Cellulase and Xylanase Production by *A. uvarum* in Solid-State Fementation Using a Packed-Bed Bioreactor

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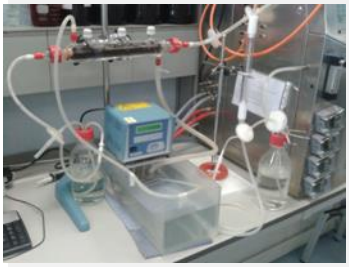

Wastes from olive oil and wine industries (as exhausted grape mark, vineshoot trimmings, olive pomace and vinasses) were used as substrate for lignocellulolytic enzymes production (as endocellulases, endoxylanase) by solid state fermentation in a packed-bed bioreactor. In previous works, *A. uvarum* was selected as suitable fungus to produce cellulases and xylanases, also composition of substrate, temperature and moisture content were optimized. An important parameter in packed-bed bioreactors is air flow rate, thus its effect on enzymes production was studied. The highest enzyme production was obtained for the lower aeration rate (0.2 L/min). It was observed that increasing air flow the enzyme production was lower.

Introduction

Packed-bed reactors can be used in solid-state fermentations and have emerged over the last 20 years as a potential alternative to other configurations, as tray and drum bioreactors [1]. Glass columns are the typical packed-bed reactors at laboratory scale. They have a perforated base plate on the bottom which allows the air flow and can be oriented vertically or horizontally. The operations of load and unload are easy in this bioreactors, additionally extraction of enzymes can be carried out into packed-bed bioreactor, reducing operations of process.

The air flow is a parameter affecting the growth of fungi and enzymes production. High aeration increases the availability of oxygen, favours the heat transfer and increase porosity of the solid [2]; however high aeration can cause effects of shear-stress on the morphology of filamentous fungi, it also can reduce the moisture of solid [2].

Fungi produce a complete set of cellulases: cellobiohydrolases (EC 3.2.1.91), endoglucanases (EC 3.2.1.4) and β-glucosidases (EC 3.2.1.21) that are necessary to efficiently hydrolyse cellulose [3].

Aspergillus uvarum is a black *Aspergillus* isolated from grape berries in the Mediterranean area [4]. It was found to be related to *Aspergillus japonicus* based on morphological data [4]. Previous studies on lignocellulolytic enzymes production by SSF with *A. uvarum* were performed by our group [5]. This strain showed a higher cellulase production than other strains as *A. niger* and *A. ibericus* on SSF of olive mill and winery wastes [5].

This work studied the use of packed-bed bioreactor for cellulase production by SSF of winery and olive mill wastes.

Material and Methods

A. uvarum was obtained from MUM culture collection (University of Minho, Braga, Portugal). It was revived on malt extract agar (MEA) plates (2% malt extract, 2% glucose, 0.1% peptone and 2% agar) from preserved glycerol stocks stored at -80 ºC. Then subcultured on MEA slants and incubated at 25 ºC for seven days to obtain inoculum for SSF.

The waste samples were collected from industries in the area in season 2011/2012 and stored at -20 ºC. The solid residues used were olive pomace (OP), exhausted grape marc (EGM) and vineshoot trimmings (VT) and the wastewaters used were vinasses.

SSF were carried out in packed-bioreactor, which was a glass water jacketed column with 2.62 cm diameter and 26.3 cm length. Column was placed horizontally, air was moistened and sterilised before entering the bioreactor, air flow was measured with a rotameter. Bioreactor was loaded with 20 g of dry solid substrate composed of OP (3.33 g), EGM (10 g) and VT (6.6 g). Moisture was adjusted to 75% with diluted vinasses supplemented with urea (0.07 g/g solid substrate). Then, bioreactor was sterilized in autoclave at 121 ºC for 15 min.

Inoculum solution (3 mL, 10^7 spores/mL) was added in different inputs of bioreactor to allow a homogenous growth of fungus. Fermentation was

performed at 30 ºC.

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. Xylanase (Endo-1,4-ß-Xylanase) activity was determined with the enzymatic kit Azo wheat arabinoxylan AWX 10/2002 (Megazyme International, Ireland). One unit of enzyme activity was defined as the amount of enzyme required to release one µmol of xylose reducing-sugar equivalents from wheat arabinoxylan in one minute at 40 ºC and pH 4.5. Both activities were expressed as unit per g of solid substrate.

Results and Conclusion

Figure 1 shows the effect of flow rate on the endocellulases and endoxylanases production by *A. uvarum* in packed-bed SSF on the olive mill and winery waste mixture. After 6 days of fermentation, solid fermented was unloaded of the bioreactor and the extraction of enzymes was carried out and their activities were measured.

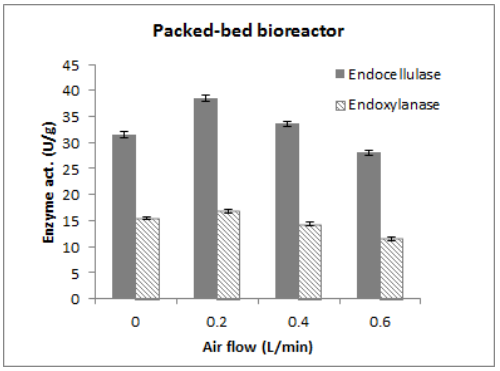

Figure 1. Effect of air flow in cellulase and xylanase production by *A. uvarum*

It was observed that increase in air flow rate from 0.2 L/min to 0.6 L/min decreases endocellulase and endoxylanase production. Low aeration (0.2 L/min) has allowed to achieve maximum endocellulase and endoxylanase activities of 38.51 ± 0.53 and 16.81 ± 0.43, respectively. The effect of air flow rate was higher in endocellulase activity than in endoxylanase activity. Endocellulase activity in SSF with 0.2 L/min of air flow was increased in 22.4%, if compared with SSF without aeration. Endoxylanase increased its activity in 9.0%. The reduction of enzyme activities was possible due to reduction of moisture and damaging effect of shear stress on fungus caused by high air flow rate [2].

Winery and olive mill wastes can be used to produce cellulases and xylanases in packed-bed bioreactor. The air flow rate affected the production of enzymes in SSF by *A. uvarum*. High air flow rates decreased enzymes production.

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