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Towards a gas-based biorefinery: purple photo-anaerobic processes for the valorization of fermentation effluents

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Highlights

- Biological processes can generate value-added products from gaseous effluents
- Purple phototrophic bacteria can turn H_2 and CO_2 into proteins at high yields
- Acetate-rich effluents can be used to fix N_2 by purple bacteria at high yields

Keywords: Resource recovery; Hydrogen; Purple phototrophic bacteria; Single Cell Protein; Nitrogen.

Introduction

Processes based on purple phototrophic bacteria (PPB) are promising for resource recovery thanks to the high biomass yields (up to 1 g COD·g COD^{-1}) that their phototrophic metabolism allows. While their photoheterotrophic capabilities for resource recovery from wastewater have been considerably studied in the last few years (Hülsen et al., 2022a, 2022b), their photoautotrophic metabolism has barely been researched for resource recovery. Autotrophic PPB growth allows the conversion of H_2 and CO_2 into single-cell protein (SCP), generating a pathogen-free value-added product applicable as feed/food. This process could be used to valorize gaseous effluents in a biorefinery (e.g. dark from fermentation), while also recovering remaining nutrients (e.g. from digestates). Spanoghe et al. (2021) realized the proof of concept of H_2 conversion by PPB using pure cultures, but yields and rates are still to be determined, particularly in enriched systems.

PPB can also fix N_2 when there is limited soluble N in the media (Capson-Tojo et al., 2020). This capability could be used to produce sustainable SCP, as an alternative to the Haber-Bosch process, which is a fast and cheap process to generate ammonia, but relies on fossil fuels. With the prices of organic-N fertilizers being 2-4 times what they were 1-2 years ago (according to the European Commission), biological N fixation processes are starting to seem like a feasible alternative.

In this study, we performed dedicated batch tests to evaluate the yields and kinetics of an enriched PPB consortia for the conversion of H_2 and CO_2 into SCP, evaluating the influence of environmental conditions (temperature, pH, light intensity). In addition, the possibility of producing SCP from N_2 was evaluated under both autotrophic and photoheterotrophic conditions.

Materials and Methods

A series of sequential batch autotrophic and heterotrophic enrichments (over 14) were performed in Schott flasks of 500 mL to obtain stable phototrophic PPB consortia. Each batch (which lasted for around 1 week) was inoculated with 20 mL from the previous culture and grown with 180 mL of fresh medium. The medium was the one proposed by Ormerod et al. (1961) for the heterotrophic tests (using acetate as C source). It was modified for the autotrophic tests, adding H_2 and carbonate instead of acetate. The bottles were closed and the headspace was flushed with N_2 prior to incubation. The initial headspace pressure was adjusted to 1.3 bar in the H_2-fed tests. The initial acetate concentration was 500 mg COD·L^{-1} in the heterotrophic tests. Illumination was provided as in Capson-Tojo et al. (2021).

The resulting stable enrichments were used to perform batch tests. Autotrophic growth was assessed at different environmental conditions (e.g. initial pH values of 6, 7 and 8.5, temperatures of 15, 25, 38 and 50 °C, and light intensities of 0, 5, 15, 30 and 50 W·m^{-2}) using triplicate batch tests, as described above. The gas composition and pressure were monitored 4-5 times per day, and liquid samples were drawn to determine biomass yields and productivities, crude protein contents, amino acids profiles and microbial communities (16S rRNA gene sequencing). A mechanistic model based on Capson-Tojo et al. (2023) was used for estimating specific uptake rates. Tests for N_2 fixation were performed as the enrichments, but using N-depleted media to allow nitrogenase activity.

