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Full Length Research Paper

Optimizing the feeding operation of recombinant *Escherichia coli* during fed-batch cultivation based on Pontryagin's minimum principle

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Recombinant *Escherichia coli* BL21 was used to produce human-like collagen in fed-batch culture. After building and analyzing the kinetic models of fed-batch cultures, the maximum specific growth rate, $Y_{x/s}$ and $Y_{p/s}$ were 0.411 h^{-1} , 0.428 $g \cdot g^{-1}$ and 0.0716 g/g, respectively. The square error of cell growth models, glucose consumption model and human-like collagen formation were almost all around 94%, which indicated that the kinetic model could describe the actual change well. According to the target, that is, to gain the highest productivity of human-like collagen, the feeding rate (F) was worked out on the basis of Pontryagin's minimum principle. In the verification experiments, the specific growth rate was controlled at 0.15 and 0.04 h^{-1} at the fed-batch and induction phase, respectively. The result showed that the concentrations of cell and human-like collagen could reach 87.6 and 6.11 $g \cdot L^{-1}$, and they were raised by 17.9 and 18.6%, respectively.

Key words: Fed-batch culture, human-like collagen, maximum specific growth rate, Pontryagin's minimum principle, recombinant *Escherichia coli.*

INTRODUCTION

Human-like collagen (HLC) is a novel biomaterial which is synthesized in recombinant *Escherichia coli* BL21 using a modified cDNA fragment transcribed from the special mRNA coding for human collagen (Guo et al., 2010a, b; Cooperman et al., 1984; Yang et al., 2004). In order to improve the productivity of human-like collagen, fed-batch culture was adopted (Huo et al., 2004). The key operation during the process of fermentation is to feed medium reasonably (Li et al., 2002). The Crabtree effect (Fuchs et al., 2002; Luli et al., 1990), due to the superfluous glucose, decreases the pH of fermentation broth and disrupts cell metabolism, and finally inhibits strain propagation and collagen synthesis. Moreover, the limited capacity of tricarboxylic acid (TCA) cycle and electron transfer system increases the accumulation of acetic acid at the maximum specific growth rate (Majewski et al., 1990).

Dynamic programming and the minimum principle were the two basic methods of seeking the optimum control (Chen et al., 2002). In the early 1950s, Bellman, a mathematician (USA), provided the dynamic programming firstly, and could be used in studying the

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Nomenclature: S, Glucose concentration, $g \cdot L^{-1}$; **P,** human-like protein density, $g \cdot L^{-1}$; S_F, flux glucose concentration, $g \cdot L^{-1}$; **t,** time, h; t_0, the starting time, h; t_f, the final time, h; **J,** the goal function; **λ(t),** Covariant vector; **H,** Hamilton function; $Y^*_{x/s}$, apparent yield, overall stoichiometric cell yield coefficient (grams of cell substrate consumed per gram of cell), $g \cdot g^{-1}$; $Y_{p/s}$, product yield coefficient, $g \cdot g^{-1}$; **μ,** specific growth rate, h^{-1}; μ_{max}, the maximum specific growth rate, h^{-1}; **σ,** specific rate of substrate disappearance, h^{-1}; **π,** specific rate of product formation, h^{-1}; **α,** yield coefficient of collagen accumulation rate associated with cell growth rate, $g \cdot g^{-1}$; **β,** variable macrokinetic parameter, yield coefficient of collagen accumulation rate associated with cell growth rate, $g \cdot g^{-1} \cdot h^{-1}$.

optimism of multi-level tactics problem. This method established a kind of inverted order algorithm which was suited to calculation of computer. But its algorism of backward was incomplete. At the same time, Pontryagin, a mathematician (Russian), provided the minimum principle when they studied the optimum control problem of engineering technology, which had the more concrete and complete algorism than the dynamic programming, and it became more convenient to acquire the optimum solution. The minimum principle had a wide use in many fields: the design of optimum program (Li et al., 2000) for the minimum time and the maximum efficiency; the evaluation of project (He, 1999); the astronavigation and rocket navigation (Chen et al., 2002). Pontryagin's minimum principle is used in optimal control theory to find the best possible control for taking a dynamical system from one state to another.

In the practical fermentation with 30 L bioreactor, many problems were found: it is hard to predict and control the growth of cells precisely; the yield of goal protein was low because of the high specific growth rate. The reaction process in microbial cells is very complicated, so the establishment of reasonable kinetic models is very difficult. In order to solve these problems, we focus on the relationship between the feeding rate and the specific growth rate on the base of Pontryagin's minimum principle (Levisauskas et al., 2003). In recent years, there were many literatures about the minimum principle used in the fed-batch fermentation (Gong et al., 2008), but the concrete and particular feeding equation was not acquired. In this study, we could control the specific growth rate at the desirable value, and the generation of organic acids such as acetic acid, formic acid, and lactic acid, a strong growth inhibitor, could be avoided. So the achievement of high cell densities and high level express of human-like protein which was produced by *E. coli* BL21 are probable.

In 2008, it was reported that Human–like collagen could also be expressed in recombinant *Pichia pastoris* expression system. Gao et al. (2008) and Niu and Yang (2008) have studied the expression of human–like collagen in *P. pastoris* GS115/Ppic9KG6 twice in 2008. The study was performed in shake flask, and the induction was aroused by the induction agentia-methanol. The final density of human–like collagen was only 0.098 g/L after the optimization with the determination method which is the same to my study. The optimization method which they adopted was orthogonal test. The productivity was well below this study in which human–like collagen was expressed in recombinant *E. coli* BL21 and the optimization method was Pontryagin's minimum principle.

MATERIALS AND METHODS

Microorganism and plasmid

Recombinant *E. coli* BL21, preserved in our laboratory (Fan et al., 2002), carried a plasmid containing a kanamycin resistance gene and human-like collagen cDNA (Luo et al., 2005). It could allow high temperature induction to synthesize human-like collagen.

Medium

Luria-Bertani medium is used as the seed medium. The compositions of batch culture medium and feeding medium are given in Table 1. The trace element solution was the same as the reported (Korz et al., 1995).

Fed-batch culture

When the initial glucose in the medium was consumed up, feeding was started. Glucose is the only limiting substrate in batch fermentation. Fed-batch cultivations were carried out in an *in situ* autoclaved fermenter (30 L, Bioengineering Co. Switzerland, L1532) containing 14 L initial medium. Cultivation temperature was 34℃, and the dissolved oxygen (DO) was maintained around a present level 30% by the fermentation controller. The pH was automatically kept at 6.8 by the addition of aqueous NH_4OH (25% w/w). When OD_{600} reached around 100, the cultivation temperature was increased to 42℃ and kept for 7 h for induction to achieve a high-level production of the human-like collagen. All experiments were performed in triplicate.

