Optimization of culture conditions involved in dissolved oxygen for production of pullulan by *Aureosidium pullulan*

서형필, 손창우, 이유정, 김성구[1], David L. Kaplan[2], 이진우*

Division of Biotechnology, Faculty of Natural Resources & Life Science,
Dong-A University, Pusan 604-714, Korea, Tel (051)200-6995 Fax (051)200-7593
Division of Food & Biotechnology, Pukyung National University, Pusan 608-737, Korea[1]
Biotechnology Center, Tufts University, medford, MA 02155, USA[2]

Abstract

Effect of carbon source and culture conditions involved in the concentration of dissolved oxygen on cell growth and the production of pullulan by *A. pullulans* HP2001 were investigated. Among those carbon sources, glucose was found to be the best carbon source for the production of pullulan by *A. pullulans* HP2001. Maximal production of pullulan by *A. pullulans* HP2001 was 26.6 g/ℓ when concentrations of glucose and yeast extract were 8% (w/v) and 0.25% (w/v), respectively. It was found that aeration rate, agitation speed and inner pressure of a bioreactor, which were some of physiological factors involved in the dissolved oxygen in the medium may affect cell growth and the production of pullulan by *A. pullulans* HP2001.

Introduction

Pullulan, an α-1,6-linked homopolymer of maltotriose, is produced by *Aureobasidium pullulans*[1]. Pullulan possesses distinctive flim and fiber-forming characteristcs which is not founded in amylose. The concentration of dissolved oxygen affect cell growth and the production of pullulan[2]. Physiological factors involved in the concentration of dissolved oxygen in the medium may be aeration rate, agitation speed and inner pressure of a bioreactor. In this study, optimal ratio of carbon to nitrogen source for the production of pullulan by *A. pullulans* HP2001 was established and effect of aeration rate, agitaion speed and inner pressure of a bioreactor was investigated.

Material and methods

Bacterial strain *Aureobasidium pullulans* HP2001 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.

Production of pullulan Starter cultures were prepared by transferring cells from agar slants to 50mℓ medium containing 2% (w/v) glucose in 250mℓ Erlenmeyer flasks. Each starter culture was used as an inoculum 5% (v/v) for 100mℓ of medium in a 500mℓ Erlenmeyer flasks. The culture were incubated for 4 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 96 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.

Analytical methods To determine biomass, the cells were washed with distilled water and dry cell weight (DCW) measured by directly weighing the biomass after drying to constant weight at 100℃ to 105℃. Desalted samples after dialysis were used for quantitation of pullulan. The concentration of pullulan was determined colorimetrically by the phenol-sulfuric acid method. A standard curve for pullulan was prepared from pullulan (Sigma, St. Louis, USA).

Results and discussion

Effect of carbon source for cell growth and the production of pullulan by *A. pullulans* HP2001 was investigated (Table 1). The carbon sources tested in this study were glucose, gluconic acid, glucosamine, fructose, sucrose, maltose, dextrin, starch and cellulose. Among those carbon sources, glucose was found to be the best carbon source for the production of pullulan. Maximal production of pullulan was 5.7 g/ℓ when carbon source was 2% (w/v) glucose and its conversion rate was 0.29. Sucrose was also found to be one of the best carbon source for the production of pullulan by *A. pullulans* HP2001.

Optimal ratio of glucose as the carbon source to yeast extract as the nitrogen source in the medium for the production of pullulan was established in Fig. 1.

Table 1. Production of pullulan by *A. pullulans* in shake-flask culture with different carbon sources

Carbon source[1)]	Pullulan (g/ℓ)	Conversion rate (%)
Glucose	5.7	28.5
Gluconic acid	1.8	9.0
Glucosamine	1.2	6.0
Fructose	3.2	16.0
Sucrose	5.6	28.0
Maltose	2.7	13.5
Dextrin	2.3	11.5
Starch	1.6	8.0
Cellulose	0.8	4.0

1) Each carbon source was 2% (w/v) in the culture medium where 0.25% (w/v) yeast extract served as the nitrogen source.

Concentrations of glucose and yeast extract ranged from 10 to 0% (w/v) and from 0.5 to 0.0% (w/v), respectively. Maximal production of pullulan by *A. pullulans* HP2001 was 26.6 g/ℓ when concentrations of glucose and yeast extract were 8% (w/v) and 0.25% (w/v), respectively whereas maximal conversion rate of pullulan occurred when those were 5% (w/v) and 0.15% (w/v), respectively. Its conversion rate of pullulan from 5% (w/v) glucose was 0.37 with 18.5 g/ℓ of pullulan.

