ENHANCED PHOTO-FERMENTATIVE BIOHYDROGEN PRODUCTION FROM CO-CULTURE OF PHOTOTROPHIC BACTERIA

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ABSTRACT

The growing demand for alternatives that contribute to the environmental pollutants reduction to achieve energy decarbonization in the current circumstances is readily apparent. Consequently, replacing fossil fuel sources with sustainable alternatives, such as hydrogen, emerges as a viable solution. Nowadays, there is great attention given to the production of biological hydrogen through photo-fermentation due to its high potential. The current study aimed to assess biohydrogen production by photo-fermentative processes using co-culture of non-sulfur purple bacteria (*Rhodospirillum rubrum* and *Rhodobacter capsulatus*). Lactose derived from milk whey permeate served as the substrate, revealing a peak productivity of 5.270 ± 0.164 mmol H_2/L.d after 120 hours.

Keywords: Hydrogen. Photo-fermentation. Co-culture. *Rhodospirillum rubrum*. *Rhodobacter capsulatus*.

1 INTRODUCTION

The increasing demand to reduce reliance on conventional energy sources and a substantial rise in the global need for energy present a formidable challenge to modern society. The planet's equilibrium is tied to reducing pollutant emissions, a direct consequence of the ongoing utilization of fossil fuels, contributing to environmental issues. Therefore, the imperative becomes the exploration of renewable energy sources that release minimal carbon into the environment.[1,2]

Hydrogen is the most compelling candidate for displacing fossil fuels because it is a clean and sustainable energy resource.[3] When hydrogen undergoes combustion or is employed in fuel cells, the sole byproduct is water, effectively eradicating the release of pollutants like carbon dioxide (CO_2).[4,5] An even more captivating option emerges with hydrogen generated through biological processes. This method leverages renewable raw materials, facilitating the repurposing of waste materials and decreasing the volume of byproducts typically generated in industrial processes.[6] Several techniques, such as the photo-fermentation process, can be employed to obtain hydrogen through these biological processes.[7]

Photo-fermentation is a method that employs photosynthetic bacteria, with purple non-sulfur bacteria (PNSB) being a prevalent choice.[8] These bacteria can transform organic substrates into hydrogen and carbon dioxide in anaerobic conditions and under illumination.[9] The PNSB's hydrogen production is linked to the existence of the enzyme nitrogenase in oxygen-deprived conditions, utilizing light energy, a carbon source, and organic acids.[10]

The current study aimed at biological hydrogen production. A co-culture of purple non-sulfur bacteria (*Rhodospirillum rubrum* and *Rhodobacter capsulatus*) was used. Lactose from milk whey permeate, a byproduct acquired through the ultrafiltration process of whey, was used as a carbon source. The experimental procedure for biohydrogen production was conducted on a small scale (50 mL).

2 MATERIAL & METHODS

The bacteria strains used in the photo-fermentation assay were the photosynthetic non-sulfur purple bacteria *Rhodobacter capsulatus* DSM 1710 and *Rhodospirillum rubrum* DSM 467, obtained from the German Collection of Microorganisms and Cell Culture, DSMZ. Microorganism cultivation was carried out in the RCV basal medium. [11]

The experimental medium, labeled as modified RCV, was formulated by dissolving the components of the basal medium and incorporating lactose from milk whey permeate as a substrate, with a concentration of 10 g/L. The malic acid suppression was intentional, as the focus was solely on investigating lactose as the carbon source.

The small-scale experiment was carried out in 50 mL bioreactors. These reactors had an operational volume of 37 mL, consisting of 32 mL of fermentable medium and 5 mL of inoculum with an initial concentration of 0.2 g/L. After inoculation, argon was introduced into the medium to sustain anaerobic conditions. The fermentations occurred within a germination chamber under 30 ± 2°C and an illumination level of 3500 lx with of cool white light-emitting diode (LED).

Periodically, samples of the medium were taken during photo-fermentation and subsequently subjected to a 12-minute centrifugation at 5,000 rpm. This process aimed to assess the composition of the fermentation medium, involving the analysis of sugars and organic acids through high-performance liquid chromatography (HPLC Shimadzu model LC-20A Prominence, column SUPELCOGEL C-610H) using the supernatant, and the sediment was used for evaluation of the cell concentration. The generated biogas was acquired using graduated syringes (10 mL) and then preserved in gasometrical ampoules. Further analysis was

performed using a Gas Chromatograph (Shimadzu GC-2014 Chromatograph), featuring a thermal conductivity detector, an oven, and a Carboxen 1010 capillary column.

3 RESULTS & DISCUSSION

The hydrogen production was evaluated using the co-culture system of bacteria *R. rubrum* and *R. capsulatus* in 50 mL reactors. The assay lasted for 192 hours. According to the data presented in Figure 1, the hydrogen productivity exhibited an upward trend until the 120-hour mark, attaining its peak at 5.270 ± 0.164 mmol H_2/L.d, accompanied by a maximum yield of 3.394 ± 0.137 mol H_2/mol of the consumed substrate.

