



A cybernetic model to describe the dynamics of myeloma cell cultivations

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ABSTRACT

A cybernetic model is developed to model the dynamics of mammalian cell growth and metabolism. Especially, the formation of the byproducts such as ammonia and alanine are taken into account in the model. The production of these byproducts is mainly regulated by the competition between transamination and deamination. In addition, amino acids utilization for protein formation and energy supply is also involved due to lysine limitation found in our experiments. The model is able to simulate the transients of the substrate and byproduct concentrations, the viable and dead biomass as well as the intracellular concentrations of the intermediates and enzymes. Myeloma cell cultivations are applied to validate the model.

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1. Introduction

Mammalian cell culture is widely used for production of many recombinant proteins, which find applications as diagnostic, analytical and therapeutic agents [1,2]. To enhance the productivity of mammalian cell culture, perfusion is often practiced which results in perturbations in culture conditions [3]. Mathematical models which are able to describe the dynamic response of cell populations to such perturbations will benefit better control of the process. The aim of this study is to develop a cybernetic model to capture the dynamics of the myeloma cell cultivations. In the literature, the cybernetic framework was developed by Ramkrishna and co-workers for the description and analysis of the metabolic network of the microorganism [4,5]. It has been found successful applications in describing the transients of bioreactions [6–10] and the metabolic pathway regulations in hybridoma cell culture [11–13].

The cybernetic approach is based on the assumption that metabolic systems have evolved optimal goal oriented regulatory strategies as a result of evolutionary pressures [5,14]. In other words, in order to realize an optimal nutrient utilization, the metabolic system is considered to be able to redirect the synthesis and activity of the enzymes in the face of environmental perturbations or genetic alternations to network structure or function. The synthesis and activity of enzymes are regulated by complicated metabolic and regulatory networks that are represented by cybernetic variables, denoted as ‘*u*’ and ‘*v*’, respectively. These cybernetic variables are derived on the basis of the matching law and proportional law [14].

Within the cybernetic framework, the metabolic system is abstracted to suitable pathways of interest. The abstracted pathway is further decomposed into four types of elementary pathways, i.e. convergent, divergent, linear or cyclic pathways, in which reactions compete to utilize or produce the same metabolite. Pathway abstraction, and the identifications of the

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Nomenclature

E_i	the <i>i</i> th enzyme
e_i	the level of the <i>i</i> th enzyme (g/gdw)
K_i	saturation constant for the <i>i</i> th reaction (g/L or g/gdw)
$K_{7,Gln}, K_{7,Lys}$	saturation constants for reaction 7 (g/L or g/gdw)
$K_{m7,Gln}, K_{m7,Lys}$	saturation constants for the saturated part of reaction 7 (g/L or g/gdw)
$K_{8,Gln}, K_{8,Lys}$	saturation constants for reaction 8 (g/L or g/gdw)
K_{M_0}	saturation constant for intracellular reaction on M_0 (g/gdw)
K_M	saturation constant for synthesis of M_0 on intermediate (g/gdw)
K_{ei}	saturation constant for the <i>i</i> th enzyme synthesis (g/gdw)
K_E	saturation constant for enzyme synthesis on intracellular lysine (g/gdw)
K_{Lys}	saturation constant for lysine uptake (g/L)
K_d	constant of cell death rate (g/gdw)
Glc, Gln, Lys_ext, Lys_int	glucose, glutamine, extra- and intracellular lysine concentrations (g/L or g/gdw)
Lac, Amm, Ala	lactate, ammonia and alanine concentrations (g/L)
M_0, M_1, M_2, M_3, M_4	intermediates of the metabolic network
C_{M_i}	the level of <i>i</i> th intermediates (g/gdw)
P_i	concentration of the <i>i</i> th species (g/L or g/gdw)
r_i	rate of the <i>i</i> th enzyme regulated reaction (1/h)
$r_{i,m}$	rate of the <i>i</i> th reaction saturated with enzyme (1/h)
r_{ei}	synthesis rate of the <i>i</i> th enzyme (1/h)
r_{min}	constitutive rate of enzyme synthesis (1/h)
$r_{e,max}$	maximum enzyme synthesis rate (1/h)
r_{Lys}	specific uptake rate of lysine (1/h)
$r_{Lys,max}$	maximum uptake rate of lysine (1/h)
r_g	specific growth rate (1/h)
r_{M_0}	formation rate of M_0 (1/h)
r_D	specific death rate
$r_{i,max}$	maximum rate of r_i (1/h)
$r_{mi,max}$	maximum rate of $r_{i,m}$ (1/h)
$r_{D,max}$	maximum rate of r_D
u_i	cybernetic variable governing the enzyme synthesis rate of the <i>i</i> th enzyme
$u_{i,c}, u_{i,d}$	u_i for convergent and divergent pathways
$u_{7,dGln}, u_{7,dLys}$	u_7 for divergent pathways of glutamine and lysine utilization
v_i	cybernetic variable governing the activity of the <i>i</i> th enzyme
$v_{i,c}, v_{i,d}$	v_i for convergent and divergent pathways
$v_{7,dGln}, v_{7,dLys}$	v_7 for divergent pathways of glutamine and lysine utilization
Glc _F , Gln _F , Lys _F	feed concentrations of glucose, glutamine and lysine (g/L)
$r_{M_0,max}$	maximum formation rate of M_0 (1/h)
Y_i	yield coefficient for the <i>i</i> th reaction
$Y_{Amm/3}, Y_{Amm/8}$	ammonia yield coefficient for reaction 3 and 8
$Y_{Ala/8}$	alanine yield coefficient for reaction 8
$F_{7,Gln}, F_{7,Lys}$	stoichiometric coefficients of glutamine and lysine in reaction 7
$F_{8,Gln}, F_{8,M_1}$	stoichiometric coefficients of glutamine and M_1 in reaction 8
$F_{M_0,M_1}, F_{M_0,M_2}, F_{M_0,M_3}, F_{M_0,M_4}$	stoichiometric coefficients of M_1 – M_4 for M_0 synthesis
X_V	viable biomass concentration (g/gdw)
X_D	dead biomass concentration (g/gdw)
F	flow rate of feeding (L/h)
V	culture volume (L)

elementary pathways as well as the corresponding competitions are generally guided by metabolic flux analysis (MFA) results [9].

In the cybernetic model proposed in this contribution, glucose and glutamine are considered as partial replaceable and complementary substrates in the metabolic network. Meanwhile, the competition between deamination pathway and transamination pathway to use glutamine to produce α -ketoglutarate (α -KG) is taken into account since these two pathways will lead to different level of glutamine utilization and different quantity of byproducts (i.e. ammonia and alanine) [15,16]. In addition, the utilization of amino acids is considered because amino acids limitation often happened in mammalian cell culture [17–21]. One rigorous batch culture and one Fed-batch culture with pulse feedings are used to validate the proposed model.

2. Materials and methods

2.1. Cell line and culture medium

The myeloma cell line X63-Ag8.653 without recombinant products expression was used as model cell line. The base medium used in flask and spinner cultures was RPMI 1640 supplemented with 10% fetal calf serum (FCS) (Gibco, UK), 2 mM glutamine and 80 µg/ml gentamicin. Two feeding media, FM1 and FM2, were used which had the following compositions: FM1 contained 18 g/L glucose and 3 g/L glutamine with 10×RPMI; FM2 contained 17.9 g/L glucose and 3.2 g/L glutamine with 1×RPMI.