Analysis methods

Cell density was measured turbidimetrically at 600 nm with spectrophotometer (UNICO Model 2082PCS, USA). The cell concentration was determined by the DCW (dry cell weight): the broth sample was centrifuged at 10000 r·min^{-1} for 10 min, washed three times with distilled water and dried to a constant mass in a 105℃ oven (Raje et al., 1998). The CO_2 and O_2 (Guo et al., 2010) concentrations in the inlet and outlet gas were measured online by a gas analyser (LKM2000A; Lokas Automation). Glucose concentration was acquired by BioProfile analyzer 300(NOVA biomedical, USA). Human-like collagen was determined with biuret reaction and electrophoretic analysis (Xiao et al., 2005). Temperature, pH, DO, rotation speed, airflow rate, zymotic fluid volume and the pressure of fermentor were controlled by the digital unit of the bioreactor.

PONTRYAGIN'S MINIMUM PRINCIPLE

In 1958, Pontryagin established the minimum principle on the base of Hamilton principle in mechanics (He, 199). It had an extensive use because of the general structure formation, and could be viewed as a strong method in the problem of the optimum control. Its subject is a system which could be controlled, and its object is to solve the problem of optimization. The controllable system contains a state equation, a dominant vector, and the goal function. When the dominant vector is confined to a constrained range, the optimum trace which is determined by the best controlling rule must acquire the minimum value in the whole range.

The states and the equation of state

The state is a group of variances which could describe the system equation of state at any time, and it is a kind of function depending on time. The system equation of state could be expressed as Equation 1

Table 1 Medium compositions

Components	Batch medium ($g \cdot L^{-1}$)	Feeding medium ($g \cdot L^{-1}$)
glucose	30.0	1000.0
yeast extract	50.0	400.0
K_2HPO_4	5.6	26.0
NaH_2PO_4	3.4	12.6
$(NH_4)_2SO_4$	4.2	16.5
$MgSO_4$	1.8	20.0
EDTA	0.8	3.0
trace element	0.6	3.0
antifoam	0.1	—

$$\frac{dx_i}{dt} = f_1(x_1, \cdots, x_n, t)$$
$$x_i(t_0) = x_i^0, i = 1,..., n \quad (1)$$

The vector is:

$$\frac{dx}{dt} = f(x,t) \quad (2)$$

$x(t_0) = x^0$

$x(t) = [x_1(t), \cdots, x_n(t)]^T$ is the state vector.

Dominant vector

The dominant vector, u(t), is important to a certain aim and contains a group of variances, and it also contains a state equation:

$$\frac{dx_i}{dt} = f_i(x_1, \cdots, x_n, u_1, \cdots, u_r, t) \quad (3)$$

$x_i(t_0) = x_i^0, i = 1, \cdots, n$

The vector formula is:

$$\frac{dx}{dt} = f(x,u,t) \quad (4)$$

$x(t_0) = x^0$

$u(t) = [u_1(t), \cdots, u_r(t)]^T$ is the dominant vector.

The goal function

The performance index change with the goal of the dominant system, the goal function could be described as Equation 5 (Liu, 2001):

$$J = \int_0^1 f_0(x,u,t)dt + g[x(t),t] \quad (5)$$

Pontryagin's minimum principle in the continuous system

Covariant vector λ(t) and Hamilton Function H

Covariant vector λ(t) is very important to the minimum principle, and it meets the needs of the canonical equations (Equations 6, 7 and 8):

$$\frac{d\lambda}{dt} = -\frac{\partial H(x,u,\lambda,t)}{\partial x} \quad (6)$$

$$\frac{dx}{dt} = -\frac{\partial H(x,u,\lambda,t)}{\partial \lambda} \quad (7)$$

$$H(x,u,\lambda,t) = f_0(x,u,t) + \lambda^T f(x,u,t) \quad (8)$$

Hamilton function H contains 2n differential equations, 2n+r unknown numbers $x_1(t),\ldots, x_n(t)$; $\lambda_1(t),\ldots, \lambda_n(t)$; $u_1(t),\ldots, u_r(t)$.

The minimum principle

The minimum principle is the function of H(x*, u, λ*, t) depending on "u", and acquires the minimum value when u(t)= u*(t):

$$H(x^*,u^*,\lambda^*,t) \le H(x^*,u,\lambda^*,t) \quad (9)$$

The methods to solve the canonical equations

The x, u and λ were contained in the canonical equations, so the solution of the best control is the solution of the canonical equations.

The boundary conditions of the canonical equations:

(1) The $x(t_0)$ equals x_1 at the point "t_0".

(2) $x_j(t_1) = x_j^1$, it is known as the boundary condition at the point "t_1"; the other boundary conditions is

$$\lambda_L(t_1) = -\frac{\partial g}{\partial x_L} t_1 \quad (10)$$

So we could have "n" boundary conditions at the point of t_1;

(3) The following formula could be used as a boundary condition if the end time "t_1" is certain.