Results and Discussion

The obtained enriched PPB cultures grew autotrophically at yields always close to 1 g $COD_{biomass}$·g $COD_{consumed}^{-1}$, corresponding to protein yields of around 4 g·g H_2^{-1}, with protein contents of 51-64%

(Figure 1). These values are on the highest end of those reported for PPB pure cultures (Spanoghe et al., 2021). Optimal autotrophic specific uptake 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}. Although these values are slightly lower than for photoheterotrophic growth (2.4-2.6 g COD·g COD^{-1}·d^{-1}; (Capson-Tojo et al., 2023)) and lower than those for hydrogen oxidizing bacteria (HOB; main competitors), they still allowed overall biomass productivities up to 378 mg COD·L^{-1}·d^{-1}, values that remain to be optimized in continuous reactors. Yields for HOB are around 0.2 g $COD_{biomass}$·g $COD_{consumed}^{-1}$, confirming the high PPB yields as their main advantage (Hu et al., 2020). Analysis of the microbial communities via 16S rRNA gene sequencing showed that PPB represented over 85% of the 16S total copies, with Rhodobacter *sp.* and *Rhodopseudomonas* sp. as dominant genera (as in the inoculum).

Regarding the N_2 fixation tests, preliminary results show that effective N_2 fixation can be achieved at reasonable rates in heterotrophic N-limited conditions (no more VFAs after 3-4 days), at yields of 1 g N·g N^{-1} and 0.88-0.92 g COD·g COD^{-1}. More research is needed to determined specific uptake rates and protein contents, and to elucidate why the biomass yields in terms of COD are lower than in soluble N rich media. Very likely, electrons from the substrate are not used for growth, but for the reduction of N_2. Light intensity did not affect the yields (Figure 1), so energy availability was not responsible for the lower yields obtained.

The non-energetic valorization of gases from fermentation (e.g. H_2, and CO_2) could improve drastically the economic and environmental performances of environmental biorefineries. PPB grown heterotrophically could valorize mixed VFAs. The ammonia could be extracted (e.g. via electrodialysis) and used for autotrophic growth, while the N-depleted VFA-rich stream could be used for N_2 fixation by PPB, generating a sustainable, clean, SCP product.

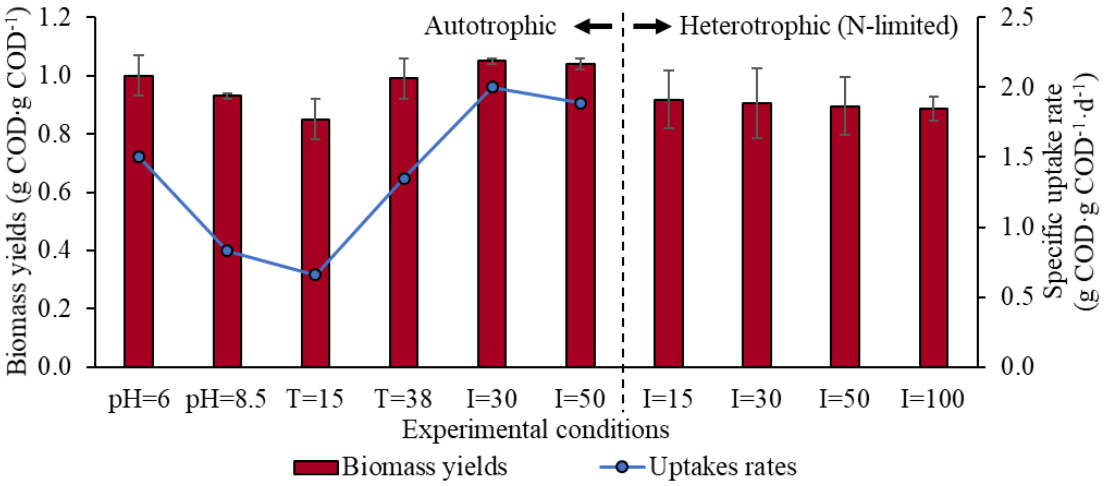

Figure 1. Biomass yields for the autotrophic and heterotrophic batch tests at different conditions. The specific uptake rates for the autotrophic tests are also shown. Temperatures (T) are expressed in Celsius and light intensities (I) in W·m^{-2}.

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