2.2. Cultivation procedures

Both experiments started with a working volume of 150 ml, and glucose and glutamine concentrations of 1.75 g/l and 0.5 g/l, respectively.

Experiment 1: Rigorous batch culture.

Experiment 2: Fed-batch culture with pulse feedings carried out three times at 54.5 h, 73 h and 113 h, respectively. At 54.5 h, 2.5 ml FCS and 3.5 ml FM2 were added to achieve the glucose concentration of 1.0 g/l. At 73 h, 8 ml FCS and 4 ml FM2 was added with the target glucose concentration of 1.0 g/l. At 113 h, 10 ml FCS and 3.6 ml FM1 was added. Before feeding, 13.6 ml supernatant was removed from the culture medium.

Both cell culture experiments were stopped at 134 h. During the cultivation, the temperature was maintained at 37 °C with a rotation rate of 120 rpm, while the concentrations of CO₂ and humidity were controlled at 5% and 80%, respectively.

2.3. Analysis method

Glucose and lactate were measured with a YSI2700 analyzer (Ohio, USA). Ammonia concentration was determined by using enzyme-based assay kits. Amino acids were analyzed by HPLC (KONTRON, Germany) and evaluated with the Kroma2000 software.

3. Metabolic pathways in myeloma cells

A simplified metabolic network in mammalian cells is depicted in Fig. 1 [12,15,16,22]. It consists of glycolysis, pentose phosphate pathway (PPP), glutaminolysis, TCA cycle and amino acid utilization pathways.

Glycolysis represents a biochemical pathway in which glucose is firstly converted into pyruvate. Pyruvate is then used for ATP generation via TCA cycle or be converted to lactate which will be secreted from the cell to the medium. The net reaction for the conversion of glucose to pyruvate is



Eq. (1) shows a net reduction of NAD⁺ to NADH such that glycolysis would not be sustainable if there were no way to regenerate the NAD⁺. Under aerobic conditions, this will be mainly balanced by oxidative phosphorylation in which NADH is oxidized to NAD⁺. Otherwise, the degradation of pyruvate to lactate will serve dominantly to regenerate the NAD⁺ as shown in Eq. (2)



The primary function of the PPP is to produce NADPH and ribose 5-phosphate. NADPH is one of the pyridine nucleotides used for reductive biosynthesis. Ribose 5-phosphate is required for the synthesis of many important compounds like DNA, RNA, CoA and NAD⁺.

Glutaminolysis is to utilize glutamine for energy production. In glutaminolysis, glutamine is converted to pyruvate with the concomitant of NADH. The carbon skeleton of glutamine enters the TCA cycle via α-ketoglutarate (α-KG) and leaves via malate which is then converted to pyruvate. The primary pathway of glutamine to α-KG is believed to go through glutamate



The conversion of glutamate to α-KG can proceed via deamination or transamination



TCA cycle has the dual roles of generating energy and providing biosynthesis precursors. The carbon atoms from glucose via Ac-CoA are oxidized completely to CO₂. This process will convert NAD⁺ to NADH and produce ATP. As stated above, glutamine enters the TCA cycle via α-KG and then follows the cycle to produce NADH which is used for ATP generation when

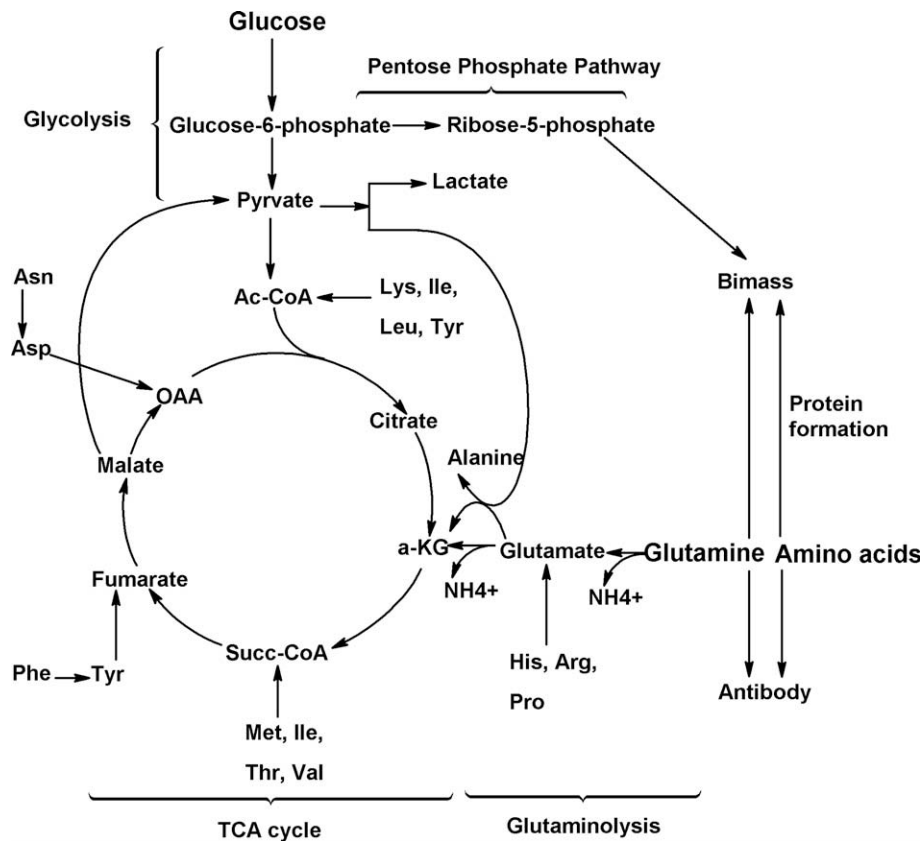


Fig. 1. Simplified metabolic pathways of myeloma cell line.

coupled with oxidative phosphorylation. Amino acids but not glutamine also participate TCA cycle via different points (Fig. 1).

Amino acids are used to produce protein, nucleic acids, and oxidation via TCA cycle. Some amino acids are synthesized and then excreted to the medium [23].

4. Cybernetic model formulation

In general, the procedure of identifying cybernetic models consists of the following steps [13]:

1. Pathway abstraction. Only the suitable pathways of interest will be considered in the cybernetic structure.
2. Identification of the elementary pathway. The abstracted network is considered consisting of convergent, divergent, linear or cyclic pathways.
3. Identification of the competitions in the elementary pathway.
4. Definition of cybernetic variables.
5. Formulation of the species balance equations.

4.1. Pathway abstraction

The metabolic network given above is abstracted according to the MFA results of the work [22,24,25], see Fig. 2. Glucose is utilized either to form pyruvate (M_1) through glycolysis or to produce intermediates (M_3) through PPP. Pyruvate may then either enter TCA cycle (M_2) for energy generating or be converted to lactate. A small part of pyruvate will participate in the pathway leading to the formation of alanine. The intermediates of TCA cycle, denoted by M_2 , contribute to the pyruvate pool. Glutamine utilization serves for both energy generating via TCA cycle and protein formation (M_4). For energy generating, the amino groups of glutamine are used either to produce alanine through transamination reaction (E_8) or to generate ammonia by deamination reaction (E_3). The growth precursors, i.e. M_1 , M_2 , M_3 and M_4 , are considered to form the rest of biomass denoted by M_0 . Lysine limitation was observed in our myeloma cell cultivations so that lysine utilization is involved in this modeling. Lysine is either channeled into TCA cycle or together with glutamine into the pathway of protein formation.