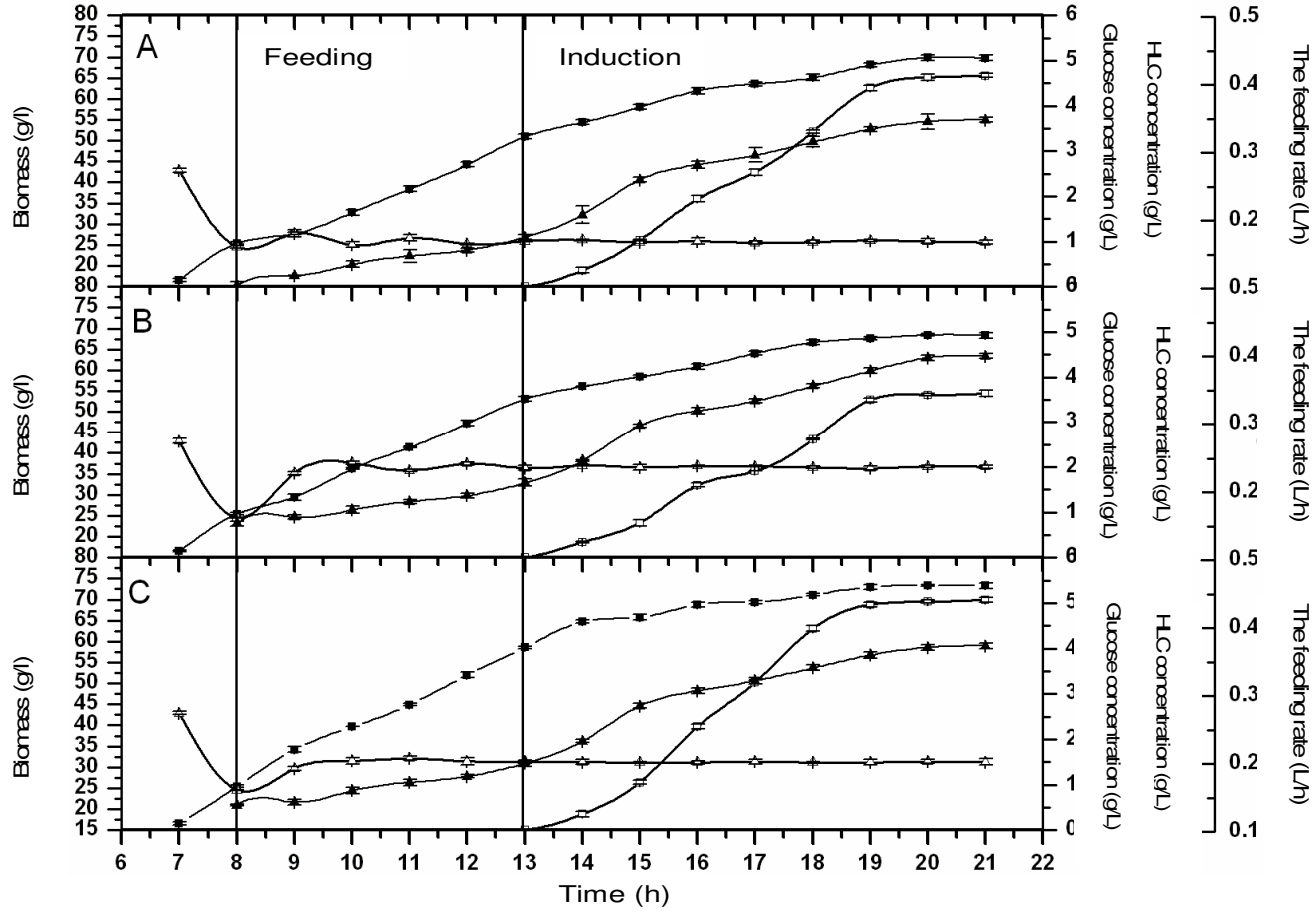

Figure 1. Comparison of fed-batch cultivation with three different glucose concentrations. ■:Biomass; ▲:The feeding rate; □: HLC concentration; □: Glucose concentration.

$$(\frac{\partial g}{\partial t} - H)t_1 = 0 \quad (11)$$

An important character of Hamilton function H

H could be thought as the function of t in the $[t_0, t_1]$, when the best control u(t), the best trace x(t) and the covariant vector λ(t) are applied to H(x, u, λ, t), where $\frac{dH}{dt} = \frac{\partial H}{\partial t}$ is correct along the best trace. The $\frac{dH}{dt} = 0$ is correct when H is not explicit function of t.

RESULTS AND DISCUSSION

Preliminary optimization of feeding operation in consideration of glucose concentration

During fed-batch cultivation, the glucose concentration has an optimal range for the growth of cell and the synthesis of the target product. The growth of cell would be very slow if the glucose concentration was low, while the organic acids synthesized greatly for the high glucose concentration. Before the establishment of kinetic models, the general range of glucose concentration must be determined.

Figure 1 shows that three different glucose concentrations were experimented at the phase of fed-batch cultivation. The final density of cell and the target product was lowest when the glucose concentrations was controlled at 2.0 g/L, the final density of cell and goal product was 68.33 and 3.64 g/L, respectively, but at the starting phase of feeding, the increase of cell density was fast. So the organic acids restricted the cell growth and the product formation. At the starting phase of feeding, the increase in cell density was lowest when the glucose concentration was controlled at 1.0 g/L. Glucose was the restrictive substance to the cell growth. The final density of cell and goal product was highest when the glucose concentrations was controlled at 1.5 g/L, the final density of cell and goal product can reach 73.27 and 5.07 g/L, respectively. So the kinetic model would be established on the base of the data when the glucose concentration

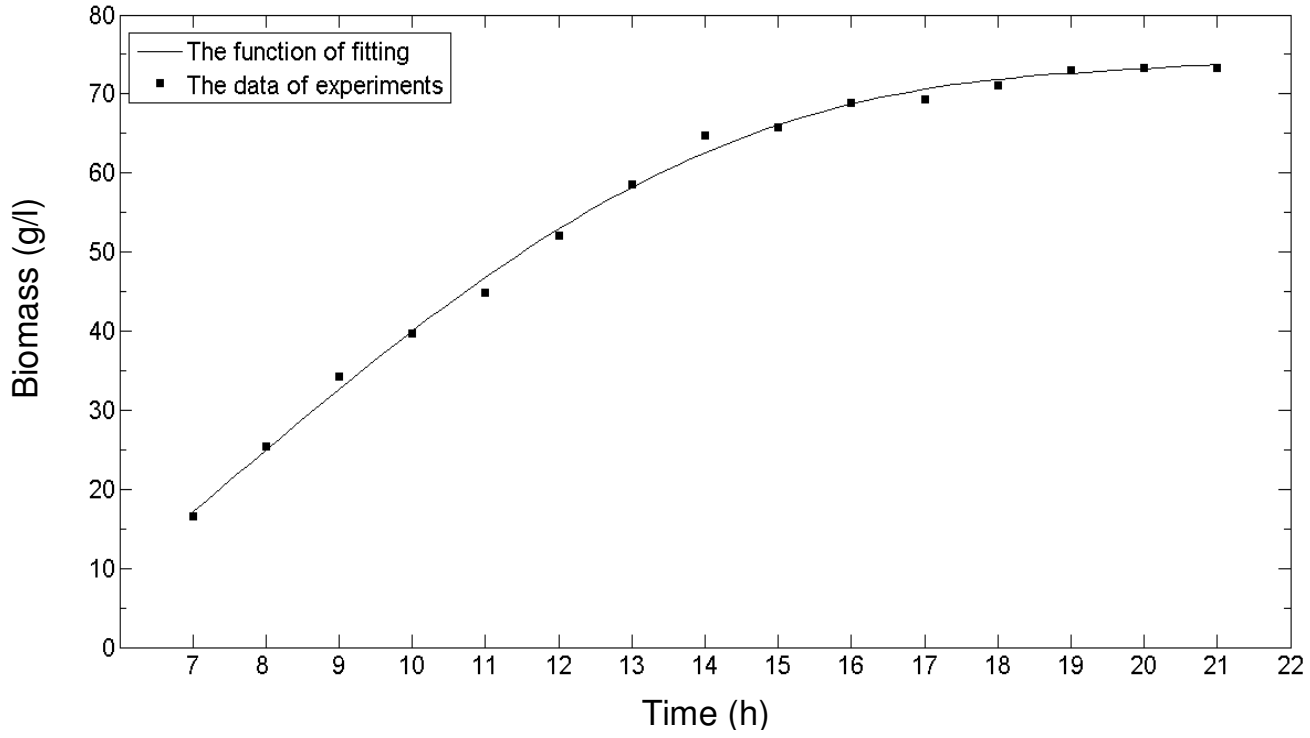

Figure 2. Biomass concentration versus time at the phase of fed-batch cultivation.

was controlled at 1.5 g/L.

