

Growth and Hydrogen Production of *Escherichia Coli* BW25113 in Mixtures of Sugar Beet Pulp and Sugar Beet Molasses

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ABSTRACT

This study examined the growth of *E. coli* BW25113 wild type in mixtures of sugar beet pulp (SBP) and sugar beet molasses (SBM) waste at various concentrations. The wastes were separately subjected to acid hydrolysis using 0.75% sulfuric acid, with 30 g L⁻¹ SBP and 100 g L⁻¹ SBM. The pH of the solutions was adjusted to 7.5 using potassium dihydrogen phosphate. Experiments were conducted with SBP/SBM ratios of 50/50, 75/25, and 25/75, in undiluted and diluted mixtures (2, 5, and 10 times), with and without 10 mL L⁻¹ glycerol. pH of the medium, oxidation-reduction potential (ORP), and optical density (OD) were measured during batch growth of *E. coli* BW25113 wild type. The studies were conducted until the 168th hour of growth, with a negative ORP values observed starting from the 3rd hour of growth. It is worth mentioning that when hydrolysates were mixed without dilution no ORP changes and hydrogen production was observed, most possibly due to high concentration of inhibiting agents. Based on the obtained data, optimal conditions for growth and activity were provided by SBP/SBM mixtures at 50/50 and 25/75 ratios, diluted 2-fold, without glycerol, where the maximum hydrogen yield was at 48th and 168th hours, respectively, making up 6.25 mmol L⁻¹ and 5.38 mmol L⁻¹. In the presence of glycerol, based on ORP values, the maximum hydrogen yield in the SBP/SBM 50/50 ratio, 2-fold diluted mixture, was 5.4 mmol L⁻¹ at 24 hours. In the SBP/SBM 25/75 ratio, 2-fold diluted mixture, and SBP/SBM 75/25 ratio, 10-fold diluted mixture, the hydrogen yield was 5.77 mmol L⁻¹ and 5.27 mmol L⁻¹ at 166 and 24 hours, respectively. To sum up 2-fold diluted mixtures with 50/50 and 25/75 ratios, both with and without glycerol, were optimal for growth and hydrogen production.

Keywords: sugar beet pulp, sugar beet molasses, *Escherichia coli*, hydrogen

References:

1. Mordini, A.L.; Giaretta, E.; Campidonico, L.; Parazza, P.; Formigoni, A. A Review Regarding the Use of Molasses in Animal Nutrition. *Animals* **2021**, *11*, 115. DOI:10.3390/ani11010115.
2. Mikoyan, G.; Vanyan, L.; Toleugazykyzy, A.; Bekbayeva, R.; Baichiyeva, K.; Bekbayev, K.; Trchounian, K. Fermentation of Sugar Beet Pulp by *E. coli* for Enhanced Biohydrogen and Biomass Production. *Energies* **2025**, *18*, 2648. DOI:10.3390/en18102648.

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