Kinetics, Experimental and Simulation Studies of Chinese Hamster Ovary Cell Growth in a Packed-Bed Bioreactor

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Abstract: To figure out the relationship between nutrient deprivation and cell growth, simulation can be effective. In the present work, the growth of Chinese Hamster Ovary (CHO) cells was simulated based on stoichiometric and kinetics calculations. The simulation results were compared to the experimental data from a packed-bed bioreactor and a good agreement was observed. The kinetic parameters ($K_{Glc}$, $K_{Amm}$, $K_{Lac}$, and $\mu_{max}$) were optimized by a genetic algorithm method using stoichiometric parameters ($Y_{X/Glc}$, $Y_{X/Amm}$, and $Y_{Lac/Glc}$). The stoichiometric and kinetic parameters were used in the simulation to study the growth of CHO cells. The concentrations of the toxic by-products, ammonium and lactate, were measured at different values of dissolved oxygen, 30% and 50% of saturated air. While dissolved oxygen was maintained at 30% of saturation, the maximum that ammonium and lactate concentrations reached were 0.099 and 2.1 g/l, respectively. At dissolved oxygen level of 50% air saturation, the maximum ammonium and lactate concentrations decreased to the values of 0.082 and 1.8 g/l, respectively. The cell density of $22 \times 10^8$ cells/l was achieved after 120 h of cultivation at dissolved oxygen of 50% in a packed-bed bioreactor.

Key words: Packed-bed bioreactor • CHO cells • Stoichiometry • Kinetics • Simulation

INTRODUCTION

Development and optimization of cell culture process is necessary to meet the demand for mammalian cells. The scale-up and optimization of mammalian cell cultures is influenced by many factors [1-3]. Since the animal cells have low productivity, a large volume of culture media is required to produce therapeutic proteins. Packed-bed bioreactors can meet the criteria as high cell density and productivity can be attained under low-volume conditions [4,5]. High cell density cultures have been developed with the aim of improving the volumetric productivity as it is proportional to both the final cell density and specific productivity, i.e. the amount of product formed per unit cell mass per unit time [6-8].

In mammalian cell cultures, cell growth and productivity can be improved by optimizing cell culture conditions. The lack of available nutrients, especially glucose, has been cited as a potential inducer of apoptosis in cell cultures [9-11]. Accumulation of toxic by-products such as ammonium and lactate are the main barriers to longevity of batch cultures. High levels of ammonium and lactate inhibit the CHO cell growth and productivity [12-16].

A mathematical model has been developed to predict the CHO cell growth according to the concentration of main nutrients and by-products [17]. Asymmetric logistic equations have been used to predict nutrient deprivation and monoclonal antibody production in hybridoma T-flask cultures [18]. Liu et al., studied the bioreactor...
behavior by using kinetic models which enabled the estimation for any given set of operating conditions. A study was carried out on using modeling techniques to determine an optimal time point for the addition of effective additives to promote the productivity in the CHO cell cultures [19]. Oxygen solubility in large-scale industrial bioreactors was known as one of the key parameters. The demand for oxygen is different depending on cell and from the supply point, the critical parameter is the volumetric transfer coefficient [20,21].

The present work studied how the CHO cell growth is influenced by the concentration of main nutrients. CHO cells are widely used in biopharmaceutical industry as they can tolerate environmental stress which made them known as stable hosts. The comparison of a simulation prediction with actual behavior usually leads to an increased understanding of the process. Oxygen supply, the key parameter for industrial-scale bioreactors, was measured in a packed-bed bioreactor. We implemented the genetic algorithm and simulator to predict the cell density and nutrient exhausting using logistic equations. Through that, the stoichiometric and kinetic parameters were measured using both experimental data and genetic algorithm. These parameters were inserted into the simulation model to study the effect of nutrient deprivation and by-product formation on the cell growth in a packed-bed bioreactor.

