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Short communication

Effect of two-stage controlled pH and temperature on pullulan production by *Auerobasidium pullulans*

Zhenqiang Xia, Shengjun Wu*, Saikun Pan*

School of Food Engineering, HuaiHai Institute of Technology, 59 Cangwu Road, Lianyungang 222005, China

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ABSTRACT

Effect of two-stage controlled pH and temperature on cell growth and pullulan production by *Auerobasidium pullulans* was investigated. Lower pH and higher temperature supported cell growth of *A. pullulans*, while higher pH and lower temperature stimulate pullulan synthesis. Maximum pullulan production (39 g/L) was obtained at initially lower pH (2.5) and higher temperature (32 °C), subsequently higher pH (5.5) and lower temperature (26 °C), and higher than those achieved at uncontrolled pH (initial pH 5.5) with two-stage temperature (initially 32 °C and subsequently 26 °C), or two-stage pH (initially pH 2.6 and subsequently 5.5) with constant temperature 26 °C. Results indicate that combination of two-stage controlled pH and temperature support mass production of pullulan.

1. Introduction

Pullulan, a linear mixed linkage α-D-glucan consisting mainly of maltotriose repeating units interconnected by α-(1 → 6) linkages, is an exocellular polysaccharide produced by *Aureobasidium pullulans*. A high value is placed on pullulan as it may be used as a coating and packaging materials, a sizing agent for paper, a starch replacement in low-calorie food formulations, in cosmetic emulsions, and in other industrial applications (Singh, Saini, & Kennedy, 2008).

Culture pH and incubation temperature play pivotal roles on pullulan production (Lacroix, LeDuy, Noel, & Choplin, 1985; McNeil & Kristiansen, 1990; Wu, Chen, Jin, & Tong, 2010). Based on the fact that lower pH of the culture medium is benefit to cell growth of *A. pullulans*, while higher pH of the culture medium supports mass pullulan production, a bistaged pH fermentation process for the production of pullulan was investigated (Lacroix et al., 1985). Similarly, a two-stage temperature fermentation process for pullulan production was established as a result of the fact that higher temperature stimulates cell growth of *A. pullulans*, while lower temperature is suitable for mass pullulan production. However, so far, the effect of combination of two-stage controlled pH and temperature on cell growth and pullulan production was not reported.

In this study, the effect of combination of two-stage controlled pH and temperature on pullulan production was investigated, and the related conditions were optimized.

2. Materials and methods

2.1. Microorganism

A. pullulans AP329, kindly supplied by Professor Qunyi Tong, School of Food Science and Technology, Jiangnan University, was used. Stock cultures were maintained on potato dextrose agar at 4 °C and subcultured every 2 weeks.

2.2. Preparation of medium

The medium contained: 50 g sucrose, 2.0 g yeast extract, 5.0 g K_2HPO_4, 0.6 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4{\cdot}7H_2O$, and 1.0 g NaCl in 1 L distilled water. The pH was adjusted to 5.5, and the medium was autoclaved at 121 °C for 15 min.

2.3. Fermentation

Seed culture was prepared by inoculating cells grown on a potato dextrose agar slant into a 250-mL flask that contained 50 mL of the medium and subsequently incubated at 26 °C for 2 days with shaking at 200 rpm. A 5-L stirred tank fermentor (5M-2002, Shanghai Baoxing Bio-engineering Equipment Co., China) with a working volume of 3 L was used for the production of pullulan in batch culture. Fermentor was consisted of a glass vessel with stainless-steel endplates and three equally spaced vertical baffles. Agitation was provided by a six-flat-blade impeller (diameter 4 cm) located 3 cm above the bottom of the vessel. The fermentor was sterilized at 121 °C for 15 min. After cooling, 3 L of production medium was

* Corresponding authors. Tel.: +86 0518 8589547; fax: +86 0518 85895428.
E-mail addresses: wushengjun008@sina.com (S. Wu), pskgx@163.com (S. Pan).