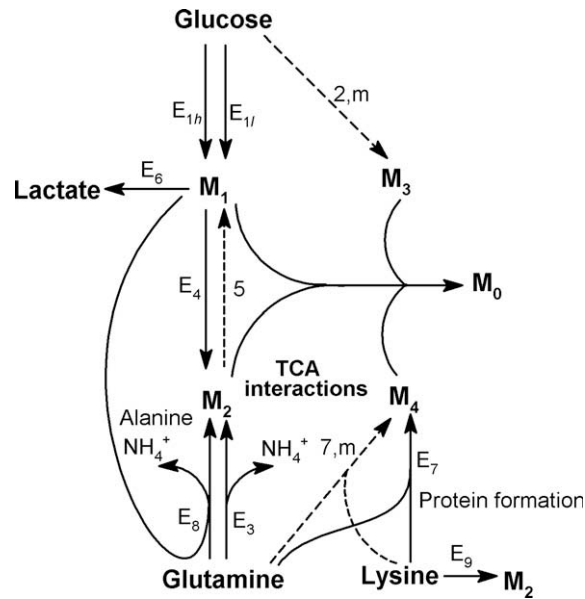


Fig. 2. Abstracted metabolic pathways used in the cybernetic model. Solid arrows indicate the pathways regulated by enzymes; dashed arrows indicate the pathways with saturated enzymes.

4.2. Identification of elementary pathways, competitions, and formulation of cybernetic variables

The abstracted metabolic network is further represented with two subnetworks, namely subnetwork (a) and (b), as shown in Fig. 3. Subnetwork (a) comprises a convergent pathway in which E_{1h} and E_{1l} compete for the utilization of glucose to produce M_1 . The conversion of M_1 to lactate is also regarded as a part of this subnetwork because the formation of pyruvate from glucose, and lactate from pyruvate occur concurrently [12]. Subnetwork (b) consists of one convergent pathway and two divergent pathways. The convergent pathway involves three enzymes E_3 , E_4 and E_8 that compete to produce M_2 . One of the divergent pathways is considered to illustrate the competition of glutamine utilization with respect to enzymes E_3 , E_7 and E_8 . The other is to demonstrate the competition of intracellular lysine utilization to form M_4 and M_2 via E_7 and E_9 , respectively.

Enzymes E_{1h} and E_{1l} compete in a substitutable manner for glucose utilization. Enzyme E_{1h} is considered to dominate the pathway under the condition of high level of glucose, while E_{1l} takes over under glucose starvation conditions. The cybernetic variable u_{1h} for the synthesis of enzyme E_{1h} is also used as a global cybernetic variable for the enzyme E_6 , for lactate formation from pyruvate. The PPP is modeled to produce the M_3 moiety which can not be made from glutamine. Also, the glutamine $\rightarrow M_4$ pathway produces protein which can not be formed from glucose. The enzymes of PPP are assumed to be saturated. Enzymes E_3 and E_8 compete to produce M_2 from glutamine. Meanwhile, these two enzymes together compete with E_7 to utilize glutamine. On the other hand, E_3 and E_8 compete with E_4 to form M_2 . The convergent competition among E_3 , E_4 and E_8 has effect on the divergent competition among E_3 , E_7 and E_8 . The processes, lysine $\rightarrow M_2$ and lysine $\rightarrow M_4$, are modeled as a divergent competition. The lysine $\rightarrow M_2$ pathway may be promoted at a high level of intracellular lysine while lysine $\rightarrow M_4$ may be promoted at a low level of intracellular lysine. The flux of lysine incorporated into the protein formation is regulated by E_7 . The flux of the reaction glutamine + lysine $\rightarrow M_4$ can be separated into two parts. One of them ensures a basal level of M_4 for biomass synthesis. Intermediates M_1 , M_2 , M_3 and M_4 leads to the formation of the rest of biomass, denoted by M_0 . All of the intracellular reactions are then considered to be catalyzed by M_0 .

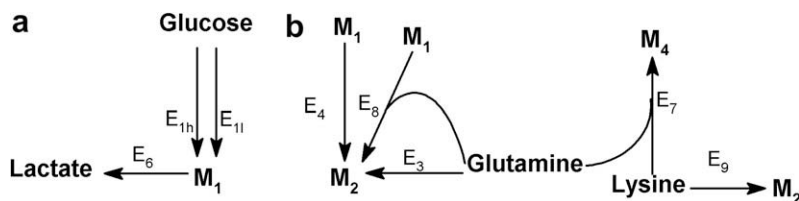


Fig. 3. Subnetworks to represent the abstracted metabolic pathways. (a) convergent pathway for glucose utilization together with lactate formation and (b) one convergent pathways for M_2 formation, and two divergent pathways for glutamine and lysine utilizations, respectively.

As proposed by Straight and Ramkrishna [14], the objective function of the divergent competition is to maximize the mathematical product of the levels of end products. The cybernetic variables in divergent competitions are therefore derived from the matching law equation with the formulations shown below:

$$\begin{aligned}
 u_{3,d} &= \frac{r_3}{r_3 + F_{7,Gln}r_7 + F_{8,Gln}r_8}, \\
 u_{7,dGln} &= \frac{F_{7,Gln}r_7}{r_3 + F_{7,Gln}r_7 + F_{8,Gln}r_8}, \\
 u_{8,d} &= \frac{F_{8,Gln}r_8}{r_3 + F_{7,Gln}r_7 + F_{8,Gln}r_8}, \\
 v_{3,d} &= \frac{r_3}{\max(r_3, F_{7,Gln}r_7, F_{8,Gln}r_8)}, \\
 v_{7,dGln} &= \frac{F_{7,Gln}r_7}{\max(r_3, F_{7,Gln}r_7, F_{8,Gln}r_8)}, \\
 v_{8,d} &= \frac{F_{8,Gln}r_8}{\max(r_3, F_{7,Gln}r_7, F_{8,Gln}r_8)}, \\
 u_{7,dLys} &= \frac{F_{7,Lys}r_7}{F_{7,Lys}r_7 + r_9}, \quad u_{9,d} = \frac{r_9}{F_{7,Lys}r_7 + r_9}, \\
 v_{7,dLys} &= \frac{F_{7,Lys}r_7}{\max(F_{7,Lys}r_7, r_9)}, \quad v_{9,d} = \frac{r_9}{\max(F_{7,Lys}r_7, r_9)}.
 \end{aligned}$$

Similarly, the objective of convergent competition is to maximize the end product. The cybernetic variables in convergent pathways are defined as follows:

$$\begin{aligned}
 u_{1h,c} &= \frac{r_{1h}}{r_{1h} + r_{1l}}, \quad u_{1l,c} = \frac{r_{1l}}{r_{1h} + r_{1l}}, \\
 v_{1h,c} &= \frac{r_{1h}}{\max(r_{1h}, r_{1l})}, \quad v_{1l,c} = \frac{r_{1l}}{\max(r_{1h}, r_{1l})}, \\
 u_{3,c} &= \frac{Y_{M_2/3}r_3}{Y_{M_2/3}r_3 + Y_4r_4 + Y_{M_2/8}r_8}, \\
 u_{4,c} &= \frac{Y_4r_4}{Y_{M_2/3}r_3 + Y_4r_4 + Y_{M_2/8}r_8}, \\
 u_{8,c} &= \frac{Y_{M_2/8}r_8}{Y_{M_2/3}r_3 + Y_4r_4 + Y_{M_2/8}r_8}, \\
 v_{3,c} &= \frac{Y_{M_2/3}r_3}{\max(Y_{M_2/3}r_3, Y_4r_4, Y_{M_2/8}r_8)}, \\
 v_{4,c} &= \frac{Y_4r_4}{\max(Y_{M_2/3}r_3, Y_4r_4, Y_{M_2/8}r_8)}, \\
 v_{8,c} &= \frac{Y_{M_2/8}r_8}{\max(Y_{M_2/3}r_3, Y_4r_4, Y_{M_2/8}r_8)}.
 \end{aligned}$$

