Structured modeling of recombinant protein production in batch and fed-batch culture of baculovirus-infected insect cells

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Abstract

The infection of insect cells with baculovirus was described in a mathematical model as a part of the structured dynamic model describing whole animal cell metabolism. The model presented here is capable of simulating cell population dynamics, the concentrations of extracellular and intracellular viral components, and the heterologous product titers. The model describes the whole processes of viral infection and the effect of the infection on the host cell metabolism. Dynamic simulation of the model in batch and fed-batch mode gave good agreement between model predictions and experimental data. Optimum conditions for insect cell culture and viral infection in batch and fed-batch culture were studied using the model.

Nomenclature

μ_{di} : specific lysis rate by viral infection.
 μ_{du} : specific death rate of viable cell.
 $[AV]_{cyt}$: total cytosolic level of adsorbed virus.
 $[ENZ1]_{cyt}$: total cytosolic level of early enzyme.
 $[ENZ2]_{cyt}$: total cytosolic level of late enzyme.
 $[ENZ3]_{cyt}$: total cytosolic level of very late enzyme.
 $[EV]_{med}$: total level of enveloped virus in medium.
 $[mRNA1]_{cyt}$: total cytosolic level of early mRNA.
 $[mRNA2]_{cyt}$: total cytosolic level of late mRNA.
 $[P10]_{cyt}$: total cytosolic level of P10 protein.
 $[PROD]_{cyt}$: total cytosolic level of recombinant product.
 $[PROD]_{med}$: extracellular concentration of recombinant product.

$[VIR]_{cyt}$: total cytosolic level of nucleocapsid.
 $[VNA]_{cyt}$: total cytosolic level of viral nucleic acid.
 $[VPC]_{cyt}$: total cytosolic level of viral protein capsid.
 Q : medium flux.
 $r_{AV,VIR}$: rate of penetration of AV into the cell.
 r_{ENZ1} : formation rate of early enzyme.
 r_{ENZ2} : formation rate of late enzyme.
 r_{ENZ3} : formation rate of very late enzyme.
 $r_{EV,AV}$: rate of AV formation from EV.
 r_{mRNA1} : formation rate of early mRNA.
 r_{mRNA2} : formation rate of late mRNA.
 r_{P10} : formation rate of P10 protein.
 r_{PROD} : formation rate of recombinant product.
 r_{REL} : rate of viral release from the infected cells.

r_{REL} :	rate of virus release from infected cell.
r_{REL} :	rate of virus release.
r_{SECR} :	specific secretion rate of recombinant product.
$r_{VIR,VNA}$:	rate of release of viral nucleic acid from nucleocapsid.
r_{VNA} :	formation rate of viral nucleic acid.
r_{VPC} :	formation rate of viral protein capsid.
r_{VIR} :	formation rate of viral nucleocapsid.
V :	culture volume.

Introduction

Baculoviruses are large, enveloped viruses that infect invertebrates, including many insects (Lepidoptera, Diptera, Hymenoptera, Coleoptera and Spondoptera) and some crustaceans and arachnids, and which carry double-stranded DNA genomes. Their host specificity implies that they can provide an environmentally friendly alternative to chemical insecticides: they can also be genetically altered so as to produce a valuable product. This technology, termed the Baculovirus Expression Vector System (BEVS), has allowed a wide range of recombinant proteins to be manufactured in insect cell culture (Miller, 1993).

Among the host systems used to produce recombinant proteins, the BEVS has been widely accepted as powerful heterologous expression system due to several benefits of the system: highly host specific (Volkman et al., 1986), relative simplicity of growing insect cells in suspension culture, the ability of insect cells to perform most desirable post-translational modifications, the ability of the vector to accommodate large foreign gene inserts, and a strong viral promoter. Though most research in the BEVS has been focused on the molecular biology and genetic engineering which, of course, is of importance, the optimization of recombinant protein expression and optimization of culture condition should be thoroughly investigated to make the BEVS more competitive with other more established expression systems. However, experimental approaches to the optimization process are time-consuming and costly.

