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EXOPOLYSACCHARIDE BIOSYNTHESIS BY A FAST-PRODUCING STRAIN OF *Aureobasidium pullulans*

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SUMMARY

The mutant strain *Aureobasidium pullulans* ICCF-68 was able to produce in batch fermentation on a glucose medium of 80 g/l, exopolysaccharide at high volumetric productivity and final concentration (1.05 g/l.h and 50.2 g/l, respectively). A specific pH pattern and very high oxygen requirement were shown.

INTRODUCTION

The yeast like fungus *Aureobasidium pullulans* synthesizes several polysaccharides, mostly pullulan. This extracellular polysaccharide, a neutral glucan, generally accepted as linear polymer of maltotriosyl units connected by α-(1-6) linkages has found increased applications to food, pharmaceutical, cosmetic and other industries (Sugimoto, 1978; Le Duy *et al.*, 1988).

Pullulan is commercially produced from hydrolyzed starch substrate by Hayashibara Biochemical Laboratories, Japan. A wide variety of carbohydrate substrates were examined for pullulan biosynthesis: D-glucose, sucrose, D-fructose, maltose, D-xylose, lactose, D-galactose, D-arabinose, L-rhamnose, peat hydrolyzate(Le Duy *et al.*, 1988). The highest yields were from sucrose and glucose and these were chosen for most studies on pullulan elaboration (Seviour *et al.*,1992).

The batch fermentation is usually carried out from initial substrate concentration of ca. 5 %, the fermentation is completed in ca. 100 h, the final concentration of polysaccharide no more than 25 g/l. From 10 % sucrose after 7 days of batch fermentation only 35 g/l were obtained (Chul Shin *et al.*, 1987), or 45 g/l after 96 h (Liu *et al.*, 1981).

This paper is concerned with the batch fermentation with a strain of *Aureobasidium pullulans* cultivated on a glucose medium of 80 g/l initial concentration, which achieves high volumetric productivity and at the same time, a high final concentration of exopolysaccharide.

MATERIALS AND METHODS

Microorganism and cultivation: *Aureobasidium pullulans* ICCF-68 is a mutant strain derived from A. pullulans YB-4515. One loop of *A. pullulans* was transferred to a 500 ml Erlenmeyer flask containing 100 ml of an inoculum medium of the following composition, in % (w/v): glucose, 2.0; Bacto-peptone, 0.2; KH_2PO_4,0.5; NaCl, 0.2; $MgSO_4.7H_2O$, 0.08. The initial pH was adjusted to 6.0-6.3 with 10 N NaOH solution. This inoculum was cultivated for 20 h at 28 °C, 200 rpm in a shaking incubator and then transferred at a ratio of 1 % (v/v) to the culture medium for fermentation experiments. Culture medium contained, in % (w/v): glucose, 8.0; KH_2PO_4, 0.5; $(NH_4)_2SO_4$,0.2; $NaNO_3$, 0.2; NaCl, 0.2; $MgSO_4.7H_2O$, 0.08. The initial pH was adjusted to 6.3-6.5 with 10 N NaOH solution.

Fermentation experiments were conducted in a 12 l fermentor (**ULTROFERM-1601 UNIT-LKB BIOTEC, SWEDEN**) (8 l working volume). The pH was measured by an **INGOLD** type probe and the dissolved oxygen level was monitored by a galvanic (Pb/Ag) probe. The fermentation was performed under the conditions: 28 °C; agitation and aeration, as follows:

Fermentation time, h	0 - 20	20 - 30	30 - 48
Agitation, rpm	400	500 - 800	900
Aeration, vvm	0.6	0.8	1.9

Analytical methods: Dry cell weight was determined by centrifuging the fermentation broth at 5,000 rpm for 30 minutes, washing the cells twice with distiled water and drying to a constant weight at 105 °C. The crude polysaccharide was determined by precipitation from the recovered supernatant with the addition of two volumes of ethanol, centrifuging at 5,000 rpm for 20 minutes, washing with ethanol and drying to constant weight at 90 °C, under vacuum. Glucose was determined by the colorimetric method with o-toluidine (Dubowski, 1962). The NH_4^+ concentration was determined by a modified Kjeldahl method (without digestion).

