Integration of filamentous fungi in ethanol dry-mill biorefinery

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Abstract

The industrial production of bioethanol as a replacement to gasoline is well-established worldwide, using starch- or sugar-rich substrates. Additionally, the bioethanol plants produce animal feeds derived from fermentation leftovers. The biorefinery character of bioethanol plants can be enhanced via process diversification. This entails the production of more value-added products, which can be accomplished by including edible filamentous fungi as the second biocatalysts while taking advantage of the available equipment for cost-effective inclusion. The process diversification can be achieved either via valorisation of the process leftovers or via inclusion of other residual substrates.

In dry-mill biorefineries, baker’s yeast is unable to consume residual pentose sugars and other more complex substrates in the process leftovers so called whole stillage and thin stillage. Edible ascomycetes and zygomycetes fungi can be used to accomplish yeast and consume those residual substrates in stillage as well as from external substrates of lignocellulosic origin, e.g. spent sulphite liquor and wheat straw. The conversion of these substrates to ethanol, and biomass rich in protein, lipids, respective essential amino acids and fatty acids as well as chitosan was investigated in this thesis.

Among the filamentous fungi studied, *Neurospora intermedia* was the best ethanol producer from thin stillage. Process developments included primary shake-flasks experiments, followed by pilot scale-up using 26 L, 2.3 m³ and 80 m³ bioreactors. The 26 L bioreactor, as a bubble column led to similar performance as an airlift bioreactor, and also a continuous mode could be successfully used instead of a batch process. By using a dilution rate of 0.1 h⁻¹, around 5 g/L of ethanol and 4 g/L of biomass rich in protein, lipids, amino acids and fatty acids essential to humans were obtained. The inclusion of the process can potentially lead to a spent medium lower in solids and viscosity which may facilitate the energy-intensive evaporation and drying steps as well as the water recycling back to the process. By applying a two-stage cultivation with whole stillage, up to 7.6 g/L of ethanol could be produced using 1 FPU cellulase/g suspended solids and 5.8 g/L of biomass containing 42% (w/w) crude protein. In the first stage (ethanol production), *N. intermedia* was used, while *Aspergillus oryzae* was the biocatalyst in the second stage for further biomass production. Both strains were able to degrade complex substrates both in liquid and solid fraction of whole stillage. The extrinsic substrates included spent sulphite liquor and pretreated wheat straw slurry. When the former was used, up to around 7 g/L of *Rhizopus* sp. could be obtained in a 26 L airlift bioreactor. The biomass was rich in protein and lipids (30–50% and 2–7% on a dry weight basis, respectively). The monomers of the latter were continuously filtered for production of biomass under simultaneous saccharification fermentation and filtration. Biomass yields of up to 0.34 g/g of consumed monomeric sugars and acetic acid were obtained.

The inclusion of the process for valorisation of thin stillage can potentially lead to the production of 11,000 m³ ethanol and 6,300 tonnes of biomass at a typical facility producing 200,000 m³ ethanol/year.

**Keywords:** airlift bioreactors, ascomycetes, biomass, bubble column, ethanol, feed, *Neurospora intermedia*, thin stillage, zygomycetes