pH 제어에 의한 전분으로부터 플루란 생산

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Production of pullulan using starch by pH control

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Introduction

Biopolymers are mainly synthesised by plant, animal, and microorganism. Biopolymers that are derived from microorganism are different from natural biopolymer such as derived from plant and seaweeds at physiologial properties. Mass production is available though proper strain selection and a method of cultivation. Biopolymers are utilized at pharmaceutical, food, and chemical industry.

Pullulan is an extracellular water-soluble microbial polysaccharide produced by the yeast-like fungus *Aureobasidium pullulans*. It consists mainly of maltotriose units interconnected via *a* (1-6) linkages. A number of potential applications have been reported for pullulan using transparent, oil resistant, and oxygen impermeable film-forming producties. *A. pullulans* is yeast-like fungus that has been used for industrial production of pullulan from starch. Pullulan may be used as a coating and packing material, a sizing agent for paper and a starch replacer in low-calorie food formulations, cosmetic emulsions, and other medicinal applications.

The effect of cell culture condition on the production of pullulan has been studied widely in respect to pH, DO, temperature, and nitrogen source.

The optimum initial pH was found to maximize the pullulan production. The pH of broth decreased continuously, since cells produces acid by products during fermentation. The production of secondary metabolites such as polysaccharide, amino acids, and proteins is different from cell mass production. The optimal condition for product formation is usually different from that of cell growth. Therefore, it is necessary to control the environmental condition optimal for cell growth and product formation. Since the optimal pH for cell growth is different from that of product formation, the systematic approach is production with *A. pullulans*.

In this study, the effects of pH on pullulan fermentation and the molecular weight distribution of pullulan during the fermentation were evaluated for determining the optimum fermentation condition for pullulan production.

Materials and methods

Micro-organism and cultivation medium : *Aureobasidium pullulans* ATCC 9348 was used as fermentation organism in this study. The strain was stored at 4℃ on PDA slant (Difco, USA).

The standard cultivation medium contained (g/L) : Starch soluble, 50; K_2HPO_4, 0.5; NaCl, 0.1; $MgSO_4 \cdot 7H_2O$, 0.02; $(NH_4)2SO_4$, 0.06; yeast extract(Difco), 0.1. The pH of medium was adjusted 7.5 before sterilization. The medium was autoclaved for 15min at 121℃.

Inoculum preparation and fermentation : The inoculum was grown for 48hr at 25℃ and 200rpm in a shaking incubator (New Bruswick Scientific Co., USA). Each culture was used as an inoculum for 200mL of medium in a 1000mL erlenmeyer flasks. Fermentations were carried out in a 4.5L jar fermenter (KFC, Korea). After every 12hr, fermentation broth from the fermenter was sampled and analysed.

pH control : The pH of fermentation broth was controlled with 0.2N HCl and 0.2N NaOH, by P-mode. The error range of pH controlled was ±0.1.

Measurement procedure : Cell growth was monitored by measuring the absorbance at 660nm using UV-spectrophotometer (Duksan, Korea). The dry cell biomass weight was expressed as g/L. Cultured broth was centrifuged at 8000rpm for 20min. to separate fungal cells. Pullulan was obtained from fermentation solution by isopropyl alcohol precipitation (1.5 volumes). The precipitate obtained was filtered through a pre-weighed Whatman No.1 filter and then dried at vaccum oven(20hr, 80℃). The dry weight of pullulan was expressed as g/L and pullulan yield was expressed as gram polysaccharide per 100g of starch consumed.

Determination of molecular weight : The average molecular weight of pullulan samples were determined by the gel permeation chromatography (Rainin 200) equipped with RI detector (Viscotek 250). The mobile phase was NaNO3 0.1M aqua on 0.5mL/min.

Results and discussion

pH shift : Cell growth, pullulan production, and molecular weight of pullulan produced from *A. pullulans* were determined at the various conditions with pH in batch cultivation. In the case of pH uncontrolled, the fermentation broth with initial pH 7.5 went down to pH 4.5 and another with initial pH 6.0 was descended to pH 2.9. Hence, pH condition was under control between 7.5 and 3.0 at intervals of 1.5.

Cell growth : The cell growth and pullulan yields were associated with the utilization of starch in the medium. Most starch was consumed and convert to pullulan for 72hr in 144hr cultivation as shown in Figure 1. The cell growth and pullulan increased steadily at the logarithm curve till 72hr in each case. The dry cell weight was maximum of 26.3g/L at pH 7.5 controlled and minimum of 13.7g/L at pH 3.0 controlled. The cell was supposed to grow well between natural pH and pH 6.0.

Pullulan and its molecular weight : The time course studies on the production of pullulan by *A. pullulans* were made for a period of 72hr in the fermentation medium by using starch. The maximum yield of pullulan was observed after 96hr. *A. pullulans* was able to produce the highest concentration of pullulan within 72-96hr of cultivation. The highest amount of pullulan was 20.6g/L wherein the initial pH of 7.5 decreased to 4.5. On the contrary, the pullulan production was extremely low reaching a concentration of 6g/L at pH 7.5 controlled. The highest molecular weight pullulan 1.6×10^6 was produced at the lowest initial pH of 3.0 and

the minimum molecular weight of pullulan was 0.2×10^6 at pH 7.5 controlled. The Table 1 is shown up that high molecular weight pullulan over 1.0×10^6 is produced under pH 6.0

Therefore, economically optimum condition for the production of the high molecular weight pullulan around 1.0×10^6 is pH 7.5 uncontrolled for 96hr.

