

# Fed-batch production of cellulases from *Trichoderma aurioviride* II growing on leached sugar beet pulp\*

Producción de celulasas de *Trichoderma aurioviride* II por lotes alimentados a partir de coseta agotada de remolacha

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Fed-batch production of cellulases through fermentation of leached sugar beet pulp (LSBP) with *Trichoderma aurioviride* II, a wild type strain from the south of Chile, is investigated. Cultures run for nine days showing a sustained increase in filter paper, endoglucanase, exoglucanase, and cellobiase activities with maximum values of 0.1, 2.2, 0.35 and 0.74 units/ml, respectively. Fed-batch cultures allow to achieve longer process times as compared to typical batch fermentation.

## INTRODUCTION

There are several factors which are necessary to manipulate in order to improve the commercial production of cellulases. Among these are the availability of an appropriate strain and the effect of the culture system (1).

Different culture collection fungi such as *Trichoderma reesei* QM 9414 and RUT C-30 (2, 3, 4, 5) and wild type strains like *Trichoderma aurioviride* (6) have been investigated in our and other laboratories trying to increase the cellulase activity.

In connection with the above the culture method, batch or fed-batch (continuous or intermittent feeding of nutrients), is of paramount importance in the obtention of a high enzymic titer.

Fig. 1 shows as schematic representation of the system and the typical behaviour of both a batch and fed-batch fermentation modality.

A fed-batch is advantageous over a batch culture when it is necessary to control the specific growth rate of the microorganism or when it is of importance to regulate the overproduction of some required metabolite (7).

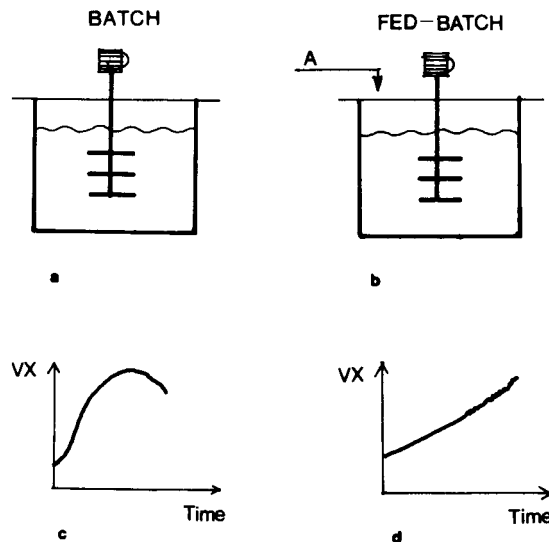


Fig. 1: Batch and Fed-batch cultures. (a) and (b) Schemes for a batch and fed-batch fermentor; (c) and (d) Typical growing profiles vs. time for batch and fed-batch fermentation. A = Substrate feed to a fed-batch fermentor; V = culture volume in fermentation vessel; X = dry cell mass concentration; VX = total microorganisms mass (dry basis) in the fermentor.

*Trichoderma aurioviride* II a wild type strain isolated from native forests in the south of Chile is capable of growing on microcrystalline cellulose as the unique carbon source (6).

In this work this strain is used to study cellulase production in an intermittent fed-batch fermentation.

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## MATERIALS AND METHODS

**Microorganism and Fermentation Conditions.** *Trichoderma aurioviride* II was maintained on potato-dextrose (DIFCO) at 4°C. Fermentations were carried out in a baffled 20 liter fermenter (BioloFitte-Gourdon, France) equipped with three four blade turbine impellers, a bottom rotary air sparger and separate units for pH and temperature control. Fermentation conditions were 28°C, pH 3.5, 1.5 Vessel Volumes per minute, 300 rpm and a working volume of 11 liters. Culture media contained LSBP (-50 +100 mesh) initially at 25 g/l; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 5 g/l; MgSO<sub>4</sub> x 7H<sub>2</sub>O, 0.75 g/l; Tween 80, 1 ml/l; KH<sub>2</sub>PO<sub>4</sub>, 2.5 g/l; CaCl<sub>2</sub> x 2H<sub>2</sub>O, 0.4 g/l; yeast extract, 1.0 g/l and 2.5 ml/l supplementary mineral salt solution. LSBP contained more than 70% (w/w) cellulosics and 1.2% lignin; its composition has been reported elsewhere (2).

After 72 hours 500 ml of a LSBP suspension were added to a level of 20 g/l in the fermentor. After 0.5 h a 500 ml harvest was taken.

Feeding and harvesting in this manner were repeated at 48 h intervals.

Inocula for the 20 liter fermentor were prepared in the same LSBP medium, by shake-flash cultivation at pH 4.0, 28°C and 200 rpm in a New-Brunswick G-25 rotary shaker. The fermentors were inoculated with 1.1 liter of a 60 h cultures. Inocula for this culture were prepared in a medium with glucose as carbon source in a 500 ml flask. This flask was inoculated with a spore suspension containing 4 x 10<sup>4</sup> spores/ml. Media were sterilized at 121°C for 30 minutes.

