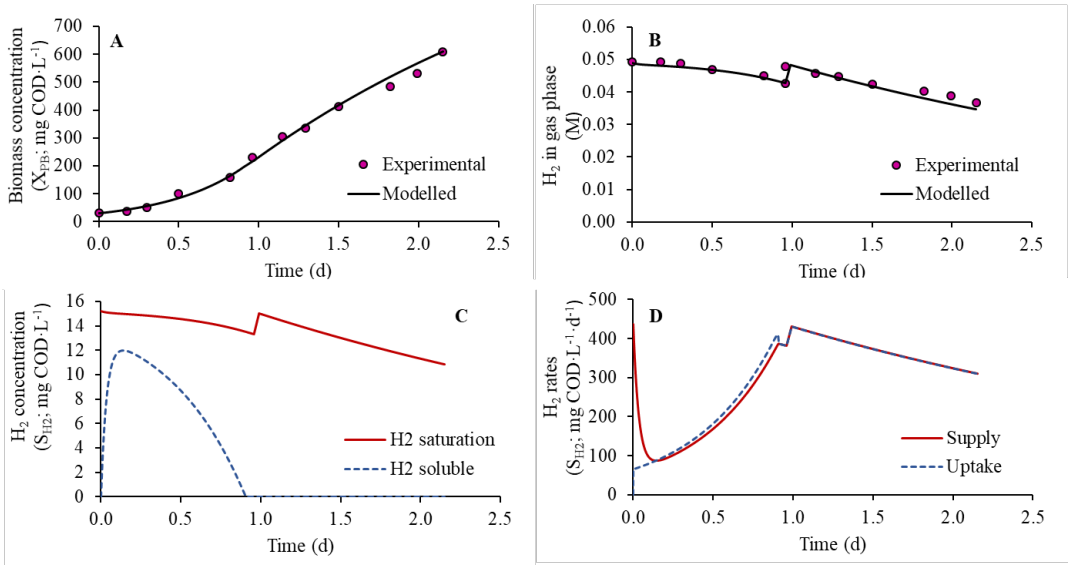

Figure 1. Results for the batch test at 150 rpm, showing (A) biomass concentrations, (B) H_2 gas concentrations, (C) H_2 liquid concentrations, and (D) H_2 supply and uptakes rates.

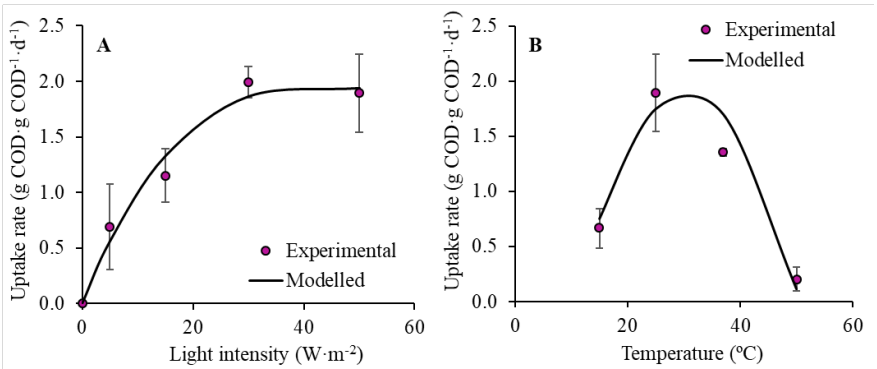

Figure 2. Influence of the (A) light intensity and (B) temperature on the specific H_2 uptake rates. Average and confidence intervals are shown (95%; n=3). The modelled results correspond to the Steele's equation (light intensity) and the cardinal temperature model with inflection (temperature).

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

	Mixing (rpm)		**pH**		**Temperature (°C)**		**Intensity (W·m^{-2})**		
	150[1]	**600**[1]	**6**	**8.5**	**15**	**38**	**15**	**30**	**50**[2]
Biomass yield (g COD$_{biomass}$· g COD$_{consumed}^{-1}$)	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
Specific uptake rates (g COD·g COD^{-1}·L^{-1})	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_La_{H2} of 28.7 d^{-1} at 150 rpm and 70.4 d^{-1} at 600 rpm.
2. These are also the results for the tests at pH 7 and 25ºC.

CONCLUSIONS

These results show, for the first time, that enriched PPB cultures can be grown autotrophically from H_2 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^{-1}·d^{-1} were reached at pH 7, 25ºC and light intensities higher than 30 W·m^{-2}. 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_2 valorisation. Volumetric production rates must be assessed and the model must be validated using continuous bioreactors.

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