MATERIALS AND METHODS

Cell Line and Culture Media: The CHO cells were obtained from the National Institute of Genetic Engineering and Biotechnology (NIGEB). The CHO cells were cultured in the basal medium consisting of DMEM (Gibco) and Ham’s F12 (Gibco) at a volume ratio of 1:1 and 10% fetal bovine serum (Gibco) in a humidified incubator at 37°C and 5% CO₂. Each instance was put into a T-75 flasks. Each set of T-flasks were inoculated from a common seed culture and 12 ml of the basal media.

Inoculum Preparation: Cells were removed from the surface of T-75 flasks using the EDTA-trypsin solution (Gibco) and inoculated into the microcarrier culture system. The initial cell density for inoculums was about 4×10⁸ cells/l. The CHO cells must be in the growth phase to shorten the lag phase.

Bioreactor: The batch bioreactor experiment was performed in a 4 l Celligen bioreactor (New Brunswick Scientific, USA) with a working volume of 3.8l at 37°C, pH of 7.2 and with an agitation rate of 50-100 rpm. In order to study the effect of dissolved oxygen (DO) on toxic by-products formation, DO content was adjusted to 30% and 50% of saturated air as a manipulated variable by the bioreactor control program. The polyester disks (134 g) purchased from New Brunswick Scientific Co., were used as microcarriers for the attachment of CHO cells present in the packed-bed bioreactor. The polyesters were removed from the bioreactor and the CHO cells were separated by EDTA-Trypsin solution. The exposure time for cell removal is 5 min at 37°C. After cells were removed from surfaces, serum was added to the culture. Harvested cells were centrifuged at 1000 rpm for 5 min at 4°C.

Sample Analysis: Cell viability was determined by the Trypan blue exclusion method. Both grids of a neubauer haemocytometer slide were loaded with the cell suspension and microscopic cell counts were performed on four large squares of each grid.

Glucose, ammonium and lactate concentrations were enzymatically measured by glucose, ammonium and lactate assay kits (ChemEnzyme Co., Iran).

Kinetic Model of CHO Cultivation: For batch cultivation process, the mass balance equations for CHO cell growth, substrate consumption and product formation are as follows:

\[ \frac{dX}{dt} = r_c \]  
\[ \frac{dC_{Glc}}{dt} = -r_{Glc} \]  
\[ \frac{dC_{Amm}}{dt} = r_{Amm} \]  
\[ \frac{dC_{Lact}}{dt} = r_{Lact} \]

Where \( X \) is the CHO concentration (10⁸ cells/l), \( C_{Glc} \) is the concentration of glucose (g/l) and \( C_{Amm} \) and \( C_{Lact} \) are ammonium and lactate concentrations (g/l), respectively.

Experimental data showed that product inhibitors are of importance for the cultivation of CHO [16]. In this study, the cell growth rate, \( r_c \), is given by:

\[ r_c = \mu_{max} \frac{C_{Glc}}{C_{Glc} + K_{Glc} + K_i X} \]  

\[ K_i = \frac{C_{Lact}}{K_{Lact}} + 1 \]

\[ K_i = \frac{C_{Amm}}{K_{Amm}} + 1 \]  


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Where $\mu_{\text{max}}$ is the maximum specific growth rate (h$^{-1}$), $K_{\text{Glc}}$ is the Monod constant for glucose and $K_{\text{Lac}}$ and $K_{\text{Amm}}$ are the parameters used to describe product inhibitors. $K_j$ is the total inhibitory constant.

The substrate consumption and product formation rates can be calculated by:

$$r_{\text{Glc}} = -\frac{1}{Y_{X/\text{Glc}}} r_x$$  \hspace{1cm} (7)

$$r_{\text{Lac}} = -\frac{Y_{\text{Lac}}}{Y_{\text{Glc}}} r_{\text{Glc}}$$  \hspace{1cm} (8)

$$r_{\text{Amm}} = \frac{1}{Y_{X/\text{Amm}}} r_x$$  \hspace{1cm} (9)

Where $Y_{X/\text{Glc}}$ is the glucose yield coefficient (10$^8$ cells/g), $Y_{\text{Lac/Glc}}$ is the lactate to glucose yield coefficients (g/g) and $Y_{X/\text{Amm}}$ is the yield of ammonium based on cell growth (10$^8$ cells/g).

