Decoupled Geometric Control of Glucose and Dissolved Oxygen Concentration for Fedbatch Methionine Production

AMALENDU P. RANJAN, JAMES GOMES
Department of Biochemical Engineering
Indian Institute of Technology, Delhi
Hauz Khas, New Delhi – 110016, INDIA

Abstract: - The dynamics of fedbatch methionine production depends on the concentration of glucose and dissolved oxygen in the reactor. The process of methionine synthesis itself is highly regulated at the genetic level by the microorganism to ensure energetically efficient allocation of resources resulting in highly nonlinear behavior. The model used to describe this process reflects this interaction. To control this process effectively, a decoupled input-output linear controller is designed using differential geometry techniques for this process. The zero dynamics of the process are shown to be stable. Simulation results demonstrate that the performance of the decoupled geometric controller is satisfactory.

Key-Words: - Decoupled geometric controller, input-output linearization, zero dynamics, fed-batch process, methionine.

1 Introduction

Methionine is a sulfur bearing amino acid that is not synthesized in the human body and must be obtained dietary sources. It is essential for the normal functioning of body and it deficiency can lead to a number of ailments. Congenital defective methionine metabolism results in debilitating diseases such cystathioninuria and homocystinuria [1]. The *L*-form of methionine is used extensively in human medicine for a variety of therapeutic purposes, including pH and electrolyte balancing, parental nutrition, pharmaceutical adjuvant, and other applications. It can be synthesized within a microorganism via highly strictly regulated mechanism at the gene and enzyme level. Since it is energy intensive and it occurs at the terminal end of the metabolic pathway of the aspartic family of amino acids, the process becomes complex and the time profiles of these changes are nonlinear. Further, oxygen being one of the terminal electron acceptors in aerobic growth, it reflects the metabolic state of the process. The change in dissolved oxygen and the glucose (substrate) concentrations greatly affects the production of methionine. Hence it is desirable to develop a process control strategy, which maintains the optimum process conditions in terms of glucose and dissolved oxygen concentrations and produces *L*-methionine in a cost effective manner.

In fed-batch fermentation, the most important aspects to be considered are the changes in process dynamics during reactor operation. Their dynamic behavior is highly nonlinear and model parameters vary in an unpredictable manner. Moreover, a lack of reliable biosensor to measure all the state variables makes the process state very difficult to characterize. Dynamics of fed-batch fermentation are commonly described by a set of ordinary differential equations arising from mass balances of the biological species involved in the process. The nature of these dynamic behaviors motivates the development of nonlinear control techniques in order to attain the desired productivity. Literature shows that several control strategies have been tried with varying degree of success to overcoming these types of problems. Among these methods, nonlinear control theories based on the concepts of differential geometry have been developed by various research groups [2-4]. Using nonlinear coordinate transformations and nonlinear state feedback, the original nonlinear model can often be transformed into an equivalent linear model and linear controller design technique can then be employed. The system model can be linearized either by input state linearization [or input-output linearization [5-7].

Although the measurement of the cell and methionine concentrations online is difficult, the dissolved oxygen (*DO*) concentration and glucose concentration can be measured accurately and quickly. Hence, the inputs selected for the process are the feed flow rate and airflow rate.

In this paper, the development of a geometric controller that decouples the interaction between glucose and dissolved oxygen is presented. The

system is input-output linearized using glucose and *DO* concentration as observation. The interaction between the glucose and *DO* concentration is decoupled using a simple matrix inversion principle. The control design ensures exponentially decaying error dynamics. Since the combined relative degree using the two observations is lower than the dimension of the system, the zero dynamics are analyzed and shown to be stable. Simulation results showing the performance of the controller in various scenarios is presented.

