Biotechnology Letters Vol 11 No 8 545-550 (1989)
Received as revised June 23rd

STUDIES ON BETA-GALACTOSIDASE PRODUCTION IN TRANSIENT OPERATION CULTURES

G. Martini, C. Mignone and R. Ertola
Centro de Investigación y Desarrollo en Fermentaciones Industriales
Facultad de Ciencias Exactas UNLP, 47 y 115 (1900) La Plata, Argentina.

SUMMARY

Beta-galactosidase production in transient operation culture was studied considering cycles of 90 and 180 min.

The results obtained after 14 cycles periods proved that significant increases in the especific enzyme activity are attained in transient operation namely 3.83 to 4.81 umg^{-1} compared with those obtained in steady state continuous culture which ranged between 1.71 to 2.2 Umg^{-1}.

INTRODUCTION

Beta-galactosidase (E.C. 3.2.1.23) is an enzyme widely used in dairy industries. Its potential importance reflects the large amounts of whey permeate available in those industries. This enzyme is normally produced in batch cultures by using various strains of yeasts such as Kluyveromyces lactis, K. marxianus and K. fragilis (Richmond et al., 1981). Beta-galactosidase is induced by lactose and related compounds.

Normally, the maximum yield and specific activity are attained at the beggining of the stationary phase of growth (Dickson and Markin, 1980). Continuous culture studies have been carried out with a strain of Candida pseudotropicalis but no significant increase in the specific enzyme activity was observed (Gómez and Castillo, 1983).

This paper deals with the preliminary results obtained in transient state operation culture, which was studied as an alternative production procedure to batch or steady state continuous cultures.

MATERIALS AND METHODS

Microorganism and medium

The microorganism used was K. lactis NRRL 1118, kindly providad by the Northern Research Regional Laboratory, Peoria, Illinois.

The strain was kept on a peptone-yeast extract lactose agar medium with the following composition (in $g.L^{-1}$): peptone (Difco) 2; yeast extract (Oxoid) 2; lactose, 4; and agar, 15.

The medium used consisted of macronutrients (in $g.L^{-1}$): lactose, 2; $H_2NH_4PO_4$. 8; H_2KPO_4, 1.67; H_2NaPO_4, 1.67; $CaCl_2.2H_2O$, 0.1; $MgSO_4.7H_2O$, 0.6; micronutrients (in $mg.L^{-1}$): H_3BO_3, 0.1; $CuSO_4$, 0.1; $FeCl_3.6H_2O$, 0.17; K I, 0.1; Na_2MoO_4, 0.1; $ZnSO_4.7H_2O$, 0.18; and vitamins (in $\mu g.L^{-1}$);

inositol, 6; biotin, 6; folic acid, 6; calcium panthotenate, 800; thiamine, 800; p-aminobenzoic acid, 400 and pyridoxine, 1600.

In shift or step change experiments, lactose concentration of the incoming medium was changed from 2 to 4 $g.L^{-1}$.

Analytical procedures

Microorganism concentration was estimated by optical density measurements and by dry weight determinations.

Lactose concentration was evaluated by the DNS method (Miller, G.L., 1959). Enzyme activity was evaluated with OPNG as substrate. Cells were harvested by centrifugation and the pellet obtained was resuspended in buffer Z (Dickson, 1980) with 2% of solvent and incubated at 37 °C in an agitated water bath during 2.5 h. After this time an alicuot of 100 µl of the treated cells (or an appropriate dilution) were mixed with 4 mL of 1.25 mM of ONPG in buffer Z at 37 °C. After 4 minutes the reaction was stopped by adding 1 M of Na_2CO_3, followed by centrifugation to separate the cells. The yellow colour of the supernatant was measured at 420 nm (ONP extintion coeficient is 4500 $cm^{-1}M^{-1}$). The activity unit is defined as the amount of Beta-galactosidase which hydrolized one micromol of OPNG per minute.

