

OPTIMIZATION OF CULTURE CONDITIONS FOR CHINESE HAMSTER OVARY (CHO) CELLS PRODUCTION USING DIFFERENTIAL EVOLUTION

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ABSTRACT

Chinese hamster ovary (CHO) cells are the most widely used mammalian cells in bioprocessing and biopharmaceutical applications. The performance of CHO cell cultivation process is significantly affected by the composition of the culture medium. Evaluating the influence of perturbations in the components of the culture medium and optimizing its composition is most important for enhancing the cell productivity. In this work, the experimentally validated macroscopic mathematical model of the cell cultivation process¹ identified under metabolic viewpoint is coupled with differential evolution (DE) to optimize the input space of the CHO cell cultivation process. The optimized culture medium composition is found to be within the range of experimental conditions for which the model is identified and offers a significant increase in cell density. The model based evolutionary optimization strategy is found effective in exploring the input search space and optimizing the initial culture conditions that enhance the cell density of the CHO cell cultivation process.

Keywords: Dynamic model, Metabolic flux analysis, CHO cells cultivation, Optimization, Differential evolution

INTRODUCTION

Chinese hamster ovary (CHO) cells are the most widely used mammalian cells in bioprocessing and biopharmaceutical applications. These cells are robust in culture and are able to produce a variety of therapeutic antibodies and recombinant glycoproteins. CHO cells are produced mostly in batch and fed-batch modes. The performance of CHO cell cultivation process is affected by several factors including the concentrations of culture medium components such as glucose, glutamine and biomass, as well as the operating conditions such as temperature, aeration rate and pH. Identifying and optimizing the significant factors that are responsible for maximizing the cell productivity is most important in view of the specialized applications of CHO cells. Optimization of batch and fedbatch processes is usually performed by using two approaches: a response surface methodology (RSM) and a dynamic optimization strategy. Response surface methodology seeks to identify and optimize the significant factors for achieving the desired responses. This approach uses statistical designs to develop empirical models that relate the responses to factors and these empirical models are used to find the important factors that enhance the process performance. This approach has been widely reported for bioprocess optimization studies²⁻⁷. Dynamic optimization finds the optimal operational profiles for the operating variables such as temperature and feed rate by using a mathematical model of the process with the support of a suitable optimization algorithm. These optimal policies that ensure the satisfaction of the product property requirements and the operational constraints can be calculated off-line, which are then implemented on-line such that the system is operated in accordance with these control policies. This approach has been employed for optimal control of various bioprocess systems^{8,9}.

The performance of CHO cell cultivation process is significantly affected by the composition of the culture medium. Identifying and optimizing the components of the culture medium is most important for maximizing the productivity of CHO cells. In this work, the experimentally validated macroscopic mathematical model of the cell cultivation process¹ that is identified under metabolic viewpoint is coupled with differential evolution (DE) to optimize the input space of the CHO cell cultivation process. In contrast to the RSM where an empirical model is generally employed to optimize the process conditions, in this work, a dynamic mathematical model of the process is considered to optimize the culture medium composition of the CHO cell cultivation process. The dynamic mathematical model of the process involves the mass balances of the extra cellular species inside the reactor with the vector of species concentrations, the vector of the macro-reaction rates, the

stoichiometric matrix of the macro reaction network and the net exchange of species with the outside environment. DE is a stochastic optimization algorithm that operates on a population of potential solutions by applying the principle of survival of the fittest to generate an optimal solution. The effectiveness and robustness of DE has been demonstrated in a variety of applications^{8,10}. In this work, the inherent optimizing feature of DE is exploited in combination with the dynamic mathematical model of the process for optimizing the culture medium composition to enhance the cell density of the CHO cell cultivation process.

MATERIALS AND METHODS

Differential evolution

Evolutionary algorithms are widely used to solve optimization problems in various fields^{11,12}. These algorithms have an advantage over conventional gradient-based search procedures because they are capable of finding global optima of multi-modal functions and searching design spaces with disjoint feasible regions. Among the evolutionary algorithms, differential evolution (DE) is a simple population based search algorithm for global optimization of real valued functions¹³. Its robustness and effectiveness has been demonstrated in a variety of applications^{10,14}. DE is similar to Genetic Algorithms (GA) in the sense that it uses the same evolutionary operators like selection, recombination and mutation as in GA, however, the significant difference is that DE uses distance and direction information from the current population to guide the search process. DE is controlled by three parameters, namely, population size (NP), crossover operator (CR) and mutation constant called scaling factor (F). The performance of DE depends on the manipulation of target vector and difference vector in order to obtain a trial vector.

