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Determination of time course of microbial specific growth rate using mathematical model and genetic algorithm

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ABSTRACT

The time course of specific growth rate (μ(t)) is essential in analyzing microbial growth characteristics and the relationships between growth and metabolites production. The conventional method for calculation of μ(t) is done by hand and has relatively large errors, which deters its applications. In this paper, a new method for calculation of μ(t) is developed using growth mathematical model and genetic algorithm for model parameter optimization. This method is practical and efficient.

KEYWORDS

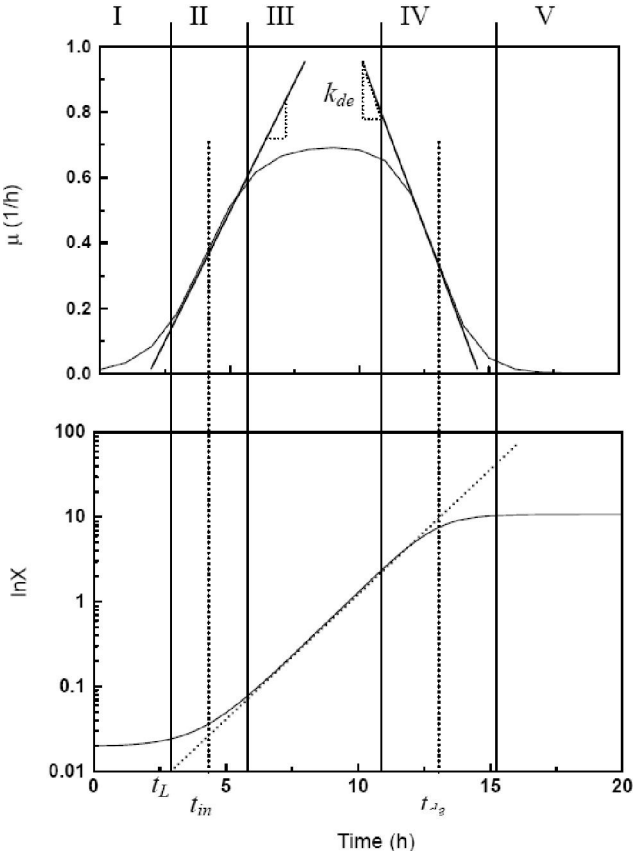
Specific growth rate;
Growth model;
Genetic algorithm;
Parameter optimization.

INTRODUCTION

Accurate calculation of time course of specific growth rate (μ(t)) is essential in analyzing the characteristics of microbial growth and growth related metabolite productions in areas of microbiology and fermentation technology[1-4]. According to Gaden (1959), microbial products are classified into three classes which are (I) the growth associated, (II) the non-growth associated, and (III) the partially growth associated types[5]. The constitutive rate expressions for these classes of product formation based on Luedeking Piret equation[6], are widely used in fermentation technology to guide the optimizations of media composition, cultivation conditions, and nutrient feeding strategies. At present, the time course of μ(t) is usually calculated by hand from the changes of cell concentration of a series of small time intervals divided by both the time interval and the cell concentration of the corresponding time interval. As the change of cell concentration during the small time interval is small, large relative error is resulted in the calculation of time course of μ(t). In order to decrease the calculation errors, data fitting and smoothing are made using polynomial equation in general cases. But, the typical "S" type growth curve of microbial batch culture can't be fitted well by polyno-

mial equation.

The typical "S" type growth curve of microbial batch culture is the result of dynamic changes of μ with cultivation time. μ(t) is small in the lag growth phase, increases gradually until it reaches μ_m in the exponential growth phase, and decreases to zero at last to reach the stationary growth phase, which leads to a "bell" typed μ curve versus time (Figure 1). The typical "S" type growth curve was well modeled in our previous study (Figure 1)[7]. Based on that model, a new method will be used in this research to calculate the time course of μ for batch culture. Genetic algorithm (GA) will be used in optimization of model parameters. GA is an optimization algorithm developed by imitating the evolution of a biological population, and it is efficient especially in optimizing nonlinear and sophisticated systems[8]. By using mathematical growth model and GA optimization methods, the time course of μ(t) is calculated in this research.



