

COMMUNICATIONS TO THE EDITOR

Production of Cellulase from Trichoderma reesei in Fed-Batch Fermentation from Soluble Carbon Sources

INTRODUCTION

To date, the best substrate for production of *Trichoderma reesei* cellulase in terms of both enzyme yield and productivity is cellulose,^{1,2} an insoluble substrate. Lactose, a soluble carbon source, will also induce cellulase from *T. reesei* to a lesser extent than cellulose.^{3,4} Cellobiose, a predominant soluble product of cellulose hydrolysis, under certain conditions, will induce cellulase from *T. reesei*.⁵⁻⁷ The soluble disaccharide, sophorose, a strong inducer of cellulase,^{3,8-11} is at present not considered a practical carbon source for cellulase production.

The use of a soluble carbon source in lieu of cellulose would allow greater control of the fermentation since growth and enzyme production would no longer be dependent upon cellulose hydrolysis. Since fed-batch has proved successful for many fermentations¹²⁻¹⁴ where carbon limitation is a requirement, this study describes cellulase production from *T. reesei* using five different carbon sources.

MATERIALS AND PROCEDURES

A 2-L BioFlo reactor, model C30 (New Brunswick Scientific) was used for the fermentation study. Carbon dioxide was measured with a Lira gas analyzer (Mine Safety Appliances). For monitoring the fermentation and control a PDP-11 minicomputer from Digital Equipment Corp. was used.

The hydrolysate used in this study was obtained from the 24-h hydrolysis of 25% BW200, a ball-milled cellulose pulp (Brown Company, Berlin, NH), using three filter-paper cellulase units/mL from *T. reesei*.¹⁵ Water was removed from the 10% sugar product by boiling to approximately 50% (w/v).

The reagent grade xylose, glucose, cellobiose, and gentiobiose used were obtained from Fisher Scientific, Baker Chemical, Eastman Kodak, and Aldrich Chemical, respectively.

Synthetic reversion syrup was obtained by incubation of a 50% (w/v) glucose syrup with 200 IU/mL of *Aspergillus phoenicis*¹⁶ β -glucosidase for 24 h, 50°C, and pH 4.8.

Protein was determined by the Lowry procedure.¹⁷ The assays for β -glucosidase¹⁸ and cellulase¹⁵ were made on the extracellular fluids.

EXPERIMENTAL

This set of experiments investigates the use of five different carbon sources for their ability to induce cellulase from the Rutgers strain,¹⁹ C-30, of *T. reesei* in a fed-batch fermentation. The five carbon sources used were 1) 50% (w/v) glucose, 2) 16% (w/v) cellobiose, 3) artificial hydrolysis syrup (reagent grade glucose, xylose, cellobiose, and gentiobiose), 4) a synthetic reversion syrup obtained from the incubation of glucose with β -glucosidase which should contain β -linked glucose dimers,²⁰ and 5) a 50% cellulose hydrolysate.

The addition of the syrups was controlled by a DEC PDP-11 minicomputer using the exhaust gas analysis (CO₂) for feedback control. The computer was programmed to control the CO₂ evolution through substrate addition. The object of the computer control was the simulation of the CO₂ profile for successful cellulose fermentations. The control scheme for

