

Biohydrogen production through dark fermentation of organic waste: evaluation of pretreatment methods

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Abstract Pretreatment of organic substrates is a critical step in enhancing the efficiency of biological hydrogen (bioH₂) production via dark fermentation. This research presents and discusses the effects of different pretreatment methods on a selected substrate, spent coffee grounds (SCGs), aiming to evaluate their influence on hydrogen yield and productivity. In addition to a control condition referred to as "non-pretreatment," all tested substrates underwent preliminary size reduction and dilution as standard treatments. The study investigates and compares the application of chemical agents (acid hydrolysis with H₂SO₄) and physical processes (ultrasound). The results show a significant influence of pretreatment on substrate biodegradability and hydrogen production. Surprisingly, the highest cumulative hydrogen volume (~280 mL H₂) was observed for the non-pretreated substrate, suggesting that the combination of size reduction and dilution alone was sufficient to enable efficient fermentation. The ultrasound-treated substrate demonstrated faster kinetics and an earlier peak in hydrogen production, though with a slightly lower total volume (~240 mL H₂). Conversely, acid hydrolysis resulted in poor hydrogen yields, likely due to the formation of inhibitory by-products or limited hydrolysis efficiency under the tested conditions. These findings highlight that not all pretreatments enhance biohydrogen production: in some cases, mild or no pretreatment may outperform more aggressive methods. The results underscore the importance of tailoring pretreatment strategies based on the specific substrate characteristics and process goals, providing valuable insights into sustainable hydrogen production from organic residues.

Keywords: biohydrogen, nutrient availability, pretreatments, residual biomass valorization, substrate biodegradability

1. Introduction

The global demand for clean and sustainable energy has driven increasing interest in biological hydrogen (bioH₂) production from organic waste (Shomope et al., 2025; Zarra et al., 2009). Dark fermentation offers a promising pathway for converting residual biomass into hydrogen under anaerobic conditions, with the added benefit of waste valorization (Mokhtarani et al., 2025). However, the efficiency of this process largely depends on the biodegradability of the substrate, which can be

significantly influenced by pretreatment (Fraiese et al., 2018; Pasquarelli et al., 2024; Shi et al., 2024). In this study, spent coffee grounds (SCGs) were selected as a model feedstock due to their high organic content and global availability. Different pretreatment strategies were investigated - including ultrasound, acid hydrolysis (H₂SO₄), and a "non-pretreatment" control (with only size reduction and dilution applied to all substrates). The aim was to evaluate how these approaches affect hydrogen yield and fermentation kinetics. Understanding the interplay between substrate structure and microbial accessibility is key to optimizing hydrogen production and developing scalable, waste-to-energy systems aligned with circular economy principles.

2. Material and methods

Spent coffee grounds (SCGs) were collected from local caf  s in Fisciano (SA), oven-dried at 80   C for 24 h. Before fermentation, SCGs were diluted in tap water to 5% w/v and homogenized with a blender for particle size reduction. All samples were filtered to remove coarse solids and subjected to a baseline treatment consisting of size reduction and dilution. Three experimental conditions were designed: (i) no further pretreatment ("none"), (ii) chemical hydrolysis with 1% v/v H₂SO₄ ("hydrolysis"), and (iii) sonication at 37 kHz, 80% power for 20 minutes at room temperature ("ultrasound").

Batch dark fermentation tests were performed in 2.1 L anaerobic bottles (working volume 800 mL), inoculated with mesophilic anaerobic sludge (20% v/v) from local a municipal WWTP. The reactors operated under mesophilic conditions (45    0.1   C) for 216 h, with continuous stirring. Biogas was collected in Tedlar   bags and analyzed for H₂ via GC-TCD. Liquid-phase analyses included pH, COD, ammonia, lipids, and carbohydrates. Hydrogen yields and productivities were calculated using the ideal gas law. All tests were conducted in triplicate to ensure reproducibility.