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added into the fermentor. The medium was inoculated with 150 mL inoculum. The fermentor was incubated at 32 °C initially for 18 h and subsequently at 26 °C for 30 h in a thermostated chamber. The impeller speed was 800 rpm and the sterile air flow 4 L/min. The pH was controlled at 2.5 initially for 18 h and subsequently at 5.5 for 30 h by feeding with either 2 M NaOH or 2 M HCl.

2.4. Isolation and purification of pullulan

The culture was centrifuged at 15,000 × *g* for 20 min to remove the microorganisms. After the supernatant was decanted, cells were dried at 80 °C for 2 h and weighed. An aliquot (3 mL) of the supernatant was transferred into a test tube, and then mixed thoroughly with 6 mL cold ethanol. The prepared mixture was left in a refrigerator (4 °C) for 12 h to precipitate the exocellular polysaccharide. Residual ethanol was removed carefully, then 3 mL deionized water was added and the mixture was heated to 80 °C in a water bath to dissolve the precipitate. The solution was dialyzed against deionized water for 48 h to remove small molecules. The polysaccharide was reprecipitated by adding 6 mL cold ethanol, and was recovered by filtering the mixture through pre-weighed Whatman GF/A filter paper. The filter paper with the recovered precipitate was dried at 80 °C to a constant weight (Badr-Eldin, El-Tayeb, El-Masry, Mohamad, & El-Rahman, 1994). The pullulan content of the ethanol precipitate was determined using the coupled-enzyme assay technique described by Israilides, Bocking, Smith, and Scanlon (1994). Cell biomass and pullulan content were expressed as g/L.

3. Results and discussion

3.1. Effect of controlled pH on cell growth and pullulan production

The pH of fermentation media can influence the morphology of the *A. pullulans* AP329, which may subsequently influence cell growth and pullulan production (Cately, 1979). Therefore, it was of our interest to investigate the effects of different pH values ranging from 1.5 to 6.5 in the media on cell growth and pullulan production by the microorganism. *A. pullulans* AP329 grew optimally at lower pH (1.5–3.5), and maximum biomass was obtained at pH 2.5, while maximum pullulan was observed at pH 5.5 (Fig. 1). These results are similar to the report by Lacroix et al. (1985). In contrast, other reports have described optimal pH for pullulan production at 5.0 (Cheng, Demirci, & Catchmark, 2010) and 6.0 (Lee & Yoo, 1993). The different optimum pH conditions reported in the literature may be due to the differences in the types of strain, composition of fermentation medium, and culture conditions used. Results indicated that the optimal pH for cell growth is not in accordance with that of pullulan production.

3.2. Effect of temperature on cell growth and pullulan production

As shown in Fig. 2, optimum cell growth of *A. pullulans* AP329 was observed at 32 °C, while the highest amount of pullulan was obtained at 26 °C. It also can be noticed that pullulan production decreased sharply when the fermentation temperature was higher than 26 °C and this observation was in agreement with those reported by Wu et al. (2010). This means that pullulan production by *A. pullulans* AP329 was sensitive to higher temperature. In contrast, other reports have described optimal conditions for pullulan production at temperature 20 °C (Roukas & Biliaderis, 1995) and 24 °C (McNeil & Kristiansen, 1990). The different optimal temperature conditions reported in the literature may be also due to the differences in the types of strain, composition of fermentation medium, and culture conditions used. Therefore, the optimal temperature of cell growth is also not in accordance with that of pullulan production.

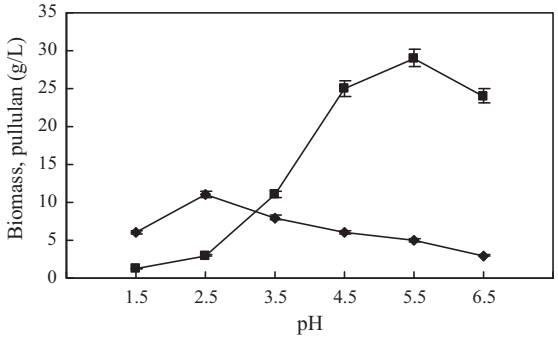

Fig. 1. Effect of controlled pH on cell growth (♦) and pullulan production (■). Fermentation conditions: temperature, 26 °C; time, 48 h. Data are shown as mean ± SD (n = 3).