According to the above descriptions, there are three parameters ($Y_{X/\text{Glc}}, Y_{\text{Lac/Glc}}$ and $Y_{X/\text{Amm}}$) estimated from the experimental data and four parameters determined by minimizing Eq 10 using optimization method (genetic algorithm). The objective of optimization is to minimize the objective function, $\theta$:

$$\theta = \sum_{i=1}^{n} \left[ \frac{X(i) - X_e(i)}{C_e(i)} + \frac{C_{\text{Glc}}(i) - C_{\text{Glc}}(i)}{C_e(i)} + \frac{C_{\text{Lac}}(i) - C_{\text{Lac}}(i)}{C_e(i)} + \frac{C_{\text{Amm}}(i) - C_{\text{Amm}}(i)}{C_e(i)} \right]$$  \hspace{1cm} (10)

where $X_e, C_{\text{Glc}}, C_{\text{Lac}}$ and $C_{\text{Amm}}$ are the measured concentrations of cell, glucose, lactate and ammonium at a given sampling time $(i)$. $X, C_{\text{Glc}}, C_{\text{Lac}}$ and $C_{\text{Amm}}$ are the concentrations computed by the model at a given sampling time $(i)$.

**Determination of $K_a$ by a Dynamic Method:**

Volumetric mass transfer coefficient ($k_an$) was calculated by a dynamic method using the following equations:

$$\frac{dC_{\text{L}}}{dt} = OTR - OUR$$  \hspace{1cm} (11)

$$OTR = k_a (C^*_L - C_L)$$  \hspace{1cm} (12)

$$OUR = qo_2 X$$  \hspace{1cm} (13)

Where $C^*_L$ is the maximum oxygen concentration in the liquid, $C_L$ is the oxygen concentration in the liquid, OUR is the oxygen uptake rate and $q_{o_2}$ is the specific oxygen respiration rate.

**RESULTS AND DISCUSSION**

It is difficult to measure the cell density in a packed-bed bioreactor during the cultivation since the cells have been immobilized onto microcarriers. Thus, the data on yield coefficients from the T-75 flask experiment were applied to the simulation model for bioreactor. Table 1 provides a summary of the yield coefficients from the T-75 flask monolayer cultivation.

The kinetic parameters were estimated using the genetic algorithm. The optimized parameters ($K_{\text{Glc}}, K_{\text{Amm}}, K_{\text{Lac}}$ and $\mu_{\text{max}}$) are shown in Table 2. Figure 1 depicts the computed profiles based on genetic algorithm for cell, glucose, lactate and ammonium under such an optimal model. In order to characterize the quality of the prediction model, the residual standard deviation (RSD) was determined [23].

$$\text{RSD(\%)} = \left( \frac{\sqrt{\sum_{i=1}^{n} (y_i - \bar{y})^2}}{\bar{y}} \right)$$  \hspace{1cm} (14)

Where $\text{RSD(\%)} = \frac{1}{y} \sum (y - \bar{y})^2$, $y_i$ is an experimental value and $\bar{y}$ is a predicted value, $\bar{y}$ is the average of experimental values and $n$ is the number of experimental points. The RSD (%) values are 3.4, 8.3, 8.2 and 6.9 for glucose, lactate, ammonium and biomass concentrations, respectively. All the RSD (%) values were below 10% indicating that the prediction model terms are significant [22].

**Table 1: Kinetic and stoichiometric parameters of CHO cells in T-75 flasks**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_{X/\text{Glc}}$</td>
<td>10$^8$ cells/g</td>
<td>4.2</td>
</tr>
<tr>
<td>$Y_{\text{Lac}}$</td>
<td>10$^8$ cells/g</td>
<td>8.9</td>
</tr>
<tr>
<td>$Y_{X/\text{Amm}}$</td>
<td>10$^8$ cells/g</td>
<td>230</td>
</tr>
<tr>
<td>$Y_{\text{Lac/Glc}}$</td>
<td>g/g</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Table 2: Kinetic parameters for the proposed simulation from genetic algorithm**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{\text{Lac}}$</td>
<td>g/l</td>
<td>1.968</td>
</tr>
<tr>
<td>$K_{\text{Amm}}$</td>
<td>g/l</td>
<td>0.173</td>
</tr>
<tr>
<td>$\mu_{\text{max}}$</td>
<td>1/h</td>
<td>0.090</td>
</tr>
<tr>
<td>$K_{\text{Glc}}$</td>
<td>g/l</td>
<td>3.457</td>
</tr>
</tbody>
</table>
Fig. 1: Experimental (closed diamond) and simulated data (represented by line) for (a) CHO cells density (b) Glucose consumption, (c) Ammonium formation, (d) Lactate formation.

