Un-structured kinetic models for rh-GCSF production by *Escherichia coli* in a fed batch reactor before and after induction

Arash Ghanbari[1], Valiollah Babaeipour[2]*, Reza Yegani[1], Mehdi Pesarn Haji Abbas[3]

[1] Department of Chemical Engineering, Sahand University of Technology, Tabriz, Iran.
[2] Group of Biochemical Engineering, Department of Bioscience and Technology, Malek Ashtar University of Technology, Tehran, Iran. P.O. Box 15875-1774, Baba1@sbmu.ac.ir,
[3] Department of Life Science Engineering, Faculty of New Technologies, University of Tehran, Iran.

Abstract. Two un-structured kinetic model for rh-GCSF production by *Escherichia coli* in a fed batch reactor are proposed. One of them predicts production of rh-GCSF before induction when concentration of glucose decreases. The other one predicts production of rh-GCSF after induction. We calculate parameters of the kinetic models by fitting models to experimental data. These experimental data have been obtained in experiments in a fed batch reactor. Suggested models are unstructured nonsegregated. R^2 of the model that predicts production before induction is equal to 0.9356 and after induction is about 0.9966.

Keywords: rh-GCSF, *Escherichia coli*, un-structured kinetic model, fed batch reactor.

1. Introduction

Escherichia coli has been the most widely used prokaryotic host because it has been characterized in terms of its molecular genetics, physiology and expression systems [1-3] and also *E. coli* has been the most frequently employed host with many available expression systems for manufacturing recombinant proteins [4]. Recent progress in the fundamental understanding of transcription, translation, and protein folding in *E. coli*, together with serendipitous discoveries and the availability of improved genetic tools are making this bacterium more valuable than ever for the expression of complex eukaryotic proteins. Commercially, *E. coli* is generally recognized as a safe organism and has proved to be an economically viable means for producing recombinant protein products [5].

The growth in the use of recombinant proteins has increased greatly in recent years. Recombinant Human Granulocyte colony-stimulating factor (rh-G-CSF or rh-GCSF) is a colony-stimulating factor hormone. It is a glycoprotein, growth factor or cytokine produced by a number of different tissues to stimulate the bone marrow to produce granulocytes and stem cells. G-CSF then stimulates the bone marrow to release them into the blood. G-CSF is also known as colony-stimulating factor 3 (CSF 3) [6].

Unstructured kinetic models are the most frequently employed for modelling microbial systems based on simplicity and technical robustness. This type of model describes the microorganisms as a black box. Unstructured models assume that all cells are in the same physiological state and can be considered as

behaving equally [7-10]. The aim of this work is the development and testing of a kinetic model able to fit our experimental data.

2. Materials and methods

2.1. Microorganism and vector system

Escherichia coli strain BL21 (DE3) (Novagen, Inc.) was used as the host for rh-GCSF expression. This strain was transformed with a commonly available plasmid, pET23a inducible expression vector (Novagen, Inc.), in which the rh-GCSF gene (Noor Research and Educational Institute, Tehran, I.R. Iran) was inserted into the *NotI* and *NdeI* sites.

2.2. Media and solutions

LB (Luria-Bertani) medium was used for plate cultivation of *E. coli* strain BL21 (DE3) and M9 medium was used for preparation of seed culture. The M9 modified medium consisted of 10 g glucose, 15 g K_2HPO_4, 7.5 g KH_2PO_4, 2 g Citric acid, 2.5 g $(NH_4)_2SO_4$, 2 g $MgSO_4.7H_2O$, and 1 ml trace element solution per litre. The trace element solution contained 2.8g $FeSO_4.7H_2O$, 2g $MnCl_2.4H_2O$، 2.8 g $CoSO_4.7H_2O$, 1.5 g $CaCl_2.2H_2O$, 0.2 g $CuCl_2.2H_2O$, and 0.3 g $ZnSO_4.7H_2O$ g per litre in 1M HCl. Batch cultivations were simultaneously carried out in two 2 l bench-top bioreactors with the working volume of 1 l.

2.3. Analytical procedure

The expression level of rh-GCSF was determined by SDS-PAGE using poly-acrylamid 12.5 % (w/v); and also, SDS-PAGE gels were blotted on PVDF membrane for further analysis. Gels were stained with Coomassie brilliant blue R250, and then quantified by gel densitometer. Total soluble protein was analyzed by Bradford method. After recombinant protein production and its determination, rh-GCSF was purified and its activity was determined by recently developed method [11, 12].