Equipment and transient operation technique

For batch and continuous culture cultivation a L.K.B. Ultraferm fermentation system was employed, with a set working volume of 1.8 liters. Temperature was set at 30 °C.

Aeration and agitation conditions were fixed at 2 $L.min^{-1}$ and 350 rpm in order to avoid oxygen limitation. After attaining and maintaining the steady state conditions for 13,5 generations (95 h), the system was subjected to square wave variations in the input of the limiting lactose concentration. Square waves in the incoming lactose concentration were achieved by connecting the bioreactor to two nutrients reservoirs, each containing the same medium but with different lactose concentration. At pre-established time intervals a timer/valve system switched the nutrient feed between both reservoirs. Samples of culture were collected in order to have intracyclic values of enzyme activity.

Two sets of experiments were carried out. In the first one, the response of the culture subjected to changes in lactose concentration from 2 to 4 $g.L^{-1}$ and from 4 to 2 $g.L^{-1}$ at 90 min cycle time was studied. The second set of experiments was conducted in the same way but with a 180 min cycle time.

The data were compared with those obtained in steady state continuous cultures carried out with media containing 3 $g.L^{-1}$ of lactose. The samples were collected for analyzing the intracycle behaviour after a kind of "steady state conditions" of the transient operation which was established by performing 14 periods of 90 and 180 min cycles in both sets of experiments.

RESULTS AND DISCUSSION

After performing the batch step, the continuous culture was started at $D = 0.111\ h^{-1}$ with the 2 $g.L^{-1}$ lactose medium and left under steady-state operation for 95 h. Table I indicates the values attained in that condition. After that time a shift in the concentration of lactose of

Table I: Data of continuous cultures of K. lactis after a shift in lactose concentration from 2 to 4 $g.L^{-1}$.

Time (h)	Dry weight ($g.L^{-1}$)	Lactose ($g.L^{-1}$)	Enzyme activity	
			Volumetric ($U.mL^{-1}$)	Specific ($U.mg\ dry\ wt^{-1}$)
0*	0.97	0.089	2.08	2.14
1	0.98	0.101	2.16	2.2
2.5	1.03	0.100	2.08	2.02
4	1.22	0.100	2.1	1.72
7	1.45	0.101	2.48	1.71
10	1.59	0.100	3.66	2.3
24.5	1.73	0.101	3.62	2.09
49	1.73	0.100	3.63	2.10
60	1.76	0.102	3.5	1.99

* The shift in lactose concentration was performed after maintaining the steady state condition during 95 hours.

Table II: Data of continuous culture of K. lactis after a pulse of lactose (3 $g.L^{-1}$ in the bioreactor).

Time (h)	Dry weight ($g.L^{-1}$)	Lactose ($g.L^{-1}$)	Enzyme activity	
			Volumetric ($U.mL^{-1}$)	Specific ($U.mg\ dry\ wt^{-1}$)
0	1.76	2.89	3.5	1.99
2	1.605	2.5	0.09	0.06
4	1.27	2.43	0.03	0.024
6	1.12	2.24	0.03	0.027
8	1.04	1.82	0.02	0.02
10	1.07	1.38	0.04	0.04

the incoming medium was initiated.

Table I shows the observed response of the culture. As expected, there was an increase in the dry weight values. Lactose concentration remained around 0.1 $g.L^{-1}$ confirming that it was still the limiting substrate. The volumetric enzyme values increased up to 3.5 $U.mL^{-1}$ but, as the specific enzyme activity remains approximately constant (values varying between 1.8 and 2.2 U.mg.dry wt^{-1}),this can only be attributed to the increase of biomass.