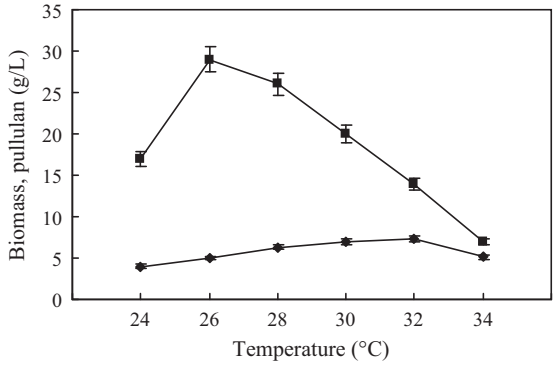

Fig. 2. Effect of temperature on cell growth (♦) and pullulan production (♦). Fermentation conditions: controlled pH 5.5; time 48 h. Data are shown as mean ± SD (n = 3).

3.3. Effect of two-stage controlled pH and temperature on cell growth and pullulan production

Based on the fact that optimal pH and temperature of cell growth is not in accordance with that of pullulan production, the effect of two-stage pH and temperature on cell growth and pullulan production was investigated. Three types of two-stage fermentation were designed to be carried out: The pH and the temperature was adjusted to 2.5 and 32 °C, respectively, for the first stage and then changed to 5.5 and 26 °C, respectively at 12 h (type 1, Fig. 3a), 18 h (type 2, Fig. 3b), and 24 h (type 3, Fig. 3c) for the second stage. Maximal pullulan production was obtained after 48 h for all the two-stage types of fermentation and was in accordance with that of one-stage fermentation (at controlled pH and temperature of 26 °C for 48 h) (Fig. 3). Of all the three two-stage types of fermentation, the type 2 of fermentation stimulated the highest yield of pullulan (39 g/L) and was 25.80% (w/w) and 18.18% (w/w) more than those achieved at two-stage pH (initially pH 2.6 and subsequently 5.5,) with constant temperature 26 °C (fermentation time: 48 h), and uncontrolled pH (initial pH 5.5) with two-stage temperature (initially 32 °C and subsequently 26 °C; fermentation time: 48 h). In the case of cell growth, the biomass continued to increase up until the end of the experimental period for the three types of two-stage fermentation (Fig. 3).

