Diazotrophic microbial protein production from volatile fatty acids, hydrogen and carbon dioxide using purple bacteria

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Abstract: the unique diazotrophic and phototrophic capabilities of purple phototrophic bacteria could provide a sustainable alternative to the current fossil-based food production systems. We show here that mixed purple non-sulphur bacteria cultures can fix at extremely high N and COD yields (average values of 0.99±0.03 and 0.89±0.02 $g_{biomass} \cdot g_{removed}^{-1}$), and with high crude protein contents (up to 58%). While the photoautotrophic rates (fed with H_2 and CO_2) were relatively low (0.85±0.29 g $COD_{substrate} \cdot g\ COD_{biomass}^{-1} \cdot d^{-1}$), the values obtained during photoheterotrophic growth (fed with volatile fatty acids) were surprisingly high, averaging 6.4±0.5 g $COD_{substrate} \cdot g\ COD_{biomass}^{-1} \cdot d^{-1}$). The latter are around three times higher than common rates reported for non-diazotrophic photoheterotrophic growth. These promising results suggest that specialised cultures of these bacteria could be effectively applied to fix nitrogen while converting reduced carbon (e.g. mixed fermentation effluents) into microbial protein.

Keywords: purple phototrophic bacteria; purple non-sulphur bacteria; nitrogen fixation

Introduction

Our current food production system relies fully on synthetic fertilisers derived from the Haber-Bosch process to generate proteins. Purple non-sulphur bacteria (PNSB) have the unique capability of performing biological nitrogen fixation while using organic compounds (such as volatile fatty acids; VFAs) as C source and taking energy from light. This strategy, named photoheterotrophic growth, might allow PNSB to grow faster than diazotrophic CO_2 fixers and at higher energetic efficiencies than non-light users (Capson-Tojo et al., 2023).

Despite their potential, the N_2 fixing capabilities of PPB remain barely explored, and rates and yields are still to be determined (Wang et al., 2019). This work aimed to assess the N_2 fixation capabilities of enriched cultures of PNSB growing on different C sources and environmental conditions.

Material and Methods

Batch tests were performed in flasks of 0.5 L, with a liquid volume of 390 mL. Different sources of C and reducing power (e.g. VFAs or CO_2 and H_2) were fed. A modified Ormerod media was used, ensuring that N_2 was the only N source, and tests were illuminated with near infrared light at different intensities (Rodero et al., 2024). The concentrations of nitrogen, ammonium, chemical oxygen demand (COD) and VFAs were measured, as well as the crude protein contents. Results were used to estimate yields, defined as the consumed substrate recovered in the product (biomass). A simplified mechanistic model was built to determine specific uptake rates, using data from dedicated batch tests using flasks of 2 L (liquid volume of 450 mL) sampled every 2-5 h (Capson-Tojo et al., 2023; Rodero et al., 2024). These conditions ensured that the estimated rates were indeed biological (not limited by gas transfer).

Results and Conclusions

After sequential enrichments (results not shown), the cultures were able to effectively assimilate N_2, growing effectively under any of the conditions tested (Figure 1A). Average N yields of 0.99±0.03 g $N_{biomass}$·g $N_{consumed}^{-1}$ imply that the consumed N was assimilated as biomass (mostly as proteins). The COD biomass yields were also remarkably high, but slightly lower than the N values (average of 0.89±0.02 g $COD_{biomass}$·g $COD_{consumed}^{-1}$). The COD yields (Figure 2) were lower than common photoheterotrophic yields of 1 g $COD_{biomass}$·g $COD_{consumed}^{-1}$ for non-diazotrophic growth (Capson-Tojo et al., 2023). The concomitant H_2 production due to nitrogenase activity in diazotrophic tests could partially explain these lower values. The amounts of H_2 produced were below detection limits, so we could not confirm this. However, assuming common ratios of 1-7 $mol_{H2,produced}$·$mol_{N2,fixed}^{-1}$, we could close COD balances. Increasing light intensities did not increase COD yields, confirming that the lack of reducing power was limiting biomass growth. Conversely, feeding more reduced VFAs (e.g. propionate or butyrate) did not increase COD yields either, suggesting a proportional relationship between H_2 production and the substrate redox state. Adding extra inorganic C did not increase the COD yields either (results not shown).