I: lag growth phase, II: increased growth phase, III: exponential growth phase, IV: decreased growth phase, and V: stationary phase. k_{in}: maximum increasing rate of μ, k_{de}: maximum decreasing rate of μ, t_{in}: the time point when μ equals k_{in}, t_{in}: the time point when μ equals k_{de}, and t_L: lag time.

Figure 1 : Illustration of growth model parameters[7]

MATERIALS AND METHODS

Microbial strains, media, and cultivation conditions

Escherichia coli SM10 and *Acidithiobacillus caldus* MTH-04 are used.

E.coli SM10 is cultivated in Luria Bertani (LB) medium containing: peptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L. Adjust pH to 7.0 and autoclave to sterilize. 100 ml of flask containing 20 ml of medium is used, cultivated at 37 °C, shaken at 200 r/min. The inoculation volume is 0.2 ml.

A. caldus is cultivated in Starky-S^0 medium containing sulfur powder of 20 g/L and the salt solution that contains $(NH_4)SO_4$ 2.0 g/L, KH_2PO_4 3.0 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $FeSO_4 \cdot 7H_2O$ 0.01 g/L, $CaCl_2 \cdot 2H_2O$ 0.25 g/L. The salts solution is adjusted to pH 2.5 using H_2SO_4, then autoclaved. Sulfur powder is autoclaved for 2 h separately and added to the salt solution before using. The flask of 300 ml containing 100 ml of medium, is cultivated at 40 °C in standstill for 5 d and then shaken at 120 r/min for 1d. The inoculation volume is 5 mL.

Growth measurement

E. coli cell concentration was measured by optical absorbance at wavelength of 600 nm. *A. caldus* cell concentration was counted using blood counter under microscope.

Mathematical model

The mathematical model of μ and cell concentration is expressed using equations (1) and (2)[7].

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

$$\frac{dx}{dt} = \mu(t) \cdot x \quad (2)$$

where, μ_m is the maximum specific growth rate, k_{in} is the rate constant for μ increase, k_{de} is the rate constant for μ decrease, t_{in} is the time point when dμ/dt is the maximum, t_{de} is the time point when dμ/dt is the minimum, and x is cell concentration.

Calculations

The calculations of the mathematical model and the model parameters were done using self-made software,

programmed using Microsoft Visual Basic, running on personal computer with Microsoft Win7. Runge-Kutta method of order four was used in solving differential equations, and GA was used in optimizing the model parameters in minimization of the sum of the squared errors between the model predicted and the measured cell growth time course data. The time course of μ was also calculated by hand using the measured experimental data, for validation of the mathematical model calculated results.

RESULTS AND DISCUSSION

Parameter optimization using genetic algorithm

GA is used in optimization of the model parameters, which is developed by imitating the evolution of a biological population[8]. In GA, the population consists of *n* individuals represented by chromosomes, one chromosome consists of *m* genes, and one gene consists of a 10 bits binary number coding for one model parameter. The gene number *m* is 5, which equals the number of model parameters. So, one chromosome consists of a 50 bits binary number coding for the whole set of 5 model parameters. The individual number *n* (also called population size or chromosome number in one generation) is 1000, providing 1000 sets of searching points in GA. The population of 1000 chromosomes consist of a 1000×50 binary matrix. In the beginning of GA optimization, the 1000×50 binary matrix is randomly initialized by randomly set "0" or "1" to each bit of the matrix. Then, hybridization is made with pairs of chromosomes randomly selected at the hybridization rate of 0.2, the hybridization sites randomly selected between 1 and 49, and the corresponding parts of the selected pair of chromosomes being exchanged. After that, mutation is made by randomly select the bits in the population matrix at the mutation rate of 0.05, and turnover the selected bits from "0" to "1" or vice versa. After above genetic operations, the value coded by each gene is decoded and changed to real by linear scaling to the range of the corresponding model parameter. By using one set of model parameter values coded by one chromosome and the growth mathematical model, model prediction of cell growth can be made and the sum of the squared errors between the model prediction and the measured cell growth time course data can be calculated. The fitness of each individual (chromosome) is defined inversely proportional to the ratio of each sum of squared errors of one chromosome to the total sum of squared errors of the 1000 chromosomes, by which the fitness of 1000 individuals (chromosomes) can be calculated. Next, natural selection is made with 1000 individuals (chromosomes) randomly selected with the selection probabilities equal to their fitness. The new generation produced by the natural selection genetic operation has higher averaged fitness than the last generation. The hybridization, mutation, and natural selection genetic operations operate continuously until the smallest model prediction error decreased smaller than the predetermined limit, and the parameter values coded by the chromosome with the smallest error are the final optimization results of GA. The diagram of the optimization software is shown in Figure 2.

