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Modeling of Typical Microbial Cell Growth in Batch Culture

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Abstract A mathematical model was developed, based on the time dependent changes of the specific growth rate, for prediction of the typical microbial cell growth in batch cultures. This model could predict both the lag growth phase and the stationary growth phase of batch cultures, and it was tested with the batch growth of *Trichoderma reesei* and *Lactobacillus delbrueckii*.

Keywords: microbial cell growth, mathematical model, specific growth rate, batch culture

Modeling and simulation of microbial cell growth is important both theoretically and practically. Although the Monod model [1] has been the most widely used for the prediction of cell growth, it only fits the exponential growth phase of the growth, without any inhibition. The lag growth phase, decreased growth phase and the stationary growth phase of a typical microbial batch culture growth curve can not be predicted using the Monod model. The typical growth curve of batch culture is the result of continuous changes of the specific growth rate, μ, with culture time. Although many efforts have been made to expand the Monod model in order to predict the typical microbial growth curve of batch culture, the time dependent changes of μ were often neglected in prediction of the lag growth phase. Efforts made to predict the lag growth phase simply defined the lag time in terms of cell growth, t_L [2,3]. The lag growth phase was related with the changes of μ and defined in the following way [3]:

$$\mu(s,t) = \mu_m \cdot \frac{s}{k_s + s} \cdot (1 - e^{-t/t_L}) \quad (1)$$

where μ_m is the maximum specific growth rate and s the substrate concentration. In equation (1), if s remains constant μ will always increase with cultivation time, which is in contradiction with the fact that μ may decrease with time in a batch culture. A structured model has been proposed to predict the lag growth phase [4], but it has too many parameters to be practical [3]. In terms of prediction of the decreased growth phase and the stationary growth phase, the Logistic model has been widely used, which relates changes of μ with changes of cell concentration. But the Logistic equation can not predict the lag growth phase. In this work, a model based on the time dependent changes of μ was developed for prediction both the lag growth phase and the stationary growth phase of microbial batch culture.

Trichoderma reesei Rut C30 (ATCC 56765) and *Lactobacillus delbrueckii* were used. The media of *T. reesei* Rut C30 contain 2 g/L $(NH_4)_2SO_4$, 3 g/L protease peptone (Difco Lab.), 0.5 g/L yeast extract (Difco Lab.), 4 g/L KH_2PO_4, 0.3 g/L $CaCl_2 \cdot 2H_2O$, 0.3 g/L $MgSO_4 \cdot 7H_2O$, 0.2 mL/L Tween/80, and 30 g/L Solka Floc (Fiber Salse and Develoment Co., Green Brook, USA). The *L. delbrueckii* media contain 20 g/L glucose, 10 g/L proteose peptone (Difco Lab.,USA),10 g/L beef extract (Difco Lab., USA), 5 g/L yeast extract (Difco Lab., USA), 1 mL/L Tween/80, 2 g/L ammonium citrate, 5 g/L sodium acetate, 2 g/L K_2HPO_4, and 0.1 g/L $MgSO_4$. Cultivation of *T. reesei* Rut C30 was carried out in 500-mL flasks containing 100 mL media. The flasks were incubated in a shaking incubator (KMC-8480SF, Vision Scientific Co., Korea) at 29°C and 200 rpm. Cultivation of *L. delbrueckii* was carried out in 50 mL test tubes containing 20 mL of broth and it was incubated in the same incubator as described for *T. reesei* above at 29°C without shaking.

Cell concentration of *T. reesei* was measured in dry cell weight using the following method [5]. One mL of culture broth and 1 mL of 1 M perchloric acid solution were mixed and boiled for 20 min. After cooling to room temperature, the sample was centrifuged and the optical density of the supernatant was measured at 260 nm. The optical density of the blank was determined using the same procedure as used for the culture filtrate. The cell concentration of *L. delbrueckii* was measured at 600 nm by optical density.

