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Introduction

Our current food production system relies fully on reactive nitrogen production via the Haber-Bosch process. This process demands high amounts of energy (500 kJ·mol N^{-1}) and accounts for approximately 2% of the total world energy consumption (Kyriakou et al., 2020). This fact, along with the extremely inefficient conversion of inorganic nitrogen into plant or animal proteins (50% and 15% respectively) and the growing demand for food, show the urgent need for the development of novel sustainable protein production systems (Matassa et al., 2015). The use of microorganisms able to fix N_2 to produce microbial protein can partially reduce the current dependence on reactive nitrogen. Among them, purple phototrophic bacteria (PPB) present the advantage of performing biological N_2 fixation using organic or inorganic compounds as a carbon source and light as an energy source. The use of light allows them to have higher yields than non-photosynthetic microorganisms, while under photoheterotrophic conditions their growth is faster than that of diazotrophic CO_2 fixers (Capson-Tojo et al., 2023). Despite its potential, the ability of PPB to fix N_2 has been barely studied and biomass yields and growth rates remain unknown. In this work, the N_2 fixation capabilities of enriched cultures of PPB have been evaluated during photoheterotrophic and photoautotrophic growth, and under different environmental conditions.

Materials and Methods

A series of eight sequential enrichments were performed to obtain a PPB consortium able to uptake N_2. These enrichments were carried out in Schott flasks of 0.5 L, with a working volume of 400 mL (heterotrophic) or 200 mL (autotrophic) under continuous illumination using infrared LED lights (850 nm). The mineral medium was based on that proposed by Ormerod et al. (1961), modified to remove the N source. The headspace of the bottles was composed by 100% N_2 at a pressure of 1.3 bar except for the autotrophic tests where H_2 (~80 % $v \cdot v^{-1}$) was also added in the headspace. Once the enrichment showed a constant performance, batch tests under similar conditions and with the appropriate C source depending on the test were performed to evaluate the influence of the carbon (C) sources (volatiles fatty acids (VFAs) versus CO_2 and H_2), the incident light intensities (15, 30, 50 and 100 $W \cdot m^{-2}$) and the pH (6, 7 and 8) on and chemical oxygen demand (COD) yields. Analyses were performed at the beginning and end of each test to measure gas composition and pressure, concentrations of nitrogen, ammonium, COD, volatile suspended solids and VFAs. In addition, dedicated batch tests using flasks of 2 L with a working volume of 500 mL were used to estimate uptake rates, ensuring that the system was not limited by gas transfer. During these tests, 5 mL of liquid samples were drawn every 2-5 hours to measure soluble and total COD. In addition, liquid samples at the end of the experiments were used to determine crude protein contents, amino acids profiles and microbial communities (via 16S rRNA gene sequencing).

Results and discussion

After some sequential enrichments, the cultures were able to efficiently grow using N_2 (results no shown). Average N yields of 1.00±0.04 g $N_{biomass} \cdot g\ N_{consumed}^{-1}$, where N consumed refers to N_2 fixed by the biomass, were obtained regardless of the condition tested, implying that all the N consumed was recovered as biomass (Fig. 1). The COD biomass yields were also high (an average of 0.88±0.01 g $COD_{biomass} \cdot g\ COD_{consumed}^{-1}$), but slightly lower than common photoheterotrophic/autotrophic yields for non-diazotrophic growth (~1 g

$COD_{biomass}$·g $COD_{consumed}^{-1}$) (Capson-Tojo et al., 2023; Rodero et al. 2024). This lower COD biomass yields were attributed to the mandatory H_2 associated to nitrogenase activity. The produced H_2 was only partially re-consumed. Increase of the light intensities or feeding more reduced VFAs (e.g. propionate or butyrate) did not impact COD yields. Adding extra inorganic C to consume the H_2 generated or working with different initial pHs (6 and 8) did not increase the COD yields either (results not shown).