Before calculation, the GA searching span of each parameter value is predetermined. The values of t_{in} and t_{de} can be estimated from the growth curve. Wider ranges can be given for the values of k_{in} and k_{de}, whose values are not easily estimated. The value of μ_m can also be roughly estimated from the physiological knowledge of the microbial strains or from the cell growth curve. Wider search ranges for the parameter values will cost longer calculation time. The optimized model parameter values are shown in TABLE 1, and the excellent fitting and predicting results shown in Figures. 3~4 show that GA is powerful in model parameter optimization.

Genetic algorithm is especially useful in solving the sophisticated optimization problems. It has been successfully used in biological model parameter optimization[9,10], medium composition optimization[11], biochemical network optimization[12], and bioprocess control[13].

Calculation of time course of μ

E. coli and *A. caldus* were cultivated in LB and Starky-S^0 salts medium, respectively. The measured and hand calculated time course of cell growth and specific growth rate as well as the model predictions for *E. coli* and *A. caldus*, respectively, were plotted in Figures. 3 and 4. The mathematical model of equations (1) and (2) and the optimized model parameter values listed in TABLE 1 were used.

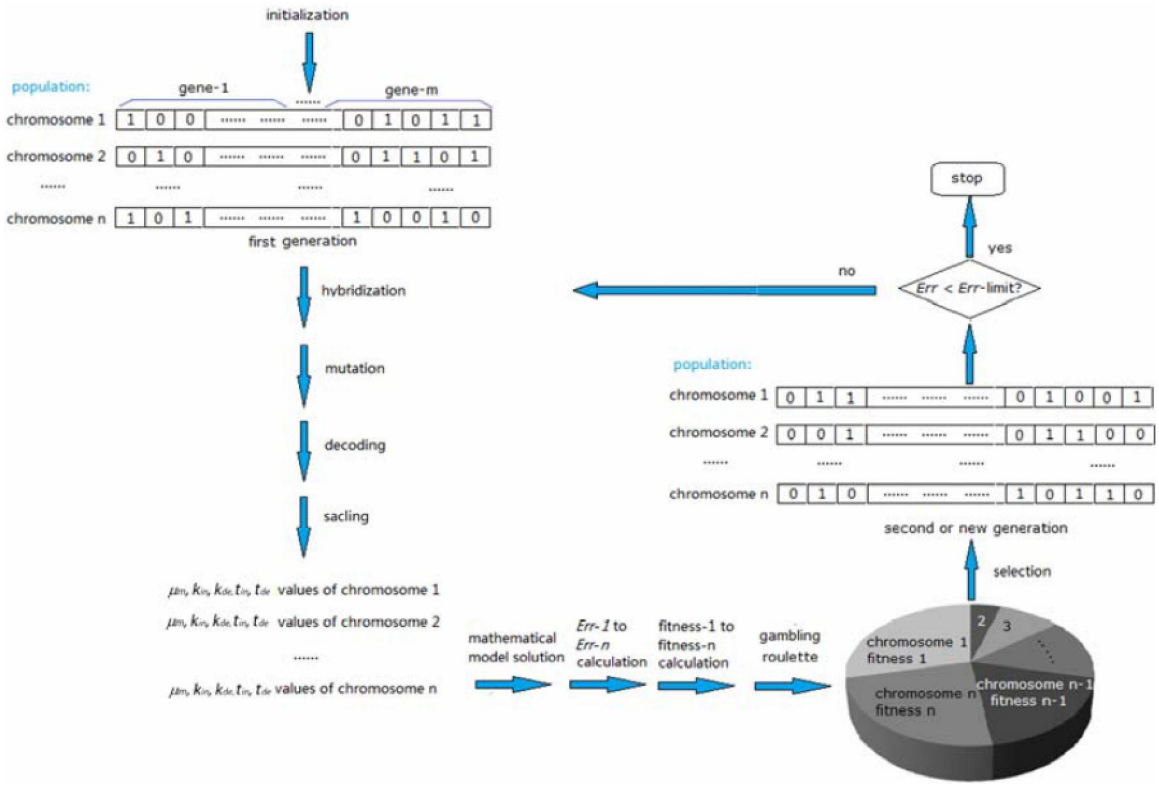


Figure 2 : The GA calculation diagram for $\mu(t)$

TABLE 1 : Model parameter values optimized using genetic algorithm

Strains	μ_m (1/h)	k_{in} (1/h^2)	k_{de} (1/h^2)	t_{in} (h)	t_{de} (h)
E. coli	0.600	7.799	0.617	0.725	4.580
A. caldus	0.022	0.065	0.036	46.380	100.000

