

AD-595

Photo-fermentation to unlock a locally available hydrogen source for biogas upgrading within two-phases anaerobic digestion processes

M. Biasiolo*, M. Turatello, P. Cerchier, G. Tassinato, C. Cavinato

* marco.biasiolo@unive.it

Abstract: Biological biogas upgrade can be achieved by supplying hydrogen in-situ or ex-situ during the anaerobic digestion process. Among the possible methods for hydrogen production, the biological processes have gained a lot of interest in recent years, as they would allow for an alternative and environment-friendly production. In this study, the VFAs-enriched fermentate produced as an intermediate in a two-phases anaerobic digestion process was used as a growth medium for Purple Non-Sulfur Bacteria (PNSB), to unlock a locally available hydrogen source within the process. Two PNSB strains, *Rhodopseudomonas palustris* and *Rhodospirillum rubrum*, were tested on suspended and immobilized cultures, fed with the fermentation broth, and compared in terms of biomass and hydrogen production. Both strains showed a consistent boost in growth yields when fed with the fermentative broth rather than with the standard growth medium (a two-fold higher biomass production for *Rps. palustris*, and eight-fold higher for *Rsp. rubrum*), and higher hydrogen productivity when testing immobilized cultures rather than suspended ones.

Keywords: Photo-fermentation; purple non-sulfur bacteria; hydrogen production

Introduction

Anaerobic digestion is a well-known biotechnology process commonly applied for the conversion of organic wastes into biogas, for energy recovery. However, biogas calorific value is strongly affected by the presence of other compounds, above all carbon dioxide which typically accounts for 30–50 % of its composition. Hydrogen can be a strategic tool to address this issue, as it can be employed for the biological conversion of carbon dioxide to methane, upgrading the biogas and enhancing its energy potential (Sun et al., 2023). The fermentate produced as an intermediate during two-phases anaerobic digestion can serve as a valuable substrate for on-site photo-fermentative hydrogen production, thus diminishing the economic and environmental cost of sourcing pressurized hydrogen externally.

This study aims to investigate the utilization of fermentate, comparing the efficiency of suspended and immobilized PNSB (Purple Non-Sulfur Bacteria) cultures, to produce locally available hydrogen for biogas upgrading in a two-phases anaerobic digestion process.

Material and Methods

The test was carried out using *Rhodopseudomonas palustris* and *Rhodospirillum rubrum*, supplied by BCCM (Belgian Coordinated Collections of Microorganisms, Ghent, Belgium). The two PNSB inocula were maintained in axenic condition in an RPN culture medium, prepared as described in Bianchi et al., (2010). The test was conducted on an RPP medium containing 4.0 g L⁻¹ of malic acid (Bianchi et al., 2010) and on a fermentative broth (FB) medium. The FB medium was prepared by processing the effluent from the fermentation phase of a two-phases anaerobic digestion process treating a mixture of food waste and sewage sludge. The fermentation broth was adjusted at 6.8 pH with NaOH 6 N, centrifuged at 9,000 rpm for 10 min, and filtered at 0.2 µm for sterilization. The fermentation broth was then diluted 1:5 with an RPP medium prepared without the addition of malic acid, thus, maintaining the overall composition of the medium and replacing with VFAs the carbon source for PNSB metabolism. After the preparation, the FB medium was characterized by an ammonium concentration of 86.8 ± 9.5 mg_{N-NH₄} L⁻¹, and was composed of (g_{COD} L⁻¹): acetic acid, 1.09; propionic acid, 0.69; butyric acid, 0.56; valeric acid, 0.75; caproic acid, 2.08; heptanoic acid, 0.54.

Both strains were tested, individually, on suspended culture using RPP and FB medium, in duplicate. Serum bottles with a 200 mL working volume were used, with a $0.020 \pm 0.003 \text{ g}_{\text{PNSB}} \text{ L}^{-1}$ initial biomass concentration. The bottles were incubated at 30 °C, under 24 h illumination (3,000 lux, incandescent lamps). The test lasted 25 d, collecting samples every two days for OD determination. Before sampling, the hydrogen production was checked by connecting each bottle gas-tight to a water displacement cylinder.

The values of biomass concentration were used to calculate the growth rate (μ , d^{-1}) and the biomass productivity (P , $\text{mg L}^{-1} \text{ d}^{-1}$), according to Eq(1) and Eq(2), respectively:

$$\mu (\text{d}^{-1}) = \frac{\ln(X_i) - \ln(X_{i-1})}{(t_i - t_{i-1})} \quad (1)$$

$$P (\text{mg L}^{-1} \text{ d}^{-1}) = \frac{X_i - X_{i-1}}{(t_i - t_{i-1})} \quad (2)$$

Afterward, *Rps. palustris* was tested on an immobilized cell reactor, consisting of a cylindrical photo-bioreactor containing an agar column as a matrix structure for entrapping and immobilizing the PNSB culture. The system was tested in batch both on RPP and on FB medium. Hydrogen production was measured through a gas trap connected gas-tight to the reactor, and the test continued until no hydrogen production was detected (6 d).