4.3. Kinetic expressions

The reaction rates of the pathways with enzyme regulation involved are assumed to follow Monod-type kinetics:

$$r_i = r_{i,max} \frac{P_i}{P_i + K_i} \frac{C_{M_0}}{C_{M_0} + K_{M_0}} \frac{e_i}{e_{max}} \quad \forall i = 1h, 1l, 3, 4, 6, 9, \tag{6}$$

$$r_7 = r_{7,max} \frac{Gln}{Gln + K_{7,Gln}} \frac{Lys.int}{Lys.int + K_{7,Lys}} \frac{C_{M_0}}{C_{M_0} + K_{M_0}} \frac{e_7}{e_{max}}, \tag{7}$$

$$r_8 = r_{8,max} \frac{Gln}{Gln + K_{8,Gln}} \frac{M_1}{M_1 + K_{8,M1}} \frac{C_{M_0}}{C_{M_0} + K_{M_0}} \frac{e_8}{e_{max}}. \tag{8}$$

The pathway glucose → M₃, and the reaction glutamine + lysine → M₄ for the basal level of protein formation are assumed saturated with enzyme. The kinetics of reaction rates therefore are

$$r_{2,m} = r_{m2,max} \frac{Glc}{Glc + K_{m2}} \frac{C_{M_0}}{C_{M_0} + K_{M_0}}, \tag{9}$$

$$r_{7,m} = r_{m7,max} \frac{Gln}{Gln + K_{m7,Gln}} \frac{Lys.int}{Lys.int + K_{m7,Lys}} \frac{C_{M_0}}{C_{M_0} + K_{M_0}}. \tag{10}$$

The conversion from M_2 to M_1 is assumed not to be regulated to compete with any other pathway. Thus the reaction rate is

$$r_5 = r_{5,\max} \frac{M_2}{M_2 + K_5} \frac{C_{M_0}}{C_{M_0} + K_{M_0}}. \quad (11)$$

Uptake rate of extra-cellular lysine is modeled as follows:

$$r_{\text{Lys}} = r_{\text{Lys},\max} \frac{\text{Lys}_{\text{ext}}}{\text{Lys}_{\text{ext}} + K_{\text{Lys}}}. \quad (12)$$

Synthesis rate of enzyme e_i denoted as r_{ei} is assumed dependent on its substrates and the limiting amino acid (lysine)

$$r_{ei} = r_{ei,\max} \frac{P_i}{P_i + K_{ei}} \frac{\text{Lys}_{\text{int}}}{\text{Lys}_{\text{int}} + K_E} \quad \forall i = 1h, 1l, 3, 4, 6, 7, 8, 9. \quad (13)$$

Formation rate of biomass M_0 is dependent on the four intermediates M_1 – M_4

$$r_{M_0} = r_{M_0,\max} \frac{C_{M_1}}{C_{M_1} + K_M} \frac{C_{M_2}}{C_{M_2} + K_M} \frac{C_{M_2}}{C_{M_2} + K_M} \frac{C_{M_2}}{C_{M_2} + K_M}. \quad (14)$$

Finally, the growth rate is obtained by

$$\begin{aligned} r_g = & Y_1 r_{1h} v_{1h,c} + Y_1 r_{1l} v_{1l,c} + Y_{m2} r_{2,m} + Y_{M_2/3} r_3 v_{3,c} v_{3,d} + Y_9 r_9 v_{9,d} + Y_7 (r_7 v_{7,d} \text{Gln} v_{7,d} \text{Lys} + r_{7,m}) + (Y_4 - 1) r_4 v_{4,c} \\ & + (Y_5 - 1) r_5 + Y_{M_2/8} r_8 v_{8,c} v_{8,d} - r_6 v_{1h,c} - F_{8,\text{Ala}} r_8 v_{8,c} v_{8,d} - Y_{\text{Amm}/3} r_3 v_{3,c} v_{3,d} - Y_{\text{Amm}/8} r_8 v_{8,c} v_{8,d} - Y_{\text{Ala}/8} r_8 v_{8,c} v_{8,d} \\ & + r_{M_0} (1 - F_{M_0,M_1} - F_{M_0,M_2} - F_{M_0,M_3} - F_{M_0,M_4}). \end{aligned} \quad (15)$$

P_i in Eqs. (6) and (13) is the reactant in the i th pathway, which is Glc for $i = 1h, 1l$; Gln for $i = 3$; Lys for $i = 7, 9$; C_{M_1} for $i = 4, 6, 8$.