Therefore, modeling and simulation work is attractive for investigating optimum strategies for the heterologous expression system. Our group has developed a detailed, structured model that is capable

of describing the behavior of animal cells growing *in vitro*. This model has been proved to be valuable both in analyzing experimental results and in designing media and feeding policies to maximize the profitability of a given system (Barford et al., 1992; Sanderson et al., 1994, 1999a, b). The basic animal cell model has been extended by our group to describe viral infection in animal cell (Sanderson et al., 1999c). In this study, the whole model was described and tested its predictability with newly obtained set of experimental data. The new model was used to simulate cell growth and viral infection dynamically in batch and fed-batch mode to prove its ability to predict previously obtained experimental data and to find out the optimum infection.

Modelled system

Basic animal cell model

The basic animal cell model includes a wide range of energy production and biosynthetic pathways and is summarized in Figure 1. This model considers the concentration of some 40 key metabolites (including fatty and amino acids, sugars and intermediates of the glycolysis and tricarboxylic acid cycle) in three regions (growth medium, cellular cytoplasm, and cellular mitochondria). These components are linked by a series of reaction and transport steps that describe cellular growth and death, glycolysis, the TCA cycle, the pentose phosphate shunt, the degradation and synthesis of amino and fatty acids and the production of protein products. The overall model is posed as a set of differential and algebraic equations and is solved using specialized dynamic simulators (SPEEDUP). A full description of the animal cell model is given by Sanderson (1997) and Sanderson et al. (1999a, b). This detailed, dynamic model has been shown to be of considerable value in simulation and initial media studies, as well as in the design of experimentally viable feeding policies.

Viral infection model

In addition to the structured model of animal cell metabolism, the model equations for viral infection were included (Sanderson et al., 1999c). Figure 2 depicts the basic model that describes the behavior of the viral system. With the structure described in Figure 2, many viral infections of animal cell cultures may be modeled. By nesting several copies of this viral model

Figure 1. Diagram of metabolic pathways included in animal cell simulation (Sanderson et al., 1999a).

into the cell simulation, genetic variations in the virus may also be included. In this way, for example, defective virions can be described (Sanderson et al., 1999c). Two key modeling assumptions are that the nucleic and cytoplasmic spaces need not be differentiated and that, on death, the cell's contents become equilibrated with the medium. The concentrations of the viral components are, therefore, calculated in just two regions – the 'medium' and the 'cytoplasm'.

Baculoviral infectious cycle

The baculoviral infectious cycle occurs in three distinguishable phases, termed early, late and very late. In the early phase, a viral particle (EV, enveloped virus) binds to the cellular membrane (AV, absorbed virus) and inserts a nucleocapsid (VIR, nucleocapsid) into the cytoplasmic space. This nucleocapsid then migrates to the cell's nucleus, to which it binds and into which it inserts its DNA (VNA, viral nucleic acid). In the nucleus, the virus replicates itself, copying its DNA and producing RNA to code for capsid formation. It is assumed that there are limited numbers of binding sites, so the rate of binding is affected both by the number of viral particles already attached to the cell and their concentration in the medium. The binding of the first virus to a given cell is taken as the point at which that cell moves from the uninfected population to the infected one.

In the cytoplasm, the modeled nucleocapsid (VIR) releases DNA into the nucleus. The model uses feedback inhibition based on the concentration of free

nucleic acid to suppress both the formation of new nucleic acid and its release from nucleocapsids.

This replication of DNA occurs at the start of the late phase of infection. Viral DNA (IVNA) is inserted into newly formed capsids (VPC), producing nucleocapsids (VIR), which may pass through the cellular membrane, acquiring a lipid envelope (EV, enveloped virus), and ultimately infecting other cells.

In the very late phase, additional viral proteins are expressed, including the polyhedron, the structural protein in which virions may become occluded, and the p10 protein, a fibrous protein that accumulates in the cellular nucleus and whose precise function is unclear. The budding of occluded particles through the cellular membrane and the filling of the nucleus with p10 protein have both been linked to cellular death. In the BEVS, one of the genes that code for these proteins, usually the polyhedron gene, is replaced by one that encodes for a valuable product.

