

## Lipase production from olive mill wastewaters by *Aspergillus ibericus*

Felisbela Oliveira, Luís Abrunhosa, Danielle Dantas, Cristiana Gonçalves, Armando Venâncio and Isabel Belo\*

IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal  
\*ibelo@deb.uminho.pt

Mediterranean countries are known to have favourable conditions for olive oil production. Spain, Italy and Greece are the most significant olive oil producers with 47%, 16% and 11% of the 2009/2010 world's production, respectively. Portugal was responsible for 2% (58700 tonnes) of the world's production in 2009/2010, and 67500 tonnes are expected for 2010/2011 (International Olive Oil Council statistical series, November 2010). Olive oil is extracted from olives by physical methods. Olive crushing, malaxation of the resulting paste and oil phase separation are the most important stages of the extraction process. Currently, this process is mainly carried out by the continuous extraction processes, which can be operated by two- and three-phase extraction technologies. From the two-phase system, results olive oil and olive cake (residual solid or pomace). The three-phase extraction technology demands the addition of hot water to the decanter, thus three phases, olive oil, olive cake and olive mill wastewater (OMW), are produced. In fact, huge amounts of this last sub-product are produced; typically, reaching 1.3 m<sup>3</sup> of OMW per ton of olives processed [1]. The OMW content in simple and complex sugars, residual oil (lipids), proteins, mineral elements and phenols, turns this effluent in a renewable resource, as it can be extracted and purified or used for fermentative production process for enzymes production [2]. In addition, the residual oil of OMW turns this waste in a potential growth medium to lipolytic microorganisms [3].

The aim of this study was to assess the ability of *Aspergillus ibericus* MUM 03.49 to grow on OMW based medium, and to produce lipase from it degrades the effluent.

OMW samples (Table 1) were collected from three-phase olive oil mills from the northern region of Portugal, and stored in the same day of collection at -20 °C.

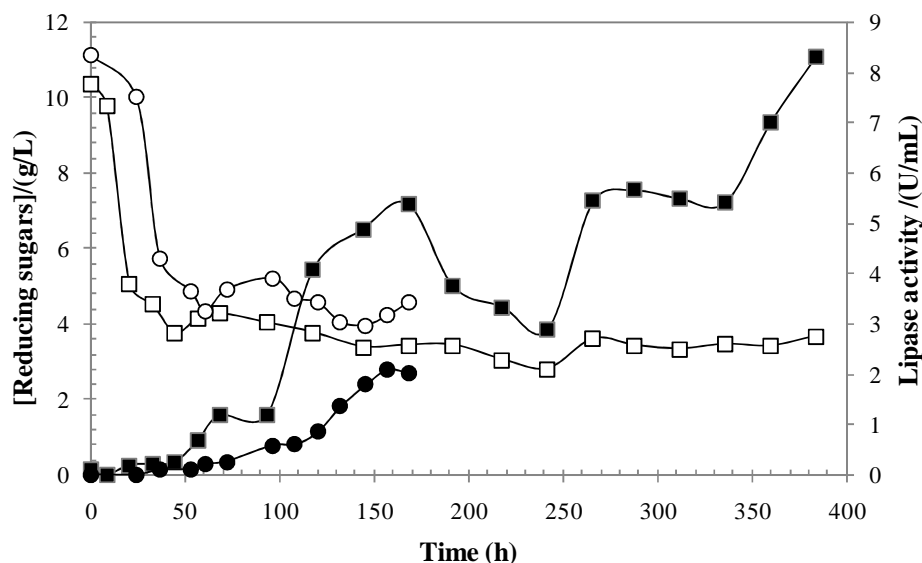
Table 1. Characterization of the OMW's used. Average values  $\pm$  confidence interval (95%).

pH	COD (g/L)	TN (mg/L)	TOC (g/L)	TS (g/L)	TVS (g/L)	TSS (g/L)	Reducing sugars (g/L)	Phenols (g/L)	Lipids (g/L)
4.9	97 $\pm$ 2	3.4 $\pm$ 0.1	15.3 $\pm$ 3.4	58 $\pm$ 1	46 $\pm$ 3	31.0 $\pm$ 0.4	16.5 $\pm$ 5.1	2.7 $\pm$ 0.2	3.5 $\pm$ 0.6