But the optimization of glucose concentration was rough and preliminary, and the level of glucose concentration before and after induction should not be consistent. So the farther optimization based on the kinetic model was necessary. Thus, we could acquire the precise equation about the feeding rate and the specific growth rate, and then the concrete feeding operation was ensured following the equation.

Kinetics of the fed-batch cultivation

Kinetics of cell growth

During the batch cultivation phase, cell concentration was increased greatly. Kinetics of cell growth at the fed-batch phase could be represented by Contois's equation (Qi et al., 2004) as Equation 12:

$$\frac{dx}{dt} = \frac{\mu_{\max} s}{k_s x + s} x \qquad (12)$$

Based on the experimental data of fed-batch cultivation, the parameters were evaluated by the lsqnonlin and Runge-Kutta (ode45) methods of MATLAB (Huang, 2006). The key kinetic parameters of fed-batch cultivation were: μ_{max}=0.411 h^{-1}, K_s=0.492g·L^{-1}. From Figures 2 and 3, we know that the kinetic model can anticipate the actual experiments well.

Kinetics of substrate consumption

At the process of cell reaction, the substrate was mainly used in three aspects: the new cell substance, the new product, and energy (Gao et al., 2006). And then, the rate of the substrate consumption was determined by three factors: cell growth rate, product formation rate, energy maintenance rate. The kinetics of substrate consumption of fed-batch phase could be represented as Equation 13:

$$\frac{ds}{dt} = \frac{FS_F}{V} - \frac{1}{Y^*_{X/S}} \frac{dx}{dt} - \frac{1}{Y_{p/s}} \frac{dp}{dt} - mx \qquad (13)$$

Based on the experimental data, the parameters were evaluated by the lsqnonlin and Runge-Kutta (ode45) methods of MATLAB. The feeding curve is shown in Figure 3. The kinetic parameters of fed-batch cultivation were: $Y^*_{x/s}$=0.428 g·g^{-1}, $Y_{p/s}$=0.0716 g·g^{-1}, m=0.0395 g·g^{-1}·L^{-1}. The kinetic model can predict the experimental data well (Figures 4, 5 and 6).

Kinetics of product formation

The kinetics of product formation was classified as three modes by Gaden on the base of the relationships

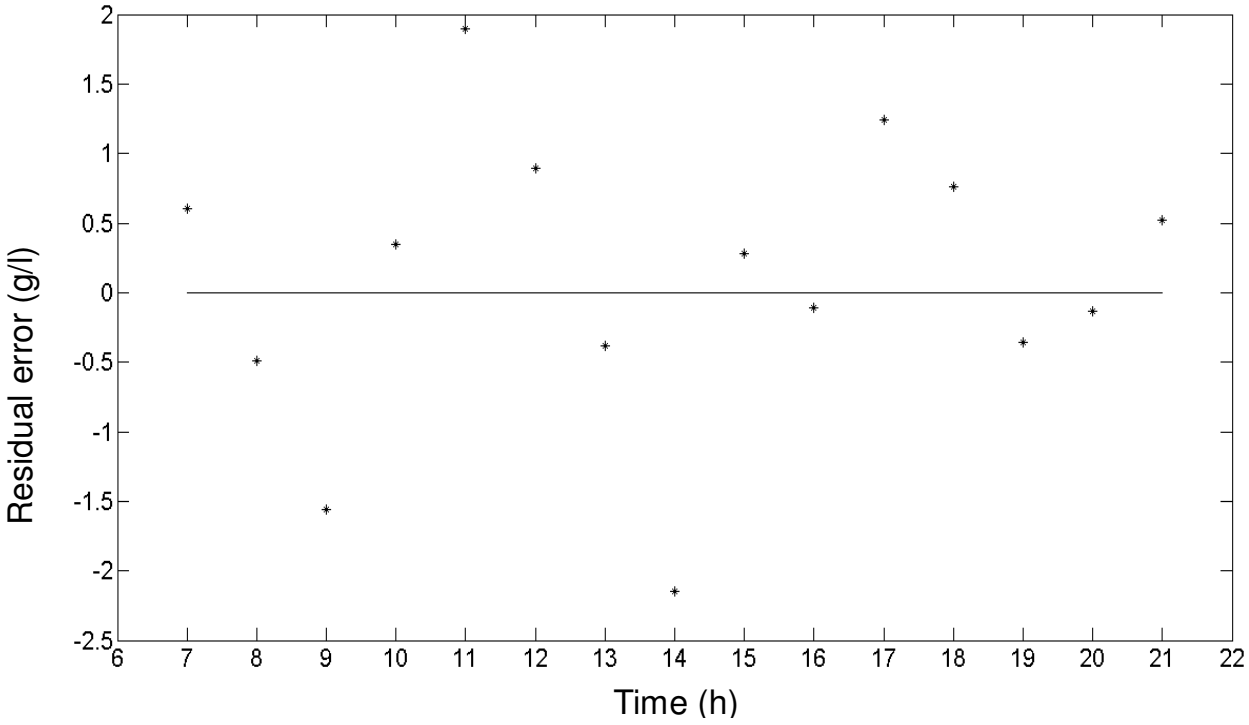

Figure 3. The fitting residual error.

