Biohydrogen Production from Wastewater-Grown Bacteria and Microalgae Consortia

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Highlights:

- Bacteria and microalgae consortia offer a promising path for biofuel production.
- The average biohydrogen production rate peaked at 0.51 mmol H_2 h^{-1}, occurring between 15 hours and 27 hours after the start of the batch process.
- Lag, exponential growth, deceleration, secondary increase, and final stationary phases indicate microbial adaptation.

Keywords: Biofuels; wastewater; bacteria-microalgae consortia.

INTRODUCTION

The indiscriminate use of fossil fuels and the resulting significant emissions of greenhouse gases into the atmosphere define the current global energy system, which is becoming increasingly dysfunctional as it contributes to the intensification of climate change. In this context, the Intergovernmental Panel on Climate Change (IPCC) (Calvin et al., 2023) emphasizes the urgent need for an immediate energy transition, advocating for the exploration of clean and renewable energy sources as alternatives to fossil fuels, particularly those with low or zero greenhouse gas emissions (GHG).

Amid the search for alternative energy sources, several potential routes have emerged, with particular attention on using biomass composed of bacteria and microalgae consortia cultivated in wastewater for biofuel production. The ability to capture carbon dioxide (CO_2) and utilize the nutrients present in wastewater – enabling simultaneous water treatment and biomass growth – are the main reasons why the biofuel pathway is considered promising in this system (Hoang et al., 2023).

Among the various bio-products that can be obtained from the biomass, which is rich in carbohydrates, lipids, and proteins, biohydrogen (bioH_2) is an attractive alternative in energy transition. It offers both sustainable production and combustion processes and a high energy content per unit of mass (Davoodabadi et al., 2021). Additionally, dark fermentation has proven to effectively convert organic substrates from biomass into bioH_2 with high energy efficiency (Iqbal et al., 2022). Therefore, this study uses wastewater-grown bacteria-microalgae consortia biomass as inoculum for bioH_2 production process through dark fermentation, evaluating production rates and accumulated bioH_2 yields.

METHODOLOGY

The biomass, composed of bacteria and microalgae consortia, was produced at the Sanitary and Environmental Engineering Laboratory of the Federal University of Viçosa, Brazil. This facility operates pilot-scale high-rate algal ponds (HRAPs) for wastewater treatment and biomass production. The HRAPs are constructed from fiberglass, equipped with steel paddles driven by a 0.5 HP electric motor, and have a surface area of 3.3 m^2.

Ultimate analysis of the biomass was performed using a Perkin Elmer Series II 2400 Elemental Analyzer, yielding the following composition: Carbon = 47.9%, Hydrogen = 7.5%, Oxygen = 27.8%, Nitrogen = 5.9%, and Sulfur = 0.5%.

A natural fermentation (NF) process was conducted to develop fermentative bacteria, maintaining the biomass at 25°C, with sample collections after ten days. BioH_2 production was performed according to Carrillo-Reyes et al. (2020). Triplicate inoculum quantities (fermentative bacteria from the NF process) were calculated to achieve a substrate-to-inoculum ratio (S/X) of 2.7 g substrate g^{-1} volatile solids (VS), with an initial carbohydrate concentration of 5 g L^{-1} in 500 mL Erlenmeyer flasks. A nutrient solution and glucose were added, and control samples were prepared without glucose. The initial pH was adjusted to 7.5, and the flasks were sealed and purged with nitrogen (N_2) gas.

The gas phase was periodically monitored using a pressure gauge, and the biogas composition was analyzed by gas chromatography. The kinetics of bioH_2 production were evaluated using the modified Gompertz equation (Zwietering et al., 1990), estimating the maximum bioH_2 production, along with the lag, exponential, and stationary phases.

RESULTS AND CONCLUSIONS

During the first 15 hours, no bioH_2 production was observed, suggesting a lag phase, where the microbial community was adapting to the fermentation conditions. The cumulative evolution of bioH_2, shown in Figure 1, was subsequently divided into five phases following this lag phase. In the first phase, there is a noticeable increase from 0.06 mmol to 6.15 mmol between 15 and 27 hours. This period corresponds to the exponential growth of bioH_2 production, reflecting active microbial metabolism and substrate utilization, with the average production rate during this 12-hour period reaching its peak at 0.51 mmol H2 h^{-1}.

In the next phase, between 27 and 45 hours, the cumulative bioH_2 production continues to increase, though at a lower average rate than the previous period (0.075 mmol H2 h^{-1}), rising from 6.15 mmol to 7.50 mmol. From 45 to 55 hours, the third phase of evolution occurs, where bioH_2 production rates become nearly stagnant, with a slight increase from 7.50 mmol to 7.98 mmol. This stationary phase indicates that microbial activity has stabilized, likely due to substrate limitations or the accumulation of inhibitory by-products.

A secondary increase in bioH_2 production is observed in the fourth phase, between 55 and 75 hours, rising from 7.98 mmol to 11.89 mmol, suggesting that fermentative bacteria may have adapted to the conditions. However, the average production rate slows again during the period between 75 and 94 hours, reaching 0.032 mmol H2 h^{-1}, with a subtle increase in accumulated H2 from 11.89 mmol to 12.50 mmol. During this period, which exhibited the lowest average production rate, it can be inferred that the fermentation process entered a final stationary phase.

These results provide a detailed characterization of bioH_2 production at each stage of the dark fermentation process using wastewater-grown bacteria-microalgae consortia biomass. Combining the findings of this study with recent and relevant literature offers the necessary support for simulating process scale-up. Future studies should focus on system modeling, as done by (Castro et al., 2023), for producing liquid biofuels and biofertilizers as by-products of wastewater-grown bacteria-microalgae consortia biomass, using software like Aspen Plus. Such modeling is essential to understand the feasibility of the dark fermentation process under these conditions on an industrial scale, confirming the system as one of the promising solutions for the imminent global energy transition.

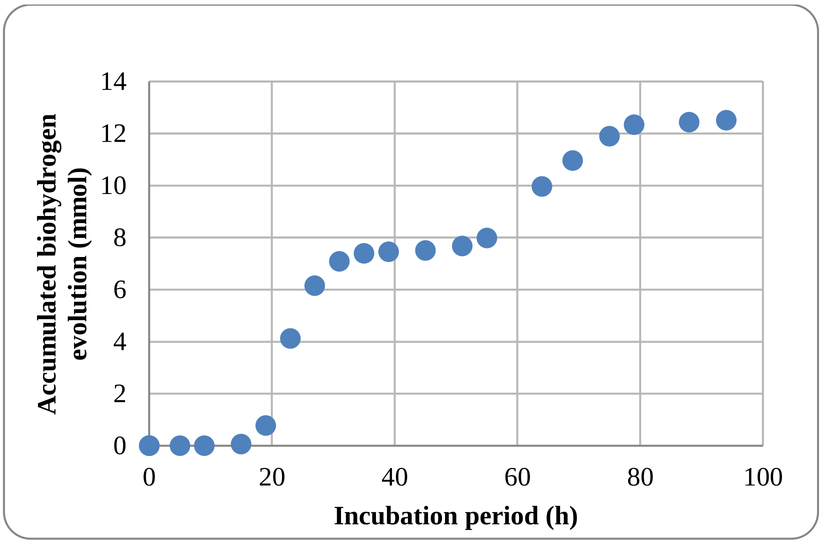

Figure 1. Cumulative biohydrogen evolution (mmol) as a function of the incubation period (hours).

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