Viral nucleic acid then promotes the formation of the early viral phase enzymes (ENZ1). The generic enzyme pool represents both viral enzymes and mRNA and, like the free nucleic acid, its concentration is prevented from rising uncontrollably in the model by implementing metabolic repression. The early phase enzymes (ENZ1) enable the formation of new nucleic acid (VNA) the late phase enzymes (ENZ2). These late phase enzymes encode for viral protein capsids (VPC) and very late phase enzymes (ENZ3), which in turn encodes for protein products (PROD) and the p10 protein, which induces cell death as its concentration increases.

acids and structural proteins in the process of repressing their own formation. The maximum concentration of these components is combined intracellularly to form nucleocapsids, which can then pass through the cell wall, to form new enveloped virions. It is estimated that each infected cell can produce several thousand new virions during a normal infection.

Each individual cell in the reactor will, in reality, experience different conditions to other cells. Treating each cell independently is, however, far too great a task to be undertaken, so modeling simplifications must be made. A key assumption is that the reactor is homogeneous and that the two cell populations, the infected and the uninfected, can be described by ‘average’ cells. This assumption works well for a virus-free culture, but has a complication when considering the infected population – infected cells that die due to active viral effects are at the end of the infection cycle and so may have substantially different concentrations of viral components to those of an average infected cell. Cells dying due to infection, therefore, have a different effect on the infected ‘pool’ as compared to those dying due to substrate depletion.

In order to overcome this difficulty, the infected cells in the model are artificially divided into sub-populations, based on the first appearance of key components, such as viral enzymes or the p10 protein. Certain components can only exist in given popula-

tions. For example the population must have freed its viral nucleic acid before any viral enzymes can be produced. These sub-populations, therefore, provide a basis for distributing viral components and estimating their concentration in cells at a given stage in the infection cycle. This component distribution information is calculated from the average internal concentrations and the population distribution of infected cells, and so provides a reasonable description of the system without adding excessively to the size of the model.

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Figure 4. Overall model trends of infected viable cell mass fraction in early (heavy line), late (solid line) and very late (dotted line) phase of viral infection cycle during batch culture.

Viral model equations

The total level of each extracellular viral subpopulation in a reactor is affected by input and output flux in the reactor system as well as supply and/or consumption by other related steps.

For example, the subpopulation of enveloped viruses (EV) which may be classified as extracellular ones, can be expressed as following equation.

$$\frac{d[EV]_{med}}{dt} = r_{REL} - r_{EV,AV} - [EV]_{med} \frac{Q}{V} \quad (1)$$

where $[EV]_{med}$: total level of enveloped virus in medium (mole l^{-1}), $r_{EV,AV}$: rate of AV formation from EV (mole $l^{-1} h^{-1}$), r_{REL} : rate of viral release from the infected cells (mole $l^{-1} h^{-1}$), Q : medium flux ($l h^{-1}$), V : culture volume (l).

On the other hand, all the other subpopulation including adsorbed viruses (AV), nucleocapsid (VIR), viral nucleic acid (VNA), early enzyme (ENZ1), early mRNA (mRNA1), late enzyme (ENZ2), late mRNA (mRNA2), very late enzyme (ENZ3), viral protein capsid (VPC), and P10 protein (P10) were assumed to be cytoplasmic ones. All these subpopulation may be affected by cell death by environmental change and cell lysis by virus maturation in the infected cells. And since the intracellular viral subpopulation was expressed in terms of extracellular concentration, its level is also affected by input and output flux in the reactor system as well as formation and/or consumption by other related steps.

$$\frac{d[AV]_{cyt}}{dt} = r_{EV,AV} - r_{AV,VIR} - [AV]_{cyt}$$

Figure 5. Comparison of model predictions (lines) and experimental data (symbols) of (a) viable cell concentration and (b) β -galactosidase production after infection of baculovirus in the batch culture of Sf9 insect cell (Dotted line in (a) represents the concentration of uninfected viable cells. Two lines in (b) denote the predictions with assumption of no secretion (dotted) and secretion (solid), respectively).

$$\left(\frac{Q}{V} + \mu_{du} + \mu_{di} \right) \quad (2)$$

where $[AV]_{cyt}$: total cytosolic level of adsorbed virus (mole l^{-1}), $r_{AV,VIR}$: rate of penetration of AV into the cell (mole $l^{-1} h^{-1}$), μ_{du} : specific death rate of viable cell due to nutrient depletion and toxic product accumulation (h^{-1}), μ_{di} : specific cell lysis rate by viral infection (h^{-1}).

