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Agitation Optimization in Submerged Fermentations of Tuber Mesentericum Truffle for High-quality Fungal Protein Production

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Abstract

The growing global demand for sustainable and high-quality protein sources for human consumption has intensified the interest in fungal biomass as a scalable alternative to conventional animal and plant proteins. Truffles (*Tuber* spp.) are ectomycorrhizal fungi that naturally grow in symbiosis with the roots of certain trees, such as oaks and hazelnuts. Their cultivation is highly challenging due to their complex ecological requirements and long growth cycles, which makes commercial production limited and difficult. Among these, truffle mycelium cultivated via submerged fermentation represents a promising proposition for alternative protein production due to its ability to generate protein-rich biomass and bioactive compounds under a controlled bioprocess condition. This study investigates the submerged fermentation of *Tuber mesentericum* truffle in 1.3L bioreactor systems, focusing on the effects of agitation rate (80, 320 and 560 rpm) under constant low aeration (0.3 VVM) at 25 °C for 44 hours. Biomass was harvested, lyophilized and analyzed for total protein content using the Kjeldahl method, while the profiles of amino acids, the building blocks of proteins that are important in human nutrition, were determined via HPLC after hydrolysis. Results indicate that agitation is a critical factor regulating both biomass and protein production. Increasing agitation from 80 to 560 rpm enhanced biomass concentration (from 4.59 to 6.71 g/L) and protein content (from 30.05 to 35.85% d.w.) were recorded, likely due to improved nutrient dispersion, oxygen distribution and mass transfer. However, the maximum biomass did not coincide with optimal amino acid composition. In low agitation (80 rpm) higher amount of essential amino acids, including lysine (83.99 mg/g protein), leucine (39.84 mg/g), arginine (55.86 mg/g) and glutamic acid (59.43 mg/g) were synthesized, indicating superior protein quality. In contrast, higher agitation appeared to shift fungal metabolism toward stress adaptation and structural maintenance, reducing the retention of essential amino acids. These findings underline the importance of optimizing agitation to balance biomass yield and protein quality. Controlled bioreactor cultivation can thus serve as a scalable strategy for alternative protein production, while further research should address scale-up, downstream processing and techno-economic feasibility for industrial implementation.

Keywords: Truffle mycelium, Bioreactor, Alternative protein, Aeration control, Amino acid profile

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