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# Scalable Mycoprotein Production by Pleurotus ostreatus through Valorization of Brewer's Spent Grain in Solid-Submerged Fermentation

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The growing demand for alternative protein sources has driven increasing interest in microbial proteins, such as mycoprotein, due to their lower environmental footprint and high nutritional value. In this context, the use of agro-food by-products as fermentation substrates in solid-submerged fermentation (SSmF) represents a valuable opportunity to enhance circularity and resource efficiency within food systems. However, challenges related to process optimization, scalability, and safety still remain, limiting large-scale industrial implementation. Therefore, the main objective of this study was to develop a scalable and efficient SSmF process for mycoprotein production using filamentous fungi and agro-food by-products.

Initially, five edible fungal species were screened under submerged cultivation conditions in shake flasks to evaluate biomass production and mycoprotein yields. The selected strain, *Pleurotus ostreatus* s1, was subsequently cultivated in media supplemented with 10% (w/v) of brewer's spent grain (BSG), cocoa bean shell (CBS), peanut peel (PP), and banana peel (BP) to assess growth performance on these by-products. The bioprocess was then scaled to stirred-tank and airlift bioreactors operating under controlled conditions with a working volume of 2.5 L. Biomass production and mycoprotein yield were used as key performance indicators to evaluate process efficiency across the different cultivation systems.

The results demonstrated that *P. ostreatus* s1 was the most suitable fungal species, combining high biomass production (16.02 ± 2.80 g/L) with a consistent mycoprotein yield (8.72 ± 0.35 g/100 g). Among the tested by-products, BSG supported the highest mycoprotein content (14.22 ± 0.95 g/100 g). Notably, both bioreactor configurations outperformed flask fermentations in terms of biomass productivity, demonstrating the positive impact of improved mixing and oxygen transfer conditions. The stirred-tank bioreactor achieved 30.52 ± 10.75 g/L of biomass, with a mycoprotein yield of 10.54 ± 1.55 g/100 g, while the airlift bioreactor reached 22.00 ± 5.12 g/L of biomass, with a mycoprotein yield of 12.23 ± 0.15 g/100 g. Scale-up experiments confirmed that both bioreactor configurations are suitable for the cultivation of *P. ostreatus* under SSmF conditions. These findings indicate that the process is robust, scalable, and adaptable, offering flexibility for future industrial implementation. In terms of safety, mycotoxin analysis revealed that fumonisin B2 (41.4 µg/kg) was the only mycotoxin detected in both the original BSG and the fermented biomass. Notably, its concentration decreased by 25% following fungal fermentation, demonstrating the potential of the process to mitigate contamination risks associated with agro-food by-products.

Overall, the results obtained validate the feasibility of producing mycoprotein from *P. ostreatus* through the valorization of BSG, supporting the transition toward a more sustainable and circular source of protein.

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