Cell death rate is assumed to be determined by the level of intracellular lysine

$$r_D = r_{D,\max} \frac{K_D}{\text{Lys}_{\text{int}} + K_D} \quad (16)$$

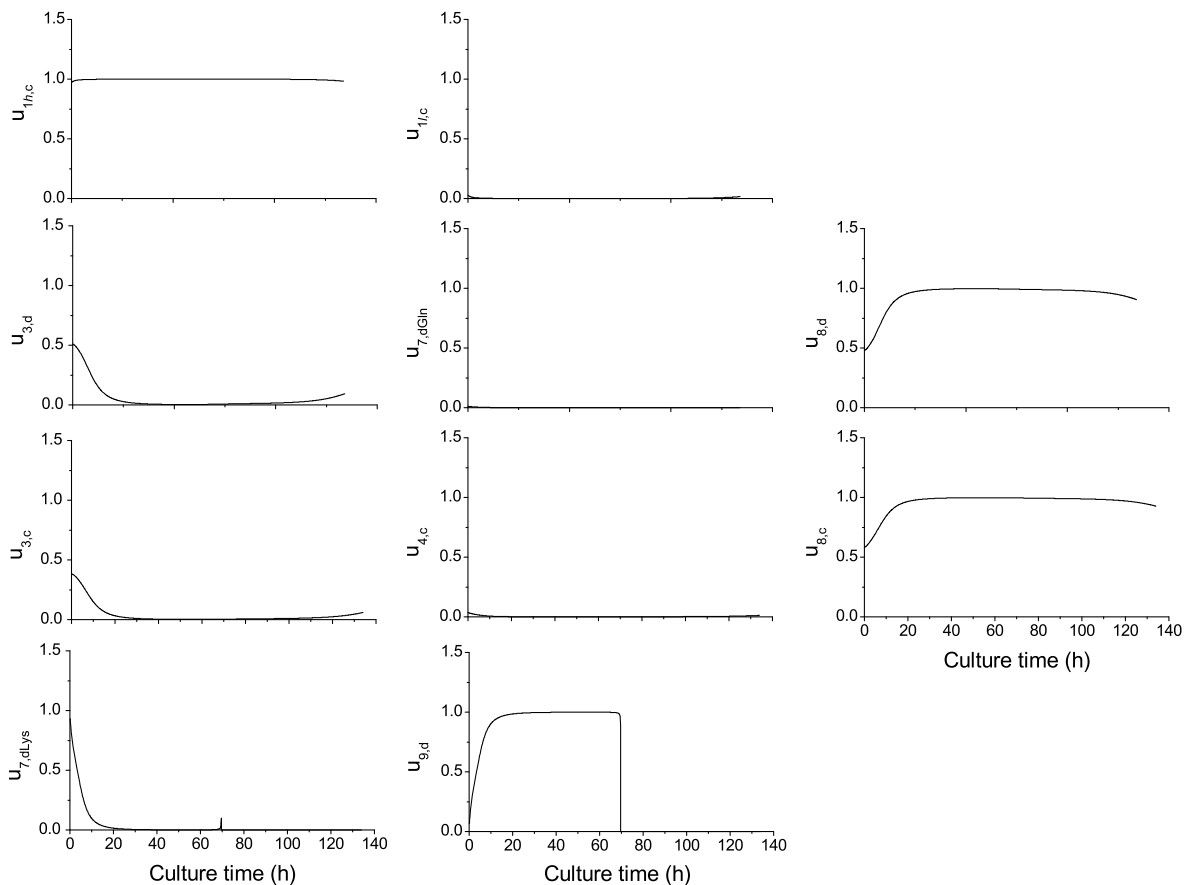


Fig. 4. Dynamics of cybernetic variables in Experiment 1.

4.4. Species balance

Balance on substrates, byproducts and biomass in the reactor:

$$\frac{dGlc}{dt} = -(r_{1h}v_{1h,c} + r_{1l}v_{1l,c} + r_{2,m})X_V + \frac{F}{V}(Glc_F - Glc), \tag{17}$$

$$\frac{dGln}{dt} = -(r_3v_{3,c}v_{3,d} + F_{8,Gln}r_8v_{8,c}v_{8,d} + F_{7,Gln}r_7v_{7,d}Gln + F_{7,Gln}r_{7,m})X_V + \frac{F}{V}(Gln_F - Gln), \tag{18}$$

$$\frac{dLys_ext}{dt} = -r_{lys}X_V + \frac{F}{V}(Lys_F - Lys_ext), \tag{19}$$

$$\frac{dX_V}{dt} = (r_g - r_D)X_V - \frac{F}{V}X_V, \tag{20}$$

$$\frac{dX_D}{dt} = r_D X_V - \frac{F}{V}X_D, \tag{21}$$

$$\frac{dLac}{dt} = Y_6 r_6 v_{1h} X_V - \frac{F}{V}Lac, \tag{22}$$

$$\frac{dAla}{dt} = Y_{Ala/8} r_8 v_{8,c} v_{8,d} X_V - \frac{F}{V}Ala, \tag{23}$$

$$\frac{dAmm}{dt} = (Y_{Amm/8} r_8 v_{8,c} v_{8,d} + Y_{Amm/3} r_3 v_{3,c} v_{3,d}) X_V - \frac{F}{V}Amm. \tag{24}$$

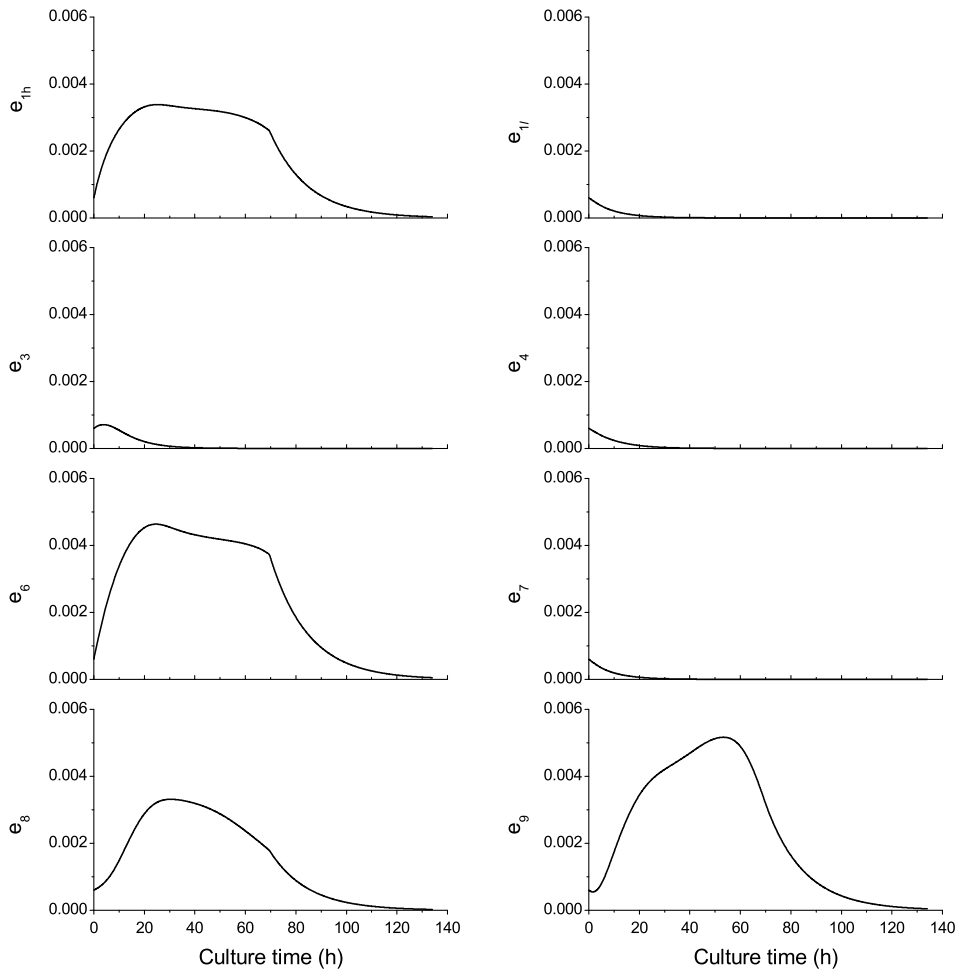


Fig. 5. Dynamics of enzymes levels (g/gdw) in Experiment 1.

Balances on intracellular intermediates and non-intermediates

$$\frac{dC_{M_1}}{dt} = Y_1(r_{1h}v_{1h,c} + r_{1l}v_{1l,c}) + Y_5r_5 - r_4v_{4,c} - r_6v_{1h,c} - F_{8,Ala}r_8v_{8,c}v_{8,d} - F_{M_0,M_1}r_{M_0} - r_gC_{M_1}, \quad (25)$$

$$\frac{dC_{M_2}}{dt} = Y_{M_2/3}r_3v_{3,c}v_{3,d} + Y_{M_2/8}r_8v_{8,c}v_{8,d} + Y_4r_4v_{4,c} + r_9v_{9,d} - Y_5r_5 - F_{M_0,M_2}r_{M_0} - r_gC_{M_2}, \quad (26)$$

$$\frac{dC_{M_3}}{dt} = Y_{m2}r_{2,m} - F_{M_0,M_3}r_{M_0} - r_gC_{M_3}, \quad (27)$$

$$\frac{dC_{M_4}}{dt} = Y_7(r_7v_{7,d}Glnv_{7,d}Lys + r_{7,m}) - F_{M_0,M_4}r_{M_0} - r_gC_{M_4}, \quad (28)$$

$$\frac{dLys_{int}}{dt} = r_{Lys} - (F_{7,Lys}r_7v_{7,d}Glnv_{7,d}Lys + F_{7,Lys}r_{7,m} + r_9v_{9,d}) - r_gLys_{int}, \quad (29)$$

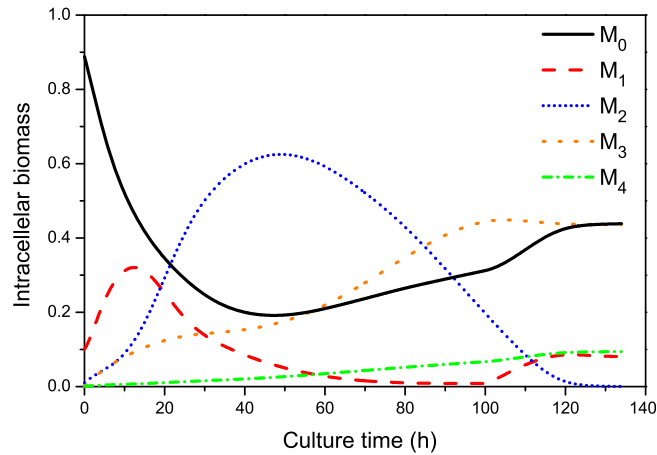


Fig. 6. Dynamics of intermediates levels (g/gdw) in Experiment 1.

