

## Production of biohydrogen from olive stones pretreated with organosolv

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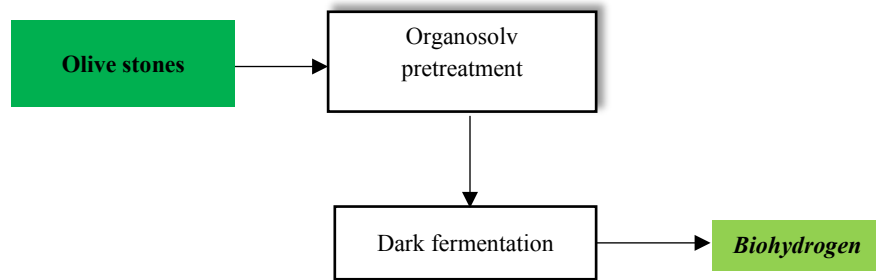
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Olive stones are the main solid residue of olive oil production, accounting from 10-12% of olive fruits by weight. During the last season 2021-2022, the global olive industry produced around 2.8 million tons of olive oil, from approximately ten million tons of olives (IOC, 2021). For this reason, the reuse of the associated wastes, significantly contributes to sustainability and caring for the environment. On the other hand, the demand on hydrogen (H<sub>2</sub>) has increased during the last years because of being a clean source of energy and an adaptable energetic vector. Therefore, the aim of this project was to obtain H<sub>2</sub> from olive stones by biological methods. Two situations were compared in terms of H<sub>2</sub> production: Organosolv pretreated olive stones' fermentation (OPF) facing natural (milling) olive stones' fermentation (NF) in same conditions.

Pretreatments alter the structure of lignocellulose biomass making it more accessible for microorganisms in fermentation (Silva Rabelo et al, 2023). These can be physical and chemical pretreatments such as milling (0.1 cm) and organosolv pretreatment (50% EtOH, 15% S/L, 190 °C, 30 min). Fermentation assays occurred in hermetic flasks with a reaction volume of 50 mL (plus 50 mL headspace) using a macronutrient solution (pH 6.8) in addition to olive stones as substrate (23.4 g/L) and *Clostridium butyricum* as inoculum (5 g-TVS/L). The flasks were incubated in a rotary shaker at 37 °C, 130 rpm for 72 h. H<sub>2</sub> concentration in headspace was determined by gas chromatography.

In this work, the advantages of pretreatments in the efficiency of the conversion of biomass into H<sub>2</sub> were discussed (Figure 1). Not only H<sub>2</sub> concentration, but also acetic and butyric acid production are significant parameters to analyse as they derive from the metabolic pathways involved in anaerobic H<sub>2</sub> production. NF produced 1.68 and 1.13 g/L of acetic and butyric acid, respectively. Whereas OPF produced 0.71 and 3.96 g/L of those products. In terms of H<sub>2</sub> production, the adjustment of Gompertz modified model helps to determine the H<sub>2</sub> potential production, which were 56.89 mL/L for NF and 99.27 mL/L for OPF (Fig. 1). Production rates were 28.0 and 50.8 mL/L.h for NF and OPF, respectively.

As a main conclusion, the application of pretreatments to biomass contributes to increase the H<sub>2</sub> production due to greater accessibility to cell fibre for hydrolytic/fermentative bacteria. Supporting the technological development of sustainable practices will make a positive impact in society and the environment.



**Figure 1.** Fractionation of olive pits to produce biohydrogen.

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