Optimization of Cho Cell Recombinant Protein Expression

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Abstract: Chinese hamster ovary cells are the most widely used cells to produce therapeutic proteins in the biopharmaceutical field. They proliferate very quickly in large-scale culture systems and can express a variety of recombinant proteins at a high level. This paper studied the optimization of CHO cell culture technology. Objective: To explore a method to improve the purity of recombinant protein in CHO cells. Methods: Different cooling time (the 5th and 6th day), temperature (33 ℃ and 31 ℃) and pH control strategy (7.00 ± 0.30 and 7.10 ± 0.10) were set to observe the changes of cell viability, protein yield and protein purity. Results: The process of cooling to 31.0 ℃ on the sixth day of culture, and pH control at 7.00 ± 0.30 after cooling, could further improve the purity of the product, and had no significant impact on cell growth, metabolism and recombinant protein yield. Conclusion: The experiment determined the appropriate protein optimization strategy, which laid the foundation for the subsequent scale-up production.

1. Introduction

The main culture parameters of CHO cell culture include temperature, pH, rotational speed, dissolved oxygen, etc.

Temperature is the basic environmental parameter of animal culture in vitro, and different cells have different tolerance limits to temperature [1,2]. CHO cells adapt to low temperature growth, so the culture is maintained at a lower temperature during the whole culture process.

PH value is another important environmental factor affecting CHO cell culture behavior. PH value has a direct impact on cell growth, metabolism, target product synthesis and quality assurance [3-5]. The optimal growth PH value of CHO cells changes around neutral, and the culture temperature affects the optimal growth and productivity of cells to a greater extent.

The current CHO cell culture technology widely uses variable temperature culture, that is, cell growth and proliferation and product expression stages are cultured at different temperatures. In general, lowering the culture temperature at the late exponential growth stage can adjust the cell cycle, delay cell death, increase the antibody production rate, extend the culture time, and achieve the effect of increasing the total expression. Similar to variable temperature culture, pH can also be adjusted to change the metabolic mode of cells from the proliferation stage to the product expression stage. It should be noted that changes in temperature and pH can affect the sugar type, polymer, charge variation, etc. of the antibody, and corresponding investigation is required [6-8].

Mammalian cell culture has very strict requirements on the culture environment of cells, as well as on the quality and reliability of the final product. Therefore, it is crucial to achieve the quality requirements of the final product by effectively regulating the cell culture process parameters.

2. Scheme and Data

2.1 Design Scheme

Cooling can control the cell cycle at the cell stop division stage/the first interval of division, improve the level of antibody protein transcription, increase the mRNA content and improve the efficiency of post translation modification; PH can promote cell metabolism.

Based on the problem that the protein purity of the expression product was not high found in the process of early process development, this topic plans to optimize the process in three aspects: cooling time, cooling temperature and pH control strategy after cooling during cell culture to further improve the purity of the recombinant protein.

2.2 Cell Lines

The cell line used in the experiment is the recombinant CHO-K1 cell line

2.3 Test Reagent

BM1 basic culture medium was prepared with water for injection, CD CHO, glutamine, hypoxanthine and thymine; BM2 basic culture medium was prepared with water for injection, ActiPro, 10M sodium hydroxide, glutamine, hypoxanthine, thymine and sodium bicarbonate; FM1 feeding medium is prepared with water for injection, Cell Boost 7a and 10M sodium hydroxide; FM2 feeding medium is prepared with water for injection, Cell Boost 7b and 10M sodium hydroxide; 400g/Kg glucose supplement medium is prepared with water for injection and glucose; 1M sodium carbonate solution is prepared with water for injection and sodium carbonate.

3. Experimental Method

3.1 Culture in Shake Flask Stage

Place BM1 culture medium in a 37 ℃ water bath to preheat, place the cryopreservation tube in a 37 ℃ water bath to thaw, and then transfer it into a biosafety cabinet. Use a pipette to absorb the cell fluid and transfer it to a flask. After mixing, take samples for counting.