A brief working of DE is as follows. First initial population of size NP vectors $[X_1^0, X_2^0, \dots, X_{NP}^0]$ are generated at random in D -dimensional search space and the fitness function values are evaluated. DE extracts distance and direction information from the current vectors and adds random deviation for diversity to generate new parameter vectors. Considering X_i^k as the target vector in k^{th} iteration, a corresponding mutant vector V_i^{k+1} is generated according to the following mutation scheme:

$$V_i^{k+1} = X_{r_1}^k + F(X_{r_2}^k - X_{r_3}^k) \quad (1)$$

where $r_1, r_2, r_3 \in \{1, 2, \dots, NP\}$ are randomly chosen integers, which are different from each other and also different from the running index i , and F is the mutation constant which controls the amplification of

the difference between two individuals. After mutation, crossover is introduced to increase the diversity of the mutant vectors. In this operation, the trial vector $U_{j,i}^{k+1}$ is developed from the elements of target vector, X_i^k , and the elements of the mutant vector, V_i^{k+1} as follows:

$$U_{j,i}^{k+1} = \begin{cases} V_{j,i}^{k+1}, & \text{if } (\text{rand}_{j,i} \leq CR), \quad (i = 1, 2, \dots, NP) \\ X_{j,i}^k, & \text{otherwise} \end{cases} \quad (2)$$

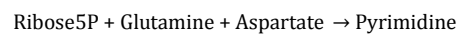
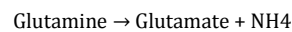
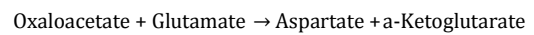
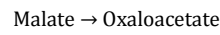
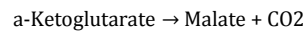
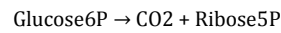
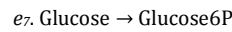
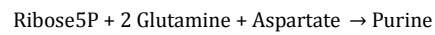
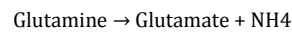
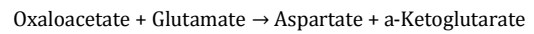
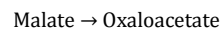
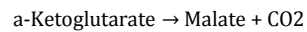
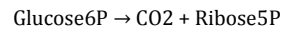
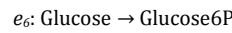
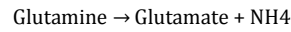
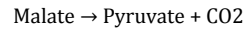
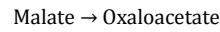
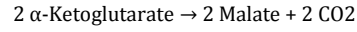
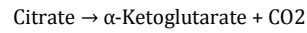
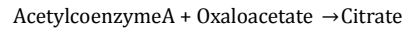
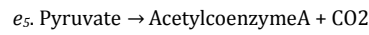
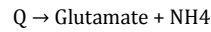
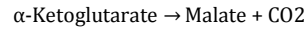
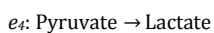
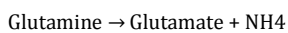
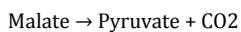
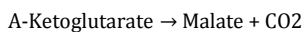
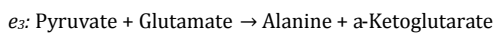
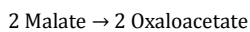
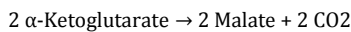
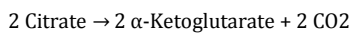
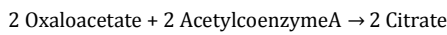
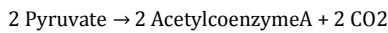
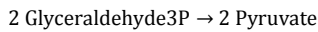
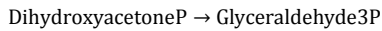
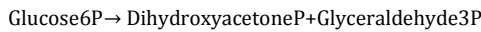
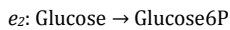
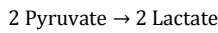
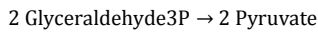
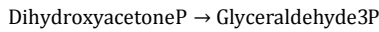
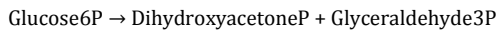
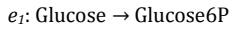
where $j \in \{1, 2, \dots, D\}$, $\text{rand } j \in [0, 1]$, and Cr is the crossover constant in the range $[0, 1]$. Finally, selection is performed by comparing the trial vector produced by the crossover operator with the target vector and the one with better fitness function is allowed to enter the next generation. The selection operation is given by

