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Unified mechanistic modelling of gas-fed purple bacteria enriched cultures: from autotrophy to diazotrophic heterotrophy

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Introduction

Thanks to their phototrophic capabilities, purple non-sulfur bacteria (PNSB) can grow at extremely high biomass yields, up to 1 g COD·g COD^{-1} (Capson-Tojo et al., 2020). This feature can be used to convert gases (e.g. H_2 and CO_2) into pathogen-free microbial protein (MP), which can be used as feed (Delamare-Debouteville et al., 2019) and, potentially, as food. If the H_2 and nutrients (e.g. N and P) are recovered from waste streams (e.g., from bioH_2 and anaerobic digestate extracts), this approach could be a game changer for resource recovery in a biorefinery. The proof of concept of H_2 conversion by PNSB has been realized using pure cultures (Spanoghe et al., 2021), and recent results from our group have shown than enriched PNSB cultures can valorize H_2 and CO_2 at higher yields and rates than pure cultures (Rodero et al., 2024).

Other than H_2 and CO_2, another gas substrate that PNSB can assimilate is N_2. Their diazotrophic growth (fixing N_2) can occur both autotrophically and heterotrophically and, opposed to common heterotrophic diazotrophs (e.g. *Rhizobium* sp.), the ability of PNSB of using energy from light allows keeping high yields and, potentially, faster rates. Despite their potential for N_2 fixation, the diazotrophic capabilities of PNSB have barely being explored, and the crucial parameters such as yields and specific growth rates (crucial for understanding the process and for technology development) are still to be determined (Wang et al., 2019).

Modelling these gas-valorization processes can allow to estimate specific rates, providing at the same time crucial insights on the system intricacies and allowing to simulate scenarios, which is extremely useful for early hypothesis testing. Recent mechanistic models have allowed to accurately represent photoheterotrophic (Alloul et al., 2023; Capson-Tojo et al., 2023) and photoautotrophic PNSB growth (Rodero et al., 2024). Nevertheless, a model comprising the different processes that can take place in a potential gas-fed reactor (e.g. autotrophic growth and both auto- and hetero-diazotrophic growth) is missing, with none considering N_2 fixation.

Here we present a unified mechanistic model considering simultaneously the different phototrophic processes that can take place in a gas-fed PNSB reactor (e.g. diazotrophic and non-diazotrophic growth, both under heterotrophy (on C2, C3 and C4 volatile fatty acids (VFAs)) and autotrophy (on H_2 and CO_2)). Dedicated experiments were used to determine COD and N yields, and the model was used to determine the specific uptake rates of enriched PNSB cultures. The model was afterwards used to simulate different scenarios.

Materials and Methods

N_2-fixing autotrophic and heterotrophic batch tests were performed as in Rodero et al. (2024), using flasks (0.19 L and 2 L of working and total volumes) fed with modified Ormerod media, either with H_2 and CO_2 (as carbonate) or VFAs (i.e. acetate, butyrate or propionate). Pure N_2 was the sole N source, provided at an initial pressure of 1.30-1.35 bar. For autotrophic tests, H_2 and N_2 were supplied at a molar ratio of ~4:1. Different infrared incident light intensities were assessed (15-100 $W \cdot m^{-2}$; LEDs at 805 nm) to ensure that the rates were not light limited. Gas and liquid samples were taken every 2-5 h to measure gas pressure and composition, and concentrations of ammonium, COD and VFAs. Non-diazotrophic data were collected from Rodero et al. (2024).

The model considered PNSB and fermenters as biomass components, and photoautotrophic (on H_2 and CO_2) and photoheterotrophic (on acetate, propionate and butyrate) growths, both under diazotrophic and non-diazotrophic conditions. Acetogenic fermentation and cell death were also included. Mass transfer and chemical equilibria were modelled as in Batstone et al. (2002), accounting for different gas solubilities and for relevant chemical species for pH calculation. The model followed IWA standards. Stoichiometric parameters were either determined experimentally (yields) or calculated. Estimation of diazotrophic kinetic parameters (e.g. specific uptakes rates (k_m) and half-saturation constants (K_S)) was performed as in Rodero et al. (2024), using results from dedicated batch tests to calibrate separately each process. Matlab was used to perform simulations.

Results and discussion

Figure 1 shows that the model was able to represent the kinetics of the different biological processes considered. Focusing on diazotrophic growth (the most novel feature here), the model allowed inferring: 1) heterotrophic diazotrophic growth is faster than growth on ammonium (e.g. 5.20±0.33 *vs.* 2.73±0.42 $COD_{substrate} \cdot g\ COD_{biomass}^{-1} \cdot d^{-1}$ on acetate at 100 $W \cdot m^{-2}$); 2) part of the H_2 produced during heterotrophic N_2 fixation can be partially consumed autotrophically by PNSB (the measured yields of 0.88±0.02 g $COD_{biomass} \cdot g\ COD_{consumed}^{-1}$ were slightly higher than the highest possible theoretical values in the model, of 0.85 g $COD_{biomass} \cdot g\ COD_{consumed}^{-1}$).