2   Methionine Model and Control Problem

The synthesis of methionine by microorganisms is strictly regulated at the genetic level because it is energetically expensive requiring 7 molecules of ATP. Its production by mutant microorganisms shows that the concentration of methionine obtained in the reactor depends on the specific growth rate and the total cell concentration. Further, there is interaction between the state variables used to describe the process. In particular, the glucose and oxygen concentration in the reactor affects the production of methionine.

An unstructured model for the fed-batch cultivation of the methionine production was developed. The prolonged lag phase and the rapid changes to environmental process, which is well described by the exponential structure of specific growth rate rather than the structure of Monod kinetics. In the aerobic fermentation, the metabolism of the micro-organisms and the synthesis of the expected compound along with the undesirable products are reported to be highly influenced by DO [8]. A switching function was incorporated to describe the change in metabolism that occurs with the changing DO concentration in the bioreactor. Another specific feature of the methionine time-profile is that it is re-utilized when the glucose concentration drops below a critical value (s_c) in the external environment. To describe this behavior, a new term is introduced in the growth and non growth associated product dynamics. These features together enable an accurate prediction of methionine production in a fed-batch bioreactor.

The fedbatch model of methionine production is described by the following equation.

$$
\begin{bmatrix} \dot{x} \\ \dot{s} \\ \dot{p} \\ \dot{cl} \end{bmatrix} = \begin{bmatrix} f(cl)\,\mu(s)\,x \\ -\gamma\, f(cl)\,\mu(s)\,x - \eta\,x \\ \beta\, f(cl)\,\mu(s)\,x + \alpha\,(s - s_c)\,px/s \\ -\phi\, f(cl)\,\mu(s)\,x - \psi\,x \end{bmatrix} \qquad (1)
$$

$$
+ \begin{bmatrix} -x & 0 \\ (s_f - s) & 0 \\ -p & 0 \\ 0 & (cl^* - cl) \end{bmatrix} \begin{bmatrix} D \\ k_L a \end{bmatrix}
$$

Where $\mu(s) = f(c_l)\mu_m \exp(-K_E/s)\exp(-s/K_I)$ and $f(c_l) = \dfrac{(c_l/cl^*)^6}{(cl^{opt}/cl^*)^* + (c_l/cl^*)^6}$ is the metabolic switching function that accounts for the dependency between specific growth rate and *DO* concentration. Here γ, η, ϕ and ψ are the constants $(1/Y_{x/s} + \delta_1/Y_{p/s})$, $(\delta_2/Y_{p/s} + m_s)$, $(1/Y_{x/o} + \delta_1/Y_{p/o})$ and $(\delta_2/Y_{p/o})$ respectively and the δ_1 and δ_2 are the growth associated and non-growth associated formation rate for all products and byproducts.

In this fed-batch process model, the dilution rate (D) and $k_L a$ are the input variables. In actual control implementation, the glucose feed rate F_S and the airflow rate F_A, are used as the real inputs. In the first case F_S is obtained from the relation

$$F_S = DV = u_1 V \qquad (2)$$

In the second case, F_A is imbedded in the term $k_L a$ and it is obtained from [9]

$$
u_2 = k_L a = 0.026 \left[\frac{0.4\rho N^3 D_i^5 N_P}{V}\right]^{0.4}\left(\frac{F_A}{A}\right)^{0.5}
$$

$$
\therefore F_A = \left(\frac{u_2 V^{0.4} A^{0.5}}{0.0018\rho^{0.4} N^{1.2} D_i^2 N_P^{0.4}}\right)^2 \qquad (3)
$$

The parameters of Eqs. 1, 2 and 3 are explained in the nomenclature.

The substrate is consumed rapidly and reaches a critical value (s_c) at the end of the batch fermentation of methionine production. Below the critical concentration, methionine is re-utilized by the microorganism to maintain an energy economy. Hence, the glucose concentration should be maintained above the critical value. This set point value is calculated from the model equation which is required to maintain during fed batch fermentation. It has been found that the specific growth rate and the rate of methionine production are strongly influenced by oxygen. Both these rates are near their maximum values when the *DO* concentration is about 40%, which has been experimentally found as optimum for methionine production [Sharma and Gomes, 2001, Kumar et al 2003, Gomes and Kumar 2005].