$$\frac{d[VIR]_{cyt}}{dt} = r_{AV,VIR} - r_{VIR,VNA} + r_{VIR} - r_{REL} - [VIR]_{cyt} \left(\frac{Q}{V} + \mu_{du} + \mu_{di} \right) \quad (3)$$

where $[VIR]_{cyt}$: total cytosolic level of nucleocapsid (mole l^{-1}), $r_{VIR,VNA}$: rate of release of viral nucleic acid from nucleocapsid (mole $l^{-1} h^{-1}$), r_{VIR} : formation rate of viral nucleocapsid (mole $l^{-1} h^{-1}$), r_{REL} : rate of virus release from infected cell (mole $l^{-1} h^{-1}$).

$$\frac{d[VNA]_{cyt}}{dt} = r_{VIR,VNA} + r_{VNA} - r_{VIR} - [VNA]_{cyt} \left(\frac{Q}{V} + \mu_{du} + \mu_{di} \right) \quad (4)$$

where $[VNA]_{cyt}$: total cytosolic level of viral nucleic acid (mole l^{-1}), r_{VNA} : formation rate of viral nucleic acid (mole $l^{-1} h^{-1}$).

$$\frac{d[ENZ1]_{cyt}}{dt} = r_{ENZ1} - [ENZ1]_{cyt} \left(\frac{Q}{V} + \mu_{du} + \mu_{di} \right) \quad (5)$$

where $[ENZ1]_{cyt}$: total cytosolic level of early enzyme (mole l^{-1}), r_{ENZ1} : formation rate of early enzyme (mole $l^{-1} h^{-1}$).

$$\frac{d[mRNA1]_{cyt}}{dt} = r_{mRNA1} - [mRNA1]_{cyt} \left(\frac{Q}{V} + \mu_{du} + \mu_{di} \right) \quad (6)$$

where $[mRNA1]_{cyt}$: total cytosolic level of early mRNA (mole l^{-1}), r_{mRNA1} : formation rate of early mRNA (mole $l^{-1} h^{-1}$).

$$\frac{d[ENZ2]_{cyt}}{dt} = r_{ENZ2} - [ENZ2]_{cyt} \left(\frac{Q}{V} + \mu_{du} + \mu_{di} \right) \quad (7)$$

where $[ENZ2]_{cyt}$: total cytosolic level of enzyme (mole l^{-1}), r_{ENZ2} : formation rate of late enzyme (mole $l^{-1} h^{-1}$).

$$\frac{d[mRNA2]_{cyt}}{dt} = r_{mRNA2} - [mRNA2]_{cyt} \left(\frac{Q}{V} + \mu_{du} + \mu_{di} \right) \quad (8)$$

where $[mRNA2]_{cyt}$: total cytosolic level of late mRNA (mole l^{-1}), r_{mRNA2} : formation rate of late mRNA (mole $l^{-1} h^{-1}$).

$$\frac{d[ENZ3]_{cyt}}{dt} = r_{ENZ3} - [ENZ3]_{cyt} \left(\frac{Q}{V} + \mu_{du} + \mu_{di} \right) \quad (9)$$

where $[ENZ3]_{cyt}$: total cytosolic level of very late enzyme (mole l^{-1}), r_{ENZ3} : formation rate of very late enzyme (mole $l^{-1} h^{-1}$).

$$\frac{d[VPC]_{cyt}}{dt} = r_{VPC} - r_{VIR} - [VPC]_{cyt} \left(\frac{Q}{V} + \mu_{du} + \mu_{di} \right) \quad (10)$$

where $[VPC]_{cyt}$: total cytosolic level of viral protein capsid (mole l^{-1}), r_{VPC} : formation rate of viral protein capsid (mole $l^{-1} h^{-1}$).

$$\frac{d[P10]_{cyt}}{dt} = r_{P10} - [P10]_{cyt} \left(\frac{Q}{V} + \mu_{du} + \mu_{di} \right) \quad (11)$$

where $[P10]_{cyt}$: total cytosolic level of P10 protein (mole l^{-1}), r_{P10} : formation rate of P10 protein (mole $l^{-1} h^{-1}$).

