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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 process configuration, the inoculum size and enzyme loading were adjusted to optimize xylitol production. Bioethanol was also produced from the glucan-rich cellulosic using a yeast strain with cellulases on the cell surface. High ethanol concentrations were achieved with the addition of wine lees or grape must, which are essential for the economic viability of distillation [2]. A synergistic alternative for reducing the amount of waste released into the environment while simultaneously converting a variety of winery wastes and by-products into biofuel and an added-value chemical is this integrated multi-feedstock valorization.

Keywords: Winery residues; Bioethanol; Xylitol; Engineered Saccharomyces cerevisiae; Integrated biorefinery

Introduction

A significant portion of global agriculture 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 it 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 lower part of wine holders after maturation. Six percent of the volume of wine is made up of settled yeast cells and residual ethanol. WL have been utilized to produce biogas and medium-chain carboxylates, extract tartaric acid and phenolic compounds, and recover ethanol through distillation. Due to their high protein and nitrogen content, as well as the presence of vitamins and essential amino acids, WL have also been evaluated for supplementation of culture media in biotechnological processes [5].

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 grown at 37 °C in Lysogeny Broth medium supplemented with 100 g/mL of ampicillin (5 g/L yeast extract, 10 g/L tryptone, pH 7.0). This work's plasmids and primers are all listed in the Supplementary Material. The procedure known as USER cloning was used to construct the plasmid. The integrative plasmid (p2909_TEF-1_GRE3) was developed by embedding the GRE3 quality from pGRE3 into pCfB2909, an EasyClone-MarkerFree integrative vector with practically no determination marker. Eurofins Genomics provided the Sanger sequencing necessary to ensure correct cloning. The lithium acetate method was utilized for the yeast transformation. In this study, the chassis strains were the industrial S. cerevisiae PE-2 and CAT-1 strains that were isolated from a first-generation bioethanol plant [6]. The PE-2 strain was changed with a Cas9-communicating plasmid before additional adjustments. Agar (20 g/L) and geneticin G418 (200 g/mL)

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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 strain expressing the Cas9 protein, resulting in the strain PE-2-GRE3-XII5. Colony PCR confirmed gene integration.

The Center of Biofuels and Bioproducts, Agrarian Technological Institute of Castilla and León (ITACyL) generously provided the raw materials Vine shoots (Vitis vinifera L.), grape must (variety White Verdejo), and wine lees (variety Red). Wine lees (WL) and grape must (GM) were kept below 20 °C until used.

The extractives, carbohydrates, and lignin in vine shoots (VS) were analyzed in accordance with the NREL (National Renewable Energy Laboratory) procedures that were previously mentioned. GM and WL were previously analyzed in another location using the described methods [7].Autohydrolysis pretreatment of vine shoots Vine shoots (VS) were subjected to an autohydrolysis-like hydrothermal treatment. The raw material was heated to a maximum temperature (Tmax) of 210–220 °C in a 2 L stainless steel reactor (Parr Instruments Company) by mixing it with water at a liquid-solid ratio (LSR) of 4 and 6 (kg water per kg of oven-dry vine shoots). Baptista et al.'s method for determining the severity prior to treatment (S0) was used. 2020). After pretreatment, strong fluid detachment was performed by filtration and the strong division (pretreated Versus) was recuperated and washed for Strong Yield (SY) assurance. NREL protocols (NREL/TP-510-42618-42622-4218) were used to analyze the chemical composition of the raw materials and pretreated vine shoots. By acid post-hydrolysis of one aliquot of liquor (4% w/w H_2SO_4 at 121 °C for 20 minutes), acetyl groups and oligosaccharides in the liquid fraction were identified. HPLC was used to measure sugars, furans, and acetic acid (the analytical conditions are described in section 2.7). The hemicellulosic hydrolysate was concentrated by a vacuum rotatory evaporator following the circumstances revealed by Domínguez et al. (2021).

Cellulase and hemicellulase activities of Cellic CTec2 were 143 FPU/mL and 626 U/mL, respectively, as determined by enzymatic activity. The enzymatic cocktail was incubated with 10 g/L of xylan from beechwood (Sigma, 90% purity) in 50 mM sodium citrate buffer (pH 5.0) for 10 min at 250 rpm orbital agitation at 30 °C for the purpose of measuring xylanase activity in Cellic CTec2. The DNS method was used to measure the amount of reducing sugar that was released from the substrate (Miller, 1959). The amount of enzyme required to release one millimol of reducing sugar per minute was considered to be one unit of xylanase activity. Following the NREL protocol (NERL/TP-510–42,628), the enzymatic cocktail's cellulase activity was measured using a filter paper assay [8].

