

Biohydrogen Production from Dark Fermentation of olive mill wastewater with a thermal dried mixture of food waste and cheese whey: Effect of HRT and pH

A. Maragkaki¹, P. Liapis¹, I. Sabathianakis¹, E. Kyzakis², N. C. Stratigakis², A. Kaliakatsos³, I.

Gounaki³, D. Venieri³, K. Velonia², T. Manios¹

¹Laboratory of Solid Waste & Wastewater Management, School of Agricultural Science, Hellenic Mediterranean University - Educational and Research Committee, Heraklion, 71401, Crete, Greece

²School of Chemical and Environmental Engineering, Technical University of Crete, 73100 Chania, Greece

³Department of Materials Science and Engineering, University of Crete, Heraklion, 70013 Greece

Hydrogen is recognized for its superior energy yield compared to fossil fuels and other emerging alternative energy carriers. As global efforts intensify to develop sustainable energy solutions, biological hydrogen production has garnered increasing attention for its potential to generate clean energy from renewable substrates. Among the various biological hydrogen production methods, dark fermentation using mixed microbial consortia has emerged as one of the most practical and scalable approaches, offering a promising route to reduce reliance on fossil fuels and mitigate climate change impacts (Jia et al., 2019).

Agro-industrial activities, particularly those associated with cheese production and olive oil processing, constitute a significant segment of the global economy, especially in the Mediterranean region. These industries generate vast quantities of wastewater and various organic byproducts, many of which remain underutilized and contribute to environmental pollution. According to official statistics and the International Olive Council, global olive oil production has tripled over the past six decades, reaching between 3.13 and 3.28 million metric tons annually during the 2017/2018 to 2020/2021 period (Tsigkou et al., 2022).

Furthermore, food waste (FW), predominantly generated by municipalities and commercial sectors, represents a substantial organic waste stream. Several studies have demonstrated its potential as a promising substrate for biohydrogen production (Yasser Farouk et al., 2020).

This study investigated the use of olive mill wastewater (OMW) combined with a thermally dried mixture of food waste and cheese whey (comprising 70% OMW and 30% dried food waste diluted in tap water) as a substrate for dark fermentation. Continuous experiments were conducted to evaluate the influence of hydraulic retention time (HRT; 24 and 12 hours) and feedstock pH (5.5 and 6.0) on biohydrogen and volatile fatty acid (VFA) production. The system demonstrated stable and prolonged reactor performance, yielding high hydrogen content in the gas phase and elevated hydrogen production rates. Hydrogen yields were recorded at 0.7 ± 0.3 L H₂/L-reactor for 24-hour HRT and 1.2 ± 0.9 L H₂/L-reactor for 12-hour HRT, indicating that shorter HRTs (12 hours) were more favorable. Optimal feedstock pH was identified as 6.0. Ethanol and VFAs, particularly acetic, propionic, and butyric acids, were the dominant fermentation products across all conditions.

REFERENCE

Jia, X., Wang, Y., Ren, L., Li, M., Tang, R., Jiang, Y., & Hou, J. (2019). Early warning indicators and microbial community dynamics during unstable stages of continuous hydrogen

production from food wastes by thermophilic dark fermentation. *International Journal of Hydrogen Energy*, 44(57), 30000–30013.
<https://doi.org/10.1016/J.IJHYDENE.2019.08.082>

Tsigkou, K., Sivolapenko, N., & Kornaros, M. (2022). Thermophilic Dark Fermentation of Olive Mill Wastewater in Batch Reactors: Effect of pH and Organic Loading. *Applied Sciences* 2022, Vol. 12, Page 2881, 12(6), 2881. <https://doi.org/10.3390/APP12062881>

Yasser Farouk, R., Li, L., Wang, Y., Li, Y., & Melak, S. (2020). Influence of pretreatment and pH on the enhancement of hydrogen and volatile fatty acids production from food waste in the semi-continuously running reactor. *International Journal of Hydrogen Energy*, 45(6), 3729–3738. <https://doi.org/10.1016/J.IJHYDENE.2019.07.236>

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