$$X_i^{k+1} = \begin{cases} U_i^{k+1}, & \text{if } (f(U_i^{k+1}) < f(X_i^k)), \quad (i = 1, 2, \dots, NP) \\ X_{j,i}^k, & \text{otherwise} \end{cases} \quad (3)$$

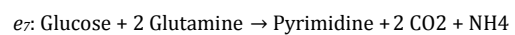
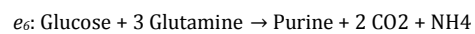
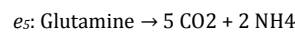
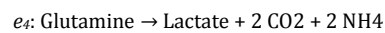
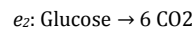
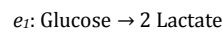
In this way, mutation, recombination and selection processes continue until some stopping criterion is met.

Mathematical model of CHO cell cultivation process

The CHO cell cultivation process involves the cell cultivation in batch mode, in which glucose and glutamine are the measured extracellular substrate species. The most significantly released metabolites are lactate, ammonia and alanine. The metabolic network representing the central metabolism of CHO cells is used to develop the elementary flux modes of CHO cells as expressed by



By eliminating the internal metabolites between the reactions, the set of macro-reactions that connect the extracellular substrates and the end-products are obtained as



On the basis of these macro reactions, a state space model incorporating Michaelis-Menton kinetics is derived. This model is represented by the following set of equations:

$$\frac{dG(t)}{dt} = -a_1 \frac{GX}{K_{G1} + G} - a_2 \frac{GX}{K_{G2} + G} - a_6 \frac{GQX}{(K_{G6} + G)(K_{Q6} + Q)} - a_7 \frac{GQX}{(K_{G7} + G)(K_{Q7} + Q)} \quad (4)$$

$$\frac{dQ(t)}{dt} = -a_3 \frac{QX}{K_{Q3} + Q} - a_4 \frac{QX}{K_{Q4} + Q} - a_5 \frac{QX}{(K_{Q5} + Q)} - 3a_6 \frac{GQX}{(K_{G6} + G)(K_{Q6} + Q)} - 2a_7 \frac{GQX}{(K_{G7} + G)(K_{Q7} + Q)} \quad (5)$$

$$\frac{dL(t)}{dt} = 2a_1 \frac{GX}{K_{G1} + G} + a_4 \frac{QX}{K_{Q4} + Q} \quad (6)$$

$$\frac{dN(t)}{dt} = a_3 \frac{QX}{K_{Q3} + Q} + 2a_4 \frac{QX}{K_{Q4} + Q} + 2a_5 \frac{QX}{(K_{Q5} + Q)} + a_6 \frac{GQX}{(K_{G6} + G)(K_{Q6} + Q)} + a_7 \frac{GQX}{(K_{G7} + G)(K_{Q7} + Q)} \quad (7)$$