Take out 1ml of the previous generation seed solution for cell counting. According to the inoculation density required by the process, suck the quantitative seed solution and fresh culture medium into the shake flask.

The number of culture generations in shake flask is 5. BM1 medium is used. The parameters of carbon dioxide shaking table are 6% carbon dioxide concentration, 36.5 ℃ temperature, and 110 rpm rotating speed.

3.2 3L Bioreactor Fed Culture

Connect the liquid inlet pipe on the tank with the culture solution storage bottle or bag, and pump the culture solution into the tank. Set and operate the reactor temperature, pH and speed according to the experimental scheme.

After correcting the dissolved oxygen electrode and pH, pump the seed solution into the tank.

During the culture, samples were taken every day to detect the density of living cells, cell viability and nutrient consumption.

Feeding and sugar supplement shall be conducted according to the nutrient consumption of cells. If the residual sugar concentration is $\leqslant$ 4.50 g/L on the 3rd to 13th day of culture, it shall be added to 6.00 g/L, and the feeding flow feeding process shall be used to add the feeding medium.

Harvesting shall be carried out within 24 hours after the 14th day of culture or when the survival rate is lower than 60%, whichever is reached first.

On the 12th to 14th day of culture, the protein yield of the reserved samples was detected; The protein purity was detected on the 13th to 14th days of culture.

3.3 Process Parameter Control Strategy

Setting of bioreactor culture parameters shows in Table 1.

Table 1 Setting of Bioreactor Culture Parameters

Reactor number	1	2	3	4
Type of reactor	Applikon 3 L			
Initial culture volume	1.6 L			
Basal medium	BM2			
Feeding medium	FM1/FM2			
Inoculation density	(0.30 - 0.50) × 10^6 cells/mLTarget 0.40 × 106 cells/mL			
Stirring speed	300 RPM			
The temperature control	Target 0.40 × 106 cells/mL Initial 36.5 ° C, cooling to 33.0 ° C on the 6th day of culture	Initial 36.5 ° C, cooling to 33.0 ° C on the 5th day of culture	Initial 36.5 ° C, cooling to 31.0 ° C on the 6th day of culture	Initial 36.5 ° C, cooling to 33.0 ° C on the 6th day of culture
PH control	7.00 ± 0.30 air			7.00 ± 0.30 before cooling, 7.10 ± 0.10 after cooling
DO control	40.0%			
Air table access	nothing			
Air underpass	0.005 VVM			
O2 underpass	Associated with DO control			
CO_2 underpass	Associated with pH control			
Replenishment strategy(CB7a/CB7b)	1.5%/0.15% FM1/FM2 per day on the 3rd to 11th day of culture			
Sugar supplement strategy	On the 3-13 days of culture, if residual sugar concentration ⩽ 4.50 g/L, supplement to 6.00 g/L; (The sugar content of CB7a should be considered on the day of feeding. The sugar content in CB7a was calculated as 80 g/L. No sugar on harvest day)			
Harvest criteria	On day 14 of culture or within 24 hours after the cell viability fell below 60.0%			

3.4 Analysis Method

Use Beckman Vi CELL XR cell counter to detect; RAPIDPoint 500 blood gas analyzer was used for detection; Detect with Roche Cedex Bio; Use Advanced 2020 osmometer for testing; SE-HPLC method.4.

4. Experimental Results and Discussion

4.1 Experimental Results

In terms of cell growth, as far as the density of living cells is concerned (Fig. 1), the density of living cells in reactors under different conditions in this round of experiment is basically the same. As far as the cell viability is concerned (Fig. 2), there is little difference in the cell viability of reactors under different conditions in this round of experiment, and the cell viability at harvest is higher than that of reactor 1(control conditions).

