Optimization of Initial Moisture and Temperature in Solid State Fermentation to Produce Cellulases by *Aspergillus Uvarum* on Olive Mill and Winery Wastes

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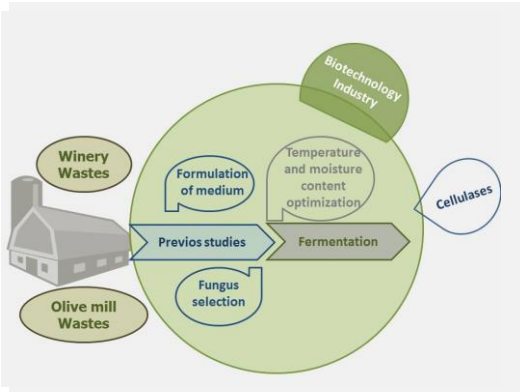

Wineries and olive oil industries are dominant agro-industrial activities in southern European regions. The use of byproducts of these industries could reduce the costs of enzymes production. Olive pomace, exhausted grape marc, vineshoot trimmings are lignocellulosic materials, thus they have potential to be used as substrate for cellulase production by solid-state fermentation. Since, moisture and temperature are important parameters on SSF, a full factorial design was planned to optimize these parameters. Optimum conditions were 29 ºC and 75% of moisture, and 33.49 U/g of cellulose activity were achieved.

Introduction

Solid state fermentation (SSF) is a convenient technology for the production of bioproducts. SSF is performed on solid substrates in absence (or near absence) of free water [1]. However, substrates need enough moisture to allow the growth and metabolism of microorganisms. Filamentous fungi are the most suitable microorganisms to grow in these conditions. In SSF, moisture is one of the most important operational parameters affecting process efficiency. The optimal moisture content depends both on the solid substrate and the microorganism used [2]. Other important parameter is temperature. Fungal growths in solid substrates are influenced by temperature and heat transfers processes [3]. Fungal can grow in a wide range of temperatures; however optimum temperature for enzymes production can be different [3].

In present study, these parameters were optimized to improve cellulase production by *Aspergillus uvarum* on olive mill and winery waste. Media composition was previously optimized; mixture of olive pomace (OP), exhausted grape mark (EGM), vineshoot trimmings (VT) and vinasses supplemented with urea were used as solid substrate to produce cellulases.

Materials and Methods

Wastes used were collected from local wineries and olive mills. OP and vinasses were used without pre-treatment. VT and EGM were ground (particle size <1 mm).

Microorganism (*A. uvarum*) was obtained from MUM culture collection (University of Minho, Braga, Portugal). Strain was preserved at 4 ºC on malt extract agar slants.

Fermentations were carried out in Erlenmeyer flasks of 500 mL with 15 g of dry substrate. Composition of media was: EGM (7.5 g), VT (5g), OP (2.5 g). Moisture of each experiment was adjusted with vinasses and water. Media were sterilized at 121 ºC, for 15 min and then each flask was inoculated with 2 mL of spore suspension (10^7 spores/mL) and incubated at different temperatures (25, 30, 35 ºC) for 7 days.

The extraction of enzymes was performed with a solution composed of 1% NaCl and 0.5% Triton X-100 in a solid/liquid ratio of 1:5 for 2 h with agitation. Following, extracts were centrifuged and filtered through filter paper.

Cellulase activity was determined with the enzymatic kit Azo-CM-Cellulose S-ACMC 04/07 (Megazyme International, Ireland). One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol of glucose reducing sugar equivalents from CM-Cellulose in 1 min at 40 °C and pH 4.5. Activity was expressed by units per g of dry solid substrate.

To optimize temperature and moisture content full factorial (3^2) design was planned. The experimental design was performed in 11 experiments. Table 1 shows conditions of each experiment.

Table 1. Full factorial design matrix.

Run	X_1	X_2	X_1 (ºC)	X_2 (%)
	Coded value		*Uncoded value*	
1	-1	-1	25	50
2	-1	0	25	62.5
3	-1	1	25	75
4	0	-1	30	50
5	0	0	30	62.5
6	0	1	30	75
7	1	-1	35	50
8	1	0	35	62.5
9	1	1	35	75
10	0	0	30	62.5
11	0	0	30	62.5

X_1: Temperarure; X_2: moisture content

Results and conclusion

The results demonstrated that *A. uvarum* was able to produce cellulases under all the experimental conditions. Both initial moisture and temperature influenced significantly the production of cellulases. The second order model describing the cellulase production (y) is described by equation:

$$y = 28.38 - 1.83\ x_1 - 6.22\ x_1^2 + 3.84\ x_2 + 0.96\ x_2^2 - 0.95\ x_1x_2$$

where x_1 and x_2 are the coded values of independent variables (temperature and initial moisture, respectively). All terms of equation were statistically significant at 99%. The coefficient of determination of the model demonstrated a good correlation between independent variables (R^2 = 0.9939), suggesting that the fitted model could explain 99% of total variation. Optimal conditions for maximum cellulase production (33.49 U/g) were determined using commercial software. The results showed that optimum temperature and initial moisture were 29 ºC and 75% moisture, respectively.

Figure 1 shows response surface of cellulase activity. As can be seen, moisture content had higher influence on cellulase production. Increasing moisture content from 50% to 75% and temperature from 25 ºC to 30 ºC resulted in an increase of cellulase activity from 19.89 to 32.53 U/g (38.9%).

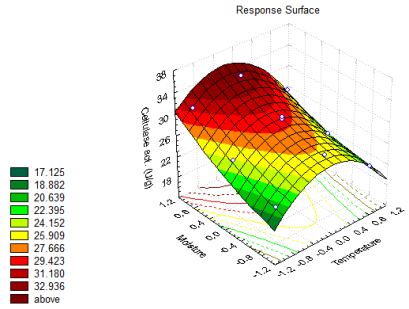

Figure 1. Response surface for cellulase production as a function of moisture and temperature according to the model.

The statistical model allowed the optimization of operation parameters improving the production of cellulases by *A. uvarum*. The variables studied had a significant effect on cellulase production by SSF of olive mill and winery wastes.

Acknowledgements

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