Cephalosporin C 발효공정의 Cybernetic Modeling

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Cybernetic Modeling of the Cephalosporin C Fermentation Process

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Introduction

Cephalosporin C is a β-lactam antibiotic synthesized as a secondary metabolite by strains of a strictly aerobic fungus *Cephalosporium acremonium*. This natural antibiotic is modified through chemical or enzymatic methods to produce different semi-synthetic cephalosporins, which are of major importance in the pharmaceutical market. In a chemically defined medium, with glucose and sucrose as major carbon sources, *C. acremonium* exhibits a biphasic growth due to successive utilization of the two carbon sources. The cells consume only the glucose as long as it is present in the system. After glucose is completely used, sucrose becomes the carbon source for cell growth and maintenance. This is a typical diauxic phenomenon, discovered by Monod, which is a well-known example of sequential utilization of two carbon substrates, with an intermediate lag phase between the two growth phases. It has been reported, on the other hand, that *C. acremonium* differentiates morphologically during fermentation. Its morphological types are classified principally into three groups: filamentous hyphae, swollen hyphal fragments and arthrospores. The production of cephalosporin C is started as soon as the glucose is depleted and the production phase is maintained while fragmenting the swollen hyphal fragments into arthrospores. Eventually, the production is ceased when swollen hyphal fragments has been completely differentiated into arthrospores.

The growth and production behavior of this process is quite complex and many researchers attempted to model the behavior in *ad-hoc* manner. Recently, it has been reported that the cybernetic modeling framework based on the hypothesis that microorganisms optimize utilization of available substrates to maximize their growth rate at all times would be appropriate to describe the complex diauxic behaviors.

In this work, a mathematical model of cybernetic viewpoints has been developed to describe the fermentation process of *C. acremonium*, resulting in a useful tool for studies in enhancing yield of cephalosporin C. With this model, the specific growth rates for different metabolic pathways are modeled according to a modified Monod rate equation, where the modification consists in the fact that each growth rate has been assumed proportional to the corresponding intracellular key enzyme

concentration. The optimal strategies for the synthesis and activity of the key enzyme are regulated by two cybernetic variables. And the morphological differentiation of cells is formulated by assuming that its rate is influenced by genetic factors and environmental conditions.

Mathematical Model

Morphological differentiation, characterized by three cell types, filamentous hyphae (X_h), swollen hyphal fragments (X_s), and arthrospores (X_a), was assumed to progress irreversibly in the following sequence.

$$X_h \rightarrow X_s \rightarrow X_a$$

The morphological differentiation should be considered since it is closely associated with the antibiotic production. The rate of differentiation of X_h into X_s, f_h, was formulated by assuming that differentiation of X_h is repressed by existence of glucose.

$$f_h = \delta_{h1} + \delta_{h2} \frac{K_{f_1}}{K_{f_1} + s_1}$$

And the rate of differentiation of X_s into X_a, f_s, was formulated by assuming that formation of X_a is enhanced by depletion of substrates.

$$f_s = \delta_{s1} + \delta_{s2} \frac{K_{f_2}}{K_{f_2} + s_1 + s_2}$$

It is assumed that substrates, glucose (s_1) and sucrose (s_2), are consumed by X_h and X_s. And their growth rates on glucose and sucrose, r_1 and r_2, are modeled according to the modified Monod rate equation, with the simple modification being that each growth rate is proportional to the intracellular concentration of e_i for a key enzyme controlling each pathway.

$$r_i = \mu_i e_i \frac{s_i}{K_i + s_i} \qquad \text{for } i = 1,2$$

Synthesis rates of the key enzymes are also modeled according to the Monod rate equation.

$$r_{e_i} = \alpha_i \frac{s_i}{K_{e_i} + s_i} \qquad \text{for } i = 1,2$$

The cybernetic modeling framework replaces the detailed modeling of regulatory processes with cybernetic variables u_i and v_i representing the optimal strategies for enzyme synthesis and activity, respectively. For the instantaneous growth rate r_i values along the two available pathways, the optimal strategies for u_i and v_i have been shown as:

$$u_i = \frac{r_i}{\sum_j r_j} \qquad \text{for } i = 1,2$$

$$v_i = \frac{r_i}{\max_j r_j} \qquad \text{for } i = 1,2$$

Simultaneous ordinary differential equations were derived for microbial growth which take into account morphological differentiation, consumption of glucose and sucrose, and synthesis of the key enzymes.

