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Optimization of *Saccharomyces boulardii* Fermentation Conditions in Sunflower Meal Hydrolysate

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Abstract

In this study, acid and enzymatic hydrolysis pretreatments were applied to sunflower meal in order to degrade its lignocellulosic structure, and the obtained hydrolysate was sterilized and used as a fermentation medium for *Saccharomyces boulardii*. Previous studies showed that 96 h fermentation in sunflower meal hydrolysate achieved comparable performance to commercial Yeast Extract-Peptone-Malt Extract (YPM) medium, despite the absence of external nutrient supplementation. Based on these findings, the present study focused on optimizing fermentation conditions. Optimization experiments were designed using the Central Composite Design (CCD) approach through Design-Expert software, and 30 different fermentation conditions were evaluated. Initial pH (2.5–8.5), temperature (25–47.5°C), aeration rate (80–150 rpm), and inoculum ratio (2.5–12.5%, v/v) were selected as independent variables. OD600 values, viable cell counts, and sugar consumption were analyzed as response variables. The results indicated that the optimum growth conditions were achieved at 25–33°C, pH 5.5–6.0, inoculum ratio of 7–9%, and aeration rate of 110–130 rpm. The highest sugar consumption (25.1 g/L) was obtained at 25°C, pH 4, 10% inoculum ratio, and 80 rpm. Viable cell counts increased from an initial range of 6.60–7.26 log CFU/mL to 6.80–8.48 log CFU/mL at the end of fermentation. The highest cell growth was observed at 32.5°C, pH 5.5, 2.5% inoculum ratio, and 115 rpm, corresponding to an increase of approximately 1.63 log CFU/mL. Model validation experiments were conducted at three selected conditions and the differences between predicted and experimental values remained low (0.12–8.14%), indicating that the model accurately predicted cell growth. Overall, the findings demonstrated that sunflower meal hydrolysate could serve as a sustainable and cost-effective substrate for probiotic fermentation processes.

Keywords: *Sunflower meal hydrolysate, Saccharomyces boulardii, Fermentation optimization, Central Composite Design (CCD)*