Hence, for controlling the glucose as well as

DO simultaneously during fed-batch fermentation, a multiple input-output controller has to be designed to maintain the rate of production of methionine throughout the fed-batch process. The controller outputs, F_S and F_A, are calculated from the input-output linearization based control law such that the glucose added to reactor keeps the residual glucose concentration above the critical value and at the same time, maintains the *DO* concentration at 40% of the saturation value.

3   Derivation of the decoupling geometric control law

The structure of the above system (Eq. 1) can be expressed in the general form

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x}) + \mathbf{g}(\mathbf{x})\mathbf{u} \tag{4}$$

$$\mathbf{y} = \mathbf{h}(\mathbf{x}) \tag{5}$$

Where the biomass concentration x, residual glucose concentration s, methionine concentration p and *DO* concentration c_l, constitute the vector of state variables $\mathbf{x} \in \mathfrak{R}^n$; the residual glucose concentration s and *DO* concentration c_l, constitute the output measurements $\mathbf{y} \in \mathfrak{R}^m$; the dilution rate D and oxygen mass transfer coefficients k_la, constitute the input variables $\mathbf{u} \in \mathfrak{R}^p$. The kinetics and the transport, $\mathbf{f}$ and $\mathbf{g}$ are smooth vector fields, and the observation $\mathbf{h}$ is smooth scalar function.

The general procedure for the controller design of a square system ($m = p$) is presented below. To obtain the input-output relation for any output y_j of the system, successive Lie differentiations are performed until for some r_j at least one of the inputs appear in $y_j^{(r_j)}$ then

$$y_j^{(r_j)} = L_{\mathbf{f}}^{r_j}h_j + \sum_i^p L_{\mathbf{g_i}}L_{\mathbf{f}}^{r_j-1}h_j u_i \tag{6}$$

With $L_{\mathbf{g_i}}L_{\mathbf{f}}^{r_j-1}h_j(\mathbf{x}) \neq 0$ for at least one $i\ \forall \mathbf{x} \in \mathfrak{R}^n$.

The above procedure is performed for each output y_j and a total of m equations in the above form was obtained, which has been written compactly as

$$\begin{bmatrix} y_1^{(r_1)} \\ \wedge \\ y_m^{(r_m)} \end{bmatrix} = \begin{bmatrix} L_{\mathbf{f}}^{r_1}h_1(\mathbf{x}) \\ \wedge \\ L_{\mathbf{f}}^{r_m}h_m(\mathbf{x}) \end{bmatrix} + \mathbf{E}(\mathbf{x})\begin{bmatrix} u_1 \\ \wedge \\ u_m \end{bmatrix} \tag{7}$$

Where $\mathbf{E}(\mathbf{x})$ is an $m \times m$ matrix defined as

$$\mathbf{E}(\mathbf{x}) = \begin{bmatrix} L_{\mathbf{g_1}}L_{\mathbf{f}}^{r_1-1}h_1 &] & L_{\mathbf{g_m}}L_{\mathbf{f}}^{r_1-1}h_1 \\ \wedge &] & \wedge \\ L_{\mathbf{g_1}}L_{\mathbf{f}}^{r_m-1}h_m &] & L_{\mathbf{g_m}}L_{\mathbf{f}}^{r_m-1}h_m \end{bmatrix} \tag{8}$$