Table 1. Changes of the molecular weight of pullulan at various culture pHs

Culture pH	Dry cell (g/L)	Pullulan (g/L)	Pullulan yield (%)	Molecular Weight
pH 7.5, uncontrolled	17.5	20.6	41.2	1.1×10^6
pH 6.0, uncontrolled	24.6	16.5	33.0	1.4×10^6
pH 7.5, controlled	26.3	6	12.0	0.2×10^6
pH 6.0, controlled	19.2	17.5	35.0	0.8×10^6
pH 4.5, controlled	15.4	13.8	30.2	1.2×10^6
pH 3.0, controlled	13.7	9.6	23.2	1.6×10^6

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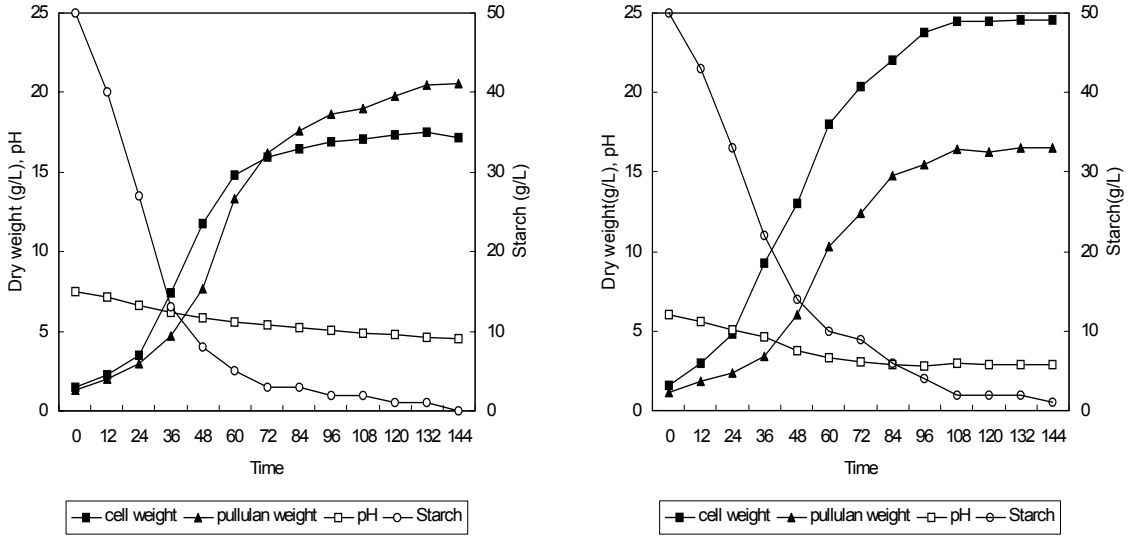

Figure 1. Time course of pullulan fermentation
(Left : pH 7.5 uncontrolled, Right : pH 6.0 uncontrolled)

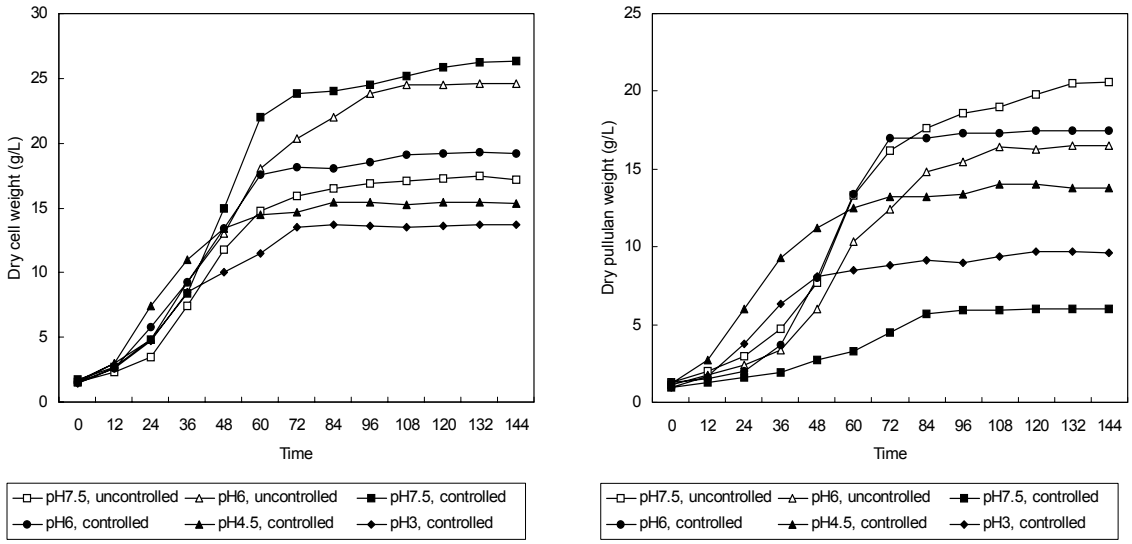

Figure 2. Effect of pH on the cell growth (Left) and pullulan production (Right)