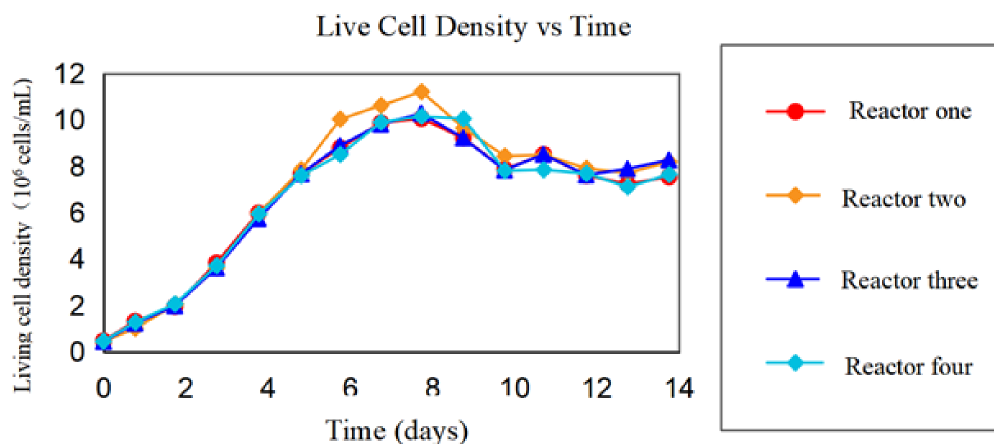

Fig.1 Curve of Living Cell Density Changing with Time

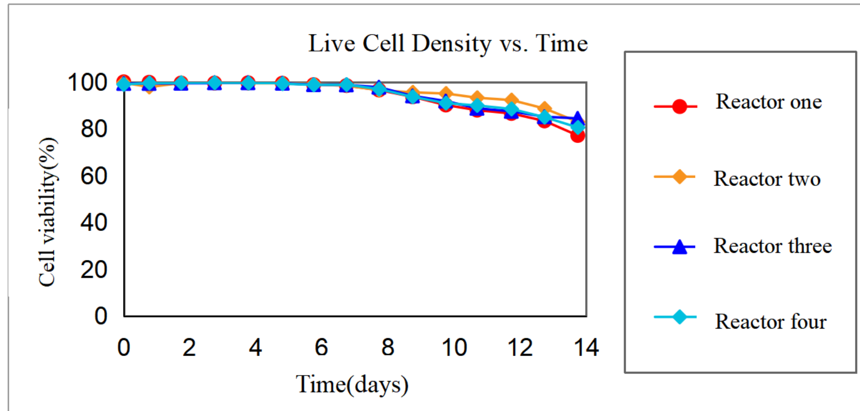

Fig.2 Curve of Change of Cell Viability with Time

In terms of cell metabolism, overall, there is little difference in cell metabolism performance in reactors under different conditions in this round of experiment, as shown in Figure 3 and Figure 4.