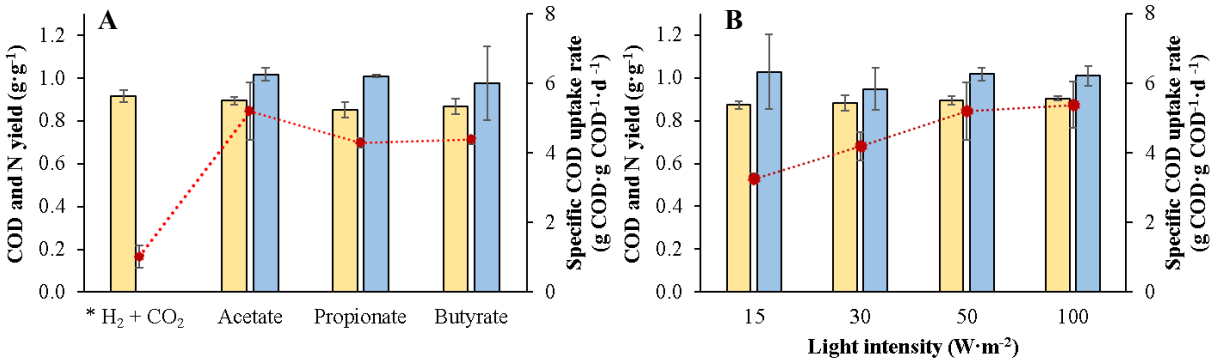

Fig. 1. Average COD yields (yellow bars), N yields (blue bars) and specific uptakes rates (red points) at (A) different substrate at light intensity of 50 $W \cdot m^{-2}$ and (B) different light intensities using acetate as substrate along with 95% confidence intervals. *N yields under autotrophic conditions were not estimable due to low N_2 consumption and the concomitant H_2 consumption.

The estimated specific uptake rates (Fig. 1A) were slightly higher using acetate as a C source (5.20±0.83 g $COD_{substrate}$·g $COD_{biomass}^{-1} \cdot d^{-1}$) in comparison with butyrate and propionate (4.38±0.13 g $COD_{substrate}$·g $COD_{biomass}^{-1} \cdot d^{-1}$ and 4.29±0.13 g $COD_{substrate}$·g $COD_{biomass}^{-1} \cdot d^{-1}$, respectively) under similar light intensities (50 $W \cdot m^{-2}$). Otherwise, light limitation was observed under intensities lower than 50 $W \cdot m^{-2}$ (Fig. 1B). In any case, the rates obtained under photoheterotrophic conditions and no light limitation were unexpectedly high when compared to previous reported values for non-diazotrophic photoheterotrophic PPB (2.4 g $COD_{substrate}$·g $COD_{biomass}^{-1} \cdot d^{-1}$ (Capson-Tojo et al., 2023)), which could be due to enhanced redox homeostasis control. Operation under autotrophic conditions resulted in much lower specific uptake rates (1.02±0.33 g $COD_{substrate}$·g $COD_{biomass}^{-1} \cdot d^{-1}$). Indeed, in this case the specific uptake rates were lower than that obtained under similar conditions using NH_4^+ as a N source (1.89±0.35 g $COD_{substrate}$·g $COD_{biomass}^{-1} \cdot d^{-1}$) (Rodero et al. 2024). This fact could be the consequence of higher energy demands for simultaneous CO_2 and N_2 fixation.

The results hereby obtained confirm that mixed PPB cultures growing under photoheterotrophic conditions can fix N_2 at high biomass yields, maximizing N and COD recovery. The biomass had crude protein contents higher than 44%, which allows its application as microbial protein. Additional amino acid analyses are being performed to further corroborate this. Sequencing data will also be presented during the conference. The presented approach could be used to valorize mixed VFAs from waste fermentation, generating a proteinaceous product (independent from fossil-derived N) that could be used as feed. Food could be produced from clean C sources.

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