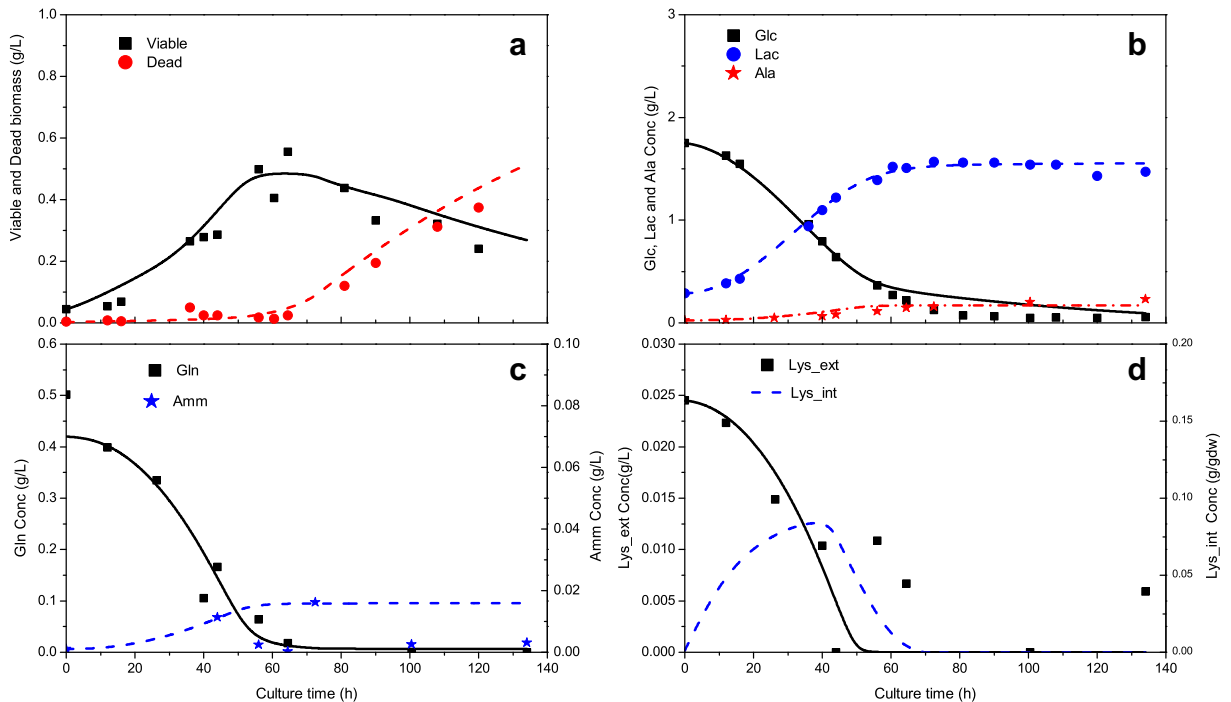


Fig. 7. Time courses of Experiment 1. (a) Viable and dead biomass concentrations, (b) glucose, lactate and alanine concentrations, (c) glutamine and ammonia concentrations and (d) extra- and intracellular lysine concentrations. Lines: model simulations; symbols: measurements.

$$\frac{dC_{M_0}}{dt} = r_{M_0} - r_g C_{M_0} \tag{30}$$

Balances on enzyme levels

$$\frac{de_i}{dt} = r_{e,\min} + r_e u_i - (r_g + b)e_i, \quad i = 1h, 1l, 3, 4, 6, 7, 8, 9, \tag{31}$$

Table 1
Parameters used in model simulations

Maximum rate (1/h)		Saturation constants		Yields and others	
$r_{1h,\max}$	0.85	K_{1h} (g/L)	0.1	Y_1	1
$r_{1l,\max}$	0.022	K_{1l} (g/L)	0.001	Y_{m2}	1
$r_{m2,\max}$	0.01	K_{m2} (g/L)	0.005	$Y_{M_2/3}$	0.68
$r_{3,\max}$	0.018	K_3 (g/L)	0.02	$Y_{Amm/3}$	0.03
$r_{4,\max}$	0.012	K_4 (g/gdw)	0.01	Y_4	0.7
$r_{5,\max}$	0.03	K_5 (g/gdw)	0.05	Y_5	1
$r_{6,\max}$	0.6	K_6 (g/gdw)	0.05	Y_6	0.7
$r_{7,\max}$	0.01	$K_{7,Gln}$ (g/L)	0.001	Y_7	0.4
$r_{m7,\max}$	0.0012	$K_{7,Lys}$ (g/L)	0.0001	$Y_{M_2/8}$	0.62
$r_{8,\max}$	0.42	$K_{m7,Gln}$ (g/L)	0.0003	$Y_{Ala/8}$	0.38
$r_{9,\max}$	0.01	$K_{m7,Lys}$ (g/L)	0.00001	Y_9	1
$r_{Lys,\max}$	0.008	$K_{8,Gln}$ (g/L)	0.08	F_{M_0,M_1}	0.2
$r_{M_0,\max}$	0.85	K_{8,M_1} (g/gdw)	0.05	F_{M_0,M_2}	0.15
$r_{e,\max}$	0.001	K_9 (g/gdw)	0.02	F_{M_0,M_3}	0.15
$r_{D,\max}$	0.002	K_{Lys} (g/L)	0.001	F_{M_0,M_4}	0.2
		K_{M_0} (g/gdw)	0.01	$F_{7,Gln}$	0.36
		K_M (g/gdw)	0.05	$F_{7,Lys}$	0.64
		K_E (g/gdw)	0.00001	$F_{8,Gln}$	0.62
		K_D (g/gdw)	0.01	F_{8,M_1}	0.38