The recombinant product (PROD) which is formed by very late enzyme may be described in terms of cytoplasmic subpopulation as following.

$$\frac{d[PROD]_{cyt}}{dt} = r_{PROD} - [PROD]_{cyt} \left(\frac{Q}{V} + \mu_{du} + \mu_{di} + r_{SECR} \right) \quad (12)$$

where $[PROD]_{cyt}$: total cytosolic level of recombinant product (mole l^{-1}), r_{PROD} : formation rate of recombinant product (mole $l^{-1} h^{-1}$), r_{SECR} : specific secretion rate of recombinant product (h^{-1}).

The recombinant product was assumed to be secreted from infected cell population with specific secretion rate, r_{SECR} , though it might be released after cell lysis by viral maturation and by cell lysis of dead cells which could be caused by nutrient exhaustion or toxic product accumulation. Thus, its level in medium can be calculated from specific secretion rate, infected cell population, the cell lysis and cell death rate.

Figure 6. Comparison of model predictions (lines) and experimental data (symbols) of extracellular amino acid profiles after infection of baculovirus in the batch culture of Sf9 insect cells. (a) Alanine (■), cysteine (◆), glutamate (▲), glutamine (●), and tryptophan (□). (b) Arginine (■), glycine (◆), histidine (▲), lysine (●), and phenylalanine (□). (c) Asparagine (■), aspartate (◆), isoleucine (▲), leucine (●), and methionine (□). (d) Proline (■), serine (◆), threonine (▲), tyrosine (●), and valine (□).

$$\frac{d[PROD]_{med}}{dt} = [PROD]_{cyt} (r_{SECR} + \mu_{du} + \mu_{di}) - [PROD]_{med} \frac{Q}{V} \quad (13)$$

where $[PROD]_{med}$: extracellular concentration of recombinant product ($\text{mole l}^{-1} \text{h}^{-1}$).

The majority of the reaction kinetics in the viral system are assumed to be first order in form. The first order viral kinetics were adjusted to account for inhibition by particular metabolites. Thus, the potential rate of a reaction decreases by a factor proportional to the fraction of the maximum possible concentration of the repressed component present in the cell. For example, mRNA1 inhibits its own formation, so its rate

of formation, r_{mRNA1} , is described by the equation (Sanderson et al., 1999a):

$$r_{mRNA1} = k_{mRNA1} [ENZ1] \left(\frac{[mRNA1]_{max} - [mRNA1]}{[mRNA1]_{max}} \right) \prod_i \left\{ \frac{[SUB]_i}{k_i + [SUB]_i} \right\} \quad (14)$$

where k_{mRNA1} is the first-order rate constant for the formation of mRNA1, $[ENZ1]$ the concentration of the early phase enzyme pool, $[mRNA1]_{max}$ the maximum allowable concentration of mRNA1, $[mRNA1]$ the concentration of mRNA1, $[SUB]_i$ the concentration of the i th precursor of mRNA1 and k_i the availability for the i th precursor of mRNA1.

Figure 8. Comparison of model predictions (lines) and experimental data (symbols) of extracellular amino acid profiles during the fed-batch culture of Sf9 insect cells with feeding at 96 h and infection of baculovirus at 132 h. (a) Alanine (■), glutamate (◆), and leucine (▲). (b) Arginine (■), histidine (◆), and isoleucine (▲). (c) Asparagine (■), aspartate (◆), and phenylalanine (▲). (d) Cysteine (■) and tryptophan (◆).

Results and discussion

Model application

A prototype model based on these assumptions has been developed in the SPEEDUP modeling environment. It includes 1710 differential and algebraic equations, describing variables such as metabolite concentrations and rates of reaction and transport.

Due to the model's complexity and the number of parameters, it was found that all available automatic parameter estimation routines were of limited value. The somewhat pragmatic approach of tuning the model manually was therefore taken. The parameters to be taken were selected heuristically.

In general, maximum rates of reaction for the first reaction step in a given pathway were favoured over

K_M and transport terms and the rates of the reactions further down the reaction pathway. Thus the rate of glycosylation can largely be controlled by adjusting the maximum rate of glucose phosphorylation – the model is relatively insensitive to the maximum rate of reaction of the subsequent steps, as long as they are high enough to prevent a bottleneck from forming. K_M terms most strongly affect the size of internal 'pools' and since these are not generally known, there is little to be gained from adjusting these parameters.

