Dark fermentation biohydrogen from olive tree pruning: mitigation of inhibitors and perspectives for LCA-TEA in the Mediterranean

J.M. Romero-García[1,3]*, M.C. García Vargas[2], I. Gómez-Cruz[3,4], M. M. Contreras[3,4], E. Castro[3,4]

[1]Department of Chemical Engineering. Universidad de Murcia. Campus de Espinardo s/n, Murcia, 30100, Spain
[2]Department of Industrial Engineering, Technological Institute of Zitácuaro/National Technological Institute of Mexico, Zitácuaro, Michoacán, 61534, Mexico
[3]Institute of Biorefineries Research (I3B); [4]Department of Chemical, Environmental and Materials Engineering. Universidad de Jaén. Campus Las Lagunillas s/n, Jaén, 23071, Spain
Keywords: olive tree pruning; dark fermentation; biohydrogen; inhibitors; life cycle assessment; techno-economic assessment
*Presenting author email: jrgarcia@ujaen.es

Context and novelty

Olive tree pruning (OTP) is an abundant lignocellulosic residue in Mediterranean regions and remains underutilized despite its potential as a renewable feedstock. Dark fermentation converts hydrolysable carbohydrates into biohydrogen without light, enabling a circular waste-to-energy route aligned with the Sustainable Development Goals (SDGs), especially SDG 7 (clean energy), SDG 12 (responsible production), and SDG 13 (climate action). The novelty challenge is not proof of concept but engineering robustness: OTP hydrolysates contain inhibitor mixtures that can sharply depress hydrogen yields, so scale-up must integrate inhibitor control and co-product recovery.

Evidence from OTP and key performance limits

In the most direct OTP-to-H_2 study, Yildirim et al. (2022) optimized oxalic and sulfuric acid pretreatments for olive tree biomass and reported 28–37 g/L total reducing sugars at optimal severity (oxalic: 114 °C, 36 min, 10.5% w/w; sulfuric: 102.5 °C, 78 min, 0.6% w/w). Mesophilic dark fermentation with a *Clostridium sp*. inoculum achieved 0.83–0.91 mol H_2 per mol total reducing sugars (Yildirim et al., 2022), i.e., roughly one-fifth to one-quarter of the 4 mol H_2/mol glucose theoretical ceiling (Thauer Limit). This ceiling is consistent with (i) incomplete conversion of released sugars to the acetate pathway and (ii) inhibition and back-pressure effects that shift metabolism.

Table 1. Minimum engineering package for scaling OTP dark fermentation.

Package element	Baseline indicator	Scale-up risk	Verification metric
Pretreatment + hydrolysis	28–37 g/L total reducing sugars (Yildirim et al., 2022)	High inhibitor load; high heat/chemical demand	Sugars (g/L); furfural, hydroxymethylfurfural, total phenolics (mg/L)
Fermentation	0.83–0.91 mol H_2/mol reducing sugars (Yildirim et al., 2022)	Low selectivity; instability from back-pressure	H_2 yield; acetate-to-butyrate ratio; gas rate vs time

Inhibitors and mitigation levers

Lignocellulose pretreatment can generate furfural and hydroxymethylfurfural and mobilize soluble phenolics. In mixed cultures, Quéméneur et al. (2012) showed that such compounds can reduce hydrogen yields from 1.67 mol H_2/mol xylose (control) to 0.34–1.39 mol H_2/mol xylose, with furan derivatives more inhibitory than tested phenolics. For OTP, mitigation must be verified with measurable markers (furfural, hydroxymethylfurfural or total phenolics, especially abundant in the OTP), and linked to yield and selectivity metrics (hydrogen yield per sugar, acetate-to-butyrate ratio). Practical levers include severity tuning, adsorption-based detoxification (biochar/activated carbon), inoculum adaptation, and process controls that lower hydrogen partial pressure (gas stripping or improved headspace management).