The matrix $\mathbf{E}(\mathbf{x})$ is called the decoupling matrix. If the decoupling matrix is nonsingular in a region around a point $\mathbf{x_0} \in \mathfrak{R}^n$, then the input transformation

$$\mathbf{u} = -\mathbf{E}^{-1}\begin{bmatrix} L_{\mathbf{f}}^{r_1}h_1(\mathbf{x}) \\ \wedge \\ L_{\mathbf{f}}^{r_m}h_1(\mathbf{x}) \end{bmatrix} + \mathbf{E}^{-1}\begin{bmatrix} v_1 \\ \wedge \\ v_m \end{bmatrix} \tag{9}$$

yields a linear differential relation between the output $\mathbf{y}$ and new input $\mathbf{v}$.

$$\begin{bmatrix} y_1^{(r_1)} \\ \wedge \\ y_m^{(r_m)} \end{bmatrix} = \begin{bmatrix} v_1 \\ \wedge \\ v_m \end{bmatrix} \tag{10}$$

The above input-output relation is decoupled.

Our case of fedbatch methionine production is a 2×2 system. The controller is designed directly from the general case. Since s and c_l can be measured online, the output vector is

$$\mathbf{y} = \begin{bmatrix} s & c_l \end{bmatrix}^T \tag{11}$$

From state space representation, the decoupling matrix is obtained as

$$\mathbf{E}(\mathbf{x}) = \begin{bmatrix} s_f - s & 0 \\ 0 & c_l^* - c_l \end{bmatrix} \tag{12}$$

The relation between the input and output is

$$\begin{bmatrix} v_1 \\ v_2 \end{bmatrix} = \begin{bmatrix} -\gamma\ f(cl)\ \mu(s)\ x - \eta\ x \\ -\varphi\ f(cl)\ \mu(s)\ x - \phi\ x \end{bmatrix} + \mathbf{E}(\mathbf{x})\begin{bmatrix} u_1 \\ u_2 \end{bmatrix} \tag{13}$$

Where v_1 and v_2 can be chosen as given below to make error is in s and c_l decay exponentially.

$$\begin{bmatrix} v_1 \\ v_2 \end{bmatrix} = \begin{bmatrix} \dot{s} \\ \dot{c}_l \end{bmatrix} = \begin{bmatrix} \dot{s}_d + k_1(s_d - s) \\ \dot{c}_l + k_2(c_{ld} - c_l) \end{bmatrix} \tag{14}$$

4 Stability of zero dynamics

The relative degree r of the system is 2 and hence there are two variables x and p whose dynamics are not observable and constitutes the internal dynamics of the process. By studying the zero dynamics, the extreme case of the internal dynamics with the outputs(s, c_l) constrained to be exactly zero, we can determine if all the states of the process will be stable under the action of the controller designed based on the measurement of s and c_l. In order to keep the outputs exactly zero, control input must be chosen as

$$\mathbf{u}^*(\mathbf{x}) = -\mathbf{E}^{-1}(\mathbf{x})\begin{bmatrix} \mathbf{L_f}^{r_1}h_1(\mathbf{x}) \\ \wedge \\ \mathbf{L_f}^{r_m}h_m(\mathbf{x}) \end{bmatrix} \tag{15}$$

For the above system equation (1)

$$\mathbf{u}^*(\mathbf{x}) = -\begin{bmatrix} \dfrac{1}{s_F - s} & 0 \\ 0 & \dfrac{1}{c_l^* - c_l} \end{bmatrix} \begin{bmatrix} -\gamma \mu(s)x - \eta x \\ -\varphi \mu(s)x - \phi x \end{bmatrix} \quad (16)$$

Substituting this into (4), and with the additional condition of outputs equal to zero we get the zero dynamics x as

$$\dot{x} = -\frac{\eta}{s_F} x^2 \quad (17)$$

Since η and s_F are positive, x is stable. The zero dynamics for p is given by

$$\dot{p} = \left[\alpha(1 - \frac{s_c}{\varepsilon}) - \frac{\eta}{s_F} \right] px \quad (18)$$

Where ε ($\varepsilon \ll s_c$) is a minimum value of the glucose concentration which is needed for the cells to survive. Since x is positive and bounded, the dynamics of p is stable. Therefore, the fedbatch methionine process is also stable.