Fig. 2: Profile concentrations in packed-bed bioreactor at different amount of dissolved oxygen. (a) Glucose consumption, (b) Ammonium formation, (c) Lactate formation, (closed square) DO 30%, (closed triangle) DO 50%.
These optimized parameters were used in the simulation. Glutamine is not essential for CHO cells having the biosynthetic glutamine synthetase. The glutamine catalyzes the following reaction [23]:

\[ \text{L-glutamate} + \text{NH}_4^+ + \text{ATP} \rightarrow \text{L-glutamine} + \text{ADP} + \text{Pi} + \text{H}^+ \]

Thus, glutamine was not taken as the limiting substrate in the simulation. The CHO cell density reached $22 \times 10^6$ cells/l in the packed-bed bioreactor after 120 h cultivation. This value was $26 \times 10^6$ cells/l based on the simulation results for the same culturing period. Without taking into account the negative effect of by-products in Monod equation, the cell density was computed as $30 \times 10^6$ cells/l at 80 h of cultivation based on the simulation. This result indicated that the accumulation of by-products had a great impact on cell density. It may be due to the reduced culture longevity because of the formation of toxic by-products.

The profile of glucose consumption in the packed-bed bioreactor is shown in (Fig. 2a). The concentration of glucose decreased to the values of 0.3 g/l at 50% DO and 0.21 g/l at 30% DO by the end of cultivation, whereas a reduction to 0.56 g/l was observed using the simulation program after 120 h cultivation. The simulation results show a relatively good agreement with the experimental data in terms of glucose consumption (Fig. 3a). No significant difference was observed in glucose utilization when DO was switched from 30% to 50%. There are lots of biochemical reactions in the metabolic pathways consuming cell energy to mitigate the adverse effects of by-products formation. For instance, the mechanism of alanine secretion to the medium by CHO cells reduces ammonium toxicity. The reaction that occurs between glutamate and pyruvate leads to the formation of alanine and decreases the interacellular pyruvate level [24]. The other reaction that needs ATP is the conversion of glutamate to glutamine which also consumes ammonium.
Fig. 4: Metabolic pathways in CHO cells. Gln is glutamine; Glu is glutamate; Pyr is pyruvate; KG is ketoglutarate; Mal is malate; Glc is glucose; Glc-6P is glucose-6-phosphate; Lac is lactate; Cit is citrate; OAX is oxaloacetate; Ac-CoA is acetyl-CoA.

Since the simulation model excluded all these reactions happening in CHO cells by means of glucose and ATP consumption, slightly higher glucose consumption observed in the bioreactor compared to the cell growth model might be due to the fact that all these reactions happening in CHO cells by means of glucose and ATP consumption were excluded from the simulation model.

The concentrations of ammonium and lactate were measured as the main by-products to provide more information on cell behavior. Fig 2b shows the data on ammonium formation from experimental runs in the packed-bed bioreactor. Ammonium concentration was below the inhibitory level. The inhibitory concentrations that have been reported from previous studies for ammonium and lactate are about 10 and 18 mM, respectively. The ammonium concentration was 0.08 g/l at 50% DO and 0.099 g/l at 30% DO after 120 h cultivation in the packed-bed bioreactor. The ammonium concentration reported for simulation program was 0.088 g/l after the same period of time. Lactate concentration reached 1.9 g/l at 50% DO and 2.1 g/l at 30% DO in the packed-bed bioreactor, whereas the computed value using the simulation program was 2.4 g/l (Fig 2c). Since detoxification of lactate occurs at low glucose concentrations [16], CHO cells can consume lactate as an alternative carbon source. The experimental data and simulation results were comparable in terms of lactate concentration, whereas for ammonium concentration a deviation observed in simulation results from the experimental data especially at lower DO (Fig. 3a,b,c).

