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Defining Fermentation Routes for Brewery–Sugarcane Residue Valorization in Aspergillus Oryzae Mycoprotein Production

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Abstract

Rising food demand coincides with increasing food and agro-industrial waste generation, emphasizing the need for circular strategies that convert residual biomass into sustainable protein sources. Brewer's spent grain (BSG), sugarcane bagasse (SB) and brewer's spent yeast (BSY) are abundant by-products with complementary carbon, fibre and nitrogen compositions (Dong et al., 2013, González-Díaz et al., 2024). These streams present strong potential for their integration into mycoprotein production using *Aspergillus oryzae,* an edible fungus with well documented hydrolytic capacity (Upcraft et al., 2020, Ahmad et al., 2022, Rousta et al., 2021). However, direct conversion of BSG and especially SB is limited by lignocellulosic recalcitrance, while BSY, despite its high protein and nitrogen content (San Martin et al., 2020), remains unexplored as direct co-substrate in fungal fermentation systems (Jaeger et al., 2020). Building on previous untreated solid-state fermentation (SSF) trials showing enhanced protein content in BSG/SB/BSY blends, this study investigates alkaline pretreatment combined with SSF and submerged fermentation (SmF) to improve substrate accessibility, fungal growth and biomass recovery. Pretreatment conditions for BSG and SB were screened based on lignin removal, glucan retention, and solid recovery yield. The selected solid fractions were then supplemented with varying BSY ratios and used as substrates for *A. oryzae* cultivation under both SSF and SmF conditions. Fermentation performance was evaluated in terms of fungal colonisation, biomass development, protein enrichment, and substrate utilisation, using untreated systems as reference. Best-performing conditions will further guide benchtop reactor scale-up and food prototype formulation to assess process feasibility. Results show that substrate composition significantly influenced fungal colonisation, with BSY supplementation supporting more homogeneous *A. oryzae* growth than single-substrate systems. Protein enrichment was highest in BSY-rich formulations, confirming its role as an effective nitrogen source. Among the pretreatment conditions evaluated, 0.5% NaOH provided the best balance between lignin removal and solid recovery, enabling the improved accessibility of polysaccharides. Preliminary results further demonstrate that pretreatment enhances fungal growth compared to untreated substrates, with 20% BSY supplementation yielding the most consistent growth in both SSF and SmF after four days of cultivation. Overall, the results so far suggest that mild alkaline pretreatment can broaden the use of lignocellulosic brewery and sugarcane residues in fungal protein ingredient production, while BSY supplementation contributes to both nutrient balance and fungal colonisation. By linking substrate modification with fermentation strategies, this work contributes to the development of scalable and sustainable approaches for producing mycoprotein ingredients relevant to future food systems.

Keywords: Mycoprotein, Agro-industrial by-products, Brewer’s spent yeast, pretreatment, Fungal fermentation

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