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Taking Advantage of Taguchi Design Method to Optimize Medium Culture Conditions for Producing Recombinant Follicle Stimulating Hormone

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Abstract

Medium culture optimization is an Effective, available and financially affordable way to improve production of recombinant proteins produced by genetic engineering. The existence of varieties of parameters and different levels for each, makes it complex, time-consuming and expensive to determine the optimum point of all parameters by applying the factorial method. To overcome these difficulties, in this study, Taguchi robust design method was employed. The environmental parameter such as temperature, pH and glutamine concentration in 4 different levels were considered. According to the design of the experiments, FSH titer was measured. In comparison with the control condition, 14.92 fold overexpression was observed. The best level for these parameters was pH=7.0, 28°C and 2 mM Glutamine concentration.

Keywords: Taguchi method; Genetic engineering; Statistical experimental design; Glutamine temperature; Optimization

Introduction

According to a released statistic from the world health organization (WHO), it had been found that 25% of the couples are affected by infertility in developing countries. For overcoming this challenge, it is essential to provide complex hormones belong to glycoprotein hormones family, using genetic engineering. One of the most important and common glycoproteins in infertility treatment of men and women, which produce with this method is FSH [1-3]. Complex post-translational modifications of this hormone such as disulfide bond and glycolization leave no choice to use mammalian cells as a host. The difficulty of cloning, low recombinant protein titer and the hard purification process of these cell lines, imposes significant costs.In this cause, lots of companies and institutes try to produce the most amount of hormones with the least price [4,5]. Environmental parameters such as temperature [6-8], pH [6,9,10] and glutamine concentration [10-12] have a significant effect on recombinant cells viability and the amount of hormone titer by this cells.

Using the traditional method for determining the optimum level of different parameters, involves the study of one variable at a time, is time-consuming and costly. In this cause to overcome the limitation and difficulty of applying this kind of methods, we used Taguchi Orthogonal array design. Taguchi method is a simple statistical method applying one-way ANOVA for analysis of variance and the tables of orthogonal arrays [13]. Using this approach, we apply a system of arrays which permits estimation of a maximum number of key effects in an orthogonal fashion with a minimum number of experimental runs [14]. This method was applied in different fields of biotechnology such as food and industrial fermentations [15-17], Molecular biology [18-20], wastewater treatment and bioremediation [16,21,22] and health care [23-25]. In this work, we explained the application of this method for optimization of medium culture parameters, for producing FSH, a complex glycoprotein hormone, in the recombinant CHO-c111 cell line.

Material and Methods

Taguchi orthogonal design

To solve our problem, the low expression of FSH hormone in rCHO cells, effective environmental parameters which influence the cell viability and recombinant protein expression opted. Essential environmental parameters which considered for designing the experiment were pH of medium culture, the temperature of culturing and the amount of glutamine concentration in medium culture. Recombinant cells according to their special features prefer to live in a different level of these parameters. Combination of these parameters affects other levels of parameters preference. For example, if cells growth in pH=7.3 at 2 mM glutamine concentration, these cells may prefer 4 mM glutamine concentration at pH=6.8. In this case, for all parameters, according to their acceptable range for culturing 4 levels were considered.(for temperature 37°C, 34°C, 31°C and 28°C, for pH 6.7, 7.0, 7.3 and 7.6 and for glutamine concentrations 0 mM/L, 2 mM/L, 4 mM/L and 6 mM/L).

Designing the experiment with these parameters and the chosen levels by applying most of the custom statistical methods, such as factorial approach, which considers all possible combination of parameters and their states needs 4*4*4=64 experiments. Considering at least 3 times repetition at least 192 experiments need to be done. Ignoring the imposes cost of these numbers of experiments, applying them is too much time-consuming and expensive. Using L_{16} orthogonal limits the numbers of an experiments to 16. (48 times considering the repetition). The parameters and their levels with designed experiment were shown in Table 1. The levels of chosen parameters are shown by 1 to 4 [26,27].

Computing the degree of freedom is vital for opting the appropriate orthogonal array. The degree of freedom calculated one less than the number of experiments. At this work, the degree of freedom is 15.

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Designing the test, practical steps of the investigation were in progress according to that. The statistical analysis and graphical analysis of the obtained data in this work were done by Minitab software (Pennsylvania University, USA)

Practical steps

In this study, recombinant Chinese Hamster Ovary C111 cells with p-VITRO expression vector contain α and β genes of FSH subunit were applied. Cells were cultured at serum-free and glutamine-free medium (LONZA, Switzerland).For cell process, cells were cultured in the T-75 flask. The equal number of cells were cultured at 37°C, 5% Co2 concentration, 2 mM glutamine concentration, pH=7.3 and 95% humidity which are the common condition for cell culture. Subsequently, after providing primary condition (cells grown in the identical environmental condition), cells were transformed to the new mediums determined by Taguchi robust design. At least 3 passages were applied for adaptation of the cells to the new conditions. FSH titer was measured by Elisa kit (FAN AZMA, Iran). Each step was repeated at least 3 time for limitation the errors of handling.

Result and Conclusion

In this study, the experiments designed and analyzed by Minitab software. The designed experiments and the obtained resulst and their analyses are shown in Table 2. Analyze of the experiments were carried out by S/N ratio and ANOVA. According to the obtained results of our study shows that the optimum levels of different parameters have altered on the production of recombinant proteins when they work simultaneously in comparison to working separately. The effect of these parameters and their best level when work simultaneously altered.

