Mixed phototrophic cultures can produce microbial protein from N2 and organic acids at high yields and rates

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MIXED PHOTOTROPHIC CULTURES CAN PRODUCE MICROBIAL PROTEIN FROM N_2 AND ORGANIC ACIDS AT HIGH YIELDS AND RATES

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1. Keywords

Purple non-sulphur bacteria, purple phototrophic bacteria, microbial protein

2. Highlights

- Mixed purple cultures can effectively fix N_2 at N and COD yields close to 1 $g \cdot g^{-1}$
- Photoheterotrophic uptake rates up to 6.8 g COD·g $COD^{-1} \cdot d^{-1}$ were obtained
- The obtained fixation rates were much higher than expected
- Crude protein contents up to 58% were obtained

3. Purpose

This work aimed to assess the N_2 fixation capabilities of enriched cultures of purple phototrophic bacteria (PPB). PPB have the unique capability of fixing N_2 while using organic compounds as C source and taking energy from light, during photoheterotrophic growth [1]. Compared to other diazotrophs, the photoheterotrophic capabilities of PPB allow them to grow faster than CO_2 fixers and at higher substrate-based energetic efficiencies than non-light users [2]. Despite their potential, the N_2 fixing capabilities of PPB remain barely explored, and rates and yields are still to be determined [3].

4. Materials and methods

Batch tests were carried out in Schott flasks of 0.5 L, with a liquid volume of 390 mL. Different sources of C and reducing power (e.g. volatiles fatty acids (VFAs) and CO_2 and H_2) were fed using modified Ormerod media, ensuring that N_2 was the only N source. Tests were illuminated with near infrared light at different light intensities [4]. Analyses were performed to measure concentrations of nitrogen, ammonium, chemical oxygen demand (COD), VFAs, and crude protein contents [4]. The results were used to estimate yields, defined as the consumed substrate recovered in the product (biomass). In addition, a simplified mechanistic model was used 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 [2, 4]. These conditions ensured that the estimated rates were indeed biological (not limited by gas transfer).

5. Results and discussion

After sequential enrichments (results not shown), the cultures were able to effectively assimilate N_2, at average N yields of 0.99±0.03 g $N_{biomass} \cdot g\ N_{consumed}^{-1}$, implying that all the consumed N was retrieved in the biomass (mostly as proteins). Concerning the COD biomass yields, they were also remarkably high, but slightly lower than the N values (average of 0.89±0.02 g $COD_{biomass} \cdot g\ COD_{consumed}^{-1}$). The COD yields (Figure 1) were lower than common photoheterotrophic yields for non-diazotrophic growth, commonly of 1 g $COD_{biomass} \cdot g\ COD_{consumed}^{-1}$ [1]. The concomitant H_2 production related to nitrogenase activity in the diazotrophic tests could explain partially these lower yields, but as the produced amounts were below detection limits, we could not confirm this. Nevertheless, assuming values of 1-7 $mol_{H2,produced} \cdot mol_{N2,fixed}^{-1}$ [5], we could close the COD balances. Increasing light intensities did not result in higher 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 the COD yields, suggesting a proportional relationship between the H_2 produced and the redox state of the substrate. Adding extra inorganic C did not increase the COD yields either (results not shown).

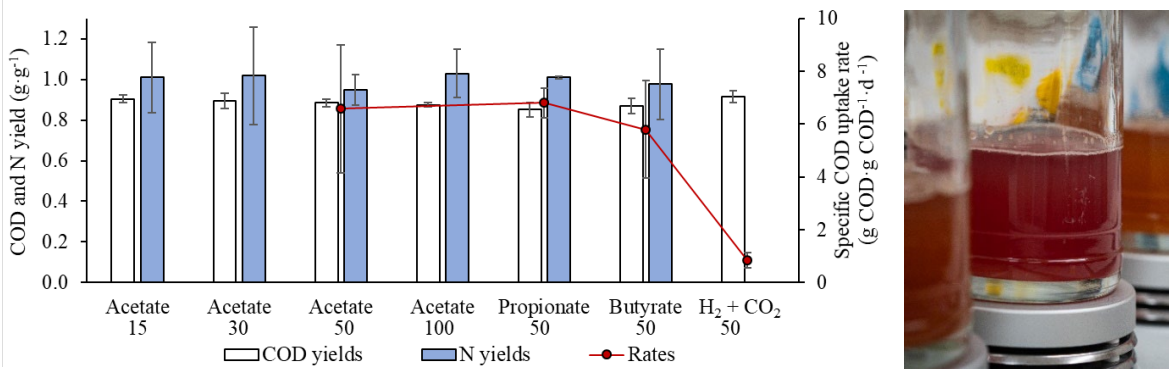

Figure 1. (Left) Biomass yields for each experiment. The substrate is indicated, together with the illumination intensity provided (in $W \cdot m^{-2}$). The specific uptakes rates for the experiments at 50 $W \cdot m^{-2}$ are also shown. 95% confidence intervals are provided. (Right) Pictures of the N_2-fixing PPB cultures.

Concerning the specific uptake rates (Figure 1), the values were similar between photoheterotrophic experiments (up to 6.8 and average of 6.4±0.5 g $COD_{substrate} \cdot g\ COD_{biomass}^{-1} \cdot d^{-1}$), but were much lower for the autotrophic test (fed with H_2 and CO_2; 0.85±0.29 g $COD_{substrate} \cdot g\ COD_{biomass}^{-1} \cdot d^{-1}$). This was caused but the higher energy requirements for simultaneous CO_2 and N_2 fixation, double compared to only diazotrophic growth. This might explain why adding inorganic C did not enhance the COD yields, as the duration of the tests (up to three days) did not allow for H_2 refixation. Compared to uptake rates for non-diazotrophic photoheterotrophic PPB (around 2.4 g $COD_{substrate} \cdot g\ COD_{biomass}^{-1} \cdot d^{-1}$ [2]), the rates obtained here are surprisingly high. The resulting N fixation rates were 0.39±0.05 mg $N_{fixed} \cdot mg\ COD_{biomass}^{-1} \cdot d^{-1}$, implying productivities up to 432 mg $N_{protein} \cdot L^{-1} \cdot d^{-1}$ (assuming 500 mg $COD_{biomass} \cdot L^{-1}$, which could be much higher). These are promising values that will be further optimised. Average crude protein contents of 49±6.7% (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 cultures.

6. Conclusions and perspectives

These results confirm that mixed PPB cultures can effectively fix N_2 at very high N and COD yields, maximising resource efficiency. The photoheterotrophic uptake rates were much higher than the non-diazotrophic rates reported previously. These resulting protein productivities are promising. 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.

7. References

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