Integration of first and second generation bioethanol processes using edible filamentous fungus *Neurospora intermedia*

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Abstract

Establishing a commercial, lignocellulose-based, second-generation ethanol process has received several decades of attention by both researchers and industry. However, a fully economically viable process still remains a long-term goal. The main bottleneck to this achievement is the recalcitrance of lignocellulosic feedstocks, although there are several other factors, such as the huge investment required for second-generation ethanol facilities. An intelligent alternative solution discussed in this thesis is an integrated approach using first-generation ethanol plants for second-generation processes.

Wheat is the major feedstock for first-generation ethanol in Europe; therefore, wheat-based lignocellulose waste, such as wheat straw, bran, and whole stillage fiber (a waste stream from first-generation wheat-based ethanol plants) was the primary focus of the integration model in this thesis. Since the major share of first-generation ethanol plant economics focuses on the animal feed DDGS *(Distillers' dried gains with solubles),* the integration of lignocellulose should be designed in order to maintain DDGS quality. An ethanol-producing edible filamentous fungus, *Neurospora intermedia*, a potential protein source in DDGS, was considered for use as the fermenting microbe. The morphological and physiological aspects of this fungus were studied in the thesis, leading to the first report of fungal pellet development.

An alternative approach of using dilute phosphoric acid to pretreat lignocellulose, as it does not negatively affect fungal growth or DDGS quality, was demonstrated in both the laboratory and on a $1m^3$ pilot scale. Furthermore, the process of hydrolysis of pretreated lignocelluloses and subsequent *N. intermedia* fermentation on lignocellulose hydrolysate was also optimized in the laboratory and scaled up to 1 m^3 using an in-house pilot-scale airlift bioreactor. Fungal fermentation on acid-pretreated and enzyme-hydrolyzed wheat bran, straw and whole stillage fiber resulted in a final ethanol yield of 95%, 94% and 91% of the theoretical maximum based on the glucan content of the substrate, respectively. Integrating the first- and second-generation processes using thin stillage (a waste stream from first-generation wheat-based ethanol plants) enhanced the fungal growth on straw hydrolysate, avoiding the need for supplementing with extra nutrients.

Based on the results obtained from this thesis work, a new model for integrated first- and second-generation ethanol using edible filamentous fungi processes that also adds value to animal feed (DDGS) was developed.

Keywords: First- and second-generation bioethanol; Integration; *Neurospora intermedia*; Edible filamentous fungi; Wheat straw; Wheat bran; Whole stillage fiber