The values of the time course of μ were calculated from the cell growth curve. The equations (2) and (3) were used for simulation of $\mu(t)$ and the cell concentration, respectively.

$$\mu(t) = \mu_m \cdot \frac{1}{1 + e^{-k_{in}(t - t_{in})}} \cdot \frac{1}{1 + e^{k_{de}(t - t_{de})}} \quad (2)$$

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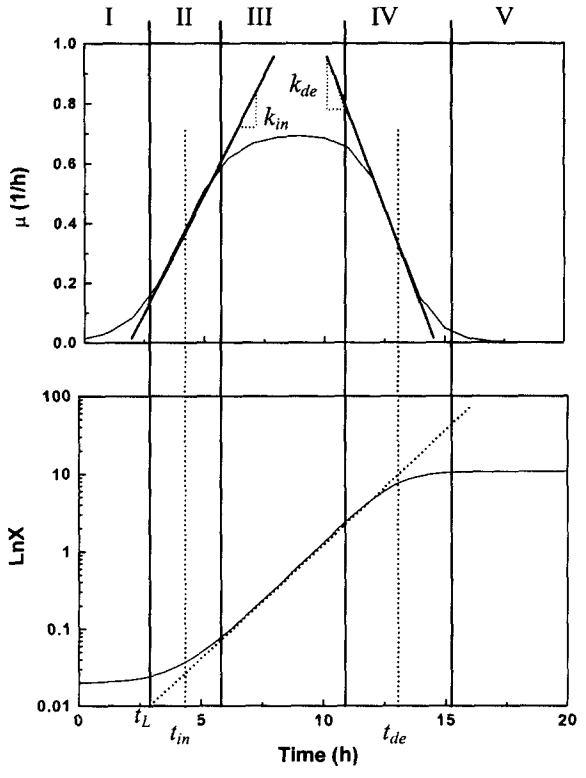

Fig. 1. Illustration of model parameters.

$$\frac{dx}{dt} = \mu(t) \cdot x \tag{3}$$

where, μ_m is the maximum specific growth rate, k_{in} is the maximum increasing rate of μ, k_{de} is the maximum decreasing rate of μ, t_{in} is the time point when the increasing rate of μ equals k_{in}, t_{de} is the time point when the decreasing rate of μ equals k_{de}, and x is the cell concentration. All the parameters used in the model can be obtained graphically (Fig. 1). The changing trends of the cell growth function with the changing of one of the parameters are shown in Fig. 2. Fig. 2 is aimed to show the characteristics of the function only, but not to predict the practical cases. Because the situation, that only one parameter changes while other parameters are fixed, will not happen in practical cases.

The experimental and simulation results of the time course of the cell growth and μ of *L. delbrueckii* and *T. reesei* are shown in Figs. 3, 4 and Tables 1, 2. μ was calculated from the two adjacent points one by one. The model parameters for *L. delbrueckii* and *T. reesei* were first roughly estimated from the figures of time course of μ in Figs. 3, 4 and then fine tuned by minimizing the total errors in fitting the corresponding $\mu(t)$ curves with the calculated values of the time course of μ. The model parameters used are shown in Table 3. μ is zero in the beginning of the cultivation, increased after the lag