2.4. Fed batch cultivation

A batch culture was initially established by the addition of 100 ml of an overnight-incubated seed culture (CDW = 0.4 –0.6 g l^{-1}) to the bioreactor containing 900 ml of M9 modified medium. The pH was maintained at 7 by the addition of 25% (w/v) NH_4OH or 3 M H_3PO_4 solutions. Dissolved oxygen was controlled at 30–40% (v/v) of air saturation by controlling of both the inlet air (which was enriched with pure oxygen) and agitation rate. Foam was controlled by the addition of silicon-antifoaming reagent. After depletion of initial glucose in the medium, as indicated by a rapid increase in the dissolved oxygen concentration, the feeding was initiated. Feeding rate was increased stepwise based on the exponential feeding strategy with maximum attainable specific growth rate during fed-batch cultivation. The exponential feeding was determined by the following equation (Eq. (1)):

$$M(t) = F(t)S_0 = [\mu(t)/(Y_{x/s}) + m]S_0V_0X_0exp(\int_{t_0}^{t}\mu(t)dt) \qquad (1)$$

Where V_0 is the volume of the medium in the bioreactor (l), X_0 is the biomass concentration at the start of feeding g(DCW) l^{-1}, t is the time (h), μ is the specific growth rate (h^{-1}), S_0 is the glucose concentration(g l^{-1}) in the feeding solution, $F(t)$ is the feeding rate (l h^{-1}), $M(t)$ is the mass feeding rate (g h^{-1}), $Y_{X/S}$ is the yield of biomass as a result of substrate (g DCW g^{-1} glucose), t_0 (h) is the starting time for each feeding step, and m is the specific maintenance coefficient (g g^{-1} h^{-1}) [13].

The coefficient yield ($Y_{X/S}$) and maintenance coefficient (m) were set at 0.5 and 0.025 g g^{-1} h^{-1}, respectively. In order to develop a simple feeding strategy with the highest attainable specific growth rate during the entire process, a maximum oxygen transfer capacity was applied to the bioreactor. Glucose concentration was maintained below 2 g l^{-1} by a gradual increase in feeding at each step.

Cells were induced by the addition of 3 mM IPTG in the all experiments. The required nitrogen source (ammonium) was supplied by the addition of 25% (w/v) NH_4OH which was also used for maintaining pH at 7; also, temperature of the process was maintained at 37 ºC. The level of phosphate added at the beginning of fed-batch was sufficient until end of process. Acetate and glucose concentrations were controlled manually at intervals 10 min.

3. Kinetic model

3.1. Model for before induction

Induction time of our experiment is about 14 h. Between 8 and 13 h before induction; rh-GCSF is produced because of concentration of glucose decreases. First, the important parameters for setting up the model that predict production before induction were determined. Cell growth $x(t)$, rate of rh-GCSF production $(dp(t)/dt)$, cell growth rate $(dx(t)/dt)$, specific growth rate $(\mu(t))$, rate of specific growth rate $(d\mu(t)/dt)$ and concentration of glucose $(Cs(t))$ were selected. Afterward the important parameters were plotted versus time (Fig.1). Plotted curves showed that behaviour of the concentration of glucose and rate of specific growth rate during the fermentation were similar to the rh-GCSF production rate nearly. We analyzed the figure and wrote the primarily kinetic model as following:

$$\frac{dp}{dt} = \alpha.\frac{d\mu}{dt} + \beta.Cs \qquad (2)$$

Where p is the rh-GCSF concentration, α and β rh-GCSF production constants associated for rate of specific growth rate and concentration of glucose respectively.

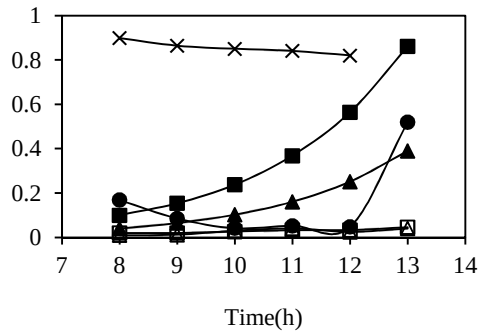

Fig. 1: Cell growth rate (■), cell growth (▲), specific growth rate (×), concentration of glucose (Δ), rate of production (□) and rate of specific growth rate (●).

Specific growth rate is an important term that must be added to equation 2 to complete the model. The specific growth rate has effect on rate of production. By adding μ to equation 2, the model will be constructed. So the kinetic model for prediction of production before induction could be written as:

$$\frac{dp}{dt} = \alpha.\frac{d\mu}{dt} + \beta.Cs + \gamma.\mu \qquad (3)$$

Where γ is rh-GCSF production constant associated for specific growth rate.

