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PRODUCTION OF PULLULAN BY A FED-BATCH FERMENTATION

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SUMMARY : In pullulan production from sucrose by Aureobasidium pullulans, a sugar concentration higher than 5 % (w/v) inhibited cell growth and the production of exopolysaccharide. By a fed-batch fermentation, the inhibitory effects of the high sugar concentration were overcome and 58.0 g/1 of exopolysaccharide were obtained from 10 % sucrose.

INTRODUCTION

Pullulan is a water-soluble neutral glucan which is a linear polymer of maltotriose units connected by α-(1-6) linkages (Bender and Wallenfels, 1961; Bouveng et al., 1963). With increased applications to food, drug, and other industries, investigations on the production of pullulan with Aureobasidium pullulans have been emphasized (Yuen, 1974). For cell growth, A. pullulans assimilates D-xylose, L-rhamnose, D-galactose, sucrose, maltose, cellobiose, lactose, inulin, soluble starch, and the non-glycosidic substrates, glycerol and acetate (Cernakova et al., 1980). Among these, glucose, fructose, xylose, maltose, and sucrose were examined for the production of exopolysaccharide (Zajic and LeDuy, 1977). Nevertheless, the utilization of high concentrations of sugar in a batch without reducing the growth rate of cells and the production rate of exopolysaccharide has not been examined, even though the increase of exopolysaccharide concentration in a batch by utilizing the high concentration of sugar has advantages for the process economy and for saving the solvent used for the recovery of exopolysaccharide.

In this paper, we aimed to utilize a high concentration of sucrose and to overcome the inhibitory effect by a fed-batch fermentation technique.

MATERIALS AND METHODS

Cultivation of microorganism : Culture medium of Aureobasidium pullulans IFO 4464 was: sucrose, 10 %; K_2HPO_4, 0.5 %; NaCl, 0.1 % ;

$MgSO_4 7H_2O$, 0.02 %; $(NH_4)_2SO_4$, 0.06 %; yeast extract, 0.3 %. Initial pH was adjusted to 7.5 with concentrated HCl. One loop of A. pullulans was transferred to 500 ml Erlenmeyer flask containing 100 ml of medium and cultivated for 3 days at 27°C, 200 rpm in shaking incubator. The 5 % culture broth of 3 day -culture was transferred to fresh medium and cultivated for 24 hr. This was used as an inoculum. The experiments on sucrose utilization were carried out in 500 ml Erlenmeyer flasks containing 100 ml medium at 27°C and 200 rpm. Fed-batch fermentation was conducted using a jar fermenter (New Brunswick Scientific, MICROFERM) containing 3 l medium under the conditions of 27°C, 400 rpm, and 1 vvm aeration.

Analytical methods : Dry cell weight and exopolysaccharide were determined by oven-drying (Lacroix, et al., 1985). The total residual sugar of culture broth was determined by the phenol-sulfuric acid method (Dubois et al., 1956). Glucose was determined using a Kit (Sigma Chem. Co.) and total reducing sugar was determined by the 3,5-dinitrosalicylic acid method (Miller,1959). The residual sucrose of culture broth was determined by subtracting the total reducing sugars from the total residual sugars. Fructose was determined by subtracting glucose from the total reducing sugars.

RESULTS AND DISCUSSION

Utilization of sucrose : Among the various mono-and disaccharides tested such as glucose, fructose, maltose, and sucrose, sucrose gave the best result for the production of exopolysaccharide. Figure 1 represents the utilization of sucrose by A. pullulans. Most of the sucrose in culture broth was rapidly converted to glucose and fructose before the late exponential stage of cells. In utilization of the monosaccharides, A. pullulans firstly utilized glucose and thereafter utilized fructose for the cell growth and the exopolysaccharide production. It seemed that A. pullulans did not take up most of the sucrose through a direct transport system but by converting it to glucose and fructose and the absorption of these

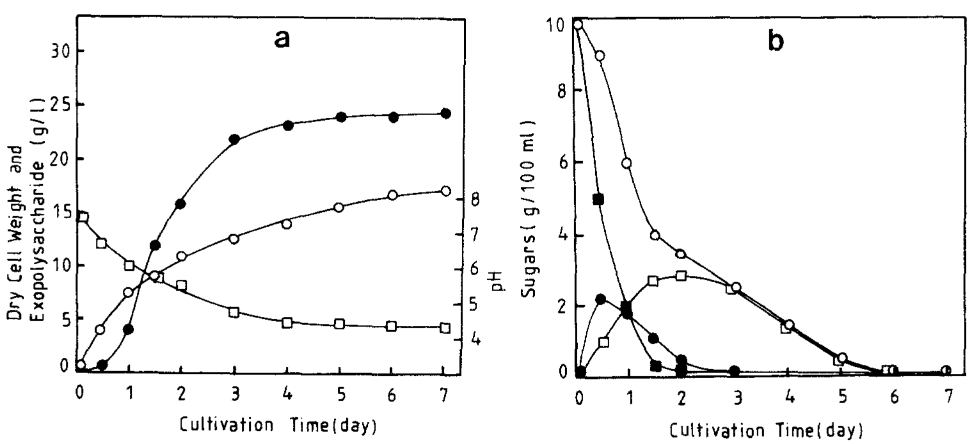

Figure 1. Kinetics of exopolysaccharide fermentation in sucrose medium with A. pullulans IFO 4464. (a) dry cell weight (○), exopolysaccharide (●), and pH (□). (b) total sugar (○), glucose (●), fructose (□), and sucrose (■).

monosaccharides. Compared with sucrose, maltose showed a different utilization pattern. Most of the maltose was apparently taken up through a direct transport system without conversion to glucose (data not shown).