By a process of trial and error, therefore, it was found that most of the data could be fitted by adjusting just 60 of 390 model parameters in case of the basic animal cell model (the model is relatively insensitive to the remaining parameters, provided that they are consistent with the core 60). This technique proved to be extremely robust, allowed apparent experimental

Figure 9. Comparison of model predictions (lines) and experimental data (symbols) of extracellular amino acid profiles during the fed-batch culture of Sf9 insect cells with feeding at 96 h and infection of baculovirus at 132 h. (a) Glutamine (■) and methionine (◆). (b) Glycine (■), tyrosine (◆), and valine (▲). (c) Proline (■) and serine (◆). (d) Lysine (■) and threonine (◆).

errors and outlying points to be identified, meant that the scaling was no longer an issue and identified subtle relationships between variables. This pragmatic approach also encouraged attempts to identify areas of experimental errors and model inaccuracy. The degree of metabolic understanding that this promoted allowed on-going model development to be performed very successfully – if the model did not fit the data, it became clear which pathways and metabolites were causing the difficulty. Additional or more detailed description of particular areas could, thus, be developed. This approach also meant that a set of rules for how to tune the model were developed, albeit at an implicit level. These rules could readily be formalised and captured in an Expert System to provide a robust parameter estimation routine (Fu et al., 1994).

This method has been used successfully to model a number of different animal cell cultivations. The basic animal cell model was previously applied to an uninfected Sf9 insect cell culture, demonstrating that, with a suitable adjustment of its parameters, a model that successfully described hybridoma cells can be also used for insect cells (Sanderson et al., 1999a, b, c). The extended model which includes viral infection cycle was also previously applied to an infected insect cell culture (Wong et al., 1994) and was found to describe the infection process well (Sanderson, 1999c).

Figures 3 and 4 illustrate the behavior of the viral sub-model for a group of cells that are assumed to be all infected at the same time. The total infected cell population, together with the virus and product con-

Figure 7. Comparison of model predictions (lines) and experimental data (symbols) of (a) viable cell concentration, (b) glucose ($\blacktriangle$) and β -galactosidase ($\square$) during the fed-batch culture of Sf9 insect cells with feeding at 96 h and infection of baculovirus at 132 h (Dotted line in (a) represents the concentration of uninfected viable cells. Two lines in (b) denote the predictions with assumption of no secretion (dotted) and secretion (solid), respectively).

concentrations are compared in Figure 3. In the figure, all the components are plotted in dimensionless units to reveal overall metabolic trends after infection (Figures 5 to 9 give detailed metabolic simulations). The cell population in each of the infection phases (early, late and very late) is shown in Figure 4. These predictions qualitatively match the expected behavior of a baculoviral infection (O'Reilly et al., 1992).

The viral infection model was run with different settings of initial condition to estimate predictability of the model for different set of experimental data,

either in batch or fed-batch mode. Model parameters except initial conditions for the simulation were the same as one previously used by our group (Sanderson et al., 1999c). Hence the model is predictive rather than simply data fitting. The results were compared with the experimental data of Chan (1998). The predictions of the viable cell concentration and β -galactosidase production after infection are compared with the experimental data in Figure 5. It was predicted that most of viable cells would get infected within 20 h after infection and that some fraction of viable cells would grow for a while after infection (Figure 5a). The model predicted relatively well the decline of overall viable cell concentration (the sum of infected and uninfected viable cells) possibly due to the cell lysis triggered by viral accumulation within the cell. However, the model predicted higher viable cell concentration after infection than the experiment. This is due to the assumption that all the infected cells are viable until lysis unless they die from nutrient exhaustion or toxic product accumulation. Therefore, more work needs to be done for the mechanism of cell death and viability.

In Figure 5b, the predicted level of β -galactosidase in the medium was fitted well to the experimental data when the product was assumed to be secreted at a defined rate from virus-infected cell population, while time lag of 50 h was predicted for the production when no secretion was assumed. Ratio of product accumulation rate in the medium by secretion and by cell lysis may result in different time lag for the product formation and may change as function of cell line and environmental conditions.

