

Molecular Hydrogen Production By *Clostridium Pasteurianum* During Utilization of Coffee Waste

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ABSTRACT

The production of hydrogen (H₂) through biological methods, particularly dark fermentation, not only enables renewable energy generation but also facilitates efficient recycling of organic waste. The production of H₂ by the *Clostridium pasteurianum* DSM525 strain was investigated using varying concentrations of untreated coffee waste (20–60 g L⁻¹) as a carbon source. Experiments were conducted both in the presence and absence of glucose to evaluate the efficiency of coffee waste (Spent coffee grounds) alone as a carbon source. *Clostridium pasteurianum* is a strictly anaerobic, gram-positive, spore-forming, mesophilic bacterium that metabolizes various carbohydrates, particularly glucose, during dark fermentation to produce H₂. The results showed that in the absence of glucose, the maximum H₂ yield was ~6 mM at 96 hour of growth when 40 g L⁻¹ coffee waste was applied, maintaining a relatively stable level until the end of fermentation. A similar trend was observed with 60 g L⁻¹ coffee waste. In conditions with 60 g L⁻¹ coffee waste, slower but stable growth was noted, with the maximum H₂ production (~3 mM) observed at 72 hour of growth. Meanwhile glucose addition lead to significantly higher yields: the highest H₂ yield was recorded at 96 hour with 60 g L⁻¹ coffee waste constituting 55mM. For 20 g L⁻¹ and 40 g L⁻¹ coffee waste, the maximum H₂ production ~36 mM was observed at 72 hour. Thus, the presence of glucose significantly enhances the growth of *Clostridium* bacteria across all groups compared to conditions without glucose. These findings indicate that coffee waste without time-consuming treatment can serve as an effective carbon source for bio-H₂ using *Clostridium pasteurianum* bacterial strain and further process optimization may further lead to cost-effective productions.

Keywords: Bio-H₂, *Clostridium pasteurianum*, Spent coffee grounds

References:

1. Wang J.; Yin Y. *Clostridium* species for fermentative hydrogen production: An overview. *Int J Hydrogen Energ.* **2021**, *46*, 34599–625. DOI:10.1016/j.ijhydene.2021.08.052

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