

Abstract

Soy whey, a nutrient-rich by-product of tofu production, has demonstrated potential as a cultivation medium for *Aspergillus oryzae*, although the optimal carbon and nitrogen supplementation remains undiscovered. This study aimed to determine the optimum carbon and nitrogen supplementation of soy whey-minerals media (SW-MM) to enhance mycoprotein production in terms of biomass yield, titer, and protein content. A One Factor At a Time (OFAT) approach was employed to investigate glucose supplementation (0-30 g/L), $(\text{NH}_4)_2\text{SO}_4$ supplementation (0-8 g/L), and different nitrogen source types, which were $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , NH_4Cl , and $\text{CO}(\text{NH}_2)_2$. Biomass production was evaluated based on yield and titer, while protein content was quantified using the bicinchoninic acid (BCA) assay and spent media sugars were analyzed by high-performance liquid chromatography (HPLC). Supplementation with 10 g/L glucose produced the highest overall production performance in terms of biomass yield and titer. Additional nitrogen supplementation did not significantly improve biomass production, although 2 g/L ammonium sulfate increased the protein content of the mycoprotein compared to other nitrogen concentrations. Different nitrogen source types did not significantly affect biomass production. These findings indicate that supplementation of SW-MM with 10 g/L glucose is optimum for biomass production by *A. oryzae*. This study further highlights the importance of standardizing soy whey composition to improve the reproducibility of complex-media fermentation and investigation of other nitrogen sources.

Keywords: *Aspergillus oryzae*; mycoprotein; soy whey; carbon and nitrogen optimization; fermentation