

ABSTRACT

Kurnia Sofi Maharani, 24020222120004. **Potential of Vinasse as a Growth Substrate for *Shewanella* sp. and Nutritional Modeling in M9 Medium.** Under the guidance of Arina Tri Lunggani and Dwi Susilaningsih.

Each liter of bioethanol produced generates approximately 9–14 liters of vinasse wastewater, posing significant environmental risks if untreated. Sugarcane vinasse from PT Madubaru Madukismo, Yogyakarta, is characterized by dark brownish-black color due to melanoidin compounds, a pH of 4–5, a BOD of 3,853.4 mg/L, and a COD of 6,435.88 mg/L. *Shewanella* sp., an exoelectrogenic bacterium, is a promising agent for Microbial Electrolysis Cell (MEC) systems targeting biohydrogen production from vinasse. This study aimed to evaluate vinasse as a growth substrate for *Shewanella* sp., identify suitable pre-treatment methods, determine supplemental nutritional requirements, and model glucose consumption kinetics in modified M9 medium. Methods included sterility testing of raw vinasse, pre-treatment approaches (sequential filtration, activated charcoal adsorption, and alum coagulation), nutrient supplementation, and two-stage cultivation at varying glucose concentrations (0%, 0.25%, 0.5%, and 1%). Quantitative analyses employed the Lowry method and phenol-sulfuric acid assay to determine the C/N ratio. Results revealed that raw vinasse was incompatible as a sole substrate due to extreme C/N imbalance, with total protein of 22.53% and total glucose of only 0.98%. Alum pre-treatment at 2,000 ppm effectively clarified vinasse color but exhibited bactericidal properties by lowering pH to 5. External carbon supplementation and nitrogen source modification failed to support consistent growth of *Shewanella* sp. In M9 medium modeling, a glucose concentration of 0.5% yielded optimal cell density with a final value of 14.02 MF-Units at hour 60. The highest substrate degradation efficiency was achieved at 0.25% glucose (96.79%), while 1% glucose induced osmotic stress inhibiting growth. It is concluded that vinasse requires pre-treatment and nutrient supplementation before use as a growth substrate for *Shewanella* sp., and 0.5% glucose in M9 medium represents the optimal condition for growth and substrate degradation. ANOVA confirmed that glucose concentration significantly affected cell density and substrate degradation rate ($P < 0.05$).

Key words: *Shewanella* sp., vinasse, melanoidin, M9 Medium, C/N Ratio