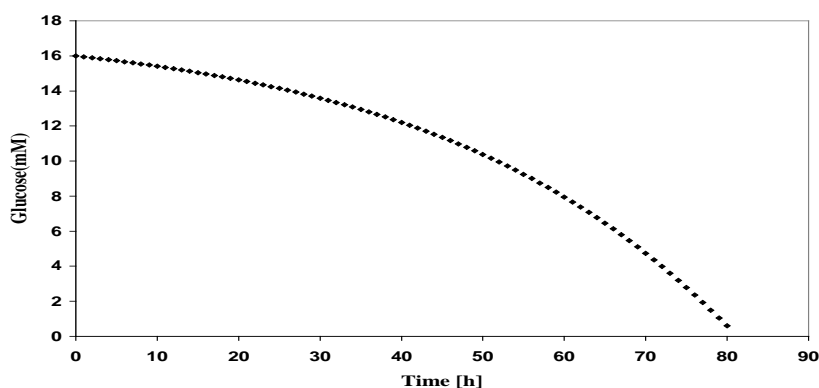
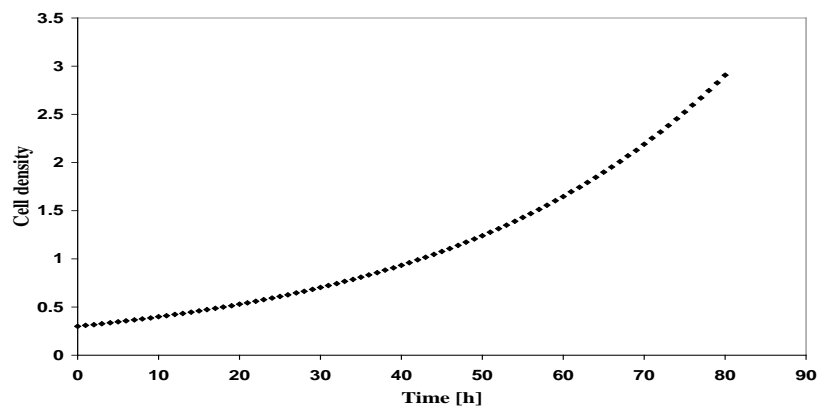
$$\frac{dA(t)}{dt} = a_3 \frac{QX}{K_{Q3} + Q} \quad (8)$$

Where X , G , Q , L , N and A are the concentrations of biomass, glucose, glutamine, lactate, ammonia and alanine, respectively. The a_i are the maximum specific reaction rates, and k_{G_i} and k_{Q_i} are the Michaelis constants. The numerical values of a_1 , a_2 , a_3 , a_4 , a_5 , a_6 and a_7 are 3.5956, 0.1736, 0.2686, 0.2038, 0, 0.1427 and 0.1427, respectively. The values of k_{G_i} ($i=1,2,\dots,7$) and k_{Q_i} ($i=1,2,\dots,7$) are taken to be 0.1 and 0.1, respectively. This model is used in combination with DE to optimize the culture conditions to enhance the cell density of CHO cell cultivation process. More details concerning the metabolic network representing the central metabolism of CHO cells and the derivation of the model can be referred elsewhere¹.

RESULTS AND DISCUSSION

The input space of the CHO cell cultivation process is represented by the initial culture medium compositions of glucose (G_i), biomass (X_i) and glutamine (Q_i). The responses are the product concentrations of cell density (X), glutamine (Q) and Lactate (L), in which the cell density (X) is the desired objective. The model equations given by eqs. (4)-(8) are solved using Eulers integration method with unit step size and the responses are shown in Figs. 1 and 2. These responses correspond to time duration of 80 hours. The differential evolution (DE) algorithm presented in the previous section is implemented to find the optimal medium composition in the input space of glucose (G_i), biomass (X_i) and glutamine (Q_i) to maximize the biomass concentration. The initial population in input space of culture medium is randomly generated with in the ranges of 15-17

for G_i , 5.2-5.4 for Q_i and 0.25-0.35 for X_i , respectively. The population size (NP), cross over operator (CR) and mutation constant (F) involved in DE are heuristically selected and set as NP=100, CR=59 and F=0.1. The population in the DE search space is of the dimension (100 x 3). The batch duration is specified as 80 hrs. The mathematical model of the process is solved for each of the population condition and the values of the process output variables are obtained at the end of the batch operation. The mutation, recombination and selection operations of DE are iteratively performed to alter the input conditions and obtain the desired cell density. The maximum value of cell density achieved due to this procedure is 3.392397 (mM) with the corresponding initial culture medium conditions as $G_i = 16.45752$ mM, $Q_i = 5.337633$ mM and $X_i = 0.3$ mM, respectively. The responses of process variables obtained from the model solution based on these optimal initial conditions are shown in Figs. 3 and 4. When the original initial culture medium composition of $G_i = 16.0$ mM, $Q_i = 5.4$ mM and $X_i = 0.3$ mM is used as such with no optimization, the cell density obtained from the model solution is 3.0 (mM). These results indicate a significant increase in cell density when the culture medium composition is configured optimally. Even though the original and optimized cell mass inoculation values are observed to be the same, the optimized values of initial glucose and glutamine concentrations are found to alter moderately from their original initial values. Thus DE is found to be simple and effective in optimizing the culture medium of the CHO cell cultivation process.