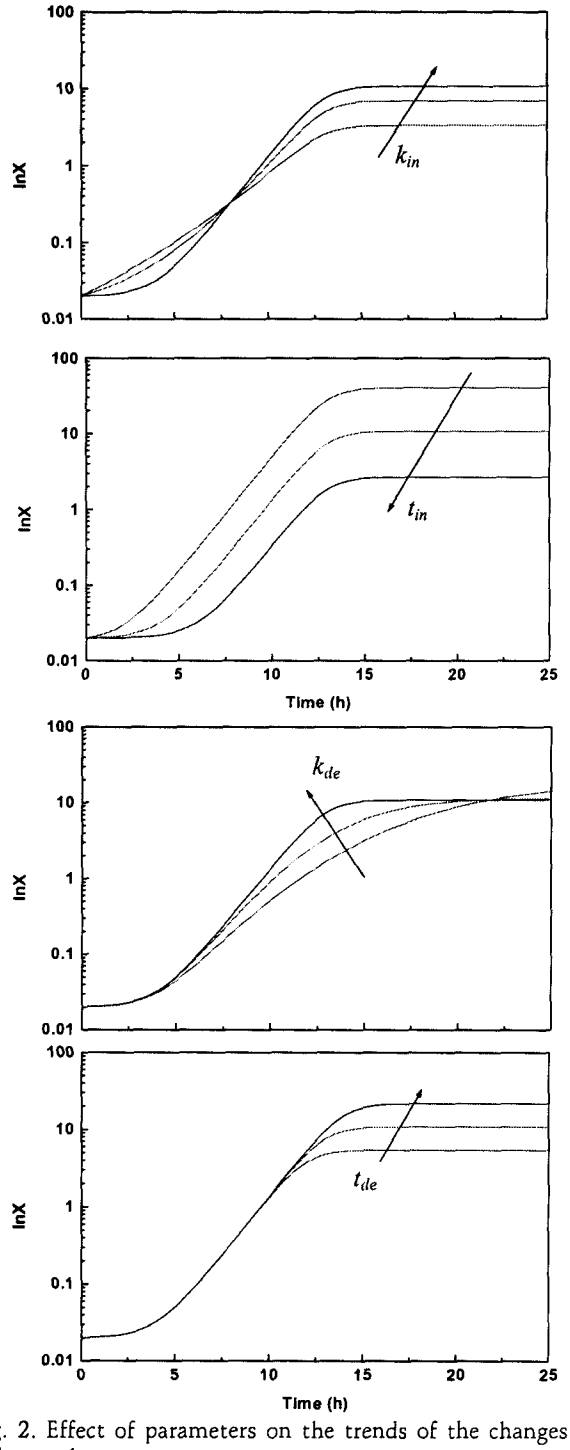

Fig. 2. Effect of parameters on the trends of the changes of cell growth curves.

growth phase, and then decreased with cultivation time (Figs. 3, 4). The model fits the data well for both μ and the cell growth for both *T. reesei* and *L. delbrueckii* (Figs. 3, 4).

The typical cell growth in batch culture includes several growth phases, which are defined as the lag, in-

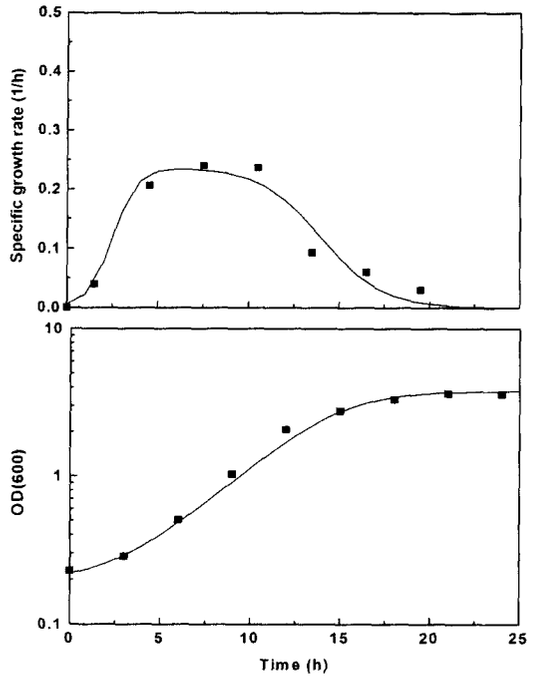

Fig. 3. Time course of the specific growth rate and the cell concentration of *Lactobacillus delbrueckii*.

