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Kinetic Studies on Production of Pullulan by *Aureobasidium pullulans*

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Abstract Kinetic studies on the production of pullulan by *Aureobasidium pullulans* using batch culture in a 15L bioreactor were carried out. The mathematical models were obtained in this study, which provided a reasonable description for the biomass, the product, and the substrate variation with time. The values from the mathematical models were satisfactorily coincided with the experimental data for the biomass of *A. pullulans*, the production of pullulan, and the utilization of sucrose as the sole carbon source.

Key words: Pullulan, *Aureobasidium pullulans*, Kinetics

Introduction

Polysaccharides are a group of macromolecules with diverse applications in the food chemical, energy production, and pharmaceutical industries [1,2]. Substantial numbers of important polysaccharides are currently obtained by microbial fermentations or extraction from natural resources. For the large scale of productions, the fermentation process is always the most attractive alternative because it can assure reproducibility and supply [3].

Pullulan has various potential applications in the cosmetic and diet food industry as a biodegradable plastic [4,5]. Due to its capability to form strong resilient film with diverse shapes, pullulan can be used in place of wraps and plastic [6]. It is anticipated that pullulan has the potential to develop into a significant market. Important parameters for the production of pullulan are carbon [7,8] and nitrogen sources [9,10], temperature [11], initial pH of medium [12], and oxygen supply [13]. Previous studies for the production of pullulan were restricted to optimize physiological conditions with the small scale of experiment [8].

To optimize the fermentation process for the mass production of pullulan, this study is focused on the kinetic study of pullulan, an interesting microbial polysaccharide synthesized by *A. pullulans*. In this study, the logistic equation has also been attempted for describing the growth of *A. pullulans*. The results of the process simulation, derived from the proposed and literature models with the experimental data, were compared and discussed [14,15].

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MATERIALS AND METHODS

Microorganism and media

Aureobasidium pullulans ZQ-01 (kept in this laboratory) was maintained at 4℃ on agar medium of the following composition, in (g/L): glucose, 50; potato extract, 0.6 and agar, 20. The medium used for cell growth and the production of pullulan contained the following components (g/L): sucrose, 50; $(NH_4)_2SO_4$, 0.6; K_2HPO_4, 5; $MgSO_4$, 0.1; NaCl, 1 and yeast extract, 0.6. The pH of medium was adjusted to 6.5 using concentrated HCl before sterilization.

Production of pullulan

Starter cultures were prepared by transferring cells from agar slants to 50mL medium in 250mL Erlenmeyer flasks. The resulting cultures were incubated for 36 h at 28℃. Of the resulting liquid 2 mL was then transferred into a fresh medium (150 mL in a Erlenmeyer flask) and incubated for 24 h at 28℃. The inoculum size used for shake flask and fermentor cultivations was 4% and 5%, respectively.

Experiments were conducted in a 15 L fermentor (Biostat C 10-3, Germany) with working volume of 9 L. The fermentor was equipped with three of six-bladed impellers and agitation speed of 400 rpm. All batch fermentations were conducted at 28℃ and 1.0 vvm. The Erlenmeyer flasks and the fermentor were sterilized at 121℃ for 20 min, respectively.

Analytical methods

Samples were periodically withdrawn from the cultures to examine cell growth and the production of pullulan. To determine biomass, the culture broth were centrifuged at 6,000 ×g for 20 min. the cells were washed twice with distilled water and placed in a preweighed aluminum dish and dried

at 80℃ to constant weight.

The recovered supernatant was then used for determination of the polysaccharide. The crude polysaccharide was precipitated with the addition of two volumes of ethanol into the supernatant. The precipitate was transferred to the preweighted aluminum dish and dried to a constant weight in an oven at 80℃. After removing cells, the culture broth was used to determine the concentration of dextrose by phenol-sulfuric acid method. The glucose was used as the standard material to construct the calibration curve.

RESULTS AND DISCUSSION

Cell growth and the production of pullulan

Cell growth and the production of pullulan with time were shown in Fig. 1. After a lag phase of about 5 h, the concentration of biomass rapidly increased to a maximum of 16 g/L. At the same time, the concentration of pullulan also increased rapidly. As expected, the concentration of sucrose decreased drastically with the growth of *A. pullulans* and the production of pullulan. The yield of pullulan was determined to be 16 g/L.