5   Performance of decoupled geometric controller

The simulations were carried out using parameter values given in nomenclature. The initial conditions of the simulations were determined based on actual fermentation runs carried out in a 15 l bioreactor (B-Braun). The initial glucose, cell mass and *DO* concentrations were 50 g/l, 1.15 g/l and 8 mg/l respectively. It was assumed that residual glucose and *DO* concentrations can be measured online. The objective of the controller was to regulate the *DO* concentration at 40% and the glucose concentration at 4 g/l. Since the glucose decreases only when consumed by the microorganism, the bioreactor was run in batch mode until the residual glucose concentration was 4 g/l. At this point, the control action for maintaining glucose concentration at 4 g/l is initiated. The concentration of the glucose in the feed solution was 25 g/l. The control action for *DO* is initiated at the beginning of the process. To mimic real time online data, 2 % random noise was added to measured data that is in glucose and *DO*. After several trial simulations, the best performance of the controller was achieved for $k_1 = 2$ and $k_2 = 40$.

The performance of the controller for maintaining the residual glucose concentration at 4 g/l, in the absence of noise and presence of 2% noise in residual glucose measurement, is presented is Figs. 1a and 1b, respectively. In the absence of noise the control action is smooth and the set point regulation of the residual glucose concentration is

accurate. In the presence of 2% noise, the glucose feed rate shows a high degree of sensitivity. The performance was studied for $0.1 < k_1 < 100$ and best results were obtained between $1.0 < k_1 < 10$. Figure 1b shows the result for $k_1 = 2.0$. The reason for this sensitivity to noise may be because the feed concentration is not optimized and has been set at 25 g/l. A decrease in the feed concentration increases the volume added in each control action and thus result in the decrease of the methionine concentration in the reactor. Whereas, increasing the feed concentration results in sensitive control action.
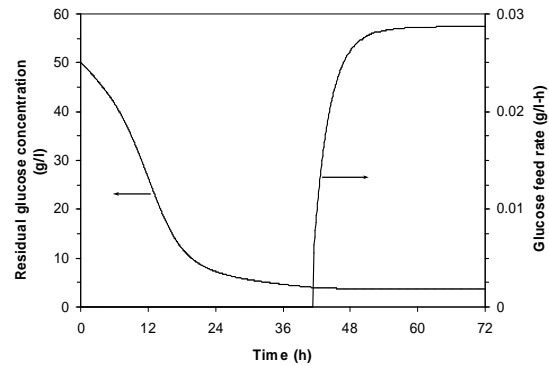
Fig. 1a. Control action showing the glucose feed rate for maintaining the residual glucose concentration at 4 g/l in the absence of noise.
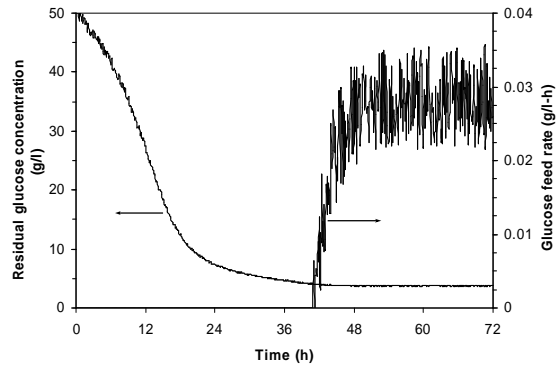
Fig. 1b. Control action showing the glucose feed rate for maintaining the residual glucose concentration at 4 g/l in the presence of 2% noise in glucose measurement.