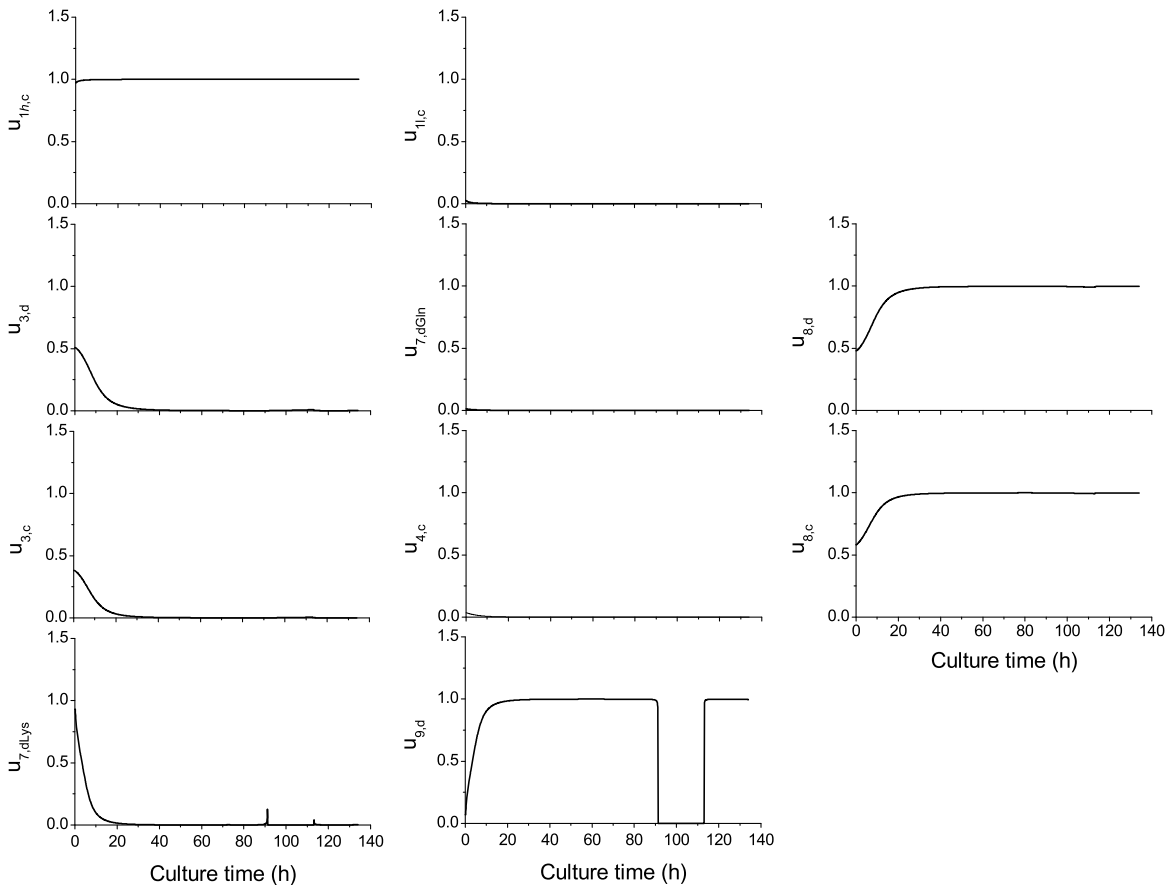


Fig. 8. Dynamics of cybernetic variables in Experiment 2.

where the cybernetic u_i variable is $u_{1h,c}$, $u_{1l,c}$, $u_{3,c}u_{3,d}$, $u_{4,c}$, u_{1h} , $u_{7,d}u_{7,c}$, $u_{7,d}u_{7,c}$, $u_{8,c}u_{8,d}$ and $u_{9,d}$ for the respective 1h, 1l, 3, 4, 6, 7, 8 and 9.

5. Results

The cybernetic model is validated by two myeloma cell cultivations. Figs. 4–7 show the time courses of Experiment 1, which was carried out in a rigorous batch mode. All enzymes are initially assumed to be expressed to 10% of the maximum level e_{max} since cells are maintained in a situation of poor substrates prior to experiments.

Fig. 4 shows the time profiles of the cybernetic variables 'u'. During the previous stage of cultivation (before 40 h), cells are growing in a medium with a high level of glucose and lysine. That means reactions 1h and 9 (Fig. 2) will dominate the pathway. As a result, cybernetic variables $u_{1h,c}$ and $u_{9,d}$ are close to 1 while variables $u_{1l,c}$ and $u_{7,d}u_{7,c}$ quickly decay to zero. For glutamine utilization, reaction (8) wins the competition which results in a large value of $u_{8,d}$, a small value of $u_{3,d}$ and a zero $u_{7,d}u_{7,c}$. This is caused by a high level of glutamine and the abundance of M_1 . Lysine is depleted at about 50 h and hence cells switch to make use of the intracellular pool. According to model simulation, intracellular lysine is exhausted at about 70 h. This makes the cybernetic variables $u_{7,d}u_{7,c}$ and $u_{9,d}$ directly drop to zero. During the last period of the cultivation, the values of $u_{1h,c}$, $u_{9,d}$ and $u_{8,c}$ show a slight decline, while their counterpart increase slowly.

The enzyme profiles are depicted in Fig. 5. At the beginning of the experiment, enzymes E_{1h} , E_8 and E_9 keep increasing till to the maximum level. At the same time, E_{1l} , E_3 and E_7 are quickly degraded. In the convergent pathway for M_2 production, E_8 also competes with E_3 , E_4 and comes out to be a winner. This causes the degradation of E_3 and E_4 . Later, E_{1h} , E_8 and E_9 are reduced with the decreasing of glucose, glutamine and lysine concentrations, respectively. Enzyme E_6 is driven by the same cybernetic variable as E_{1h} so that it shows a similar trend as E_{1h} . Finally, all the enzymes hit zero because enzyme synthesis is hampered due to lysine limitation.

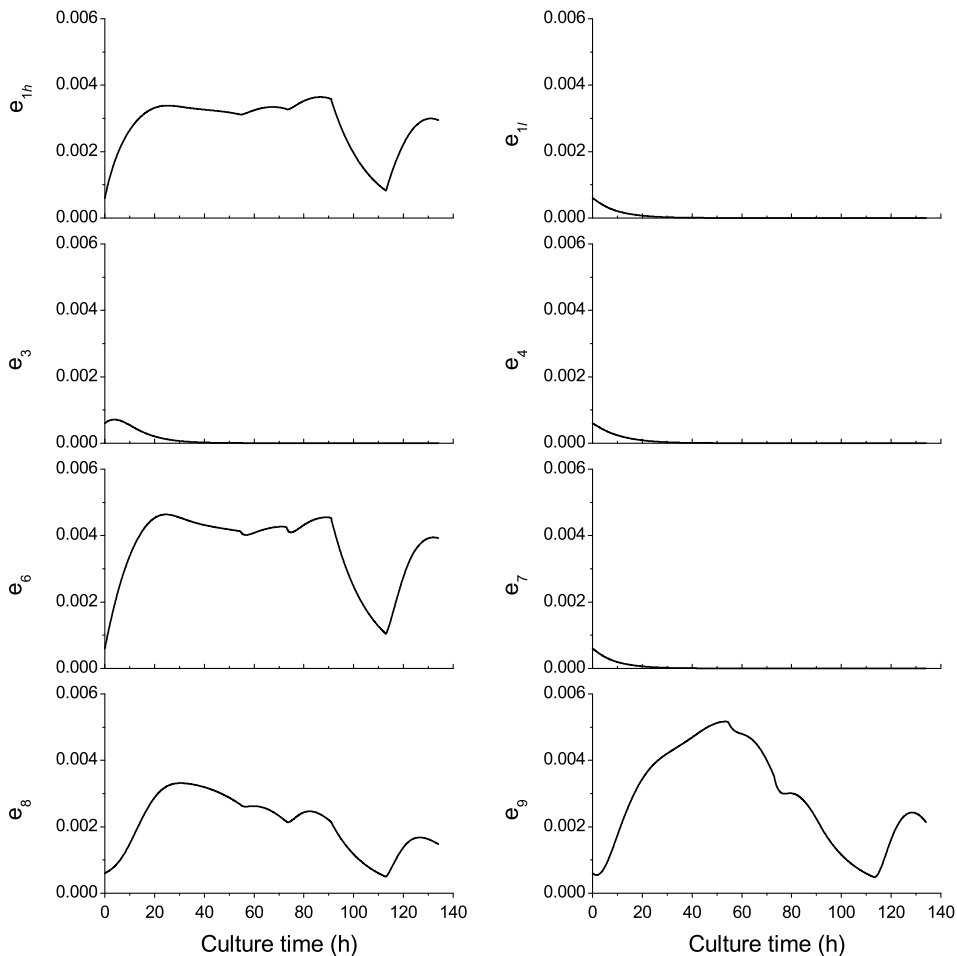


Fig. 9. Dynamics of enzyme levels (g/gdw) in Experiment 2.