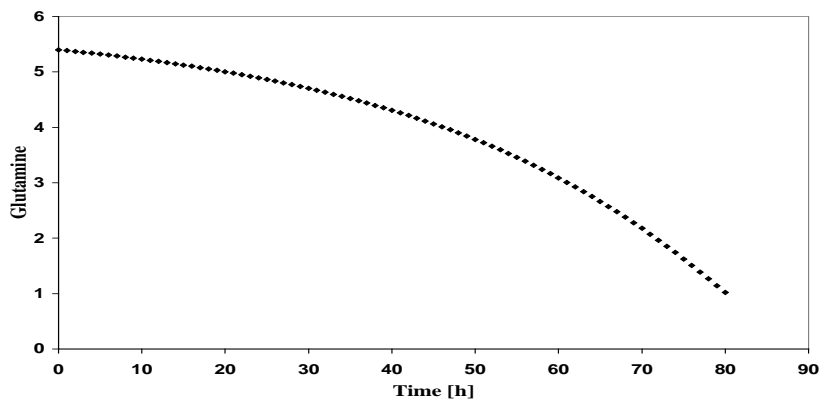


Fig. 1: Dynamic responses based on original initial culture medium composition.

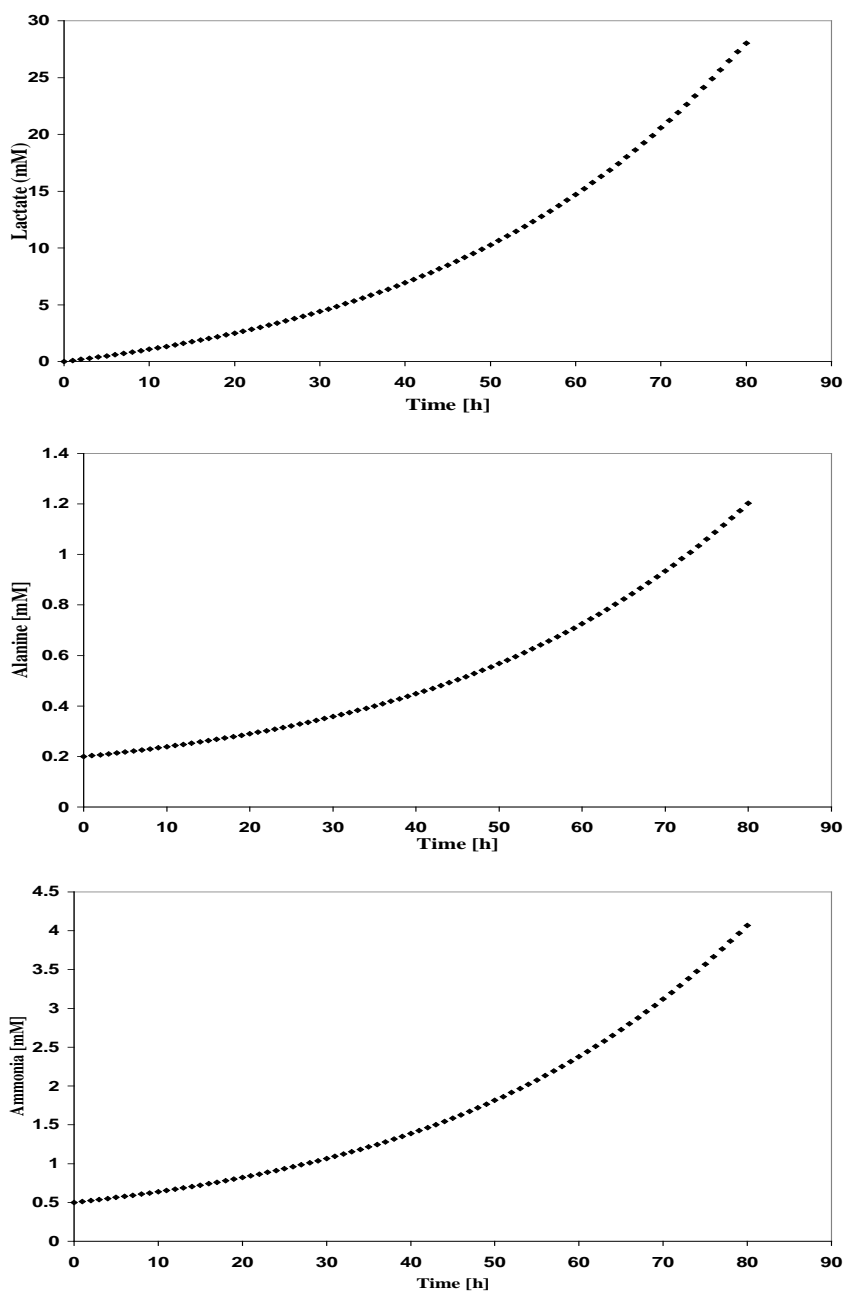