## ANALYTICAL METHODS

Soluble protein was determined by the method of Lowry (8). Reducing sugars were measured by the dinitrosalicylic acid method (9). Exoglucanase (EXG) and endoglucanase (ENG) activities were determined as previously reported by Illanes and Rossi (10). Combined activity or filter paper activity (FPA) was determined by the method of Mandels (11) at 50°C in citrate buffer 0.05 M at pH 4.8. Cellobiase activity (CA) was determined on cellobiase at 50°C, citrate buffer 0.05 M, pH 4.8.

Enzymic activity units (U) were defined as one unit (U) corresponds to the production of one micromole of glucose equivalent per minute from the cellulosic substrate.

## RESULTS AND DISCUSSION

Results for a fed-batch run under the above feeding strategy are shown on Figs. 2 and 3.

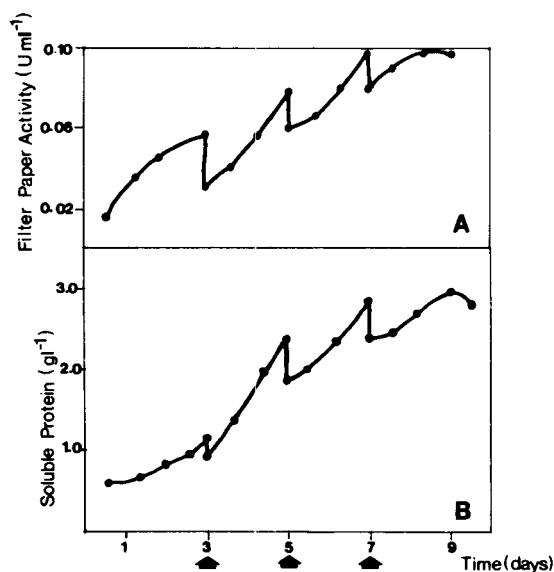


Fig. 2: Kinetics of filter paper activity ( $\text{U ml}^{-1}$ ) and soluble protein concentration ( $\text{g l}^{-1}$ ) during fed-batch production of cellulases on leached sugar beet pulp (LSBP) with *Trichoderma aurioviride* II in a 20 liter fermentor at 28°C, pH 3.5, 1.5 vessel volume per minute and 300 rpm. Arrows indicate addition of LSBP to a level of 20 g/l in the fermentor.

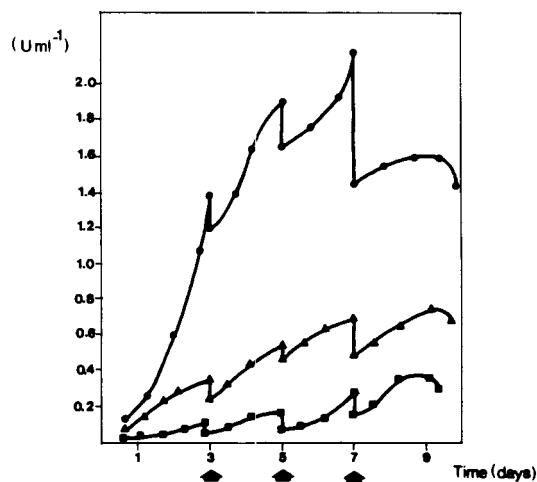


Fig. 3: Kinetics of cellulases fraction activities during fed-batch fermentation of leached sugar beet pulp (LSBP) with *Trichoderma aurioviride* II in a 20 liter fermentor at 28°C, pH 3.5, 1.5 vessel volume per minute and 300 rpm. Arrows indicate addition of LSBP to a level of 20 g/l in the fermentor. Endoglucanase (●); Exoglucanase (■); Cellobiase (▲).

Solid lines have been drawn to facilitate interpretation only and are not the result of any statistical or theoretical analysis.

The enzymic activity curves are not continuous at the feeding points as a result

of both a dilution rate effect and the adsorption of some enzymic fraction on the solid cellulose substrate. This adsorption effect has been demonstrated previously (12).

Results indicate a sustained increase in all enzymic activities, and protein concentration up to the ninth day. Ultimate values of FP, ENG, EXG, cellobiase and soluble protein of 0.1 U/ml, 2.2 U/ml, 0.35 U/ml and 0.74 U/ml and 0.28 g/l, respectively were obtained.

Having the fed-batch process a much longer operation time than a batch culture, it allows to maintain enzymic activities at the higher range values for extended periods; on the contrary *Trichoderma* sp. growing on LSBP in a 20 liter batch culture runs for no more than 120 h (2, 13, 14).

This longer fermentation time for the fed-batch process would allow to harvest a broth with a much higher enzymic activity at each partial culture withdrawal.

The purpose of this paper has been to point out the effect of some variables in the production of cellulases through fed-batch culture. An optimization of the feeding policy, the length of the initial batch run and the volume of broth to be replaced in each substrate addition can lead to significant improvements in process performance. These and other variables such as aeration rates, agitation and micronutrient concentrations are under experimentation in our laboratories.

#### ACKNOWLEDGMENTS

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