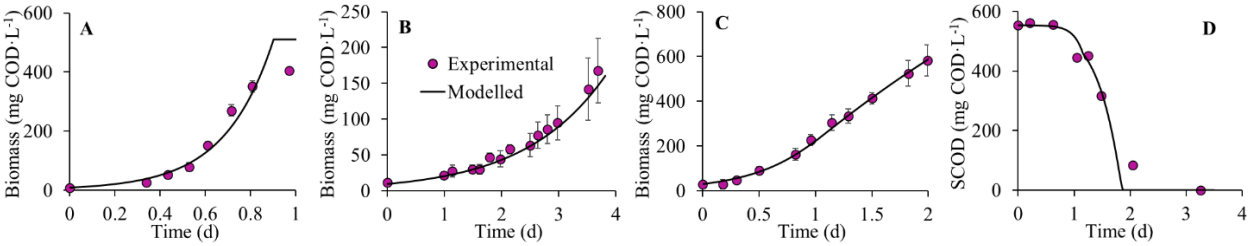

Fig. 1. Experimental and modelling results from batch tests for (A) heterotrophic-diazotrophic growth, (B) autotrophic-diazotrophic growth, (C) autotrophic growth on NH_4^+, and (D) heterotrophic SCOD consumption on NH_4^+.

Simulations were performed using the calibrated model to represent a continuous system changing dynamically between growth modes (i.e. (I) diazotrophic growth on a VFA mixture, (II-III) alternate diazotrophic and non-diazotrophic heterotrophic growth, (IV-V) simultaneous heterotrophic and autotrophic growth, (VI) autotrophic growth; Figure 2). The results from I-III confirm the faster diazotrophic growth and the higher yields on NH_4^+. Thanks to the lack of NH_4^+, fermenters were washed-out despite the presence of propionate/butyrate on the feed, resulting in a pure PPB culture (data not shown). Simultaneous H_2 and VFA supply increased the biomass concentrations (best on NH_3 due to the higher yields), but single H_2 supply resulted in biomass wash-out at the applied HRT do to the much slower autotrophic growth rates. PNSB could fix N_2 consuming simultaneously VFA and H_2 at high rates, provided that VFA are not limiting and allow biomass growth.

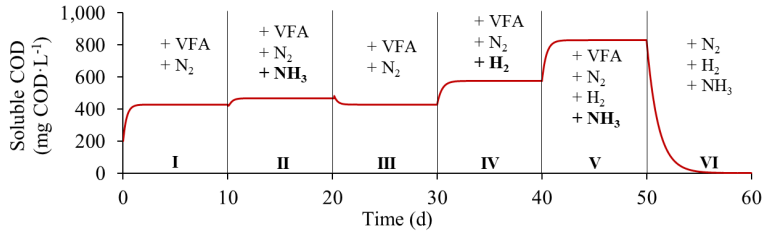

Fig. 2. Simulation results for different feeding periods: (I-III) alternate diazotrophic and non-diazotrophic heterotrophy on mixed VFA, (IV-V) simultaneous heterotrophy-autotrophy, (VI) autotrophy. It was assumed that there was no light nor gas transfer limitations.

Research is on-going using a continuously gas-fed bubble-column. The resulting data will be used for model validation under similar conditions to those simulated above. Validation results will be included in the final work.

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References

Alloul, A., Moradvandi, A., Puyol, D., Molina, R., Gardella, G., Vlaeminck, S.E., De Schutter, B., Abraham, E., Lindeboom, R.E.F., Weissbrodt, D.G. (2023). A novel mechanistic modelling approach for microbial selection dynamics: Towards improved design and control of raceway reactors for purple bacteria. Bioresour. Technol. **390**, 129844.

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).

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., Hülsen, T. (2023). Expanding mechanistic models to represent purple phototrophic bacteria enriched cultures growing outdoors. Water Res. **229**, 119401.

Delamare-Debouteville, 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.

Rodero, M. del R., Magdalena, J.A., Steyer, J.-P., Escudié, R., Capson-Tojo, G. (2024). Potential of enriched phototrophic purple bacteria for H2 bioconversion into single cell protein. Sci. Total Environ. **908**, 168471.

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.

Wang, B., Xiao, K., Jiang, Z., Wang, J., Yu, J.C., Wong, P.K. (2019). Biohybrid photoheterotrophic metabolism for significant enhancement of biological nitrogen fixation in pure microbial cultures. Energy Environ. Sci. **12**, 2185–2191.