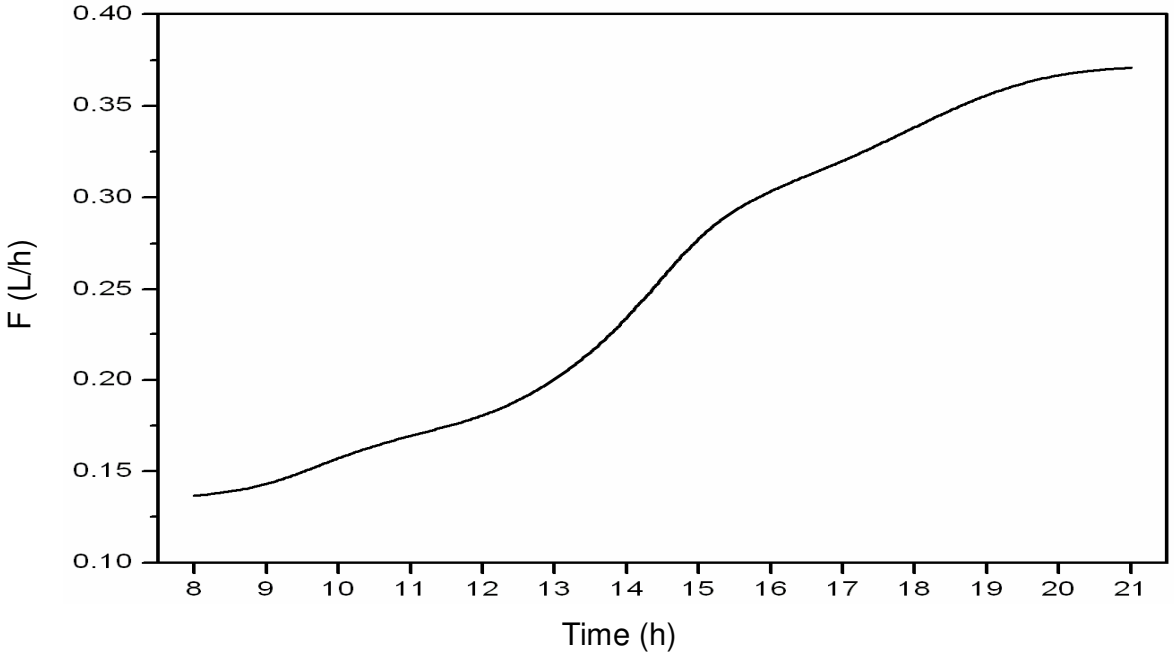

Figure 4. The feeding curve of glucose during the phase of fed-batch culture.

between product formation rate and cell growth rate. The kinetics of product formation of fed-batch phase could be represented by Luedeking-Piret (Qi et al., 2004) equation as Equation 14:

$$\frac{dp}{dt} = \alpha \frac{dx}{dt} + \beta x \quad (14)$$

Based on the experimental data of the induction phase,

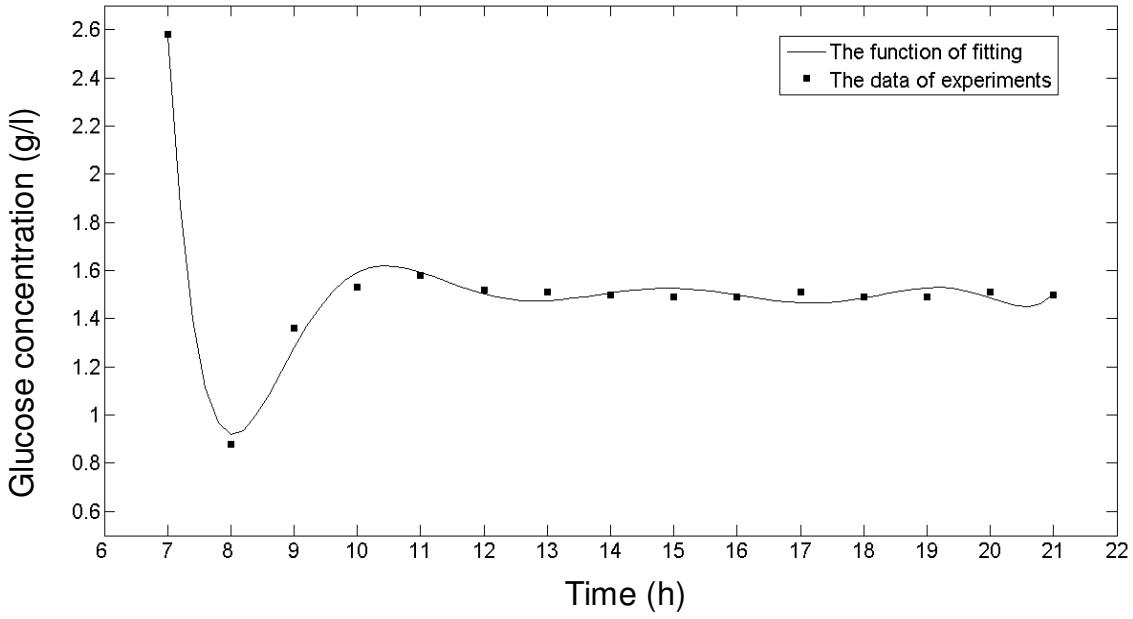

Figure 5. Glucose concentration versus time during the phase of fed-batch culture.