Firstly, agitated batch fermentations in 500 mL Erlenmeyer's flasks were carried out at 27 °C for seven days using culture media prepared with different concentration (10, 50 and 100 %) of centrifuged OMW. Daily changes on the concentration of reducing sugars, protein, phenolic and aromatic compounds, COD, colour and pH were determined. Extracellular lipase was assessed using p-NPB as substrate using an improved method adapted to OMW samples [4]. When 10% of OMW was used, *A. ibericus* was effective in the degradation of the effluent and reductions on OMW colour (55%), aromatic compounds (39%), phenolic compounds (37%) and COD (39%) were obtained. In those same conditions, the maximum lipase activity obtained was 291 U/L. Moreover, *A. ibericus* was able to growth on undiluted OMW and to produce reductions on OMW colour and COD of 97% and 45%, respectively. Additionally, a 10-fold increase of lipase production was obtained attaining the maximum value of 2927 U/L.

Lipase production by *A. ibericus* using OMW was subsequently tested in a 2-L fermenter and different strategies of medium inoculation and fungal biomass development were evaluated. Two batch experiments were conducted with 1.5 L of OMW. One was inoculated with 5 mL of a spore suspension of *A. ibericus* (10<sup>6</sup> spores/mL); the other, with active mycelium obtained by growing the fungus in 250 mL of YPD medium. Fermentations were conducted at 27 °C with pH control at 5.5, low agitation and 1 L/min of aeration rate. In the second experiment, after 170 h, further 1.25 L of OMW was added to the fermenter in order to extend the fermentation time. Results of sugars consumption and lipase

production are depicted in Figure 1.



**Figure 1.** Time course of reducing sugars consumption (open symbols) and extracellular lipase activity (closed symbols) obtained in batch experiments conducted in the 2-L fermenter. (●) batch started with the addition of the spore suspension; (■) batch started with preculture of the fungus in YPD. In this last experiment a second batch was repeated adding a second 1.25 L of OMW at the end of the first batch (170 h).

The production of *A. ibericus* lipase using OMW was successfully scaled-up from Erlenmeyer flasks to a 2-L bioreactor since similar yields of lipase were obtained in flasks trials and in bioreactor experiments when the conditions were identical. The values of lipase activity obtained are similar to the ones obtained by Gonçalves et al. [5] using *C. cylindracea* CBS7869.

The lipase production at bioreactor scale was improved by using a different inoculation procedure. When active mycelium of the fungus was used, a 2.7-fold increase in the lipase activity was obtained. Moreover, it was possible to further improve the lipase production by adding fresh OMW to the fermentation, leading to a final lipase activity in the medium of 8300 U/L.

Both batch cultures resulted in OMW degradation but no significant differences between both strategies were found that led to a COD reduction of around 50%, and reducing sugars degradation of around 70%, slightly higher for the repeated batch procedure.

This work leads to new perspectives on *Aspergillus ibericus* applications for bioprocess development and agro-wastes pre-treatment and valorisation.

#### References:

- [1] Vitolo, S., Petarca, L., Bresci, B., Treatment of olive oil industry wastes. *Bioresour. Technol.* 67 (1999) 129–137
- [2] Ertugrul, S., Doenmez, G., Takac, S., Isolation of lipase producing *Bacillus* sp. from olive mill wastewater and improving its enzyme activity. *J. Hazard. Mater.* 149 (2007) 720–724
- [3] Brozzoli, V., Crognale, S., Sampedro, I., Federici, F., D'Annibale, A., Petruccioli, M., Assessment of olive-mill wastewater as a growth medium for lipase production by *Candida cylindracea* in bench-top reactor. *Bioresour. Technol.* 100 (2009) 3395–3402
- [4] Gomes N., Gonçalves C., García-Román M., Teixeira J. A., Belo I., Optimization of a colorimetric assay for yeast lipase activity in complex systems, *Anal. Methods.* (2011) DOI:10.1039/C0AY00680G
- [5] Gonçalves C., Lopes M., Ferreira J.P., Belo I., Biological treatment of olive mill wastewater by non-conventional yeasts, *Bioresour. Technol.* 100 (2009) 3759–3763

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