$$\frac{dX_h}{dt} = (r_1 v_1 + r_2 v_2) X_h - f_h X_h - k_{D,h} X_h$$

$$\frac{dX_s}{dt} = f_h X_h + (r_1 v_1 + r_2 v_2) X_s - f_s X_s - k_{D,s} X_s$$

$$\frac{dX_a}{dt} = f_s X_s - k_{D,a} X_a$$

$$\frac{ds_i}{dt} = -\frac{r_i v_i}{Y_i}\left(X_h + X_s\right) - m_i r_i v_i \left(X_h + X_s\right) \qquad \text{for } i = 1, 2$$

$$\frac{de_i}{dt} = r_{e_i} u_i - \left(r_1 v_1 + r_2 v_2 + \beta_i\right) e_i + \alpha_i^* \qquad \text{for } i = 1, 2$$

For the production phase, it was assumed that, among three cell types, only X_s is able to produce the enzyme (e_3) of the antibiotic biosynthetic pathway that are crucial to production of cephalosporin C.

$$\frac{de_3}{dt} = \alpha_3 \frac{K_{e_3}}{K_{e_3} + s_1} X_s - \beta_3 e_3$$

And the production rate of cephalosporin C was assumed to be proportional to the concentration of the key enzyme, but to be repressed when the concentration of cephalosporin C, P, is increased over a certain critical value.

$$\frac{dP}{dt} = \frac{1}{Y_3} \mu_3 e_3 \frac{K_3}{K_3 + P} X_s - k_{D,P} P$$

Results and Discussion

The simulation was carried out for a batch process with glucose and sucrose as main carbon sources. The simulated results were illustrated in Fig. 1, and compared with experimental data from literatures (Cruz *et al.*, 1999).

The diauxic growth is well described by the proposed model. Experimental data in Fig. 1 show that high growth rate take places when glucose is preferentially consumed. After depletion of glucose, sucrose starts to be metabolized at a slower rate. During this phase, cephalosporin C begins to be produced.

Some typical phenomena observed in the fermentation process of cephalosporin C by the fungus *Cephalosporium acremonium* such as diauxic growth, repression of production of cephalosporin C, and morphological differentiation are well described by the proposed model derived from point of view of cybernetics.

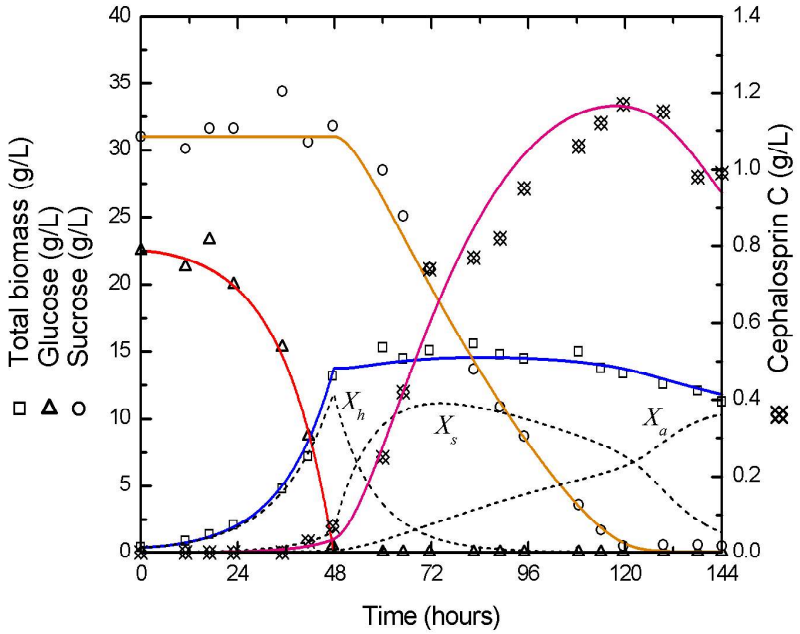

Fig. 1. Simulation results by the cybernetic model and experimental data of Cruz et al. (1999).

Conclusions

The proposed model, which takes into account the diauxic behavior of substrate consumption and the morphological differentiation simultaneously, has been tested on the experimental data from the literatures. The results obtained show that the model can adequately describe the morphological differentiation of cells, the sequential utilization of carbon sources and the production of cephalosporin C, so it should be useful to optimize the production process of cephalosporin C by the fungus *Cephalosporium acremonium* in batch or continuous operations.

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