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Fermentation of Molasses by *Clostridium* spp. for Biohydrogen and Biomass Production: A Step Toward Renewable Energy and Circular Bioeconomy

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ABSTRACT

Molasses, a carbohydrate-rich by-product of the sugar industry, it presents an abundant and low-cost substrate for microbial cultivation. This study evaluates the potential of molasses for biohydrogen (H_2) and biomass production using pure cultures of four obligate anaerobic *Clostridium* species: *C. pasteurianum* DSM525, *C. beijerinckii* DSM791, *C. acetobutylicum* DSM792, and *C. intestinale* DSM6191. Batch fermentations were performed at 4%, 8%, and 10% (w/v) molasses concentrations within an initial pH range of 6.0. Throughout a 168-hour incubation period, microbial growth and metabolic performance were assessed by monitoring optical density at 600nm (OD_{600}), pH fluctuations, oxidation-reduction potential (ORP), gas-phase hydrogen production, ethanol accumulation, total carbohydrate (TC) consumption, and volatile solids (VSs) reduction. Notably, all strains exhibited robust fermentative activity, with the efficient biomass and hydrogen yields recorded at 4% and 8% substrate concentrations. Maximal microbial biomass was achieved with OD_{600} values up to 1.6, indicating efficient carbon utilization and cell density increase. Among the strains, *C. beijerinckii* demonstrated the most promising biohydrogen output, producing up to 1093 ± 10 ml L^{-1} H_2, alongside measurable ethanol production and substantial carbohydrate degradation. A marked decline in ORP (as low as -508 ± 10 mV) corresponded with increased hydrogen output, underscoring the strong anaerobic nature of these metabolic pathways. Carbohydrate removal efficiencies varied by species, reflecting diverse metabolic strategies and fermentation profiles across the tested *Clostridium* spp. The simultaneous generation of biohydrogen and biomass signifies a dual valorization pathway for molasses, with implications for both clean energy and microbial-based bio-products. This research highlights raw molasses as a promising low-cost substrate for integrated biohydrogen and biomass production, advancing sustainable waste-to-energy technologies. The application of *Clostridium* spp. in bioconversion processes demonstrates a viable route for agro-industrial residue utilization, reinforcing the circular bioeconomy model and contributing to green energy development.

Keywords: *Clostridium* spp, biohydrogen, molasses fermentation, anaerobic digestion, circular bioeconomy, microbial biomass

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