Optimization of C/N ratio for production of pullulan

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Abstract

The production of pullulan by *Aureobasidium pullulans* HP-2001 was investigated under various ratios of glucose as carbon source and yeast extract as the nitrogen source. Highest conversion rate (productivity) of glucose to pullulan was 40.0% when concentrations of glucose and yeast extract were 5% and 0.15%, respectively. Maximal production of pullulan was 29.3g/l when the concentration of glucose was 8%(w/v) and that of yeast extract was 40:1. On basis of the result that production of pullulan was found in a medium which concentration of glucose as carbon source was up to 20%(w/v), *Aureobasidium pullulans* HP-2001 seemed to overcome the catabolite repression. Conversion rate of pullulan from 20%(w/v) of glucose was 11.1%.

Introduction Pullulan, an α-1,6-linked homopolymer of maltotriose, is produced by *Aureobasidium pullulans*, a member of the *Fungi imperfecti*. *Aureobasidium pullulans* is yeast-like fungus that has been used for industrial production of pullulan from starch substrates. Pullulan possesses distinctive flim and fiber-forming characteristcs which is not founded in amylose. Water-soluble flims can be formed that are impermeable to oxygen ; partial or complete water insolubility may be obtained by controlled esterification or etherification. In this paper, the effect of glucose and yeast extract in medium on growth and production of pullulan was studied.

Material and methods

Bacterial strain *A. pullulans* ATCC 42023 was obtained from the American Type Culture Collection and was transferred monthly to the nutrient agar medium. The medium used for cell growth and exopolymer production contained the following components(g/l): K_2HPO_4, 5.0; NaCl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; $(NH_4)_2SO_4$, 0.6; yeast extract (Difco Lab., Detroit. MI), 2.5. The concentration of

glucose as a carbon source in the main culture was 2%, 5%, 8%, 10%, 15%, 20%(w/v). The pH of medium was adjusted 6.8 to 7.0 before sterilization. Carbon source was autoclaved separately for 15 min at 121℃ and added to the medium under aseptic conditions.

Production of pullulan Starter cultures were prepared by transferring cells from agar slants to 50㎖ medium containing 2%(w/v) glucose in 250㎖ Erlenmeyer flasks. The resulting cultures were incubated for 2days at 30℃ and 200rpm. Each starter culture was used as an inoculum 5%(v/v) for 100㎖ of medium in a 500㎖ Erlenmeyer flasks. The culture were incubated for 5 days under the same condition used to prepare the starter cultures. Samples were periodically withdrawn from the cultures to examine cell growth and pullulan production.

Purification of pullulan Cultured broth after 120 hr was centrifuged at 8000×g for 15 min to remove fungal cells. Supernatant was mixed with 2 vol of isopropyl alcohol and incubated at 4℃ for 24 hr to precipitate the crude product, which were separated by centrifugation at 8000×g for 20 min. the precipitated material was repeatedly washed with acetone and ether, dissolved in deionized water, and dialyzed against deionized water by using dialysis tubing with a molecular weight cut off 12,000 to 14,000. After dialysis for 2 to 3 days with four or five change of deionized water, the solution was lyophilized and the exopolymer yield was determined by weighing. To determine biomass, the cells were washed with distilled water and dried at 100 to 105℃ until the weight was constant.

Results and discussion

The effect of yeast extract as the nitrogen source with 2% glucose as the carbon source on cell growth and production of pullulan was shown in Fig.1. The production of pullulan increased as the concentration of yeast extract increased up to 0.1%(w/v) and the ratio of glucose to yeast extract in the medium was 20:1. The maximal production of pullulan was 5.5g/l and its conversion rate was 27.5%. The effect of yeast extract with 5% glucose as carbon source on cell growth and production of pullulan was shown in Fig.2. The maximal production of pullulan was 19.9g/l and its conversion rate was 40.0%.

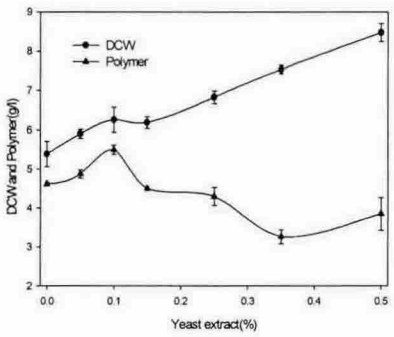

Fig. 1 Effect of yeast extract concentration on the dry cell weight and pullulan productivity of *A.pullulans* in shake flasks (medium contain 2% glucose as carbon source)