Where n is the total number of the experiments in the array and Yi is the mean percentage of FSH titer for the ith experiment, S/N rate calculated by this formula.

$$SN_{L} = -10\log\left(\frac{\sum_{i=1}^{n} 1/y_i^2}{n}\right)$$

As it comes from the formula, "the larger the best" strategy were applied in this research. The goal of this study was introducing the condition which in that the most amount of FSH titer obtaines. After calculation of S/N ratio for each experiment, the delta for each parameter was determined according to the following formula.

D= delta= S/Nmax_S/Nmin

The variation of delta showed the importance and effectiveness of each parameter in the maximization of FSH titer. As it is shown in Table 3, the greatest variation belongs to temperature. Decreasing the temperature to 28°C is around 16 times more effective than altering

Parameters / Levels	Temperature (°C)	pH	Glutamine concentration (mM)
1	28	6.7	0
2	31	7.0	2
3	34	7.3	4
4	37	7.6	6

Table 1: Parameters and their levels.

Experiments	Temperature (°C)	L-Glutamine concentration (mM)	pH	MEAN 1	SNRA1
1	1	1	1	106.3	60.0545
2	1	2	2	1122.8	61.0060
3	1	3	3	956.4	59.6128
4	1	4	4	901.7	59.1012
5	2	1	2	803.8	58.1030
6	2	2	1	839.2	58.4773
7	2	3	4	705.1	56.9650
8	2	4	3	729.6	57.2617
9	3	1	3	168.5	44.5320
10	3	2	4	181.3	45.1680
11	3	3	1	196.8	45.8805
12	3	4	2	170.2	44.6192
13	4	1	4	50.4	34.0486
14	4	2	3	68.7	36.7391
15	4	3	2	75.2	37.5244
16	4	4	1	55.8	34.9327

Table 2: The designed experiments by Taguchi method and the obtained data.

Level	Temperature (°C)	L-Glutamine concentration (mM)	pH
1	59.94	49.18	49.84
2	57.70	50.35	50.31
3	45.05	50.00	49.54
4	35.81	48.98	48.82
Delta	24.13	1.37	1.49
Rank	1	3	2

Table 3: Response table for signal to noise ratios.

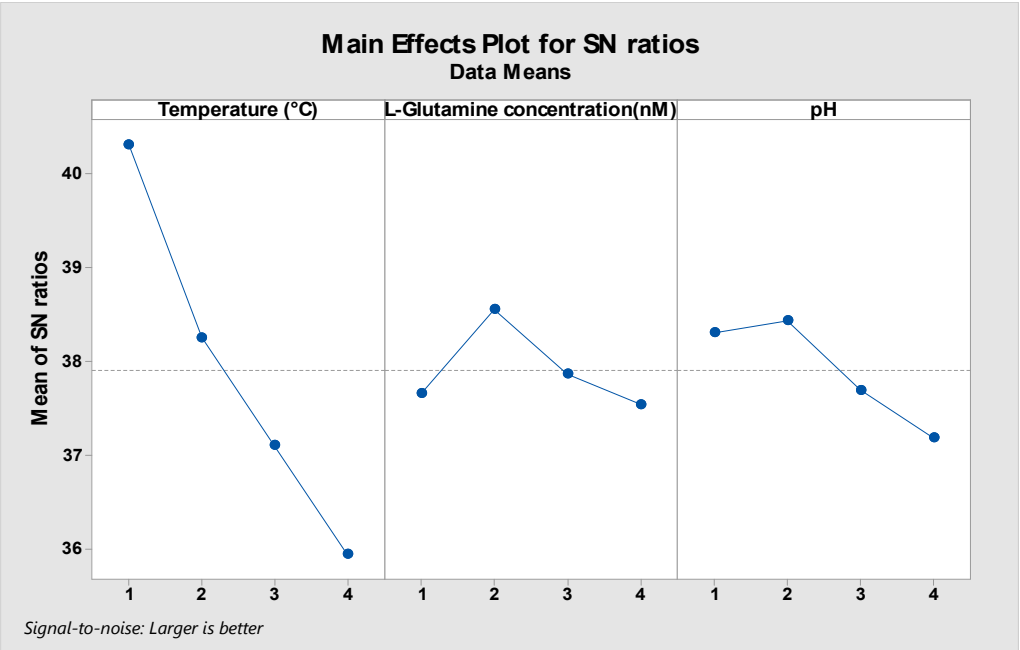

Figure 1: Mean of signal to noise ratios for parameters.

two other parameters. Altering the pH value of medium culture is more effective than glutamine concentration but it's not considerable in comparison to temperature.

Their best level of each parameter and the order of their importance and effectiveness were determined (Figure 1). The common condition for mammalian cells growth is 37°C at pH=7.3 and 2 mM glutamine concentration. As it is shown in Table 2, 14.92 fold overexpression was observed in suggested condition obtained by Taguchi method. Future more, Interestingly the optimum level of temperature, pH and glutamine were 28°C, 7.3 and 4 mM when the parameter examined separately(Data not shown), But in our experiment, while exam all parameters simultaneously it changed to 28°C, 7.0 and 2 mM. It means when the effect of parameters investigates simultaneously their effect changes. The interplay of these parameters changes their result and influence. The most effective parameter is temperature and there is no such difference between the effectiveness of pH and glutamine concentration on the expression of rCHO (Table 2). As shown in Table 2, when the optimum level of each parameter which is obtained from separate tests applied simultaneously (28°C, 7.3 and 4 mM) on the cell culturing, the expression rate decrease 15% in comparison with the optimum condition obtained using the Taguchi method (28°C, 7.0, 2 mM). In comparison with other studies, the effect of the different parameter in variety levels was examined by applying Taguchi methods. Using that we demonstrated the best condition for producing rFSH. This amount of production is considerably more than another experiment. With no more cost, the production of this important medicine in reproductive considerably increased.

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