Fig. 2: Dynamic responses based on original initial culture medium composition.

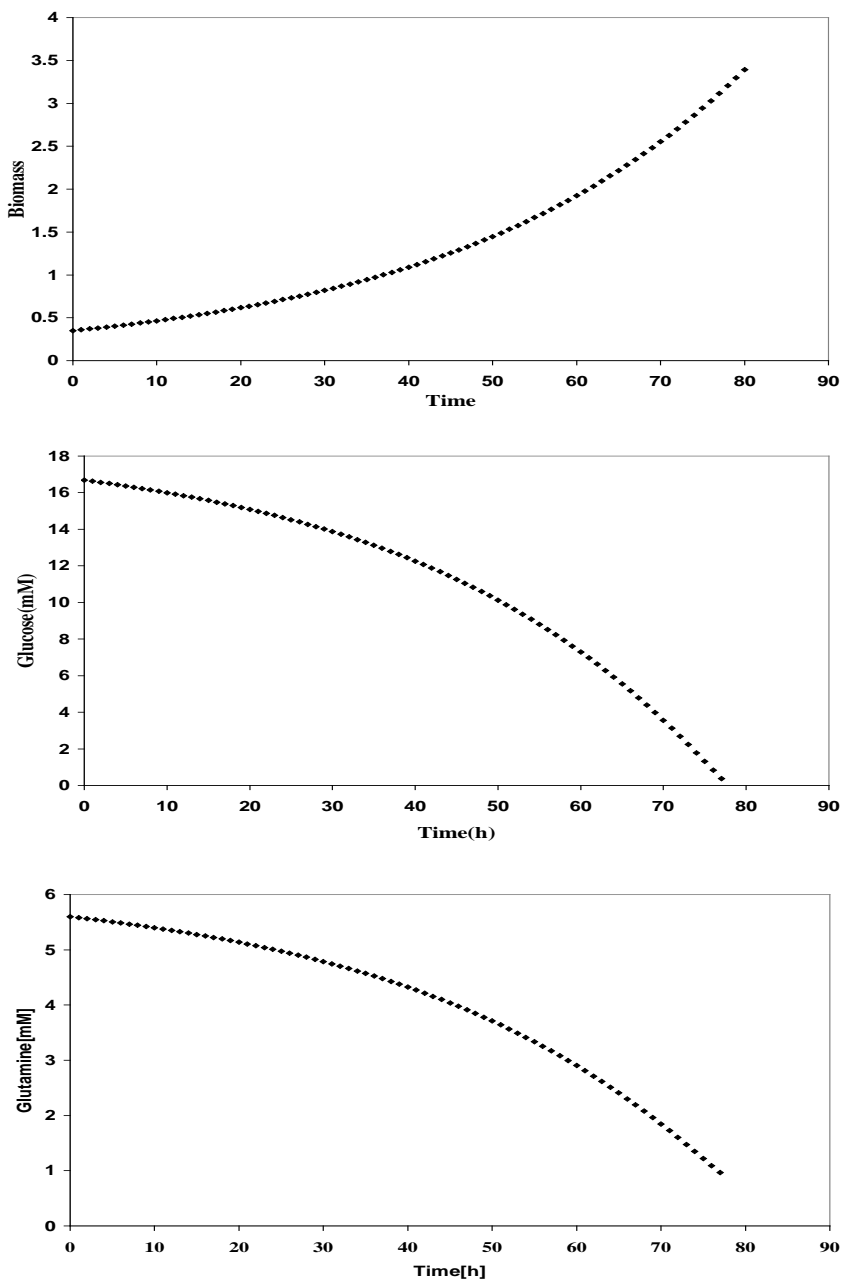
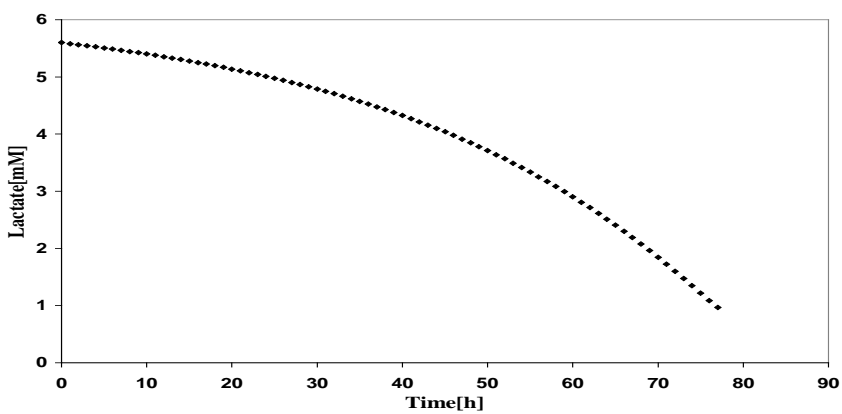


