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IBS 2024 Abstract

Influence Of The Experimental Design On Shake Flask Culture Of *Acidithiobacillus* (*At.*) *ferrooxidans* Using Sulfur As Substrate

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In bioleaching field, shake flask is a common experimental device that has been used in numerous research works to cultivate microorganisms. However, none of these studies has considered if the agitation and aeration conditions applied to these cultures were sufficient to avoid O₂ transfer limitations and to promote efficient solid particles dispersion, two key mechanisms in bioleaching experiments. Our study aimed at assessing the influence of the design of shake flask experiments on the growth of *At. ferrooxidans* using elemental sulfur (S₀) as substrate. Cultures of *At. ferrooxidans* in OKm medium with 1% sulfur were incubated at 30°C for 10 days in duplicates. Different culture volumes (V , 75 to 150 mL), agitation rates (N , 101 to 350 rpm) and orbit diameters (D_0 , 19 to 25 mm) were used to vary the gas-liquid mass transfer coefficient (k_{LA} , from 8 to 41 h⁻¹) and the power dissipation per unit of volume (P_0/V , from 21 to 982 W/m³) from one culture to another. Daily monitoring of the cultures included the measurement of pH, dissolved oxygen concentration and biomass (concentration of bacteria in the liquid phase). Dissolved sulfate concentration was also measured by IEC at the beginning and the end of the culture. *At. ferrooxidans* growth (characterized by μ_{max}) was improved with the increase of k_{LA} and P_0/V while S⁰-biooxidation rate was increased. Moreover, whatever the experimental conditions, dissolved oxygen concentration was always above 85% of saturation, indicating that there was no limitation of the gas-liquid mass transfer in the range of k_{LA} used for the study. Increasing P_0/V for a given value of k_{LA} improved the growth rate while it remained the same for a fixed P_0/V and different k_{LA} . As an example, for a k_{LA} equal to 16 h⁻¹, μ_{max} increased from 0.028 to 0.035 h⁻¹ when P_0/V increased from 34 to 198 W/m³. On the contrary, for P_0/V equal to 200 W/m³, μ_{max} remained close to 0.035 h⁻¹ when k_{LA} was increased from 16 to 23 h⁻¹. It was also observed that μ_{max} reached a plateau when P_0/V was above 600 W/m³. Interestingly, at the plateau, the agitation rate was higher than the theoretical critical rate N_C required to achieve a complete suspension of the sulfur particles in the liquid, which was not the case for the other cultures. These results show that microbial growth of *At. ferrooxidans* on S⁰ was mainly influenced by particles dispersion and

not by gas-liquid mass transfer. In this case, P_0/V and N_c are two key factors that must be considered in the design of the experiments. The study confirms that operating conditions such as N , Do and V , which influence the aeration and homogenization of the culture in shake flask, must be carefully selected to avoid limiting bacterial growth.