Results and Discussion

Biorefinery scheme and chemical composition of winery wastes

To lay out a sugar stage in light of the utilization of side-effects created during winemaking handling and viticulture rehearses, plant shoots (Versus), the overflow of grape must (GM), and wine dregs (WL) were the waste assets considered as sustainable carbon-based feedstocks [9]. An overview of the design that this study proposes for the integral valorization of these winery wastes into bio-based products (xylitol, ethanol, and biocatalysts) employing the diverse sugars (xylose, fructose, and glucose) found in these feedstocks is shown in d. For the production of whole-cell biocatalysts, GM (mainly fructose and glucose; Supplementary Material) was evaluated as a cost-effective carbon source. In order to produce xylitol and ethanol, these biocatalysts are required for the subsequent conversion steps that involve the native aldose reductase and the heterologous cellulases [10]. On the other hand, WL are nitrogen-rich residues (see Supplementary Material) that can be used in place of peptone and yeast extract to reduce overall production costs at a lower cost. As a result, the production processes for ethanol and xylitol could be applied to GM and WL with readily available carbon and nitrogen sources without the need for additional processing. Even though VS (Supplemental Material) has a fascinating composition, extracting fermentable sugars from its recalcitrant lignocellulosic structure necessitates a pretreatment. Glucan and xylan are the primary sources of glucose and xylose in VS, which has a polysaccharide content of 46% (Supplementary Material). The scientific literature is consistent with this chemical composition [11].

Grape must surplus as feedstock for whole-cell biocatalysts production

In order to achieve optimal yeast product performance in subsequent industrial applications and maximize yields and productivity in industrial production of yeast cell biomass, cells must replicate effectively. To ensure good process economics, substrates that are inexpensive and readily available in large quantities are required. These substrates ought to have a lot of carbon compounds that can be completely transformed into cell biomass [12]. Sugar-rich grape must (GM) fits these necessities to turn into a favored substrate for the creation of modern yeast biomass. In any case, states of proliferation have been displayed to influence the cell execution of S. cerevisiae. Yeast cells were grown in aerated cultures with 10, 25, and 50 percent grape must to see if it was possible to use GM as a source of carbon. The provided data clearly demonstrate that the growth kinetics of the three tested conditions was comparable, and that the biomass yield increased with the concentration of grape must. In 42 hours of cultivation, yeast cells consumed approximately 80% of the sugar in the GM-based media Supplementary Material, regardless of the consumption of GM sugar. After 24 hours of cultivation with 50% GM, the maximum biomass concentration of 5.2 g/L was reached, indicating that high substrate concentrations did not hinder microbial growth. The viability of these cells as biocatalysts in bioproduction processes must be verified, despite the effective utilization of GM to produce biomass from yeast [13].

Ethanol creation from pretreated Versus

The cellulolytic S. cerevisiae Feline 1-C strain, showing the Aspergillus aculeatus β-glucosidase 1 (BGL1), Trichoderma reesei endoglucanase II (EGII), Talaromyces emersonii cellobiohydrolase I (CBH1) and Chrysosporium lucknowense cellobiohydrolase II (CBH2) on the cell surface, was assessed for ethanol creation in a SSF measure utilizing pretreated Versus as substrate. Hydrothermal treatment of hardwood biomass (such as eucalyptus, paulownia, or vine shoots) is remarkable for its high solubilization selectivity for hemicelluloses [14]. In order to achieve high ethanol yields, SSF experiments with the recombinant strain CAT-1-C require supplementation with Cellic Ctec2 at a low concentration of 6 FPU/g of solids. However, for high cellulose-to-glucose conversion, the appropriate conditions for XOS production are insufficient. A delignification process is typically used to increase the glucan content and improve the enzymatic convertibility of glucan into glucose in order to achieve high glucose concentrations and, consequently, high ethanol yields from hardwood. To increase the sugar concentration and, as a result, the concentration of the final ethanol, GM-derived sugars can be utilized as an alternative to this costly and risky delignification process [15].

Conclusions

A novel integrated biorefinery concept aimed at maximizing the value of winery waste is presented in this work. It has been demonstrated that yeast propagation using excess grape must is feasible and may reduce overall process costs. The hydrothermal processing of vine shoots resulted in the conversion of hemicellulosic and cellulosic fractions into xylitol and ethanol, respectively, in order to maximize the conversion of all polysaccharides in the lignocellulosic biomass. A robust industrial isolate was genome-engineered for enhanced xylitol production in the hemicellulosic-to-xylitol conversion process. The experimental design demonstrated that enzyme loading and inoculum size had a significant impact on the production process. In addition, it was demonstrated that supplementing with nutrients further overcame acetic acid inhibition. In the cellulosic-to-ethanol change process, the mix of plant shoots with surplus grape must empowered a maintainable creation of ethanol, with a monetarily possible refining process. Additionally, the amount of enzyme that was added to the process was reduced by employing a robust yeast chassis that displayed cellulases on the cell surface. Nevertheless, the xylitol and ethanol titers of 37 and 50 g/L, respectively, achieved in two distinct streams contrast favorably with single-product wine waste valorization processes.

Acknowledgement

None

Conflict of Interest

None

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