Fig. 3: Dynamic responses based on optimized initial culture medium composition.



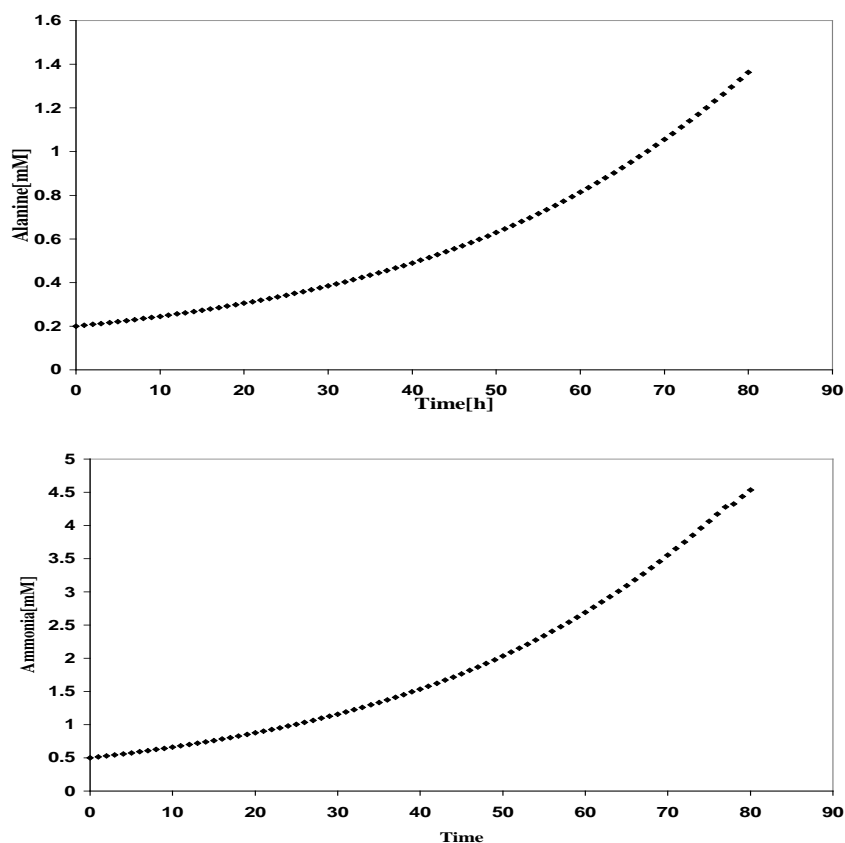


Fig. 4: Dynamic responses based on optimized initial culture medium composition.

CONCLUSIONS

Identifying and optimizing the compositions of the initial culture medium is most important for maximizing the productivity of CHO cells. A dynamic mathematical model of the CHO cell cultivation process is combined with the differential evolution (DE), and this strategy applied to optimize the initial culture conditions of the CHO cell cultivation process. This method is found to be simple and effective in optimizing the culture medium and enhancing the productivity of the CHO cell cultivation process.

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