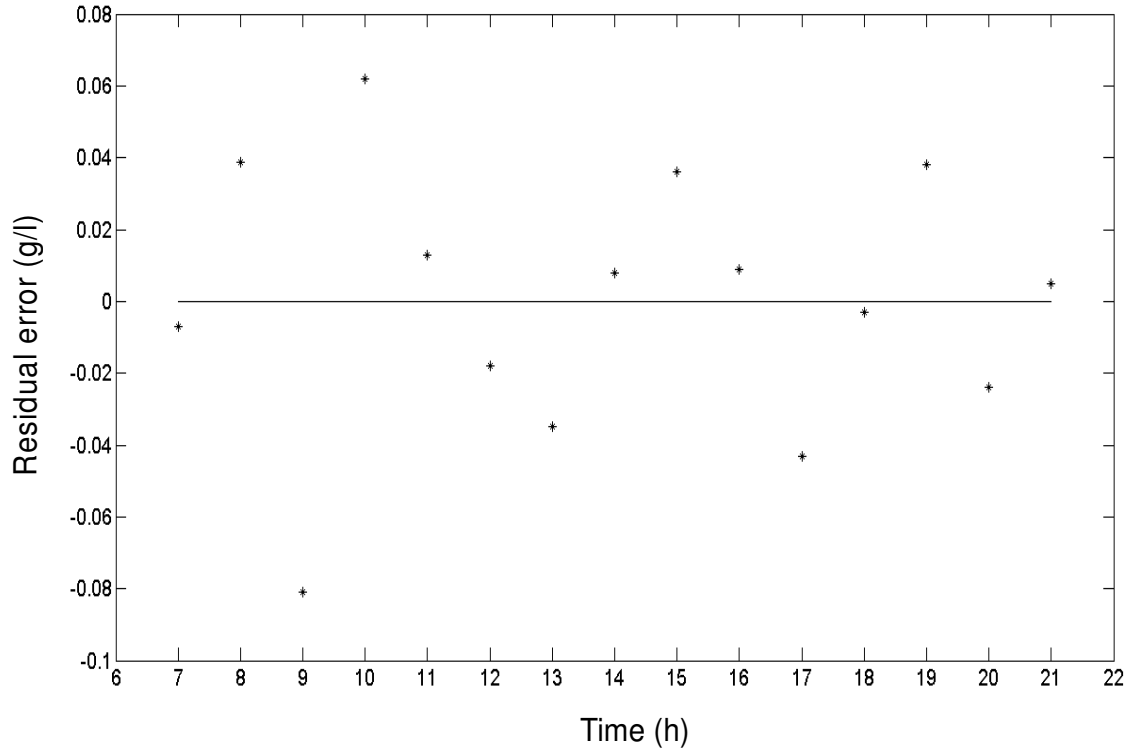

Figure 6. The fitting residual error.

the parameters were evaluated by the lsqnonlin and Runge-Kutta (ode45) methods of MATLAB. The kinetic parameters of fed-batch cultivation after high-temperature induction were: $\alpha=1.37\times10^{-3}\ g\cdot g^{-1}$ and $\beta=6.34\times10^{-2}\ g\cdot g^{-1}\cdot h^{-1}$. The kinetic model can agree well to the experimental data (Figures 7 and 8).

In general, the parameters were calculated and shown in Table 2. And the three models describing cell growth,

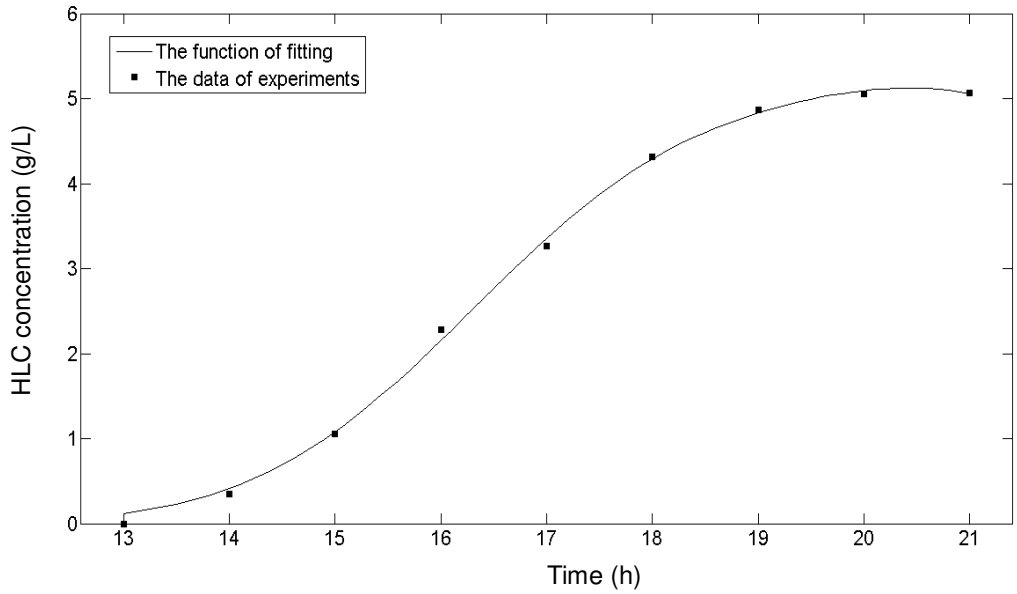

Figure 7. HLC concentration versus time in fed-batch cultivation phase.

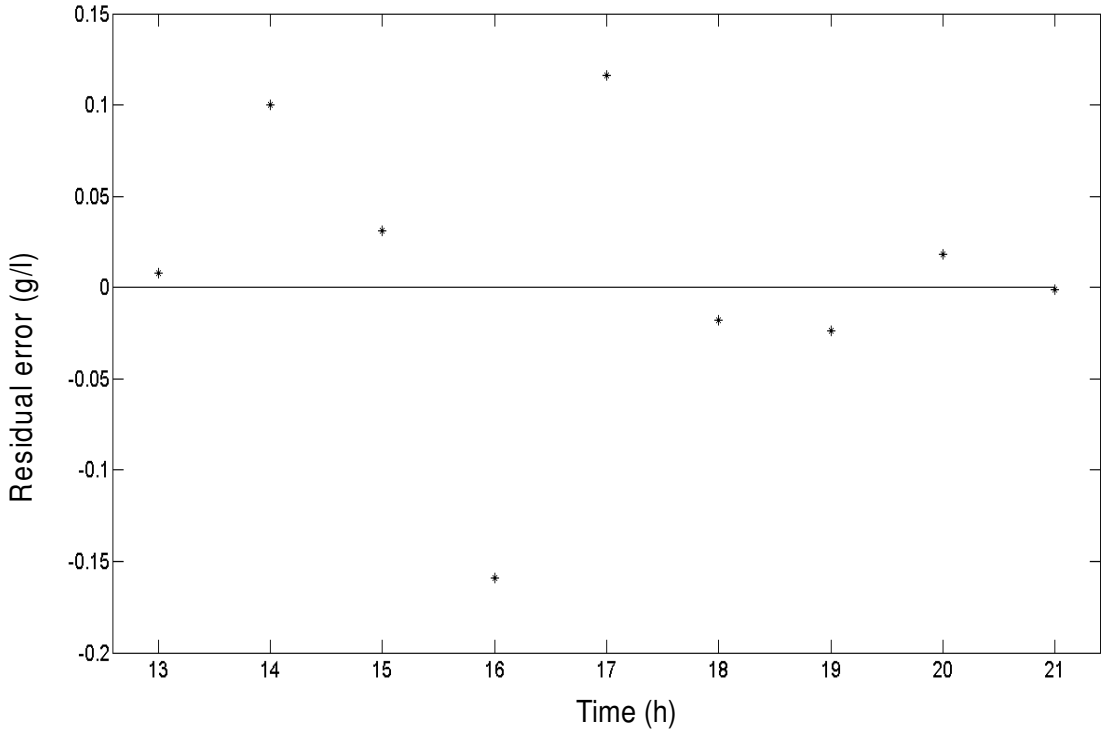

Figure 8. The fitting residual error.