The performance of the controller for maintaining the *DO* concentration at 40% of the saturation value is presented in Figs. 2a and 2b. These figures show respectively, the performance in the absence of noise and in the presence of 2% noise in *DO* measurement. The control action is smooth and regulation of *DO* concentration at 0.0032 g/l (40% saturation) is satisfactory, both in the absence and in the presence of noise. The airflow rate does

exhibit some sensitivity during the peak growth phase when the demand for oxygen by the microorganism is highest.
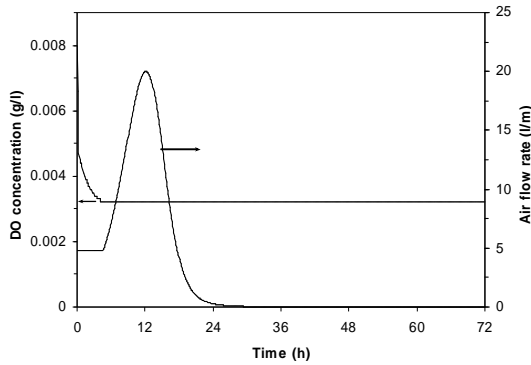

Fig. 2a. Control action showing the airflow rate for maintaining the *DO* concentration at 40% of the saturation value in the absence of noise.
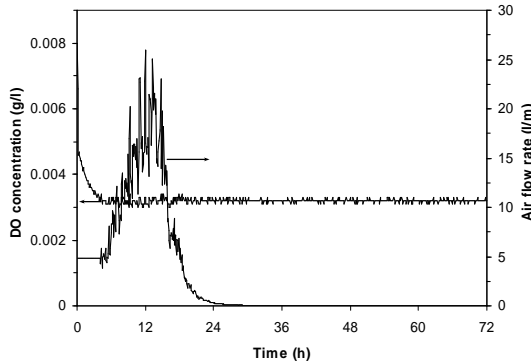

Fig. 2a. Control action showing the airflow rate for maintaining the *DO* concentration at 40% of the saturation value in the presence of 2% noise in *DO* measurement.

The time-profiles of methionine, residual glucose and cell mass concentration, when the fedbatch production is carried out under the action of the geometric controller is presented in Fig. 3. A final methionine concentration of about 4.3 g/l is obtained in the reactor. The experiment assumes that the mutant microorganism will possess the desirable characteristics, such as analogue resistant, so that this target is achievable.

The performance of the controllers was also tested set point changes. These results are presented in Figs. 4a and 4b. In the case of the residual glucose concentration, the set point was changed from 4 g/l to 4.5 g/l at the instant of 50 h. The response of the controller is immediate. Although the control action is sensitive to noise, its performance is acceptable. The inset shown in Fig. 4a, shows the performance

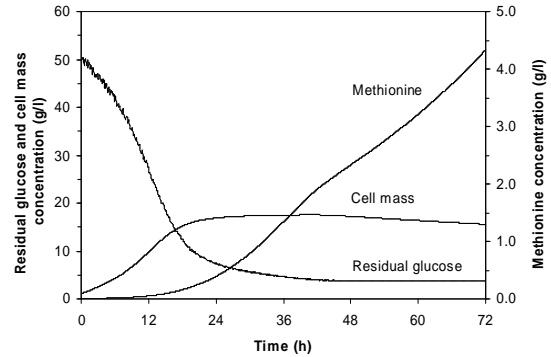of set point tracking of the residual glucose concentration.

Fig. 3. The time-profiles of methionine, residual glucose and cell mass concentrations, when the decoupled geometric controller is used to maintain the residual glucose concentration at 4 g/l and the *DO* concentration at 40% of the saturation value in the presence of 2% noise in both measurements.
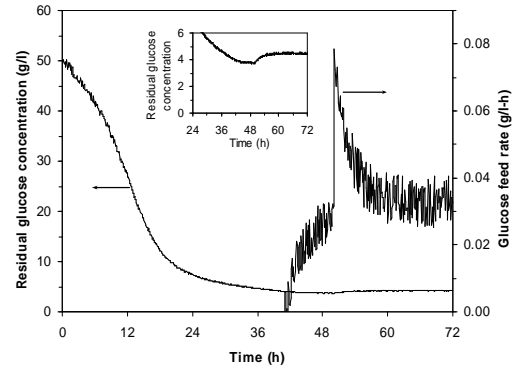

Fig. 4a. Control action for set point change in the residual glucose concentration from 4 g/l to 4.5 g/l at 50h. The inset shows the residual glucose response between 24h and 72 h.