In this research, only the measured time course of cell growth data is used in the model parameter optimization by GA while the calculated data of time course of μ is not used. The perfect fit of the model prediction with the measured data of cell growth shown in Figures. 3a and 4a provides the basis for the accurate calculation of μ(t). In addition to *E. coli* and *A. caldus*, the feasibility of the model in prediction of cell growths of the fungi *Trichoderma reesei* Rut C30 (ATCC 56765) and the bacteria *Lactobacillus delbrueckii* is also being verified[7]. Above results confirm that the model calculation method for calculation of μ(t) developed in this research is reliable and widely applicable.

The widely used growth models for example, Monod (1949)[14], Tessier (1942)[15], Moser (1958)[16], and Contois (1959)[17] growth models, which use the limiting substrate concentration as the independent variable, are not fit for the purpose for calculation of μ(t). Moreover, these models can not predict the lag or the stationary growth phases or both of them. Logistic equation is another widely used growth model[18], but it can not predict the lag growth phase and unable be used in calculating the typical "bell" typed μ curve of batch culture. The mathematical model used in this research correctly models the typical "S" type growth curve and "bell" type μ(t) curve of microbial growth, is appropriately applied as a general tool for calculation of (t) of typical microbial growth.

Analysis of metabolite production characteristics

As introduced in the section of instruction, according to Gaden (1959), microbial products are classified into the growth associated, the non-growth associated, and the partially growth associated types[5], and the relationship between the specific product production rate ($q_p(t)$) and μ(t) can be described by Luedeking Piret equation (3)[6]:

$$q_p(t) = \alpha \cdot \mu(t) + \beta \quad (3)$$

Where, α, β the constants, with $\alpha \neq 0$ and $\beta = 0$ for growth associated, $\alpha = 0$ and β '™0 for non-growth associated, and $\alpha \neq 0$ and $\beta \neq 0$ for partially growth associated production types, respectively. Assuming α=8

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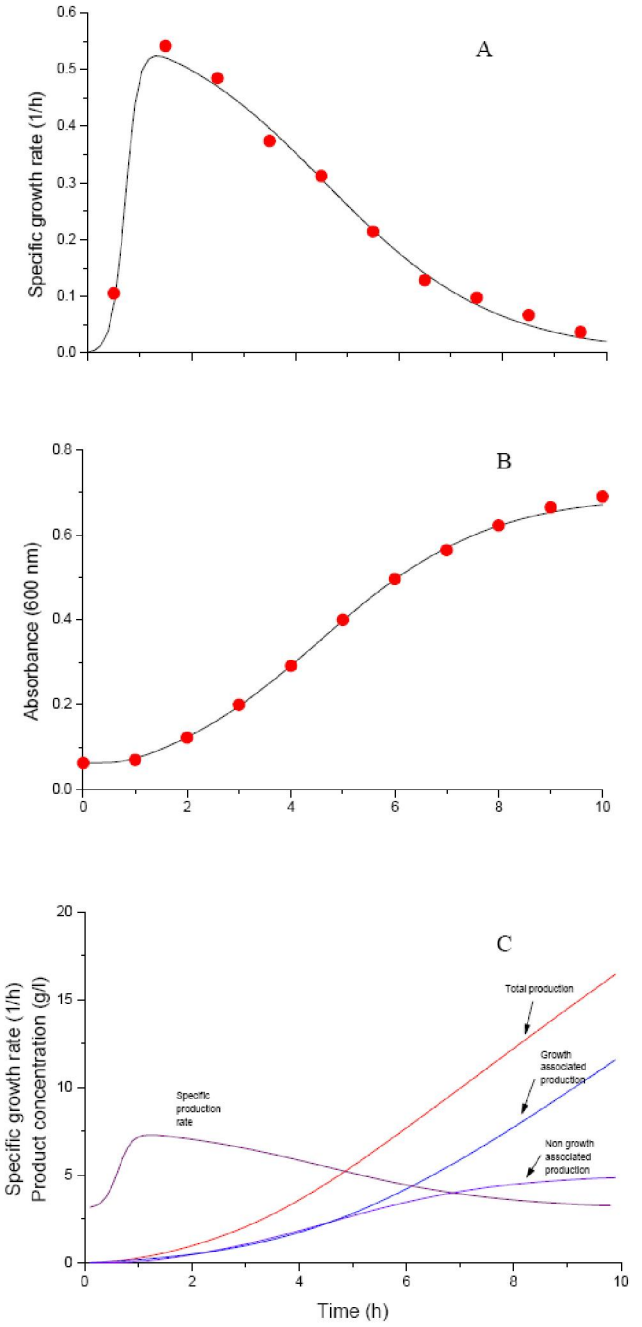

