Enhanced microalgal growth and hydrogen generation by addition of iron-based nanoparticles

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The generation of hydrogen as a by-product of waste effluent treatment may not be far off in the future, just as methane is today. Biological processes that can lead to hydrogen production from an organic-rich waste stream are well known. Among them, fermentative processes, photolytic processes (direct and indirect) and photofermentative processes stand out for their novelty, expectations and interest for the scientific community (Hidalgo *et al*., 2022). However, the major handicap to be overcome in all cases is that the efficiency and cost of these processes still need to be improved in order to achieve their general and large-scale implementation. This is why research is needed to make these processes economically viable and their integration into the energy system a reality.

Biohydrogen generation from microalgae has been reported as a highly attractive approach that can achieve carbon neutrality and bioenergy sustainability by producing a benign clean energy carrier (Li *et al.,* 2021). Microalgae fix CO_2 and produce H_2 naturally by biophotolysis. *Chlorella sp*., *Scenedesmus sp*. and *Chlamydomonas sp*. have been reported to be very versatile algae because are able to produce H_2 through direct or indirect photolysis mechanisms (Goswami et al., 2021). Among them, Chlorella is the most promising microalga for biohydrogen production, considering that is the most commonly used microalga for animal food and human nutrition and there are already functioning Chorella production facilities in several countries around the world that could be adapted for this new process (Jiménez-Llanos and Ramírez-Carmona, 2021).

It is generally accepted among the scientific community that the ability of microalgae to produce H_2 by biophotolysis depends on the selected species, environmental factors, enzymes, and metabolic pathways (Nagarajan *et al*., 2021). However, recently, some researches have illustrated that biohydrogen generation can be dramatically enhanced with the presence of metal or metallic oxide nanoparticles, considering they may improve the bioactivity of hydrogenase and ferredoxin oxidoreductase as well as electron transfer. Metal oxide nanoparticles can be accumulated inside of cells as well as interact with intracellular substances through physical, chemical or biological mechanisms (Shanmugam *et al*., 2020). Also, the formation of microalgae-bacteria consortium could be a strategy to enhance H_2 production at large scale. Since the high sensitivity of hydrogenases to O_2 is one of the major limitations to algal photolysis-mediated H_2 production, the removal of O_2 by the bacteria present in the mixed culture may contribute to reduce media toxicity for algae and, thus, improve H_2 production (Ban *et al*., 2018). Furthermore, working with microalgae-bacteria consortia may be the best strategy from an economic point of view when working with wastewater streams, as it avoids the need to work under sterile conditions to avoid contamination. Finally, previous studies have also indicated that the yield and efficiency of biohydrogen could be improved by the integration of wastewater and microalgae with photosynthetic and non-photo synthetic bacteria in fermentation with the participation of electricity (Kim *et al*., 2018).

The present work, developed in the frame of the H24NEWAGE project, proposes a novel hybrid biological H_2 generation approach (Figure 1) which consists of an integrated microalgae-based H_2 production process, with the focus on *Chlorella sp*., using low toxicity wastewater as carbon and nutrients source, which will provide a possible avenue for commercialisation in the near future. Three advanced approaches will be jointly implemented: 1) microalgae-bacteria consortia as a strategy to advance H_2 production; 2) electro-bio-hydrogenation as a new method for algal biohydrogen; and 3) Fe^0 nanoparticles addition for increasing enzyme stability and hydrolytic efficiency. Furthermore, the integration of these three approaches for the improvement of bio-H_2 production and its economic feasibility will be evaluated.

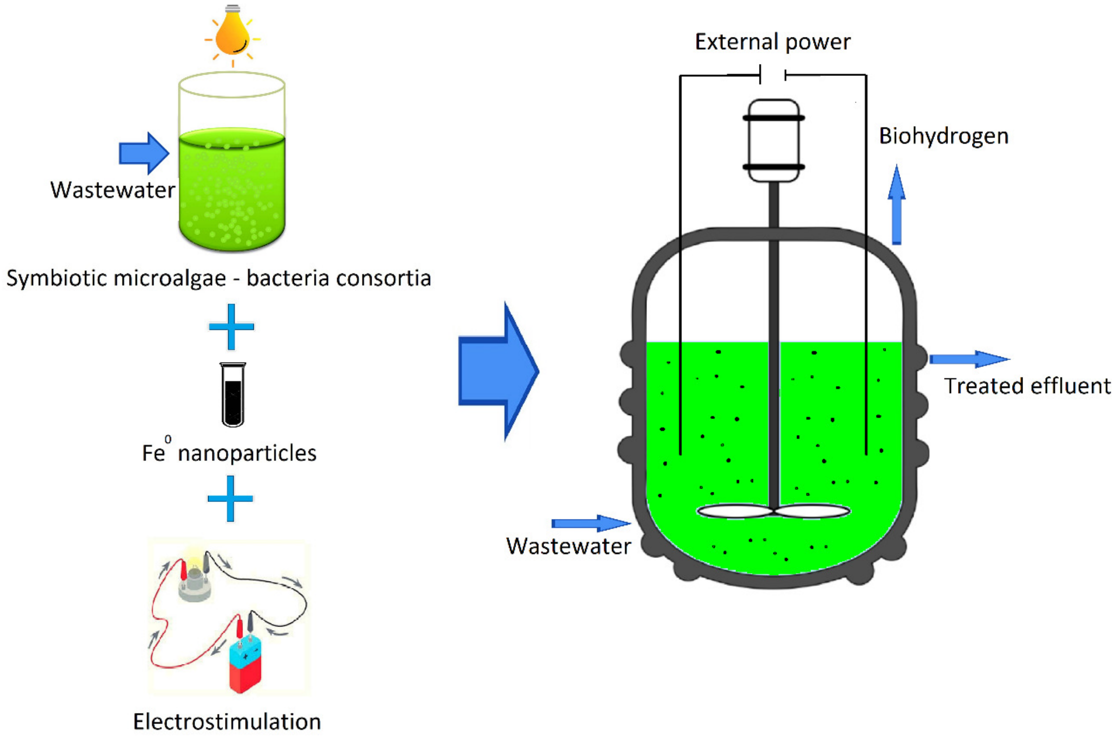

Figure 1. Schematic diagram of the experimental set-up.

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