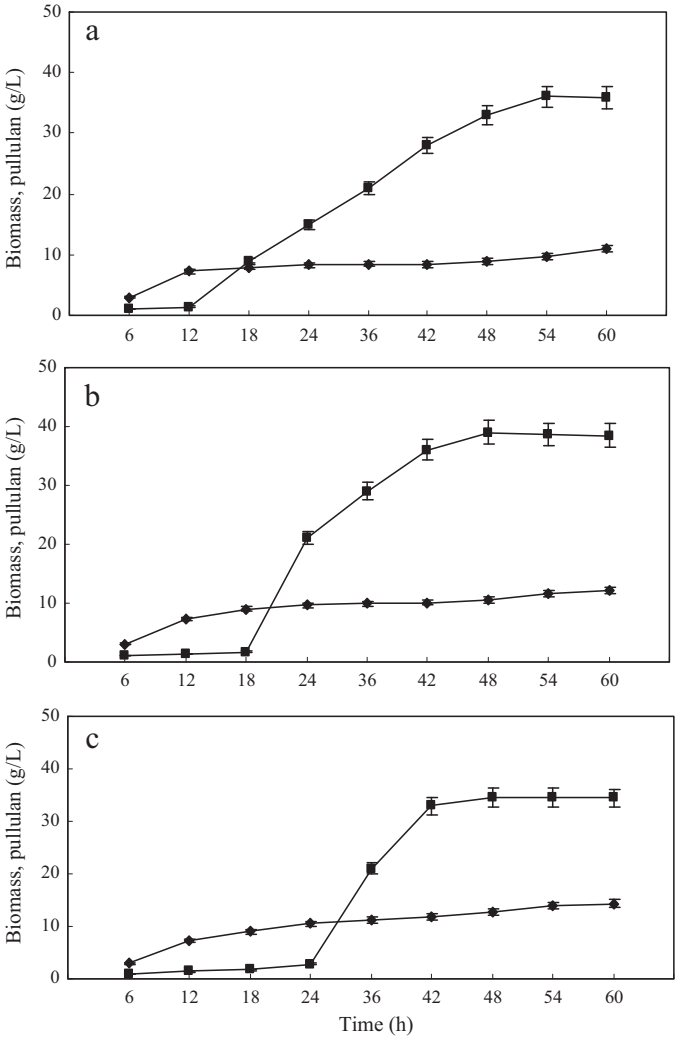

Fig. 3. Effect of two-stage of pH and temperature on cell growth (♦) and pullulan production (♦). Fermentation conditions: The pH and the temperature was adjusted to 2.5 and 32 °C, respectively, for the first stage and then changed to 5.5 and 26 °C, respectively at 12 h (a), 18 h (b) and 24 h (c) for the second stage. Data are shown as mean ± SD (n = 3).

4. Conclusions

A. pullulans AP329 grew well at lower pH of 2.5 and higher temperature of 32 °C, but did not synthesize pullulan well under this condition. A two-stage controlled pH and temperature process for the production of pullulan has been developed based on this study, and pullulan production significantly increased––compared to two-stage controlled pH (initially pH 2.6 and subsequently 5.5) with constant temperature 26 °C, and two-stage temperature (initially 32 °C and subsequently 26 °C) with uncontrolled pH (initial pH 6.5).

References

Badr-Eldin, S. M., El-Tayeb, O. M., El-Masry, E. G., Mohamad, O. A., & El-Rahman, O. A. A. (1994). Polysaccharide production by *Aureobasidium pullulans*: factors affecting polysaccharide formation. *World Journal of Microbiology and Biotechnology*, *10*, 423–426.

Cately, B. J. (1979). Pullulan synthesis by *Aureobasidium pullulans*. In R. C. W. Berkeley, G. W. Gooday, & D. C. Elwood (Eds.), *Microbial Polysaccharides and Polysaccarases* (pp. 69–84). New York: Academic Press.

Cheng, K. C., Demirci, A., & Catchmark, J. M. (2010). Effects of plastic composite support and pH profiles on pullulan production in a biofilm reactor. *Applied Microbiology and Biotechnology*, *86*, 853–861.

Israilides, C., Bocking, M., Smith, A., & Scanlon, B. (1994). A novel rapid coupled enzyme assay for the estimation of pullulan. *Biotechnology and Applied Biochemistry*, *19*, 285–291.

Lacroix, C., LeDuy, A., Noel, G., & Choplin, L. (1985). Effect of pH on the batch fermentation of pullulan from sucrose medium. *Biotechnology and Bioengineering*, *27*, 202–207.

Lee, K. Y., & Yoo, Y. J. (1993). Optimization of pH for high molecular weight pullulan. *Biotechnology Letters*, *15*, 1021–1024.

McNeil, B., & Kristiansen, B. (1990). Temperature effects on polysaccharide formation by *Aureobasidium pullulans* in stirred tanks. *Enzyme and Microbial Technology*, *12*, 521–526.

Roukas, T., & Biliaderis, C. G. (1995). Evaluation of carob pod as a substrate for pullulan production by *Aureobasidium pullulans*. *Applied Biochemistry and Biotechnology*, *55*, 27–44.

Singh, R. S., Saini, G. K., & Kennedy, J. F. (2008). Pullulan: Microbial sources, production and applications. *Carbohydrate Polymers*, *73*, 515–531.

Wu, S. J., Chen, H. Q., Jin, Z. Y., & Tong, Q. Y. (2010). Effect of two-stage temperature on pullulan production by *Aureobasidium pullulans*. *World Journal of Microbiology and Biotechnology*, *26*, 737–741.