substrate consumption and human-like collagen synthesis were listed as Equations 15, 16 and 17:

$$\frac{dx}{dt} = \frac{0.411\ \ s}{0.492\ x + s} x \quad (15)$$

$$\frac{ds}{dt} = \frac{937.5F}{15 + F\Delta t} - \frac{1}{0.428}\frac{dx}{dt} - \frac{1}{0.0716}\frac{dp}{dt} - 0.0395x \quad (16)$$

$$\frac{dp}{dt} = 1.37 \times 10^{-3}\frac{dx}{dt} + 6.34 \times 10^{-2} x \quad (17)$$

Table 2 Kinetic parameters of fed-batch culture

μ_{max}, h^{-1}	K_s, $g \cdot L^{-1}$	$Y^*_{x/s}$, $g \cdot g^{-1}$	$Y_{p/s}$, $g \cdot g^{-1}$	m, $g \cdot g^{-1} \cdot L^{-1}$	α, $g \cdot g^{-1}$	β, $g \cdot g^{-1} \cdot h^{-1}$
0.411	0.492	0.428	0.0716	0.0395	1.37×10^{-3}	6.34×10^{-2}

Those models would be a very useful reference to design expert control systems. Fed-batch experimental results were close to model predictions, which proved that the developed model could be used to design fed-batch fermentation. It could also be used as a reference for other feeding strategies of fed-batch fermentation.

Optimizing the feeding operation on the base of Pontryagin's minimum principle

Optimization of fed-batch fermentation system

The dominant vector (Wang et al., 2002) of state equation is linear in the fed-batch cultivation, and the final optimization model of this system could be represented as Equation 18:

The goal function: $$J = \phi[x(t_f)] \quad (18)$$

State equation: $$x = f(x) + g(x)u, 0 \le t \le t_f \quad (19)$$

The functions f(x), g(x) and ϕ(x) have n dimensions, and for all x:

$$g(x) \neq [0, \cdots, 0]^T \quad \frac{\partial \phi}{\partial x} \neq [0, \cdots, 0]^T \quad (20)$$

According to Pontryagin's minimum principle, the goal function J has the minimum value when Function H has the minimum value. We could gain the following equations when Function H is described as the formation of u:

$$H(x, \lambda, u) = \lambda^T f(x) + \lambda^T g(x)u \quad (21)$$

Considering the product formation, the concrete formation of f(x), g(x) and u are represented as Equations 22, 23 and 24:

$$f(x) = [\mu x_1, -\sigma x_1, \pi x_1, 0, 1] \quad (22)$$

$$g(x) = [0, s_f, 0, 1, 0] \quad (23)$$

$$u = F \quad F_{\min} \le F \le F_{\max} \quad (24)$$

Control algorism of the optimization in fed-batch fermentation system

The substrate was added into the fermenter continuously with no outflow, the optimization problem of fed-batch fermentation is the determination of the optimum feeding rate. The series of equations based on the material balance (Zhou et al., 2009) were:

$$(xV) = \mu x V; \; x(0) = x_0$$

$$(sV) = -\sigma x V + s_F F - mxV - [(\pi + \beta)/Y_{p/s}]xV; x(0) = s_0$$

$$(pV) = (\pi + \beta)xV; p(0) = p_0$$

$$V = F; V(0) = V_0$$

The restrictive conditions for the final volume and the feeding rate were:

$$V(t_f) = V_f \quad (25)$$

$$0 = F_{\min} \le F(t) \le F_{\max} \quad (26)$$

The key problem of fed-batch fermentation is how to feed substrate reasonably. According to Pontryagin's minimum principle, the goal function I(F)=g(x_f, p_f, s_f, V_f, t_f) which is dependent on the final fermentation results could have the minimum value. The x_5 could be introduced to denote the fermentation time, so the state variances could be represented as follows: x_1=X, x_2=S, x_3=P, x_4=V and x_5=t.

$$x = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \\ x_5 \end{bmatrix} = \begin{bmatrix} xV \\ sV \\ pV \\ V \\ t \end{bmatrix} = \begin{bmatrix} \mu x_1 \\ -[\sigma + (\pi + \beta)/Y_{p/s} + m]x_1 \\ (\pi + \beta)x_1 \\ 0 \\ 1 \end{bmatrix} + \begin{bmatrix} 0 \\ S_F \\ 0 \\ 1 \\ 0 \end{bmatrix} F$$

$$x_0 = \begin{bmatrix} (xV) \\ (sV) \\ (pV) \\ (V) \\ t \end{bmatrix}_{t=0}$$

Or

$$x = a(x) + bF \quad (27)$$

$$x(0) = x_0 \quad (28)$$

$$J[F] = g[x(tf)] \quad (29)$$

The system which is optimized could be denoted as the formulas (27) and (29), the restrictive conditions are the formulas (25) and (26), and the problem of optimization is to determine the dominant vector F(t), then the goal function could have the minimum value.

The determination of dominant vector F(t) in the fed-batch fermentation system is a standard vector problem, hence the Hamilton function could have the minimum value by Pontryagin's minimum principle (Qi et al., 2004):

$$H = \lambda^T [a(x) + bF] \quad (30)$$

$$\lambda^T = \left[0,1,-\frac{1}{t},1,\frac{P}{t^2}\right] \quad (31)$$

λ(t) must cater for the following condition:

$$\lambda(t) = -\frac{\partial H}{\partial x} = -\left[\frac{\partial a}{\partial x}\right]^T \quad \lambda = -(a)_x^T \lambda \quad (32)$$

The final condition could be represented as Equation 33:

$$\lambda_i(t_f) = \frac{\partial g}{\partial x_i(t_f)}; i = 1,2,3,5 \quad (33)$$

Along the optimum trace, the Hamilton function is the constant, the constant is called H* (Duan, 2007), and the Hamilton function could be rewritten as follows:

$$H^* = \lambda^T a + \lambda^T bF = \lambda^T * \begin{bmatrix} \mu x_1 \\ -\left(\sigma + \dfrac{\pi + \beta}{Y_{p/s}} + m\right) x_1 \\ (\pi + \beta) x_1 \\ 0 \\ 1 \end{bmatrix} + \lambda^T bF$$

$$= \left[0,1,-\frac{1}{t_f},1,\frac{P}{t_f^2}\right] * \begin{bmatrix} \mu x_1 \\ -\left(\sigma + \dfrac{\pi + \beta}{Y_{p/s}} + m\right) x_1 \\ (\pi + \beta) x_1 \\ 0 \\ 1 \end{bmatrix} + \left[0,1,-\frac{1}{t_f},1,\frac{P}{t_f^2}\right] * \begin{bmatrix} 0 \\ S_F \\ 0 \\ 1 \\ 0 \end{bmatrix} F$$

$$= -\left(\sigma + \frac{\pi + \beta}{Y_{p/s}} + m\right) x_1 - \frac{(\pi + \beta) x_1}{t_f} + \frac{P_f}{t_f^2} + S_F F + F = 0$$

$$\Rightarrow F = \left[\frac{\sigma + \dfrac{\pi + \beta}{Y_{p/s}} + m}{1 + S_F} + \frac{\pi + \beta}{(1 + S_F) t_f}\right] x_1 - \frac{P_f}{(1 + S_F) t_f^2}$$