Results and Conclusions

In Figure 1, the biomass growth evolution of *Rps. palustris* and *Rsp. rubrum* both in RPP and FB medium is reported. Both PNSB strains reached a higher biomass concentration when fed with the FB medium (1.08 and $1.04 \text{ g}_{\text{PNSB}} \text{ L}^{-1}$, for *Rps. palustris* and *Rsp. rubrum*, respectively) compared with RPP medium (0.54 and $0.13 \text{ g}_{\text{PNSB}} \text{ L}^{-1}$, respectively). Maximum productivity and maximum growth rate (Table 1) also showed improvements in the FB medium. Gas production was detected only for *Rps. palustris* on RPP medium, but the amount produced ($< 30 \text{ NmL}$ during the whole experiment) was too low for the determination of hydrogen composition. *Rsp. rubrum* on the RPP medium showed no gas production, as well as both strains on the FB medium.

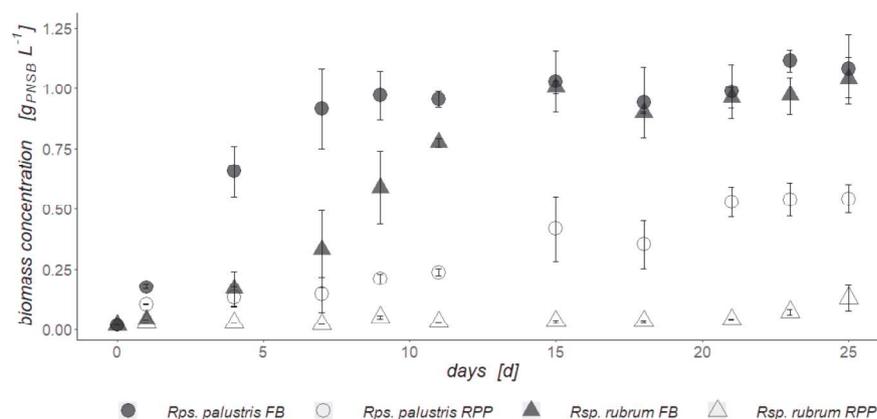


Figure 1 *Rps. palustris* and *Rsp. rubrum* biomass concentration on RPP and FB media on suspended culture.

Table 1 Growth parameters of the two PNSB strains in RPP and FB medium.

	Medium	Biomass Concentration ($\text{g}_{\text{PNSB}} \text{ L}^{-1}$)	P_{max} ($\text{mg}_{\text{PNSB}} \text{ L}^{-1} \text{ d}^{-1}$)	μ_{max} (d^{-1})
<i>Rps. palustris</i>	RPP	0.54 ± 0.06	84.31	4.43
	FB	1.08 ± 0.14	159.53	5.05
<i>Rsp. rubrum</i>	RPP	0.13 ± 0.05	28.09	2.16
	FB	1.04 ± 0.08	128.32	3.76

The test on immobilized culture was conducted only on *Rps. palustris* strain, both on RPP and on FB medium. On immobilized cultures, both media resulted in the photo-evolution of hydrogen, with a 172.4 and 97.9 NmL_{H2} hydrogen production and a 2.58 and 1.23 mmol_{H2} mmol_{VFA}⁻¹ specific hydrogen production on RPP and FB medium, respectively. Despite higher overall and specific production were observed on the standard medium, the FB medium resulted in a higher hydrogen productivity (1.06 mmol_{H2} L⁻¹ h⁻¹) compared to 0.76 mmol_{H2} L⁻¹ h⁻¹ obtained on the RPP medium. The yields obtained using this medium, characterized by mixed VFAs as an organic source, were consistent with those obtained by other authors for immobilized cultures fed with a synthetic organic source: 0.75-1.03 mmol_{H2} L⁻¹ h⁻¹ for *Rhodospseudomonas capsulatus* fed with acetate (Elkahlout et al., 2019).

Acknowledgments

This research was funded by the European Space Agency (ESA) for contract No. 4000137190 “Purple-B: Hydrogen production from immobilized cells in photo-bioreactors”. The Green Propulsion Laboratory of VERITAS S.p.a. is gratefully acknowledged for its support and hospitality.

References

- Bianchi, L., Mannelli, F., Viti, C., Adessi, A., & De Philippis, R. (2010). Hydrogen-producing purple non-sulfur bacteria isolated from the trophic lake Averno (Naples, Italy). *International Journal of Hydrogen Energy*, 35(22), 12216–12223. <https://doi.org/10.1016/j.ijhydene.2010.08.038>
- Elkahlout, K., Sagir, E., Alipour, S., Koku, H., Gunduz, U., Eroglu, I., & Yucel, M. (2019). Long-term stable hydrogen production from acetate using immobilized *Rhodobacter capsulatus* in a panel photobioreactor. *International Journal of Hydrogen Energy*, 44(34), 18801–18810. <https://doi.org/10.1016/j.ijhydene.2018.10.133>
- Sun, Z.-F., Zhao, L., Wu, J.-T., Wang, Z.-H., Wu, K.-K., Chen, C., Xing, D.-F., Liu, D.-M., Yang, S.-S., Wang, A., & Ren, N.-Q. (2023). Exogenous hydrogen supply improves in-situ biogas upgrading of sewage sludge: Performance and mechanisms. *Chemical Engineering Journal*, 477, 147307. <https://doi.org/10.1016/j.cej.2023.147307>