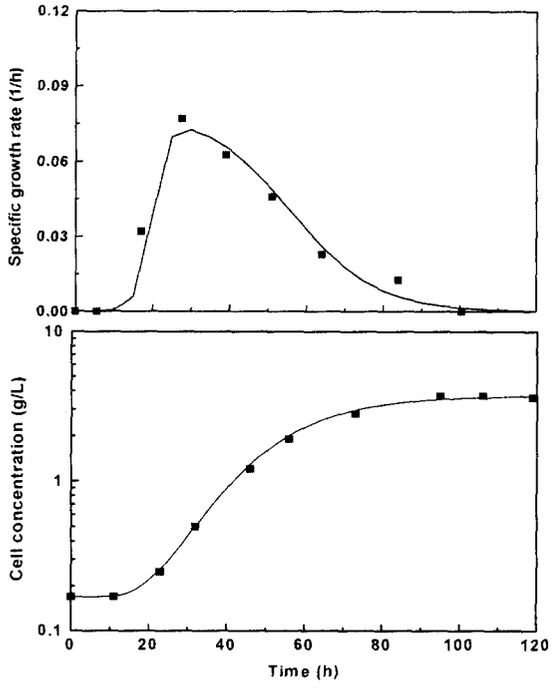

Fig. 4. Time course of the specific growth rate and the cell concentration of *Trichoderma reesei*.

creased growth, exponential growth, decreased growth, stationary growth and death phases. The lag growth phase occurs during the initial period of cultivation

Table 1. Data for *Lactobacillus delbrueckii* growth

Time (h)	X (g/L)	LnX (g/L)	Time (h)	μ (1/h)
0	0.229	-1.474	0	0
3	0.286	-1.252	1.5	0.040
6	0.503	-0.687	4.5	0.206
9	1.035	0.034	7.5	0.240
12	2.070	0.728	10.5	0.237
15	2.770	1.019	13.5	0.093
18	3.320	1.200	16.5	0.060
21	3.650	1.295	19.5	0.030
24	3.610	1.284	22.5	-0.003

Table 2. Data for *Trichoderma reesei* growth

Time (h)	X (g/L)	LnX (g/L)	Time (h)	μ (1/h)
0	0.17	-1.771	0	0
11	0.17	-1.771	5.5	0
23	0.25	-1.386	17	0.032
32	0.50	-0.693	27.5	0.077
46	1.20	0.182	39	0.063
56	1.90	0.641	51	0.046
73	2.80	1.029	64	0.023
95	3.70	1.308	84	0.013
106	3.70	1.308	100.5	0
119	3.60	1.280	11.25	-2.4E-4

Table 3. The values of the parameters

	Lactobacillus delbrueckii	*Trichoderma reesei*
μ_m (1/h)	0.237	0.080
k_{in} (–)	5	0.5
t_{in} (h)	2.5	20
k_{de} (–)	0.6	0.09
t_{de} (h)	14	56

when the value of μ is zero or near to zero. The physiological explanation of the lag growth phase is that when the cells are transferred into a new environment the cells need some time to synthesize new enzymes for adaptation to the new environment. The cell adaptation involves many physiological and metabolic changes and is a gradual process [6,7]. μ increases gradually from zero or a small value, enters into increased growth phase and then exponential growth phase reaching its maximum value μ_m. In batch culture, limitations to cell growth will occur after some time of exponential growth by the accumulation of intracellular toxins or depletion of some nutrients and it leads to the reduction in μ. With the decrease of μ from μ_m to zero, cell growth enters the decreased and stationary growth phases (Figs. 3, 4). As a result of the typical changes of μ, cell growth curve turns out to show the lag, increased growth, exponential growth, decreased growth and stationary growth phases. This model can not predict the

death phase as μ will not decrease below zero using equation (2).

In conclusion, this cell growth model for batch culture is based on the premise of the time dependent changes of μ. It can predict the typical microbial cell growth of both fungi and bacteria in batch culture.

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