Kinetics for the growth of *A. pullulans*

The logistic equation was used to represent the experimental data obtained in this study. The logistic equation is as follows:

$$\frac{dC_x}{dt} = \alpha \cdot C_x \cdot (1 - \frac{C_x}{\beta}) \tag{1}$$

$$C_x(t) = \frac{C_{x0} \cdot e^{\alpha t}}{1 - (\frac{C_{x0}}{\beta}) \cdot (1 - e^{\alpha t})} \tag{2}$$

The model parameters α, β were evaluated by solving equation (2), where α=0.1298 (h^{-1}), β=16.2773 (g/L). The model for the growth of microorganisms is as follows:

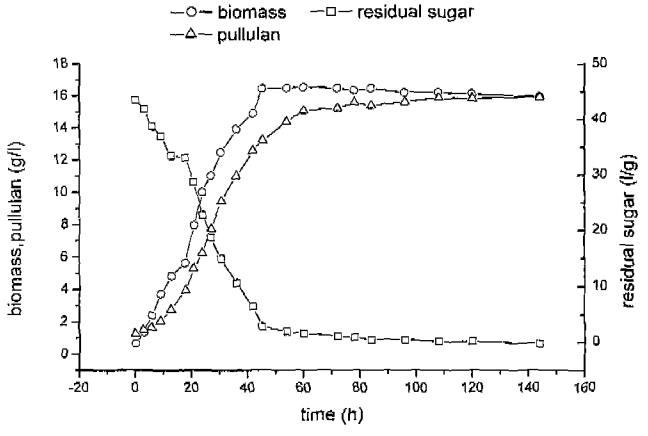

Fig. 1. Cell growth and the production of pullulan by *A.pullulans* with batch fermentation process

$$C_x(t) = \frac{C_{x0} \cdot e^{0.1298t}}{1 - (\frac{C_{x0}}{16.2773}) \cdot (1 - e^{0.1298t})} \tag{3}$$

The experimental data was compared with the theoretical vales (Fig. 2). The relative error between them was calculated as 3.3%. There was a satisfying agreement between the model and experimental data.

Kinetics for the yield of pullulan

According to the course of batch fermentation of *A. pullulans*, the following equation was used:

$$\frac{dC_p}{dt} = Y_{p/x} \frac{dC_x}{dt} \tag{4}$$

Combined with the equation (2), the function of concentration of pullulan (C_p) was as follows:

$$C_p(t) = C_{p0} + Y_{p/x}(C_x(t) - C_{x0}) \tag{5}$$

$$C_p(t) = C_{p0} + Y_{p/x} \cdot C_{x0} \left[\frac{e^{\alpha t}}{1 - \left(\frac{C_{x0}}{\beta}\right) \cdot \left(1 - e^{\alpha t}\right)} - 1 \right] \tag{6}$$

The model for te production of pullulan is determined as the equation (7):

$$C_p(t) = C_{p0} + 0.8797 \cdot C_{x0} \left[\frac{e^{0.1298t}}{1 - \left(\frac{C_{x0}}{16.2773}\right) \cdot \left(1 - e^{0.1298t}\right)} - 1 \right] \tag{7}$$

The experimental data was compared with the calculated data from the model equation in Fig. 3. The relative error between them was calculated as 0.4%.

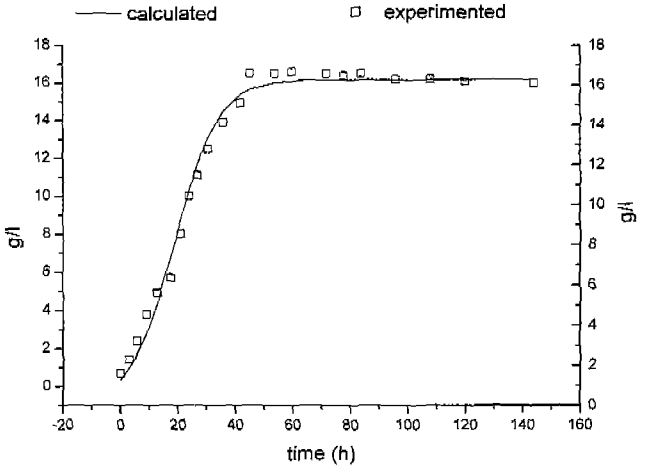

Fig. 2. Comparison between the model and experimental data for growth of microorganisms