Effect of sucrose concentration : The effects of various sucrose concentrations on the cell growth and exopolysaccharide production were investigated. The kinetics of the cell growth and the exopolysaccharide production fitted the equations developed by Weiss and Ollis (1980) (regression coefficient greater than 0.930) : $dX/dt = \mu X (1 - X/Xmax)$ for the cell growth and $dP/dt = m\, dX/dt + n X$ for the exopolysaccharide production. By using these equations, the specific growth rate and the specific production rate of exopolysaccharide were calculated for the various concentrations of sugar from 4 to 12 % (w/v). As shown in Figure 2, both the specific growth rate and

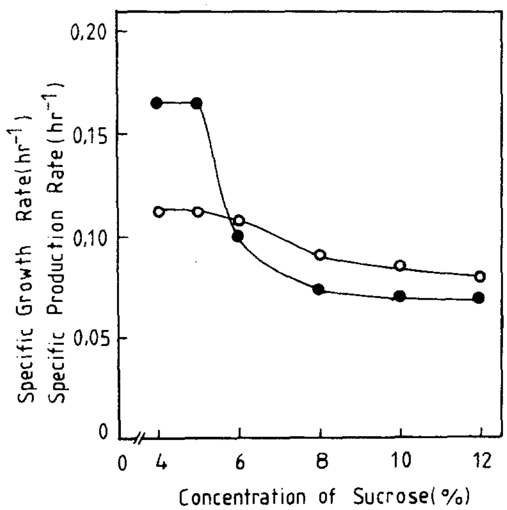

Figure 2. Effect of the various concentrations of sucrose on the specific growth rate (○) and the specific production rate of exopolysaccharide (●).

especially the specific production rate of exopolysaccharide were decreased at sucrose concentrations higher than 5 %. For the utilization of a high concentration of sucrose in a batch without inhibitions of the cell growth and exopolysaccharide production, the application of a fed-batch fermentation or the isolation of an osmotolerant mutant is indicated.

Fed-batch fermentation : As shown in Figure 3, 30 (g/l) and 36 (g/l) of the exopolysaccharide were obtained in batch fermentations, when using 5 % and 10 % sucrose as a carbon source. The utilization of 10 % sucrose by a batch fermentation showed a large decrease in the exopolysaccharide yield as expected from Figure 2. By using a fed-batch fermentation with an initial 5 % of sucrose and two subsequent additions of sucrose, however, 58.0 (g/l) of the polysaccharide was

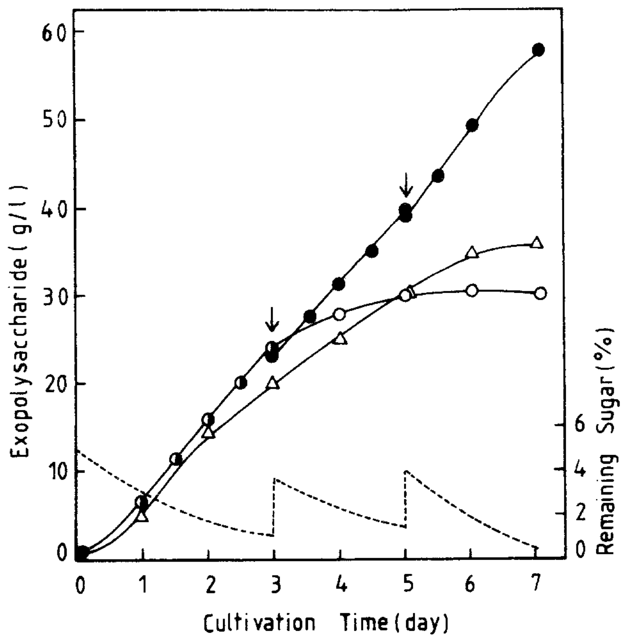

Figure 3. Production of exopolysaccharide by batch and fed-batch fermentations. In batch fermentations, 5 % sucrose(○) and 10 % sucrose (△) were used. In fed-batch fermentation, total 10 % sucrose (●) was used. The dotted line represents the change of sucrose concentration in culture broth and the arrows indicate the addition of 150 ml of sucrose solution (0.5 g/l) in fed-batch fermentation.

obtained. With different feeding modes (intermittent, continuous, exponential), significant differences in polysaccharide production were not observed. By using this fed-batch fermentation, it may be possible to reduce the cost of the precipitation and the recovery of exopolysaccharide.

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ABBREVIATIONS : m, n = relationship parameters for the growth and non-growth associated product formation ; X, Xmax = biomass and maximum biomass concentration (g cell/l) ; P = product concentration (g exopolysaccharide/l) ; μ = specific growth rate of cell (hr^{-1})

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