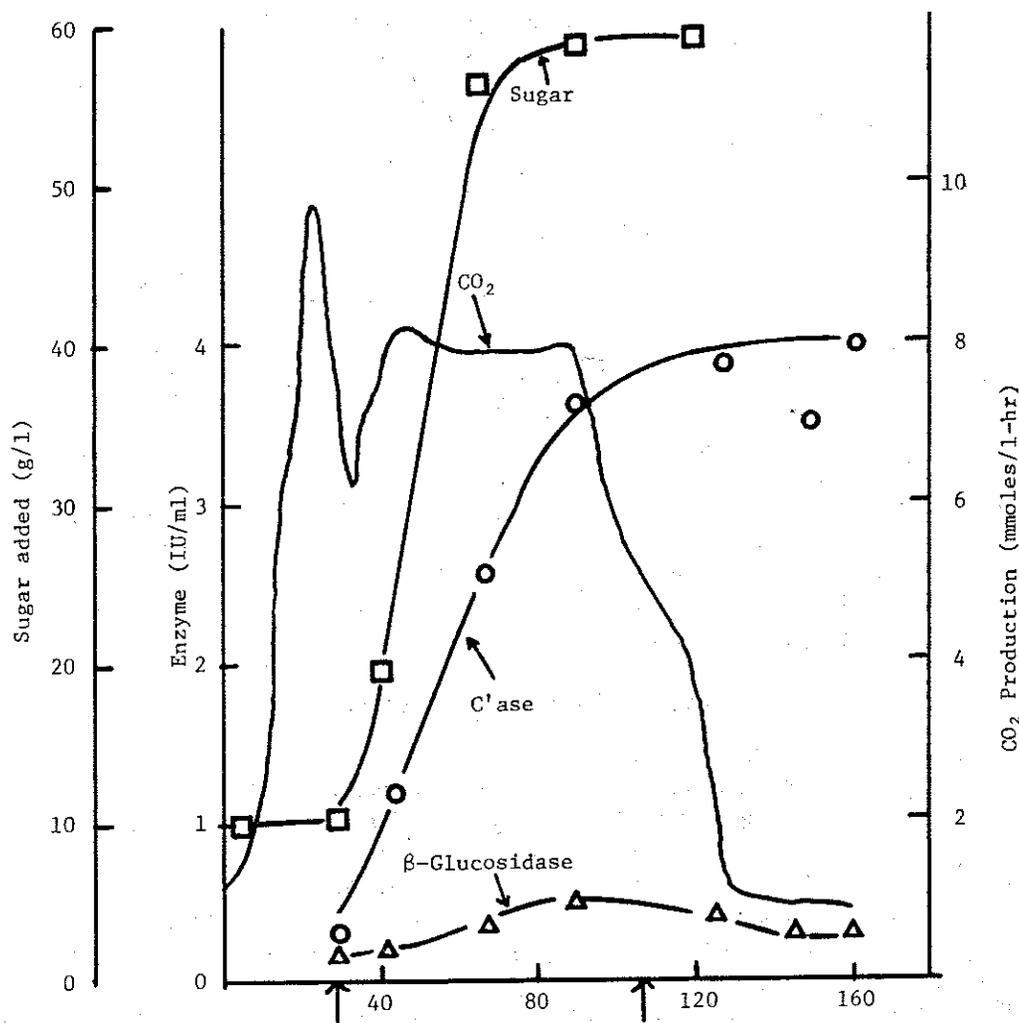


Fig. 1. Production of *T. reesei* cellulase in fed-batch fermentation from hydrolysis syrups. Total sugar consumed equals 60 g in a 1-L fermentor. (□) Sugar, (○) Case, (△) β-glucosidase, (no symbol) CO₂.

substrate addition approximated proportional control; i.e., substrate addition was proportional to the negative deviation of the set point (CO₂ evolution rate). No control was exerted for positive deviations from the CO₂ set point. The computer program also ensured carbon limitation by not allowing substrate addition if the dissolved oxygen fell below 30% of the saturation level. With the exception of one fermentation, the CO₂ profile for all the other fermentations resembled that shown in Figure 1. All fermentations were run at 30°C, with pH controlled at 4.0 using ammonium hydroxide.

Unless otherwise noted, the volume increase, due to substrate addition, was less than 100 mL (10%). The average dilution rate for all fermentations was ca. 0.002 h⁻¹.

Each fermentation was started by spore inoculation of the fermentor which contained 1% glucose with Mandels salts.²¹ After the 1% glucose was consumed, yielding approximately 5 g/L of cells (dry wt.), sugar feeding commenced under computer control.

RESULTS

Figure 1 shows the results of feeding a 50% cellulose hydrolysate. The arrows indicate when the sugar addition started (≈ 30 h) and stopped (≈ 105 h). The computer was pro-

