

Exploring the possible effect of magnetic nanoparticles addition on biohydrogen production by dark fermentation

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Keywords: Bioaugmentation, two-phase anaerobic digestion, Fe nanoparticles, green hydrogen.

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The search of alternatives for the sustainable production of fuels is, undoubtedly, one of the most important research activities in the world today. Waste treatment facilities are becoming, in this sense, an opportunity for the generation of renewable energy carriers. Until now, methane production was commonplace in biomass waste treatment processes, but the interest that hydrogen is awakening is turning to the generation of this other renewable gas with a great future (Hidalgo *et al.*, 2022).

In recent years, dark fermentation has been established as the most promising method of biological hydrogen production (Show *et al.*, 2019) motivated, on the one hand, by its higher productivity and biohydrogen yield compared to other biological methods, and, on the other hand, by its greater versatility in treating different substrates, from simple sugars to lignocellulosic biomass, food waste and high loaded wastewater streams, or glycerol (Soares *et al.*, 2020). The use of these substrates brings a competitive advantage to this production route with added value in terms of its role in waste valorization, which is key in the transition to a circular economy. Hydrogen production by dark fermentation takes place by anaerobic bacteria that grow in the dark and use carbohydrate-rich substrates. The dark fermentation process to produce hydrogen has not yet been implemented on an industrial scale and is currently under investigation because the process is sometimes unstable because the bacteria involved are very sensitive, especially to the partial pressure of hydrogen. The focus is now on the development of suitable microbial consortia, new bioreactors and new operating strategies for the process to take place in a stable and continuous manner. Based on *Clostridium sp.*, the most efficient known producer of bio-H₂, an artificially engineered microbial consortium has been able to produce 5.6 moles of H₂/mol of glucose in the laboratory (Ergal *et al.*, 2020), with very promising results.

Dark fermentation is a technology based on the recovery of hydrogen that is generated during the first stages of anaerobic digestion, preventing it from being consumed by methanogenic bacteria. It is commonly carried out in two-stage anaerobic digestion processes, consisting of physically separating the microbiological stages of the process: in the first digester, the acidogenic reactor, hydrolysis and acidogenesis of the organic matter are carried out, while in the second digester, the methanogenic reactor, fed with the effluent from the previous one, the other two stages, acetogenesis and methanogenesis, are mainly carried out. Depending on the specific operating conditions applied in the first (acidogenic) system, such as the type of microorganisms inoculated, pH, hydraulic retention times, temperature, hydrogen partial pressure, etc., the production of biohydrogen from the organic waste can be optimised and dark fermentation take place. However, the amount of biohydrogen produced by dark fermentation in practice is low and far from the theoretical values (theoretical stoichiometric maximum that could be obtained from the degradation of 1 mole of glucose is 12 moles of hydrogen). Many factors are involved in this low productivity, such as the emergence of alternative metabolic pathways in the process that consume hydrogen instead of producing it (Sivagurunathan *et al.*, 2016). To increase the quantity and quality of the search product, one strategy is to alter the conventional fermentation conditions, for example with microbial enrichment or bioaugmentation procedures to favour the growth of those species favourable to the target process, in this case biohydrogen production (Villanueva-Galindo and Moreno-Andrade, 2021). Recently, nanotechnology has also been introduced to improve the rate of dark fermentation. Nanoparticles, specifically inorganic ones such as iron, can increase the rate of biohydrogen production (Kumar *et al.*, 2019).

The present work, developed in the frame of the H24NEWAGE project, focuses on exploring operational strategies that include harnessing the potential of nanotechnology in the activation of microorganisms of interest that favour biohydrogen production by dark fermentation, analysing the mechanisms involved, the influence of the substrate used and the operational changes that must be made to increase the overall efficiency of hydrogen production by this fermentative route. To do so, wet waste biomass (animal manure and high load effluents) will be subjected to treatment by anaerobic digestion in two phases, the first being a dark fermentation phase in which novel iron nanoparticles encapsulated in carbon will have been added. Initially, laboratory scale tests will be carried out to optimise the dark fermentation process with direct generation of biohydrogen and inhibition of hydrogenotrophic bacteria. Finally, scale-up tests will be carried out in a 1.2 m³ two-phase digestion pilot plant (Figure 1) in order to optimise the overall process and make it transferable to companies.

Preliminary lab-scale results show an increase in hydrogen production by dark fermentation of up to 15% using the nanoparticles under study.



Figure 1. Pilot plant for biohydrogen production by dark fermentation

Acknowledgements

The authors gratefully acknowledge support of this work by CYTED (Ibero-American Program of Science and Technology for Development) in the frame of the H2TRANSEL network (Ref. 721RT0122) and by the CDTI-Spanish Ministry of Science and Innovation in the frame of the project H24NEWAGE (Ref. CER-20211002).

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