

Abstract

Mycoprotein is an alternative protein source that is considered environmentally sustainable due to its ability to utilize agricultural by-products such as soy whey and molasses as fermentation substrates. Conventional batch fermentation is widely used for mycoprotein production but may experience declining productivity due to nutrient depletion and metabolite accumulation over time. Repeated-batch fermentation offers a potential alternative by periodically replacing a portion of the culture medium while retaining active biomass within the system. This study evaluated the effect of different medium harvest ratios on biomass production by *Aspergillus oryzae* cultivated in soy whey–molasses medium. Repeated-batch fermentation was conducted for four consecutive 72-hour cycles using harvest ratios of 45%, 60%, and 75%, followed by replenishment with fresh sterile medium. Titer, productivity, and sugar content were analyzed throughout the fermentation process. The results showed that final-cycle titers ranged from 22.80 to 34.92 g/L, while productivity values ranged from 2.10 to 2.91 g/L/day. ANOVA showed that the effect of harvest ratio on biomass production performance was not significant. Sugar analysis indicated fructose as the dominant residual sugar; however, quantitative sugar consumption and biomass yield could not be reliably determined due to limitations in sugar characterization. These findings demonstrate that repeated-batch fermentation can maintain biomass production comparable to repeated batch fermentation while reducing the need for repeated inoculation.

Keywords: Repeated Batch Fermentation, *Aspergillus oryzae*, Soy whey, Molasses