Table II shows the results obtained after a pulse of lactose, which increased its concentration up to 3 $g.L^{-1}$ in the reactor. It can be observed that biomass decreases considerably from 1.76 to 1.07 $g.L^{-1}$ in 10 h. An abrupt decrease in the volumetric and specific enzyme activity is also noted. The results of this experiment, which was repeated several times, may be explained by postulating a strong catabolite repression of the enzyme formation produced by the sharp increase in the lactose concentration, as reported by Elander et al. (1981).

Table III shows the data obtained in the transient-state operation which were taken, as already mentioned, after 14 operation periods. These results are compared with those obtained under steady state conditions with an incoming lactose concentration of 3 $g.L^{-1}$. As it can be seen, biomass and lactose concentration remained constant in the two sets of experiments. In both cases, however, either during the 90 min or 180 min cycles experiments, a significant increase of the volumetric and specific enzyme activity was observed. Although variations in macromolecular composition with increase of protein or RNA values can be expected according to Pickett et al. (1979) in transient operation cultures the reason for these results is not clear. A possible explanation is related to a modification of the proportion of Beta-galactosidase content due to alterations in the regulation of the enzyme biosynthesis produced by maintaining the culture during a long time under a constant periodic oscillation in the input of lactose.

Although these results are preliminary it is evident that the transient operation culture technique deserves further investigation in order to prove its technological potential for attaining higher productivity in enzyme production.

Table III: Steady state and transient intracycle data of K. lactis

a) Averaged data of continuous culture in steady state (incoming lactose concentration: 3 $g.L^{-1}$).

Dry weight ($g.L^{-1}$)	Lactose ($g.L^{-1}$)	Enzyme activity: Volumetric ($U.mL^{-1}$)	Enzyme activity: Especific ($U.mg\,dry\,wt^{-1}$)
1.35	0.101	2.7	2

b) Transient intracycle data

Time (min)	Dry weight ($g.L^{-1}$)	Lactose ($g.L^{-1}$)	Enzyme activity: Volumetric ($U.mL^{-1}$)	Enzyme activity: Especific ($U.mg\,dry\,wt^{-1}$)
			Cycle time 90 min.	
4	1.28	0.096	5.5	4.29
19	1.27	0.095	5.15	4.05
34	1.18	0.092	4.96	4.22
49	1.23	0.093	4.7	3.83
64	1.18	0.091	4.83	4.1
79	1.18	0.090	4.9	4.15
			Cycle time 180 min.	
17	1.21	0.094	5.5	4.55
47	1.21	0.094	5.84	4.83
77	1.29	0.092	5.77	4.47
107	1.25	0.093	5.98	4.78
137	1.21	0.095	5.8	4.79
167	1.42	0.09	5.84	4.11

Acknowledgements

The authors wish to thank CONICET and SECYT for the support given and also Jorge Argemi for the technical assitance.

References

- Dickson, R.C. and Markin, J.S. (1980), Physiological Studies of Beta-galactosidase Induction in Kluyveromyces lactis. J. of Bact. 142, 3, 777-785.
- Elander, R.P. and Demain, A.L. (1981). Genetics of Microorganisms in relation to Industrial Requirements. In: Biotechnology, Vol. I, 259. Ed. by Rehm, H.J. and Reed, G.. Verlag Chemie, Weinheim; Deerfield Beach, Florida; Basel.
- Gómez, A. and Castillo, F. (1983). Production of Biomass and Beta-D-galactosidase by Candida pseudotropicalis Grown in Continuous Culture on Whey. Biotech. Bioeng. XXV, 1341-1357.
- Miller, G.L. (1959) Anal. Chem. 31, 426-428.
- Pickett, A.M.; Topiwala, H.H. and Bazin, M.J. (1979). A new method of industrial Bio-reactor operation: the transient operation technique. Process Biochem. Vol. 14 (11), 10-16.
- Richmond, M.L.; Gray, J.I. and Stine, C.M. (1981). Beta-galactosidase: Review of Recent Research Related to Technological Aplication, Nutritional Concerns, Immobilization. J. Dairy Sci., 64, 1759-1771.