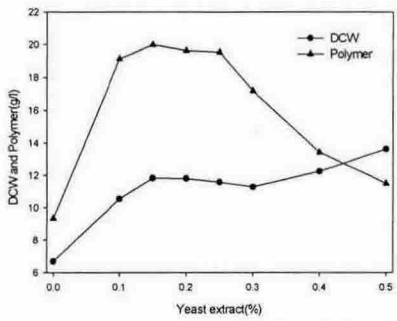

Fig. 2 Effect of yeast extract concentration on the dry cell weight and pullulan productivity of *A.pullulans* in shake flasks (medium contain 5% glucose as carbon source)

The effect of yeast extract with 8% glucose as the carbon source on cell growth and production of pullulan was shown in Fig.3. The maximal production of pullulan was 29.3g/l and its conversion rate was 36.7%. The effect of yeast extract with 10% glucose as the carbon source on cell growth and production of pullulan was shown in Fig.4. The maximal production of pullulan was 26.3g/l and its conversion rate was 26.3%.

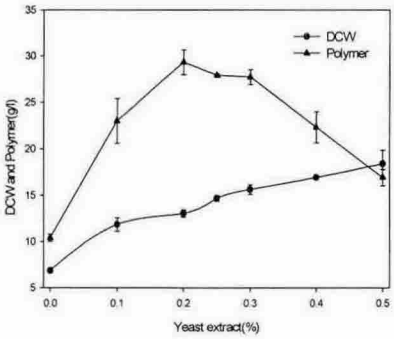

Fig. 3 Effect of yeast extract concentration on the dry cell weight and pullulan productivity of *A.pullulans* in shake flasks (medium contain 8% glucose as carbon source)

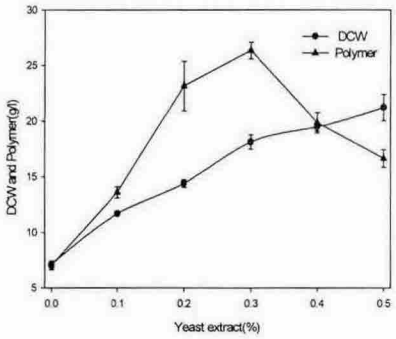

Fig. 4 Effect of yeast extract concentration on the dry cell weight and pullulan productivity of *A. pullulans* in shake flasks (medium contain 10% glucose as carbon source)

The effect of yeast extract with 15% glucose as the carbon source on cell growth and production of pullulan was shown in Fig.5. The maximal production of pullulan was 27.3g/l and its conversion rate was 18.2%.

The effect of yeast extract with 20% glucose as the carbon source on cell growth and production of pullulan was shown in Fig.6. The maximal production of pullulan was 22.1g/l and its conversion rate was 11.1%. The catabolite repression was not shown until 20%(w/v) glucose concentration and ratio of glucose and yeast extract was 40:1 (Table. 1).

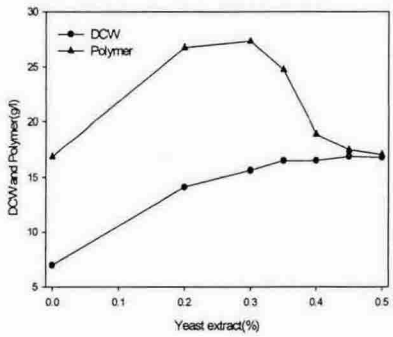

Fig. 5 Effect of yeast extract concentration on the dry cell weight and pullulan productivity of *A. pullulans* in shake flasks (medium contain 15% glucose as carbon source)

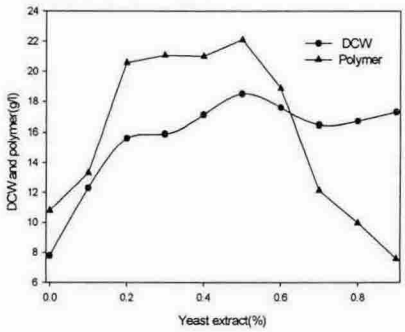

Fig. 6 Effect of yeast extract concentration on the dry cell weight and pullulan productivity of *A.pullulans* in shake flasks (medium contain 20% glucose as carbon source)

From these results, it was concluded that proportional ratio of glucose and yeast extract in the medium was indispensable condition for elaboration of pullulan production.

Table 1. Effect of various ratios of glucose as the carbon source and yeast extract as the nitrogen source on growth and production of pullulan

Glucose (%)	Yeast extract(%)	DCW (g/l)	Pullulan (g/l)	Conversion rate(%)	Specific yield
2	0.10	6.26	5.50	27.5	0.88
5	0.15	11.83	19.98	40.0	1.69
8	0.20	13.02	29.32	36.7	2.25
10	0.30	18.09	26.32	26.3	1.45
15	0.30	15.63	27.30	18.2	1.75
20	0.50	18.50	22.10	11.1	1.19

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