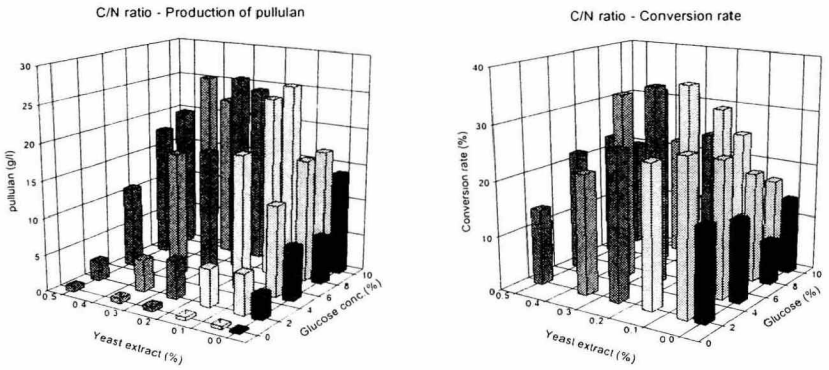

Fig. 1. Production of pullulan with various ratios of glucose and yeast (4 days cultivated at 30℃, 200rpm in shaking incubator)

Effect of aeration and agitation on the production of pullulan with a 7L bioreactor was investigated (Fig. 2). Aeration rate and agitation speed in a 7L bioreator ranged from 1.5 to 0.5 vvm and from 450 to 150 rpm, respectively.

Generally speaking, production of pullulan with relatively high agiation speed was better than low one and higher aeration rate at each a fixed agitation speed showed higher production of pullulan. It seems that higher aeration rate and agitation speed maintain relatively higher concentration of dissolved oxygen in the medium, which enhances the production of pullulan by *A. pullulans* HP2001. Effect of the pressure in a 100L bioreactor on cell growth and the production of pullulan was investigated (Fig. 3). The inner pressure of a 100L bioreactor ranged from 0.8 to 0.0 kgf/cm^2. Production of pullulan increased with increased pressure up to 0.6 kgf/cm^2. Maximal production of pullulan was 28.0 g/ℓ and its conversion rate was 0.35 when the inner pressure of bioreactor was 0.6kgf/cm^2. Whereas production of pullulan was 19.3 g/ℓ without inner pressure. The inner pressure of a bioreactor is one of physiological factors involved in the dissolved oxygen in the medium may affect cell growth and the production of pullulan by *A. pullulans* HP2001

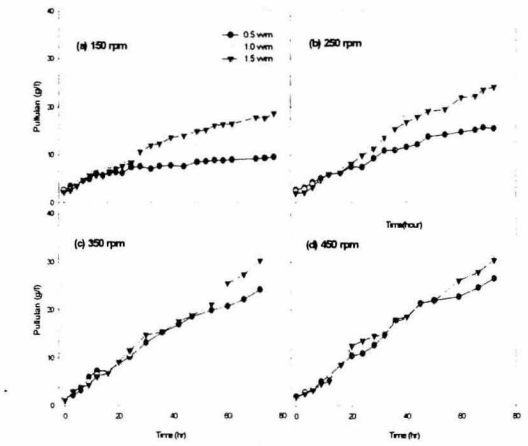

Fig. 2. Effect of aeration and agitation on production of pullulan by *A. pullulans* HP2001

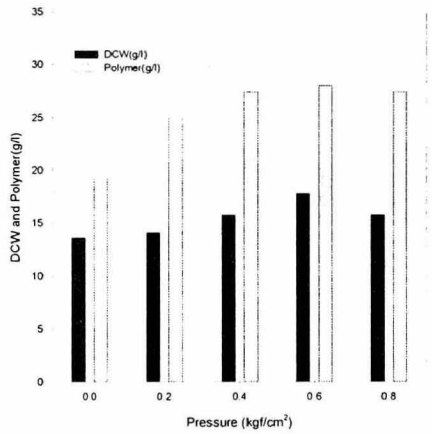

Fig. 3. Effect of inner pressure of bioreactor on cell growth and production of pullulan by *A. pullulans* HP2001

References

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2. Wecker, A., and Onken, U., "Influence of dissolved oxygen concentration and shear rate on the production of pullulan by *Aureobasidium pullulans*"(1991), *Biotech. Lett.*, 13, 155-160.