Innovation options: mixed consortia and nanoparticles/adsorbents

Mixed consortia can improve tolerance to variable pruning hydrolysates, provided hydrogen consumers are suppressed via heat-shock, pH selection, or short hydraulic retention times. Nanoparticle-enabled systems have reported yield increases in OTP matrices: ferrite nanoparticles ($NiFe_2O_4$ or $CoFe_2O_4$) dosed at 200 mg/L have been associated with ~0.27–0.29 m^3 H_2 per kg glucose-equivalent sugars in *Clostridium*-based dark fermentation, suggesting enhanced electron transfer and hydrogenase activity (Morán-Alarcón et al., 2026). For OTP, a deployable strategy is to pair recoverable (magnetically separable) ferrites with carbonaceous adsorbents to buffer inhibitors and accelerate reducing power transfer.

Mediterranean LCA–TEA outlook and waste-to-energy economics

A Life Cycle Assessment (LCA) model of a biohydrogen biorefinery reported a gate-to-gate fossil global warming potential of 24.4 kg CO_2-eq/kg H_2 and identified conversion steps as dominant hotspots (Gamero et al., 2024). Techno-economic feasibility for OTP is therefore expected to depend on a cascade configuration, where a first stage of recovery of phenolic compounds with antioxidant capacity turns into high value-added products, recovery of sugars for a dark fermentation acts as an acidogenic stage and the effluent is valorized (anaerobic digestion to methane for onsite heat and power, or recovery of volatile fatty acids). With OTP valued around 0.03 €/kg in some markets, and with practical yields corresponding to only a few kilograms of H_2 per tonne of dry OTP at current performance levels, feedstock logistics and co-product monetization become decisive. Mediterranean scale-up is most defensible in clustered, modular plants (5–50 t/d) near olive-groves to reduce transport, stabilize feedstock supply, and capture avoided open-burning impacts as part of the SDG narrative.

Illustrative cost framing (assumptions stated): at 30 €/t delivered OTP and 4 kg H_2/t of dry OTP (calculated according to the results of Yildirim et al. (2022)), feedstock alone contributes 7.5 €/kg H_2 (30 €/t ÷ 4 kg/t). The heat, chemicals/enzymes, gas upgrading/compression, and wastewater management would all contribute to the overall cost, making a business model based solely on hydrogen unlikely to be viable. Therefore, a viable value-added design is a multiproduct biorefinery that generates value through: (i) the recovery of high-value antioxidant compounds, (ii) on-site energy recovery from the effluent (biogas for utilities), and/or (iii) the recovery of products from volatile fatty acids, while simultaneously considering the avoidance of open burning and rural logistics as measurable co-benefits.

Acknowledgements

I.G.-C. thanks the grant DGP_POST_2024_00733 from the Andalusian Government. The authors thank the project PID2023-149614OB-C21, funded by the “Ministerio de Ciencia, Innovación y Universidades” MICIN/AEI/10.13039/501100011033 and by FEDER, UE, and the project M.1.B.A TA_000701 funded by the “Programa Operativo FEDER Andalucía 2021-2027” (BOJA nº 248, de 29 de diciembre de 2023). The project from the “Instituto de Estudios Giennenses” (research projects in the area of knowledge of Natural Sciences and Technology in accordance with the provisions of the Call Bases BOP No. 42, dated 29 February 2024) is also appreciated. Thanks to the "Chemical and Environmental Engineering" Research Group (IQUIMA) at the Universidad de Jaén.

References

Gamero, E., Ruppert, S., Miehe, R., and Sauer, A. (2024) Process model and life cycle assessment of biorefinery concept using agricultural and industrial residues for biohydrogen production. Energies 17, 4282.

Morán-Alarcón, L.C., Galán-Martín, Á., Contreras, M.d.M., and Castro, E. (2026) Recent advances and future perspectives in the valorization of olive biomass for sustainable biorefinery applications. Renewable and Sustainable Energy Reviews 226, 1364032125011116.

Quéméneur, M., Hamelin, J., Barakat, A., Steyer, J.-P., Carrère, H., and Trably, E. (2012) Inhibition of fermentative hydrogen production by lignocellulose-derived compounds in mixed cultures. International Journal of Hydrogen Energy 37(4), 3150–3159.

Yildirim, Ö., Tunay, D., Özkaya, B., and Demir, A. (2022) Optimization of oxalic and sulphuric acid pretreatment conditions to produce bio-hydrogen from olive tree biomass. International Journal of Hydrogen Energy 47(62), 26316–26325.