Valorization of Bourbon Stillage through Production of Tunable Pure Mycelium Materials

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Kentucky's produces 95% of the world's bourbon and whiskey distilleries have expanded production by approximately 250% over the last decade. However, this increased production has given rise to more concerning issues regarding the management of stillage, the most important byproduct of bourbon production. Therefore, finding potential solutions to upgrade stillage into value-added products is of great importance. Filamentous fungi are saprophytes that can utilize stillage to grow as threadlike mycelium. The structure and composition of the filamentous mycelium have shown promise for the production of pure mycelium materials (PMM), which present an innovative alternative to traditional leather materials with improved environmental impact profiles. PMM production can be enhanced by controlling the direction of growth perpendicular to the substrate to create aerial mycelium. Basidiomycota fungi (true mushrooms) species are presently used in industry to create PMM due to the ease of suppressing sporulation and encouraging a vegetative growth mode. Other fungal phyla such as Ascomycota (many molds) offer benefits of faster growth but suppression of sporulation is often more difficult. The production of PMM is a relatively new area and sufficient quantitative data is lacking on the effective cultivation and processing steps required to optimize the materials for different potential applications.

In this study, a cultivation system capable of producing pure mycelium materials with solid-state fermentation of stillage substrate by Ascomycota fungus *Rhizopus oligosporus* was designed and tested. The influence of important operational parameters including 1) substrate packing density, 2) external support geometry, 3) substrate C/N ratio, and 4) aerial delivery of aerosolized humectants on the aerial mycelium length and morphology was studied. The results showed that stillage was a favorable substrate for production of PMM and that the studied operational parameters allowed for effective control of the mycelium length, density, and moisture content. *R. oligosporus* displayed rapid growth, enhanced by an order of magnitude compared to Basidiomycota fungus *Pleurotus ostreatus* (oyster mushroom). Increasing substrate packing density and the length of external supports was found to encourage longer aerial mycelium fibers while aerial delivery of humectants was found to have limited effects on fiber length but significantly influenced mycelium density and moisture content. It was also found that the low C/N ratio found in un-processed stillage solids is effective at delaying the sporulation of Ascomycota fungi. Together, these results indicate promise for the efficient production of tunable pure mycelium materials from cheap organic substrates.