High oxygen concentration usually has adverse effects on mammalian cell growth. However, since oxygen limitations within the microcarriers is critical, for immobilized cells high levels of oxygen is nontoxic and can improve the performance of fixed-bed bioreactors [21,22]. To determine the effect of dissolved oxygen on by-products formation, the packed-bed bioreactor with common seed was adjusted at two different values of DO. The 20% reduction in DO led to an increase in ammonium and lactate production from 0.08 and 1.9 g/l at 30% DO to 0.0999 and 2.1 g/l at 50% DO, respectively. Major metabolic pathways in CHO cells including those of by-products formation and glucose consumption are shown in Fig 4. Glucose and glutamine are the major sources of carbon and energy in animal cell cultures. Increase in lactate formation at lower DO is because lactate forms under anaerobic conditions, i.e. oxygen limitations. More energy produced through oxidative pathway (38 mol ATP) compare to oxygen limitation conditions (2 mol ATP), which is the reason behind higher glucose consumption
at high level of DO. Furthermore, the increased level of ammonium might be related to the mechanism through which glutamine consumes to furnish the energy deficit (Fig. 4).

The volumetric mass transfer coefficient (k_a) is difficult to predict, although it can be measured and the value reported for 120 h of cultivation was 8.006 (1/h). The specific respiration rate was calculated by equation (13). According to the cell density this amount was 0.4 mmol O_2/l-h/10^6cells/ml.

In summary, this research was focused on mathematical calculations for the prediction of CHO cell growth in a packed-bed bioreactor. To our knowledge, no empirical models have yet been developed to simulate the experimental data of CHO cells in a packed-bed bioreactor. In this study, the kinetic parameters were optimized by genetic algorithm. Then the CHO cell growth was simulated based on Monod kinetics which showed a good agreement with the laboratory results, hence it can be used to predict the CHO cell growth and determine the values of important factors expressing nutrient consumption and some metabolites production. Improved understanding of the process leads to the promotion of cell density and improvement of productivity which has a great impact on the industrial production of recombinant proteins by mammalian cell cultures.

ACKNOWLEDGEMENTS

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REFERENCES


Nomenclature

\begin{align*}
Amm & : \text{Ammonium} \\
Glc & : \text{Glucose} \\
Lac & : \text{Lactate} \\
C_{Glc} & : \text{Glucose concentration (g/l)} \\
C_{Lac} & : \text{Lactate concentration (g/l)} \\
C_{Amm} & : \text{Ammonium concentration (g/l)} \\
C^* & : \text{Maximum oxygen concentration in the liquid (mg/l)} \\
C_L & : \text{Oxygen concentration in the liquid (mg/l)} \\
K_{Gl} & : \text{Glucose Monod constant (g/l)} \\
K_{Lact} & : \text{Inhibition constant for lactate (g/l)} \\
K_{Amm} & : \text{Inhibition constant for ammonium (g/l)} \\
\varphi & : \text{Specific oxygen consumption rate} \\
r_{Gl} & : \text{Glucose consumption rate} \\
r_{Lac} & : \text{Lactate formation rate} \\
r_{Amm} & : \text{Ammonium formation rate} \\
Y_{X,Glc} & : \text{Glucose yield coefficient (10^8 cells/g)} \\
Y_{X,Lac} & : \text{Lactate yield coefficient (10^8 cells/g)} \\
Y_{X,Amm} & : \text{Ammonium yield coefficient (10^8 cells/g)} \\
Y_{Glc,Glc} & : \text{Glucose yield coefficient (g/g)} \\
Y_{Amm,Glc} & : \text{Glucose yield coefficient (g/g)} \\
\mu_{max} & : \text{Maximum specific growth rate (1/h)}
\end{align*}