3.2. Model for after induction

The same as previous section, the important parameters were plotted versus time (Fig. 2). This model predicts production of rh-GCSF after induction (14 h). The analyzing of the figure result the following model:

$$\frac{dp}{dt} = \alpha.\frac{d\mu}{dt} + \beta.\frac{dx}{dt} \qquad (4)$$

Where p is the produced rh-GCSF, α and β are rh-GCSF production constants associated for rate of specific growth rate, the cell growth rate respectively.

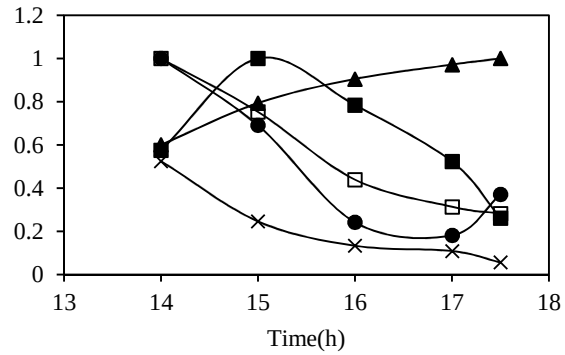

Fig. 2: Rate of production (■), cell growth (▲), specific growth rate (×), cell growth rate (□) and rate of specific growth rate (●).

Specific growth rate is an important term that must be added to equation 4 to complete the model. The specific growth rate has effect on rate of production. By adding μ to equation 4, the model will be constructed. So the kinetic model for prediction of production after induction could be written as:

$$\frac{dp}{dt} = \alpha.\frac{d\mu}{dt} + \beta.\frac{dx}{dt} + \gamma.\mu \qquad (5)$$

Where p is the produced rh-GCSF, α and β and γ are rh-GCSF production constants associated for rate of specific growth rate, the cell growth rate and specific growth rate respectively.

4. Analytical method

The parameters of the models are calculated by fitting models to experimental data. Experimental data fitting has been accomplished by a nonlinear regression method.

5. Results and discussion

α, β and γ in equation 3 are calculated by fitting and R^2 of this model is equal to 0.9356. The parameters of the model that predict production after induction (α, β and γ) are calculated by fitting the same as previous model and R^2 of this model is about 0.9966. The results of fitting are shown in Table 1 and 2. You can see the experimental data and the models prediction of rate of production after and before induction in Figure 3 and 4 respectively. As can be seen in Figure 3 and 4 the models prediction is in accordance with the data experimental data.

Table 1. The parameters of equation 3 (model of before induction) calculated by fitting of experimental data.

Parameters	α	β	γ
Optimal value	-1.1909	0.4315	0.1867

Table 2. The parameters of equation 5 (model of after induction) calculated by fitting of experimental data.

Parameters	α	β	γ
Optimal value	71.5892	1.0477	-40.8363

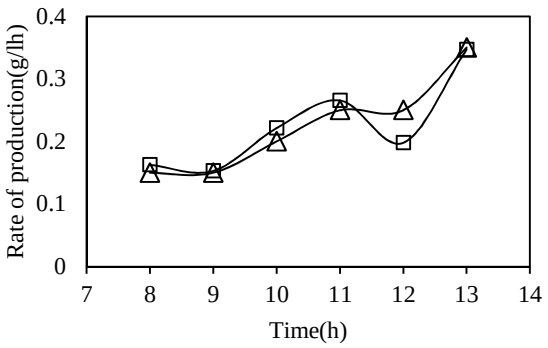

Fig. 3: Comparison between experimental data of rate of production (Δ) and model prediction of rate of production before induction (□).

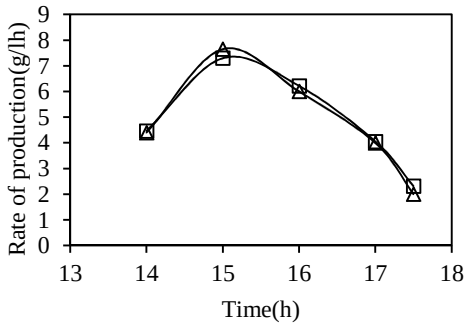

Fig. 4: Comparison between experimental data of rate of production (Δ) and model prediction of rate of production after induction (□).

6. Conclusions

Two simplified unstructured nonsegregated kinetic models have been proposed for rh-GCSF production from *E. coli* in the fed batch reactor before and after induction. The model of before induction describes production of rh-GCSF that occurred because of decreasing of glucose concentration. The models are able to fit the experimental data given in this work. The parameter values of the kinetic models calculated by fitting of experimental data using a nonlinear regression given in Table 1 and 2.

7. References

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