3. Results and discussion

The results (Figure 1) demonstrate that pretreatment significantly influenced the hydrogen production potential of spent coffee grounds (SCGs). Among the three tested scenarios, the ultrasonic pretreatment resulted in the highest cumulative hydrogen production,

reaching a peak of approximately 186 mL H₂ after 160 hours of fermentation. The “none” scenario, which included only particle size reduction and dilution, produced a comparable amount of hydrogen (~175 mL H₂), albeit with a slower initial rate and later peak (around 180 hours). Conversely, the acid hydrolysis pretreatment showed limited efficacy, with hydrogen production remaining below 60 mL H₂ over the entire fermentation period.

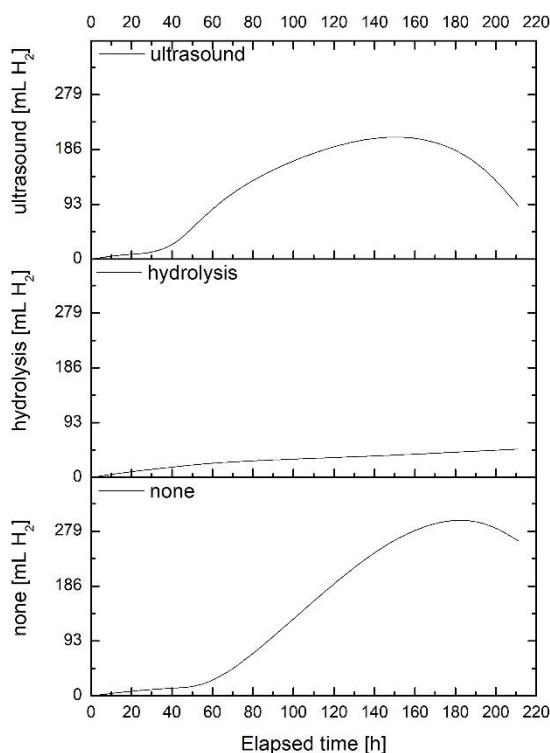


Figure 1 Pretreatment influence on H₂ production via dark fermentation

These findings suggest that physical disruption via ultrasound enhances substrate solubilization and microbial accessibility more effectively than chemical hydrolysis under the tested conditions. The poor performance of the hydrolysis condition may be attributed to the formation of inhibitory by-products or insufficient carbohydrate release, which could have suppressed microbial activity. Notably, the “none” scenario performed better than expected, highlighting that simple mechanical processing and dilution alone can ensure moderate biohydrogen production from SCGs.

Hydrogen production was quantified in moles using the ideal gas law. Cumulative yields were compared across the three pretreatment scenarios. The untreated SCGs achieved the highest cumulative production, reaching 85.12 mmol H₂ at 168 h. Ultrasound pretreatment led to faster kinetics, with a peak of 60.90 mmol H₂ at 120 h, followed by a decline. Hydrolysis showed the lowest and most gradual production, reaching only 13.04 mmol H₂ at 216 h. These results were used to evaluate the effect of pretreatments on hydrogen generation kinetics and fermentability. Overall, the study confirms the critical

role of pretreatment in dark fermentation and underscores the importance of selecting methods that maximize substrate digestibility while minimizing inhibitory effects, with ultrasound emerging as a promising, non-chemical approach for SCG valorization.

4. Conclusions

This study investigated the effect of different pretreatment strategies on hydrogen production from SCGs via dark fermentation. Experimental results demonstrated that pretreatment significantly influences both hydrogen yield and production kinetics. Among the tested conditions, the untreated SCGs (no pretreatment) achieved the highest cumulative hydrogen production, reaching 85.12 mmol H₂ at 168 h, suggesting that mild mechanical treatment alone may preserve substrate integrity and favor microbial activity over time. Ultrasound pretreatment accelerated early hydrogen release (60.90 mmol at 120 h), but was followed by a sharp decline, indicating rapid substrate exhaustion or inhibition. Acid hydrolysis, while increasing solubilization, resulted in the lowest hydrogen yield (13.04 mmol). These findings underscore the importance of tailoring pretreatment approaches to substrate characteristics to optimize biohydrogen production and support waste-to-energy strategies based on resource recovery and circular bioeconomy principles.

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