

Concept for A Multi-Feedstock Bio-refinery that uses Engineered Yeast to value winery waste

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Abstract

Without proper management, the wine industry produces a lot of byproducts and residues, which are bad for the environment. Vine shoots, wine lees, and surplus grape must have the potential to be utilized as renewable resources for the production of chemicals and energy. *Saccharomyces cerevisiae* is now recognized as an effective microbial cell factory for biorefineries thanks to efforts in metabolic engineering. The bioeconomy would clearly benefit if these biorefineries could effectively convert multiple feedstocks, but the current biorefineries designed for producing multiple products frequently rely on just one feedstock. Additionally, a biorefinery ought to be able to supplement the production of biofuel with the production of high-value products in order to maximize production economics and minimize the impact on the environment of fossil fuel consumption [1]. Through the biosynthesis of xylitol and ethanol, this study proposes an integrated strategy for the valorization of various wastes from winemaking processes. The xylose-rich hemicellulosic fraction of hydrothermally pretreated vine shoots was turned into xylitol with genetically modified *S. cerevisiae* strains, and the cellulosic fraction was used to make bioethanol. Additionally, sugar-rich grape must was successfully utilized as a low-cost source for yeast propagation. In a Simultaneous Saccharification and Fermentation

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Introduction

A significant portion of global wine production is dedicated to the wine industry, which has cultural and commercial significance. One of the most widely grown fruit crops is grapes, with an estimated 7.3 million hectares of vineyard land in 2021. The top six countries that grow vines are Spain (13%), France (11%), China (11%), Italy (10%), Turkey (6%), and the United States (5%)—each of which accounts for 56% of the total area planted with vines. Over the past few years, global wine production has remained relatively stable. It is anticipated to be 260 million hectoliters (mHL) in 2021, a decrease of approximately 3 mHL (1 percent) from 2020 [3].

Vine shoots, grape pomace (seeds, stalks, and skins), and wine lees are all byproducts of viticulture and winemaking. Wineries also produce an excessive amount of grapes and a significant amount of wastewater in addition to these waste products. Up to 93% of winery leftovers are made up of vine shoots (VS) from pruning, an agronomic practice. Xylitol, cellulose, hemicellulose, and lignin make up VS, which can be thought of as a platform for the synthesis of numerous biobased products like proteins, oligosaccharides, lactic acid, bioactive compounds, biosurfactants, and biofuels like ethanol and biogas.

Therefore, utilizing VS as a source of energy and value-added products rather than burning or dumping it on the ground where it will decompose is more environmentally friendly and cost-effective [4].

Wine dregs (WL) are a typical winery squander that structures at the

Material and Method

For cloning and plasmid maintenance, *Escherichia coli* DH5/NZY5 (Nzytech, Portugal) strains, plasmids, and engineered strain

construction were utilized. For transformant selection, *E. coli* cells were evaluated for supplementation of culture media in biotechnological processes [5].

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were added to the YPD media, which contained 10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose, to select the transformants. After that, the constructed integrative vector (p2909_TEF-1_GRE3) and the guide RNA (gRNA) helper vector (pCFB3050) were transformed into the