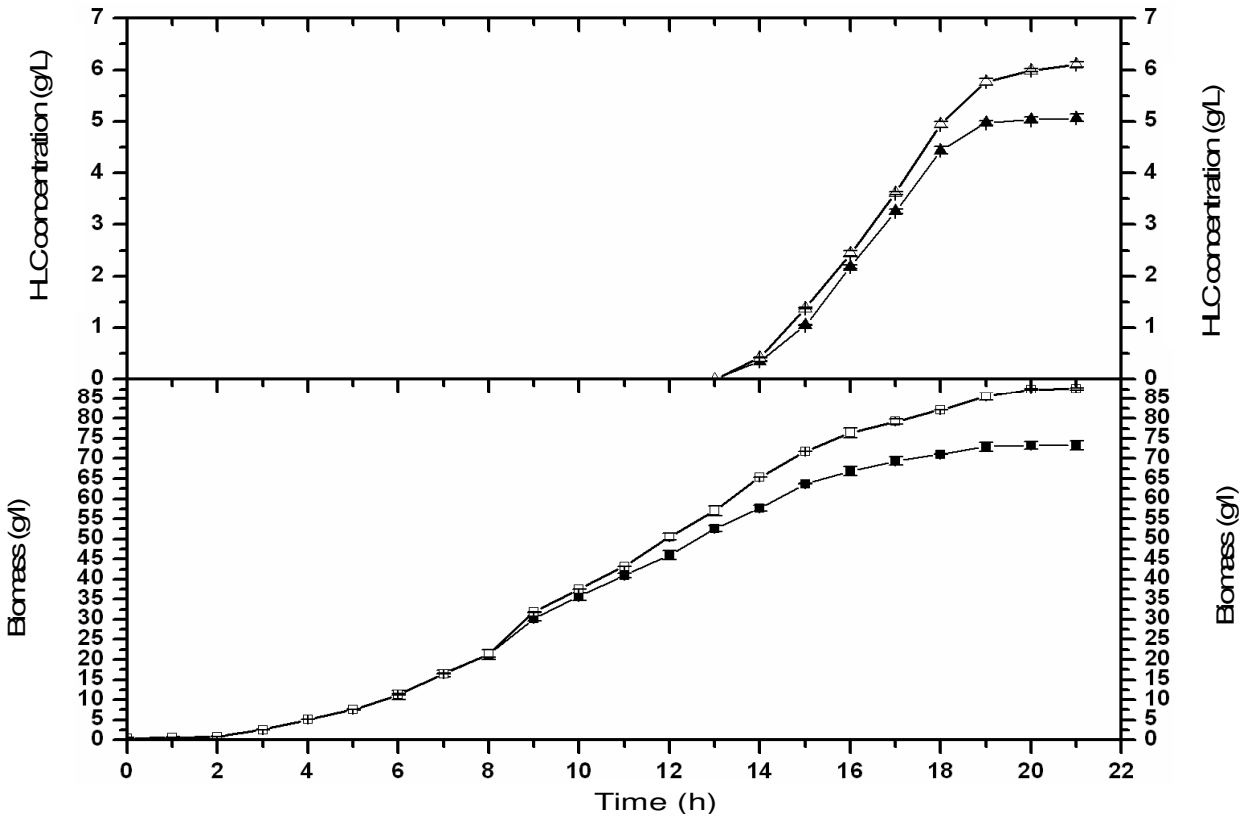

Figure 9. The process curve of cell growth and product formation. Δ&□: after the optimization by the Pontryagin's minimum principle; ▲&■: before the optimization by the Pontryagin's minimum principle.

Then

$$\frac{dx}{dt} = \mu x$$

$$\Rightarrow \quad \frac{dx}{x} = \mu\, dt$$

$$\Rightarrow \quad \int \frac{dx}{x} = \int \mu\, dt$$

$$\Rightarrow \quad \ln x = \mu t + C \qquad C = \ln x_0$$

$$\Rightarrow \quad \ln x_1 = \mu t_f + \ln x_0$$

$$\Rightarrow \quad x_1 = e^{\mu t_f + \ln x_0}$$

So

$$F = \left[\frac{\sigma + \dfrac{\pi+\beta}{Y_{p/s}} + m}{1+S_F} + \frac{\pi+\beta}{(1+S_F)t_f} \right] e^{\mu_f + \ln x_0} - \frac{P_f}{(1+S_F)t_f^2}$$

Before the induction:

$$F = (4.399\times10^{-2} + \frac{6.755\times10^{-5}}{t_f}) e^{\mu_f - 0.6733} - \frac{5.4\times10^{-2}}{t_f^2} \qquad (34)$$

After the induction:

$$F = (4.368\times10^{-1} + \frac{1.6488\times10^{-3}}{t_f}) e^{\mu_f - 0.6733} - \frac{4.32\times10^{-1}}{t_f^2} \qquad (35)$$

The verification experiments

According to the experimental data which was used in the kinetic model, the specific growth rate (μ) was controlled at 0.15 and 0.04 h^{-1}, respectively, before and after induction. The practical feeding was following Equations 34 and 35. Then, the results of cell growth and product formation could have a comparison with the previous results.

The situation of cell growth and product formation are shown in Figure 9. The result showed that after the

optimization by the principle minimum, the final concentrations of cell could reach 87.57g·L^{-1}, and they were raised by 17.78%. The final concentrations of product could reach 6.11 g·L^{-1}, and they were raised by 18.60%.

Conclusions

In this study, the kinetics models of the cell growth, substrate consumption and product formation were firstly determined on the base of experimental data by the lsqnonlin and Runge-Kutta (ode45) in MATLAB. The intrinsic relationship between feeding rate (F) and the specific growth rate (μ) could be obtained by the Pontryagin's minimum principle. When the specific growth rate (μ) was controlled at 0.15 and 0.04 h^{-1} before and after induction respectively, the final concentrations of cell and the target product could reach 87.57 and 6.11 g·L^{-1}, they were raised by 17.78 and 18.60% respectively. Pontryagin's minimum principle proves to be very useful in optimizing the feeding rate in the fed-batch cultivation.

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