The estimated specific uptake rates (Figure 2) were similar between photoheterotrophic experiments (up to 6.8 and average of 6.4±0.5 g $COD_{substrate}$·g $COD_{biomass}^{-1}$·d^{-1}), but were much lower for the autotrophic test (0.85±0.29 g $COD_{substrate}$·g $COD_{biomass}^{-1}$·d^{-1}). This might be due to the higher energy requirements for simultaneous CO_2 and N_2 fixation, double compared with only diazotrophic growth. The rates obtained here were surprisingly high compared to values for non-diazotrophic photoheterotrophic PNSB (2.4 g $COD_{substrate}$·g $COD_{biomass}^{-1}$·d^{-1} (Capson-Tojo et al., 2023)). Resulting N fixation rates of 0.39±0.05 mg N_{fixed}·mg $COD_{biomass}^{-1}$·d^{-1} imply productivities up to 432 mg $N_{protein}$·L^{-1}·d^{-1} (at 500 mg $COD_{biomass}$·L^{-1}, which could be much higher). These promising values will be further optimised. Crude protein contents up to 58% suggest the biomass applicability as microbial protein. We are currently waiting for 16S rRNA sequencing results to identify the microorganisms in our PNSB cultures (Figure 3).

These results confirm that mixed PNSB cultures can fix N_2 at very high N and COD yields, maximising resource efficiency. The photoheterotrophic uptake rates were much higher than non-diazotrophic rates reported previously, with promising protein productivities. This approach could be used to valorise mixed VFAs from waste fermentation, generating a sustainable proteinaceous product (independent from fossil-derived N) that could be potentially used as feed.

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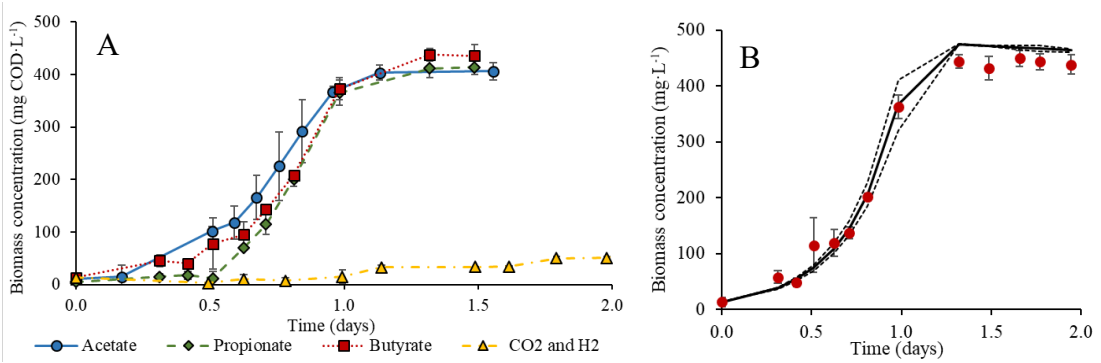

Figure 1.1. (A) Growth curve of the diazotrophic batch tests. (B) Modelling and experimental results for the batch tests fed with butyrate. Standard deviations are shown for experimental values. Modelling results include 95% confidence regions.

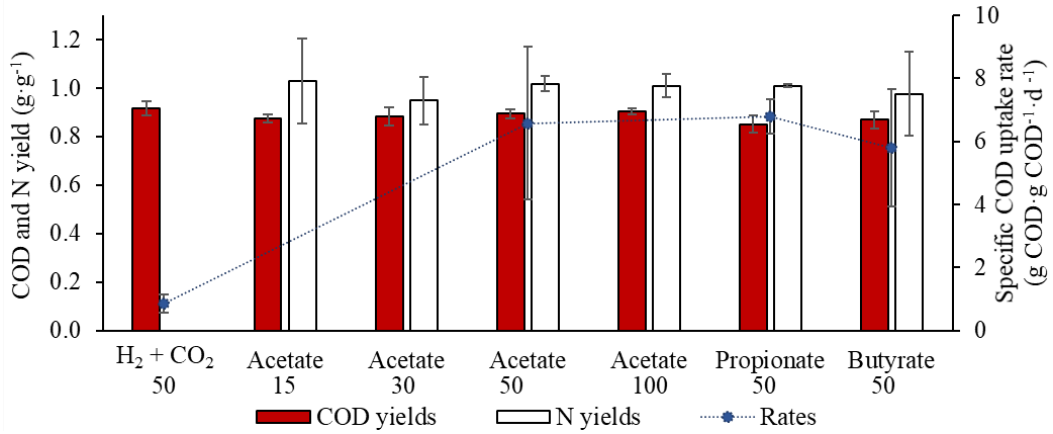

Figure 1.2. Biomass yields for each experiment. The substrate is indicated, together with the illumination intensity provided (in $W \cdot m^{-2}$). The specific uptakes rates for the experiments at 50 $W \cdot m^{-2}$ are also shown. 95% confidence intervals are provided.

Figure 1.3. Pictures of the N_2-fixing PNSB cultures.