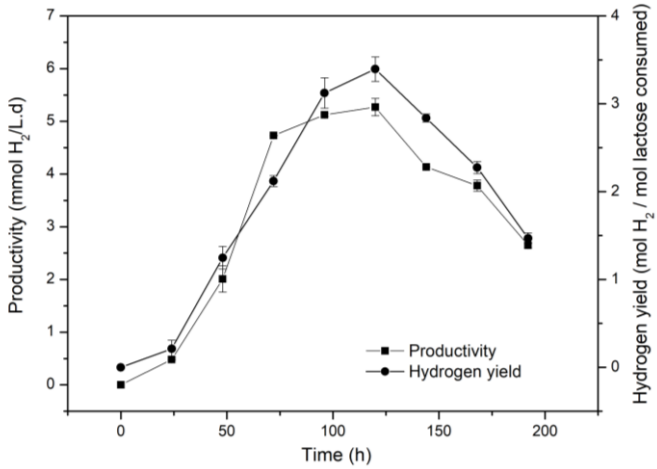

Figure 1 Hydrogen Productivity (■) and Yeld (●) profile obtained during photo-fermentation.

The co-culture (1:1) initial cell concentration was 0.20 g/L. A significant rise in biomass was noted, reaching 0.530 ± 0.003 g/L during the process (Figure 2). The observed growth indicated a 2.65-fold increase in cell concentration compared to the starting point, reaching its highest point at 96 hours, followed by a transition to the stationary phase.

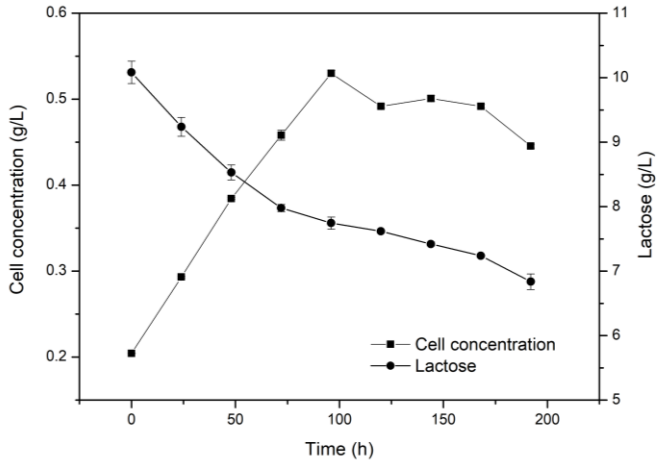

Figure 2 Cell concentration (■) and Lactose concentration (●) profile obtained during photo-fermentation.

The relationship between cell growth and substrate consumption can be established. The lactose concentration stood at 6.863 ± 0.122 g/L, suggesting incomplete utilization. However, as observed in Figure 2, a rise in cell concentration is concurrent with ongoing substrate consumption.

Upon reviewing Figures 1 and 2, it becomes apparent that following the rise in cell concentration and peak hydrogen production, there is a decline in productivity associated with the cessation of cell growth, linked to the entry into the stationary phase.[12]

Figure 3 displays the concentration profiles of organic acids generated during the fermentation process. The primary metabolites identified include lactic, acetic, propionic, and butyric acids, with lactic acid registering the highest concentration. Lactic acid is detected from the early stages, peaking at 2.710 ± 0.004 g/L. Acetic acid is produced throughout fermentation but in smaller amounts, concluding with a final concentration of 0.835 ± 0.005 g/L. The propionic acid concentration reaches 1.930 ± 0.004 g/L at 192 hours, while butyric acid attains its maximum production of 0.250 ± 0.001 g/L at 120 hours.

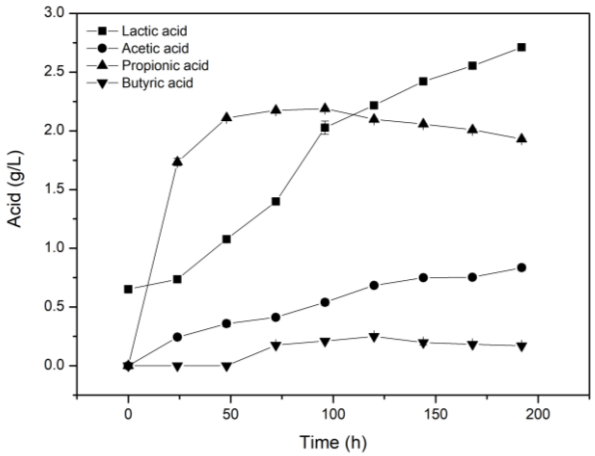

Figure 3 Concentration of acids obtained during photo-fermentation.

It is possible to assess hydrogen production with the generation of organic acids. Fermentation processes yielding acetic and butyric acids can provide high hydrogen yields, while lactic and propionic acids were associated with lower hydrogen yields.[13]

4 CONCLUSION

The findings showed that hydrogen production through photo-fermentation shows potential. The assay using the co-culture of bacteria *R. rubrum* and *R. capsulatus* with lactose as the substrate yielded outcomes. There was an increase in cell concentration and hydrogen productivity (with an attractive peak of 5.270 ± 0.164 mmol H_2/ L.d) until the process's stationary phase. The metabolites were monitored. The lactic acid stood out from the others. And it exhibited an inhibitory effect on H_2 production.

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