RESULTS

Fermentation kinetics: Figure 1 depicts a typical kinetics of batch fermentation with *A. pullulans* ICCF-68. After a lag phase of less than 10 h, the biomass concentration rapidly increased and kept rising during the fermentation time until the total glucose was consumed. The production of exopolysaccharide appeared to be growth associated. As expected, the glucose concentration remained almost constant during the lag phase and declined after.

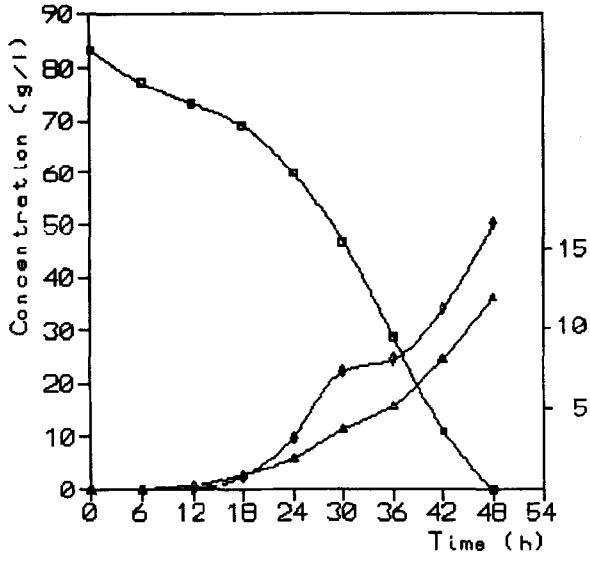

Fig. 1. Typical batch fermentation with A.pullulans ICCF-68

pH dynamics.: Figure 2 represents the pH dynamics, and shows a quite interesting variation. Remaining almost constant during the lag phase, it dropped rapidly from 5.6 to 2.9 within

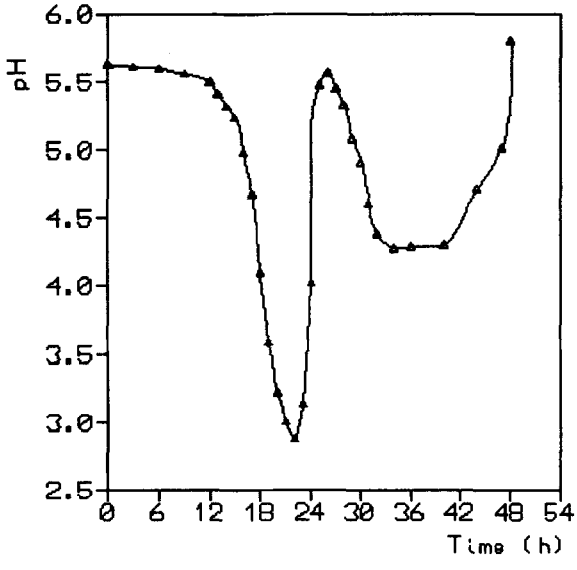

Fig. 2. pH dynamics during exopolysaccharide synthesis by A.pullulans ICCF-68

12-22 h, rose faster back to 5.5 within 22-28 h, decreased to 4.25 and remained almost constant within 33-40 h, then increased to the initial value during the time when glucose concentration drew nearer to zero.

Dissolved oxygen concentration: The dissolved oxygen concentration drastically dropped from the saturation level to lower than 20 % within 12-20 h (corresponding to the pH dropping), and was maintained very low after (lower than 10 %). Only within the last period of fermentation (42-48 h), the p_{O2} value started to increase (figure 3).

