

Evaluation of olive tree pruning biomass as feedstock to produce hydrolytic enzymes and antioxidant compounds through solid-state fermentation

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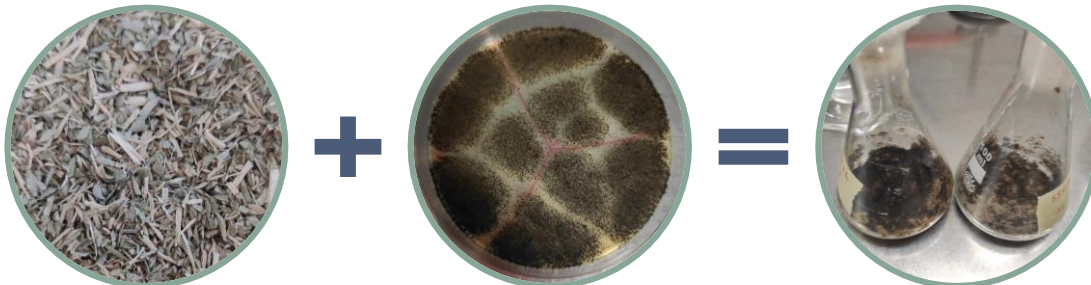
INTRODUCTION

The **olive oil industry** produces high number of by-products such as **olive tree pruning** (OTP). The OTP is obtained after the pruning of the olive tree, which is performed every two years after the fruit harvesting. This biomass is usually burned, which implies the loss of biomass that can be reused to obtain bioproducts since its composition is mainly cellulose, hemicellulose and lignin.

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 and optimize the production of enzymes through SSF of OTP with *Aspergillus niger*.

MATERIAL AND METHODS

Enzymes were produced by solid-state fermentation.



Olive tree pruning

Aspergillus niger

SSF

Cellulase activity was optimized with a **Box-Behnken design**:

- Moisture content: 60-80%
- Fermentation time: 24-72 hours
- Spores concentration: 10^6 - 10^8 spores/g

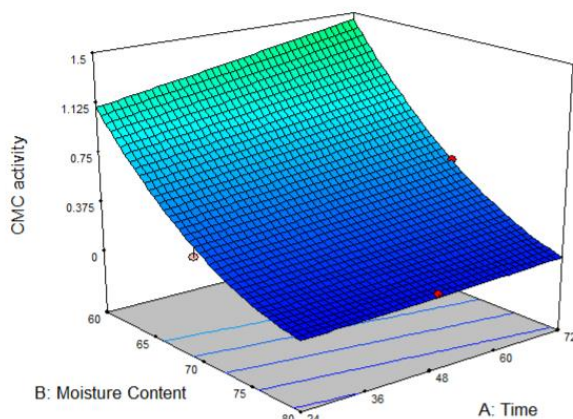
RESULTS

The **SSF with *Aspergillus niger*** showed **higher growth** when the fermentation was performed **with the addition of a nutrient solution** instead of distilled water. Thus, **cellulase activity** produced after 5 days of fermentation, without the optimization of the process, was **20 times higher** when the fermentation was performed with the nutrient solution.

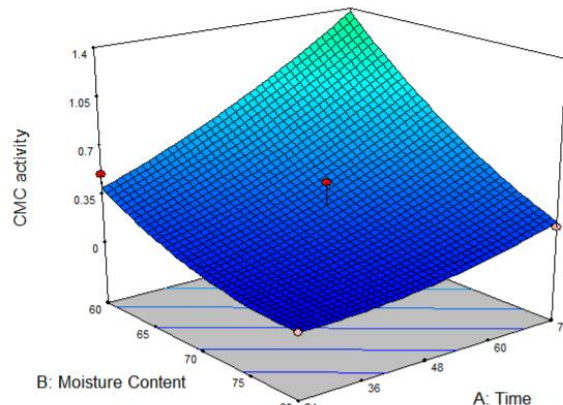
To increase the cellulase production, the moisture content, the fermentation time and the inoculum concentration were optimized using a **box-Behnken design**.

- **Fermentation time** and **moisture content** showed **higher impact** on the production of CMC than the spores concentration.
- Maximum activity was obtained at **60 % of moisture content** and **72 h of fermentation**.

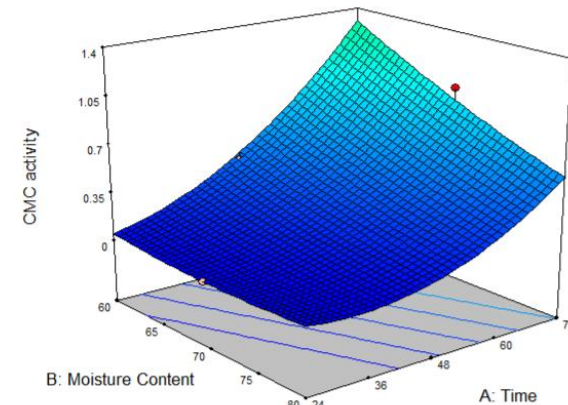
Spores = $1.0 \cdot 10^6$ spores/g



Spores = $5.05 \cdot 10^7$ spores/g



Spores = $1.0 \cdot 10^8$ spores/g



CONCLUSION

- **Olive tree pruning biomass** is a good option to produce cellulase.
- The highest CMC production was obtained with $1.0 \cdot 10^6$ spores/g at 60 % and 72 h of fermentation

WORK IN PROGRESS

- Determination of **antioxidant activity** of the extracts
- **Central composite design** considering the time and the moisture content.
- **Enzymatic hydrolysis** of OTP with the enzymes produced by SSF

References

- Dahdouh, A., Khay, I., Le Brech, Y., El Maakoul, A., & Bakhouya, M. (2023). Olive oil industry: a review of waste stream composition, environmental impacts, and energy valorization paths. *Environmental Science and Pollution Research* 30:16, 30(16), 45473–45497. <https://doi.org/10.1007/S11356-023-25867-Z>
- Filipe, D., Fernandes, H., Castro, C., Peres, H., Oliva-Teles, A., Belo, I., & Salgado, J. M. (2020). Improved lignocellulolytic enzyme production and antioxidant extraction using solid-state fermentation of olive pomace mixed with winery waste. *Biofuels, Bioproducts and Biorefining*, 14(1), 78–91. <https://doi.org/10.1002/BBB.2073>

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