Amino acid profiles after infection in batch culture were simulated using the model and are shown in Figure 6. The predictions of amino acid metabolism are relatively well fitted to experimental data. Alanine was the only amino acid which was predicted to be produced by the model and it agrees well with experimental result. Alanine was predicted to be produced until cells started to lyse and fitted relatively well the experimental data during the period before lysis. Only glutamine and cysteine were predicted to decrease significantly after infection and cysteine level was found to be nearly limiting to cell growth. Other amino acids gave no significant changes after infection in both model predictions and experimental data.

It has been reported that some nutrient might limit insect cell growth in batch culture (Radford, 1997). Nutrient exhaustion in batch culture may be easily overcome either by fortifying the initial batch medium

Figure 10. Comparison of (a) viable cell concentration and (b) β -galactosidase production after infection of baculovirus in the batch culture of Sf9 insect cells, which were predicted virus model with different MOI values (0.5, 5, 50, 500, 5000).

or by using fed-batch systems. Chan (1998) performed a fed-batch culture of insect cells and found a significant increase in the cell density and β -galactosidase production compared with batch data. In this study, our model simulated the fed-batch mode with initial conditions and feed conditions reported in Chan's experiment. All the parameters used in the fed-batch simulation were adopted from the model parameter estimation in batch culture.

The prediction of viable cell concentration and β -galactosidase level during the fed-batch simulation

was relatively well fitted to the experimental data. The product level was predicted to increase earlier than experimental data when the product was assumed to be secreted into medium before cell lysis, while time lag was predicted when no secretion was assumed (Figure 7). As mentioned in batch case previously, ratio of product accumulation rate in the medium by secretion and by cell lysis may result in different time lag for the product formation and may change as function of cell line and environmental conditions. Maximum viable cell concentration in fed-batch culture was significantly increased, when compared with batch data. This result confirms that fed-batch can relieve growth limitation. Glucose profiles in fed-batch culture was well predicted by the model simulation (Figure 7b). Amino acid profiles in model predictions matched those of experimental data closely (Figures 8 and 9). Alanine production was well predicted compared to the experimental data for most of culture time except time after 180 h, possibly due to the earlier death of infected viable cells in the model prediction compared with measured data.

Both lactate and ammonia have been reported to accumulate to significant levels in the batch culture of uninfected insect cells but not in the batch culture with infection (Radford et al., 1997). The accumulation of such products may inhibit insect cell growth to give less viable cells available for the baculovirus infection. The infected insect cells may, therefore, die either from accumulation of toxic end-products or the expression of baculovirus. The productivity of recombinant protein in the fed-batch culture of recombinant virus expression system, therefore, could be increased if the policies for feeding and infection are optimized.

The model with virus expression system may be used to estimate the effect of MOI (multiplicity of infection), TOI (time of infection), and TOF (time of feed) on the productivity of recombinant viral protein. In this work, the effect of MOI on the insect cell growth and β -galactosidase production in batch culture was investigated by the simulation of the model (Figure 10). From the results of the simulation, maximum viable cell concentration was predicted to increase with lower MOI, and β -galactosidase production was predicted to be higher with lower MOI. A higher MOI above a certain level (500 in the model simulation) did not give any further changes. If the prediction by the simulation is proven correct, it may be better to use lower MOI to increase the productivity. On the other hand, it was reported that the final β -galactosidase was constant for the infection with

different MOI and the specific β -galactosidase yield from viable cells decreased with increasing final cell concentration (Wong et al., 1996). They explained the decline of the product yield at higher final cell concentration by substrate limitation. Therefore, if no substrate is limiting during the infection process, the model prediction might be expected to give further increase of the product titer.

Conclusions

A structured model for virus infection in animal cell culture has been developed and has proved to predict closely the baculoviral infection and the production of recombinant viral protein in the batch and fed-batch culture of insect cells. The model describes most of key features of viral infection in animal cell culture and predicts intracellular processes in the cells infected with baculovirus. Predictions of the medium components such as recombinant protein, glucose and 20 amino acids by the model were relatively well fitted to the experimental data, which implies that the model simulates closely the real metabolic reactions occurring during the culture and infection processes.

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