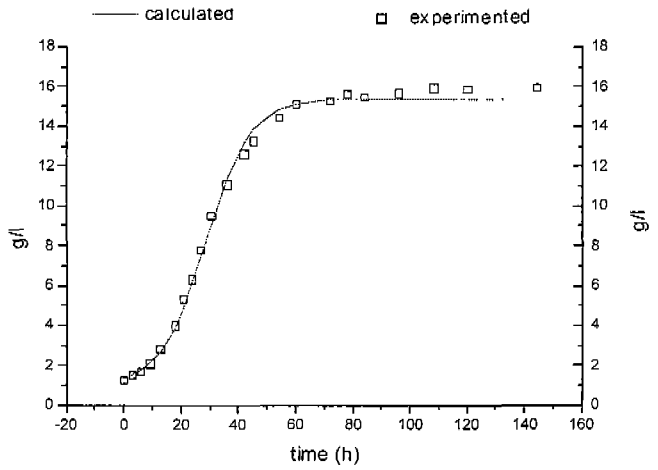

Fig. 3. Comparison between the model and experimental data for the production of pullulan

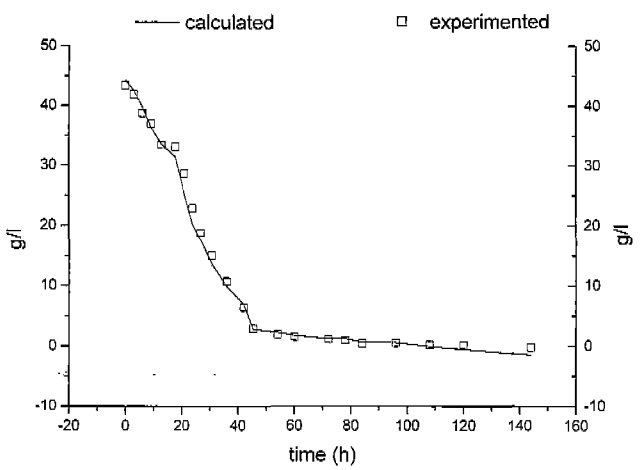

Fig. 4. Comparison between the model and experimental data for the utilization of substrate

Kinetics for utilization of substrate

The kinetics for the utilization of substrate was related to the growth of *A. pullulans* and the production of pullulan, so the concentration of substrate could predict as follows:

$$-\frac{dC_s}{dt}=\frac{1}{Y_{x/s}}\cdot\frac{dC_x}{dt}+m\cdot C_x+\frac{1}{Y_{p/s}}\cdot\frac{dC_p}{dt} \quad (8)$$

Combined with the equation (4),

$$-\frac{dC_s}{dt}=\frac{1}{Y_{x/s}}\cdot\frac{dC_x}{dt}+m\cdot C_x+\frac{1}{Y_{p/s}}\cdot Y_{p/x}\frac{dC_x}{dt} \quad (9)$$

$$C_s(t)=C_{s0}-\delta[C_x(t)-C_{x0}]-\gamma\{(\beta/\alpha)\ln[1-(C_{x0}/\beta)(1-e^{\alpha t})]\} \quad (10)$$

The model parameters δ, γ = 0.0035 could be obtained by nonlinear estimate. The function of the concentration of substrate was as follows:

$$C_s(t)=C_{s0}-2.5263[C_x(t)-C_{x0}]-0.4389\ln[1-(C_{x0}/16.2773)(1-e^{0.1298t})] \quad (11)$$

The concentration of substrate from the model and experimental data was compared in Fig. 4. The relative error between them was calculated as 3.0%.

Similar to the production of other polysaccharides, the culture broth of *A. pullulans* showed typical characteristic of the non-Newtonian liquid. Due to increased cell growth and the production of pullulan with time, the viscosity of the culture broth increased. It seemed to result in inhibition of the oxygen transfer and the cell growth on basis of the experimental data and the constructed mathematical model. In this study, the logistic models used to predict cell growth, the production of pullulan, and the utilization of sucrose by *A. pullulans* with batch fermentation were obtained.

NOMENLATURE

C_p —— concentration of pullulan (g/L)
C_s —— concentration of substrate (g/L)
C_x —— concentration of microorganisms (g/L)
$Y_{x/s}$ —— yield of biomass based on substrate
$Y_{p/x}$ —— yield of product based on biomass
$Y_{p/s}$ —— yield product based on substrate
α, β —— constant in equation (2)
δ, γ —— constant in equation (10)

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