Figure 3 : The experimental and model calculation results of (A) μ, (B) cell concentration, and simulated $q_p(t)$, the growth associate and non-growth associate production contributions to the total production of *E. coli*. (●) experimental results; (—) calculation results.

g/OD$_{600}$, β=3.1 g/h/OD$_{600}$, the growth associated and the non-growth associated product production, can be evaluated separately in view of fermentation process optimization (Figure 3C). For example, maintenance of high μ(t) value and longer growth phase is preferred if the growth associated production takes a major part, while maintenance of high cell concentration for longer time is preferred if non-growth associated production takes a major part. The total production can be regarded as the sum of the growth associated and non-growth associated productions as described in equation (4):

$$\frac{dP}{dt} = q_p(t) \cdot X = \alpha \cdot \mu(t) \cdot X + \beta \cdot X \qquad (4)$$

Where, P is the product concentration. The illustration of q_p(t), the contributions of growth associated and non-growth associated productions to the total production are shown in Figure 3C.

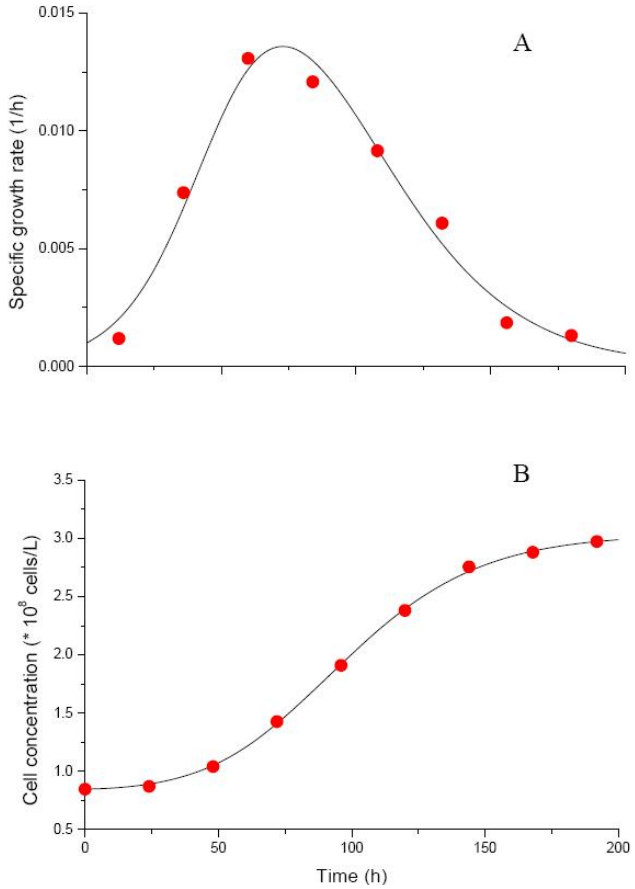

Figure 4 : The experimental and model calculation results of μ and cell concentration of *A. caldus*. (●) experimental results; (—) calculation results.

CONCLUSION

By using the growth mathematical model and GA, μ(t) of microbial growth can be calculated. While, most of the currently used microbial growth models are not suitable for above purpose. The growth model based GA optimization method can be used as a general

method for calculation of μ(t). And the usefulness of μ(t) in analysis of product production and optimization of the fermentation process are illustrated.

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