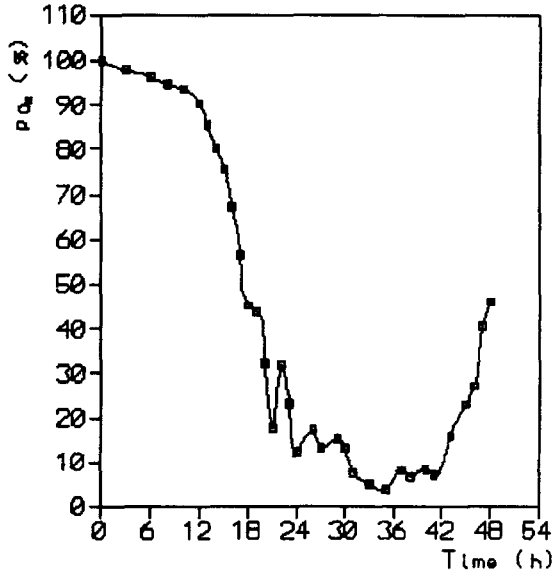

Fig. 3. Dissolved oxygen level during batch fermentation with A.pullulans ICCF-68

In fermentation experiments conducted under a modified agitation-aeration schedule from that mentioned above, mostly a lower level of oxygen-transfer possibilities after the first 25 h, there were changes in cell growth, glucose utilization and polysaccharide production patterns: cell growth stopped at 42 h, the rates of glucose utilization and polysaccharide production were somewhat reduced, the fermentation time extended to 54-56 h (data not shown).

Ammonium ion concentration: As can be seen in figure 4, ammonium ion concentration dropped drastically to negligible values after the first 12 h of cultivation.

Note. During the whole course of fermentation, no black pigment (melanin formation) was occured, the broth colour remaining creamy.

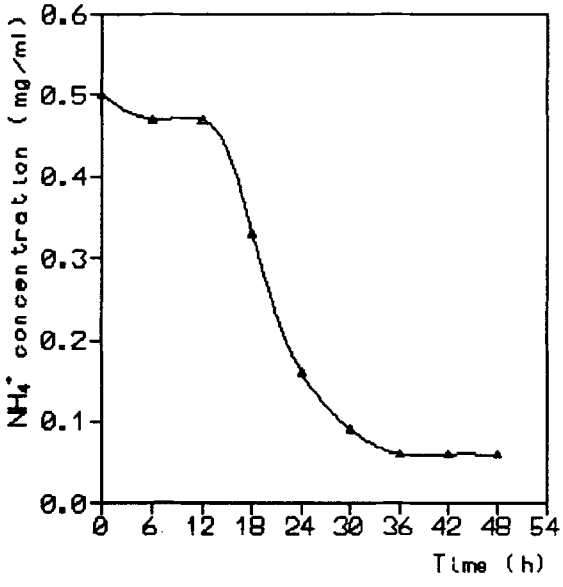

Fig. 4. Ammonium ions concentration during exopolysaccharide synthesis by A.pullulans ICCF-68

DISCUSSION

In spite of the relatively high initial glucose concentration (about 80 g/l), the lag period was less than 10 h, followed by a rapid increase of cell growth and exopolysaccharide production. This start was specifically marked by a deep and sharp minimum in the pH curve, which rose back to 4.0-5.0 values during the maximum polysaccharide production.

This specific aspect of the pH curve was probably due to the presence in the culture medium of the both nitrogen sources: ammonium and nitrate.

The residual dissolved oxygen level was very low during the

maximum pullulan synthesis and growth rate , even while a major increasing of oxygen transfer rate by agitation-aeration parameters occured. This proved a very high oxygen requirement.

Oxygen limiting conditions corresponding to p_{O2} values under 8 % (30-36 h) were accompanied by a temporary cessation of polysaccharide production. However, at p_{O2} of 10-20 %, the pullulan synthesis rate was high. These remarks are in good agreement with previous results (Rho *et al.*, 1988).

The start of polysaccharide synthesis was marked by a drop of ammonium ion concentration to negligible values, maintained during the time of biosynthesis. The pullulan production at negligible ammonium ion concentration was reported (Bulmer *et al.*,1987, Auer and Seviour, 1990).

As could be seen from the polysaccharide concentration dynamics (figure 1),at 48 h of fermentation, the final concentration of polysaccharide was 50.2 g/l, corresponding to an average volumetric productivity of 1.05 g/l.h.

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