Solid-state fermentation of vine shoots to produce hydrolytic enzymes

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Keywords: pretreatment, cellulase, solid-state fermentation, fungi

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Introduction

The winemaking industry is widespread throughout the world, producing 166 million of hectolitres of wine in 2021. However, this industry also generates large amount of by-products (Castro et al., 2023). Among them, vine shoots (VS) are generated yearly and have no practical application (Cardoza et al., 2024). So, they are usually burned on the fields, which implies the loss of biomass rich in carbohydrates that can be considered as a promising alternative source. VS is composed mainly of 33.9 % cellulose, 18.5 % hemicellulose and 22.1 % acid-insoluble lignin (Castro et al., 2023).

Due to the composition of VS, this biomass can be valorised through enzymatic hydrolysis and fermentation processes to produce value-added products. On the other hand, a pretreatment step before the enzymatic hydrolysis is usually required when the lignin content of the solid is high. Thus, pretreatments such as steam explosion or acid has been studied on VS (Cardoza et al., 2024). Also, some studies have shown an increase of enzyme activity produced by solid-state fermentation when the solid is pretreated with steam explosion (Souza Filho & dos Santos, 2023)

The fungal solid-state fermentation (SSF) is the most used technic to produce hydrolytic enzymes such as cellulases and hemicellulases. These enzymes are essential to produce fermentable sugars from lignocellulosic biomass. Thus, the purpose of this work is to evaluate the production of enzymes through solid-state fermentation of VS with *Aspergillus niger* and *Trichoderma reesei*. Additionally, the effect of steam explosion pretreatment on the production of enzymes will also studied. Afterwards, the fermentation conditions will be optimized to produce the maximum enzyme activity based on statistical experimental design considering the moisture content, the inoculum concentration and the fermentation time as the main variable to optimize.

Material and methods

Vine shoots (VS) were collected after pruning of the vineyards. The VS was milled using a blade mill Retsch SM 100 (Haan, Germany), obtaining a particle size of 1 mm. Before the SSF, the solid was autoclaved at 121ºC and 20 min.

VS were pretreated by steam explosion in a custom-built pilot unit equipped with a 4 L capacity vessel. The reactor was filled with 400 g of dry biomass, soaked for 12 h in 2 L of diluted sulfuric acid, and heated with saturated steam to reach the working temperature. The pretreatment time was fixed at 5 min, and once the time had elapsed, the reactor was rapidly depressurised to atmospheric pressure. After pretreatment, the resulting slurry was vacuum filtered to separate the two phases. The pretreated solids were washed for acid removal until neutral pH. dried at 40 ◦C, and used as raw material for the subsequent solid-state fermentation step.

The SSF was performed with the two fungi: *Aspergillus niger* and *Trichoderma reesei*. Each fungus was grown in a petri dish with potato dextrose agar and incubated at 28ºC for 5 days. After that, spores were collected with NaCl 0.1 M, and the concentration of spores were counted in a Neubauer chamber. The fermentation was carried out in a petri dish with 8 grams of solid with or without pretreatment. A nutrient solution or distilled water was used to adjust the moisture content of the fermentation. Enzyme production was optimized by studying the moisture content between 60 - 80 % and the fermentation time between 0 - 76 h.

After the fermentation, the medium was extracted with NaCl 0.1 M by adding 10 mL per gram of dry solid. The mixture was mixed at 150 rpm for 30 min at room temperature. After that, the extract was centrifuged at 5500 rpm for 15 min, and filtered through 45 µm. The enzyme extract was frozen at -25 ºC until the analysis of enzyme activity was performed.

Results and discussion

Preliminary results obtained from the solid-state fermentation with *Aspergillus niger* showed higher growth when the fermentation was performed with the addition of a nutrient solution when it was compared with the fermentation performed with water. However, the growth of *A. niger* was lower than in other feedstocks such as olive tree pruning biomass. So, VS were pretreated with steam explosion with the aim of break the structure of the biomass and enable the microorganism growth.

After optimizing the moisture content, the fermentation time and the inoculum concentration, it is expected to obtain a crude enzyme cocktail with a high content of cellulases and hemicellulases which can be used to hydrolyse VS. In this way, it can be obtained a broth rich in sugars that could be fermented to different by-products such as ethanol or lactic acid.

Acknowledgment

Authors thanks to Ministerio de Ciencia e Innovación and Agencia Estatal de Investigación for the financial support through the Juan de la Cierva fellowship financed by MCIN/AEI/10.13039/501100011033 and the European Union "NextGenerationEU"/PRTR", being JDC2022-049264-I the reference of the grant as specified in the concession resolution. This research was funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe" Grant PID2020-112594RB-C31, PID2020-112594RB-C33.

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