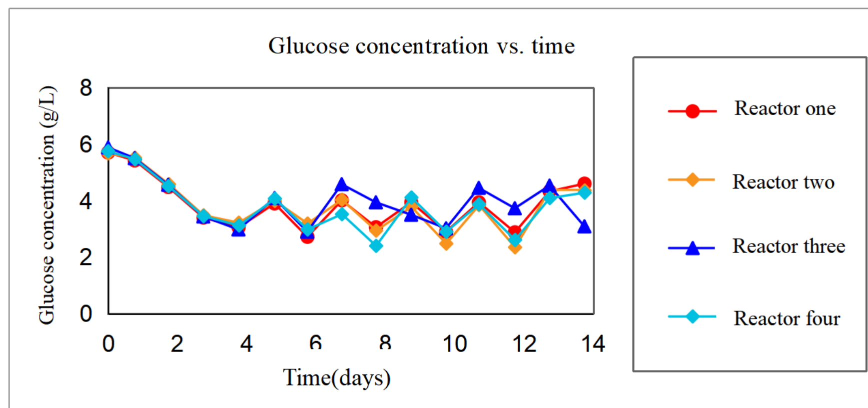

Fig.3 Glucose Concentration Curve with Time

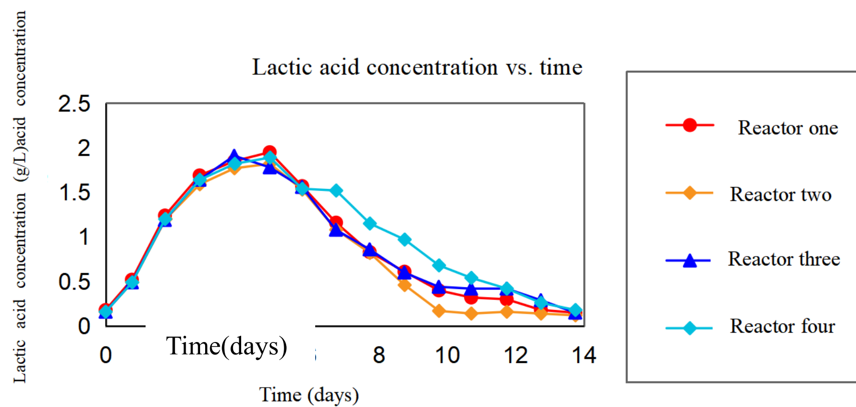

Fig.4 Curve of Lactose Concentration Changing with Time

In terms of protein output, it can be seen from Table 2 that the recombinant protein output of reactors under all conditions in the last three days of culture shows a continuous accumulation state, and the overall difference of the recombinant protein output at harvest is small, and it is equal to or slightly higher than that of reactor 1 (control reactor).

Table 2 Production of Recombinant Protein in Bioreactor

Bioreactor	Recombinant protein output (g/L) # 1		
	Day 12	Day 13	Day 14
Reactor one	1.05	1.24	1.35
Reactor two	1.13	1.33	1.49
Reactor three	1.07	1.16	1.37
Reactor four	1.02	1.10	1.28

In terms of protein purity, samples cultured in reactors under different conditions on the 13th and 14th days were purified in one step and then tested for SE-HPLC purity. See Table 3 for the results. It can be seen from the table that the proportion of the main peak of SE-HPLC in reactor 3 (cooled to 31.0 ℃ on the sixth day of culture) at harvest was the highest, 82.6%, and it still showed a trend of continuous increase in the last two days of culture. The proportion of main peak of SE-HPLC in reactors under other conditions at harvest time was lower than 80.0%, and showed a decreasing trend in the last 2 days of culture, which was basically consistent with reactor 1 (control reactor).

Table 3 Results of Purity of Recombinant Protein in Bioreactor

Bioreactor	Training days	SE-HPLC		
		Main Peak (%)	HMWs (%)	LMWs (%)
Reactor one	Day 13	78.3	4.5	17.1
	Day 14	73.0	6.4	20.5
Reactor two	Day 13	76.6	6.3	17.1
	Day 14	73.1	8.0	18.8
Reactor three	Day 13	81.2	3.3	15.6
	Day 14	82.6	4.8	12.7
Reactor four	Day 13	77.8	4.8	17.4
	Day 14	75.7	6.0	18.2

4.2 Discussion

The optimization experiment of process parameters was carried out through cooling time, cooling temperature and pH control strategy. It was found that the cell viability and protein purity of reactor 3 at harvest time were higher than those of the other three reactors, and the yield of recombinant protein at harvest time was higher.

It can be seen that the process of cooling to 31.0 ℃ and pH 7.00 ± 0.30 in reactor 3 on the sixth day of culture can further improve the purity of the product, and has no significant impact on cell growth, metabolism and recombinant protein output.

In this experiment, only two groups of cooling temperature are set, and the temperature is reduced to 33 ℃ and 31 ℃ respectively to observe the effect of temperature control. Considering that temperature has a great impact on CHO cells, the interval gradient will be reduced in the next step, and further attempts will be made near the current conditions.

To sum up, this experiment found that CHO could adapt to growing at lower temperatures and harvesting higher recombinant proteins, and determined appropriate protein optimization strategies, laying the foundation for subsequent large-scale production.

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