A similar set point change experiment was carried out for the *DO* concentration. The set point changed from 40% to 50% at the instant of 12 h and then from 50% to 30% at he instant of 18 h. The result is presented in Fig. 4b. Clearly, the set point tracking of *DO* is acceptable. The air flow rate responds to the change quickly. In the case of the step down from 50% to 30%, the airflow rate drops to a small value within 4 time steps. The reason for the air flow rate to remain at this low value is that the demand for oxygen (as required for growth) is primarily over by the first 20 hours (see Fig. 2a). Further, the reduction in *DO* occurs only by consumption by the microorganism; consequently a low demand translates to low supply rate for the controller.
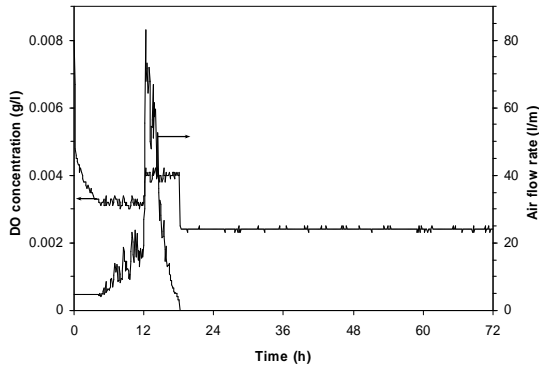
Fig. 4b. Control action for set point change in the *DO* concentration from 40% to 50% at 12 h followed by a change from 50% to 30% at 18 h..

6 Conclusions

The development of a nonlinear controller for methionine production has been successfully presented. The control objective was to regulate residual glucose concentration and the *DO* concentration at predetermined set points. The performance of the decoupled geometric controller was also evaluated fro set point changes. The control action shows sensitivity to noise; however, the control action remains satisfactory. The sensitivity problem may be addressed by using filtering techniques in actual implementation. An overall evaluation shows that the decoupled geometric controller is successful in meeting the performance criteria of the fedbatch methionine production process.

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Nomenclature

Model Parameter	Description	Units	Value
δ_1	Growth associated formation rate for all products and by-products	g/g	1.49×10^{-4}
δ_2	Non-growth associated formation rate for all products and by-products.	h^{-1}	5.5×10^{-4}
$Y_{x/s}$	Biomass yield based on glucose	g/g	0.38
$Y_{p/s}$	Product yield based on glucose	g/g	0.105
$Y_{x/o}$	Biomass yield coefficient based on oxygen	g/g	2.33
$Y_{p/o}$	Product yield coefficient based on oxygen	g/g	3.26
s_c	Critical glucose concentration	g/l	3.25
γ	$1/Y_{x/s} + \delta_1/Y_{p/s}$	g/g	2.633
η	$\delta_2/Y_{p/s} + m_s$	g/gh	0.0052
α	Non-growth associated product synthesis coefficient	$g^{-1}lh^{-1}$	0.0139
β	Growth associated product synthesis coefficient	g/g	0.0039
K_e	Exponential equivalent of the Monod constant	g/l	27.99
K_i	Glucose inhibition constant	g/l	400.26
k_La	Mass transfer coefficient	h^{-1}	50
c_l^*	Saturation value of dissolved oxygen concentration	g/l	0.008
ϕ	$\left(1/Y_{x/o} + \delta_1/Y_{p/o}\right)$	g/g	0.4292
ψ	$\left(\delta_2/Y_{p/o}\right)$	g/gh	0.000258
μ_m	Maximum specific growth rate	h^{-1}	0.7