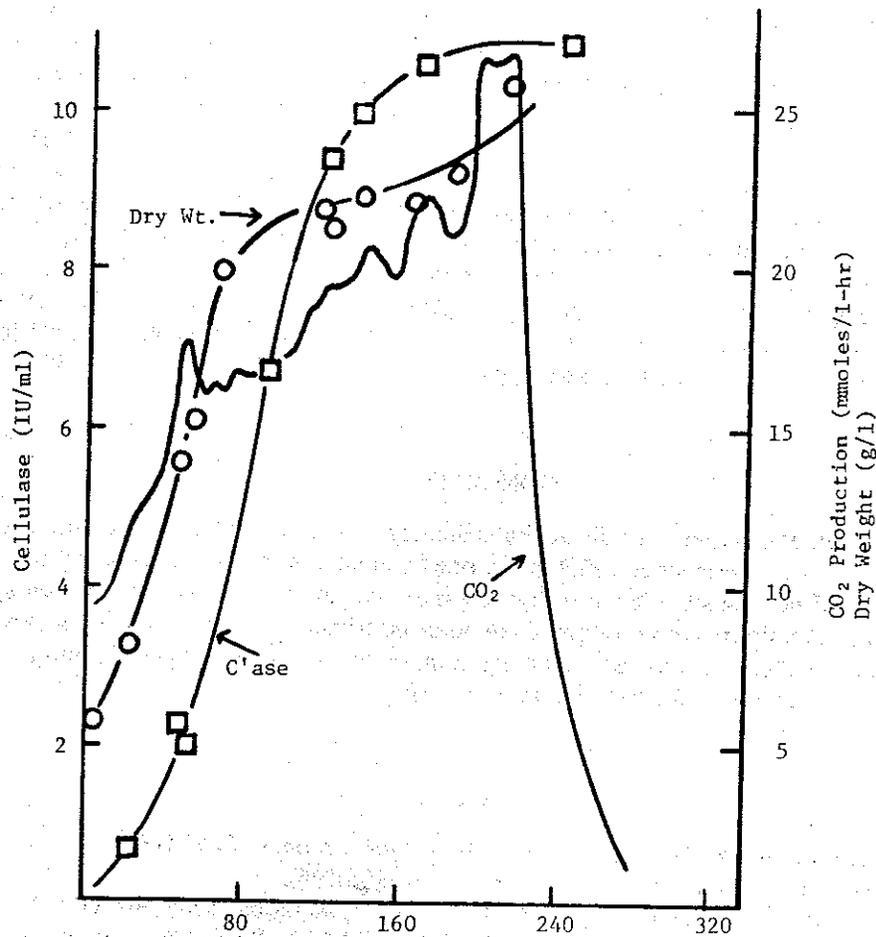


Fig. 2. Production of *T. reesei* cellulase in fed-batch fermentation from hydrolysis syrups. Total sugar consumed equals 115 g in a 1-L fermentor.

TABLE I
Summary of *T. reesei* Fed-Batch Fermentation

Substrate	Sugar consumed (g/L)	Cellulase titer (IU/mL)	Extracellular β -glucosidase (IU/mL)
Glucose	60	0	0.37
Cellobiose ^a	60	0	0.94
Artificial hydrolysis syrup ^b	60	0	1.0
Synthetic reversion syrup ^c	60	1.8	0.60
Hydrolysis syrup ^d	60	4.0	0.35
Hydrolysis syrup ^d	115	10.7	1.8

^a Feed syrup contained 16% cellobiose.

^b Feed syrup consisted of reagent grade xylose, glucose, cellobiose, and gentiobiose in the weight ratio of 6:25:17:2 with a total concentration of 50% (w/v).

^c Feed syrup was obtained from a 50% (w/v) glucose syrup incubated with 200 IU/mL of β -glucosidase for 24 h, 50°C, pH 4.8.

^d Feed syrup was obtained from the enzymatic hydrolysis of 25% BW200, 3 IU/mL Case for 24 h at 50°C, pH 4.8, and concentrated to 50% (w/v).

grammed to raise the respiration slowly ($\mu \approx + 0.02$ h) for 24 h, maintain it for 48 h, and slowly lower it ($\mu \approx - 0.02$ h) for 24 h. The data indicate that cellulase was produced soon after the feeding had started and production ceased when the carbon source was exhausted.

Although the respiration profiles were essentially identical for all the fermentations, appreciable levels of cellulase were produced only from the cellulose hydrolysate (4 IU/mL) and the synthetic reversion syrup (2 IU/mL) which had been previously incubated with β -glucosidase. The glucose syrup, cellobiose syrup, and the synthetic hydrolysis syrup (xylose, glucose, cellobiose, and gentiobiose) produced very little cellulase (< 0.05 IU/mL).

When the 1-L fermentor was fed 105 g of hydrolysis sugars instead of 50 g, the cellulase titer approached 11 IU/mL representing 22 mg/mL of extracellular protein (Fig. 2). This level of activity is comparable to that obtainable from cellulose fermentations.²² Table I gives the results of the fermentations using the various carbon sources.

CONCLUSION

These fermentations seem to indicate that cellobiose is not the natural inducer for cellulase production from *T. reesei* since cellobiose alone or in admixture with glucose, xylose, and gentiobiose did not induce. Cellulose hydrolysates and the β -glucosidase reversion syrup are known to contain transfer products (β dimers and trimers of glucose) and it appears that one or more of these may be the inducing compound. Sophorose appears a more likely candidate than cellobiose for this role in *T. reesei*.

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