Fig. 6 shows the profiles of the intermediates that act as connections between various reactions. The dynamics of biomass, substrate and byproduct are demonstrated in Fig. 7.

Parameters used in model simulation of Experiment 1 are given in Table 1. The reaction rate constants are determined by tuning the model simulations to the experimental data. The yield coefficient of the reaction is the ratio between the weight of the reactant and product, while the enzyme constants are obtained from literature [12,23]. The values of saturation constant K_{ei} are all equal to the corresponding K_i , except for $K_{e1h} = 20K_{1h}$. Maximum enzyme amount is calculated as $e_{max} = (r_{e, in} + r_{e,max}) / (r_{M_0,max} + b)$, where $r_{e,min} = 1e-07$ g/(gdw h) and $b = 0.1/h$ [11]. All these parameters are further used to simulate Experiment 2.

Fig. 8–11 show the time profiles of Experiment 2 in which three doses of feeding medium were added at 54.5 h, 73 h and 113 h, respectively. During the whole cultivation, cybernetic variables $u_{1h,c}$ dominate $u_{1l,c}$ since glucose are maintained at a high level. Time courses of enzyme are similar to Experiment 1 before the first feeding. After the first feeding, E_{1h} , E_6 , E_8 and

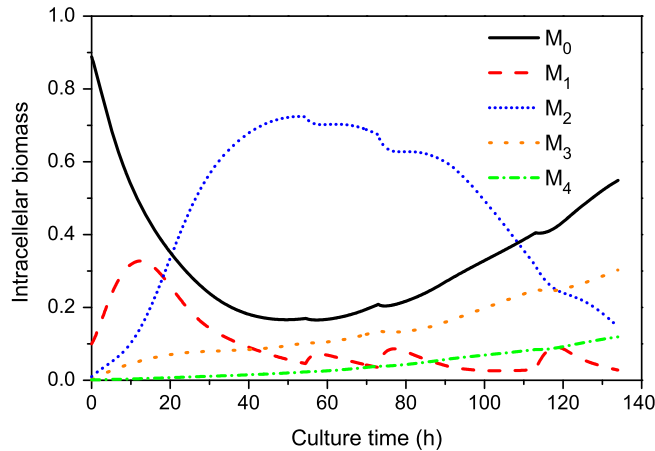


Fig. 10. Dynamics of intermediates levels (g/gdw) in Experiment 2.

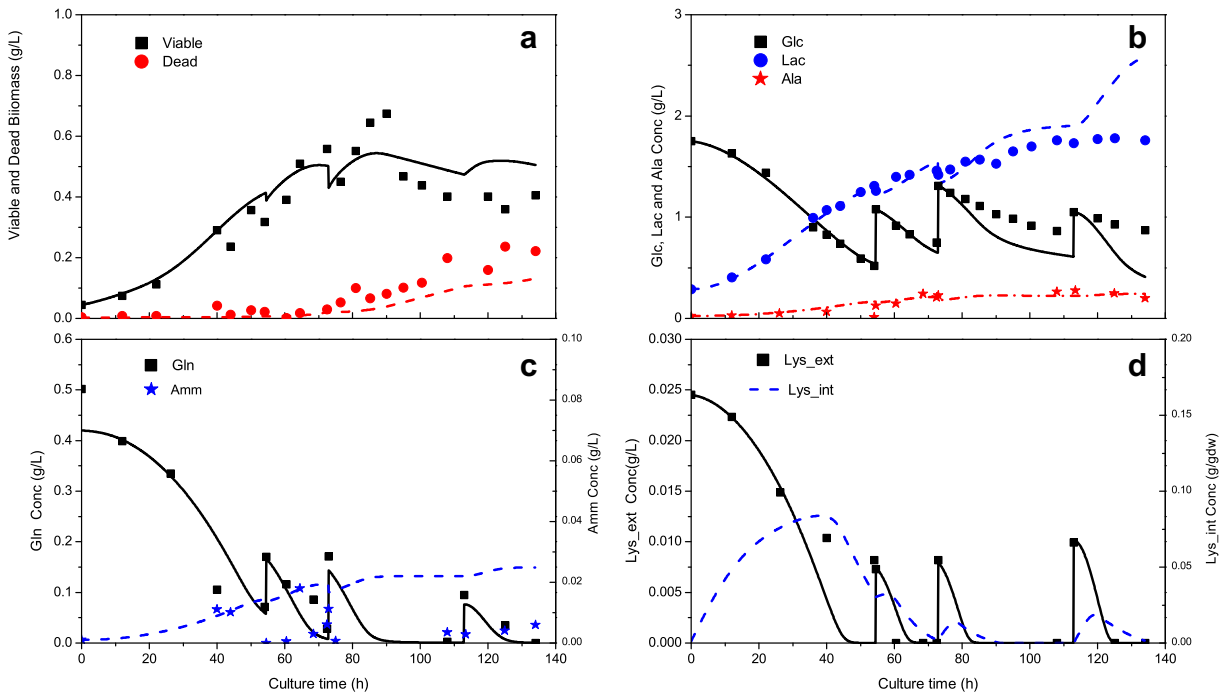


Fig. 11. Time courses of Experiment 2. (a) Viable and dead biomass concentrations, (b) glucose, lactate and alanine concentrations, (c) glutamine and ammonia concentrations and (d) extra- and intracellular lysine concentrations. Lines: model simulations; symbols: measurements.

E_9 are maintained at a high level, as indicates that three doses of medium feeding have prevented these four enzymes from decreasing. Obviously, the model prediction and the measurements largely agree with each other (Fig. 11).

6. Discussion and conclusion

The cybernetic models found in the literature are mostly based on the steady state balance of the metabolic network [26–28], where the transients of the state variables are excluded. As a comparison, the cybernetic model proposed in this paper captures the dynamic regulatory behavior of cell growth so that it is able to depict the transient behavior traversing through multiple domains of metabolic shifts. By considering glutamine utilization through transamination and deamination to produce α -KG, the production of ammonia and alanine is modeled. At the same time, lysine is modeled as the energy supplier and protein building block. Model validation is performed with myeloma cell cultivations without product expression. The model simulations are found to be consistent with the measurements with a reasonable accuracy.

Although cybernetic models have been proved successful for dealing with the diverse effects of metabolic regulation in numerous microbial processes, they are seldom used for mammalian cell culture modeling. The extremely complicated internal structure of mammalian cells presents a challenge to determine the objective functions in the cybernetic framework. On the other hand, pathway abstraction as well as the identification of element pathways and competitions relies on metabolic flux analysis (MFA) to a considerable extent. Further development of MFA technologies will be beneficial to the application of the cybernetic modeling approach.

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