• BelloCell-500 Technical Report X •

Cultivation of suspend CHO cells for protein secretion in HyQ-PF-CHO

Description BelloCell-500 provides a powerful cell culture tool to achieve high cell density and high productivity of target bioproducts because of its unique feature of offering high oxygen transfer and low shear stress culture environment. Users can easily collect highly concentrated cells, virus or secreted products from one 500 ml BelloCell-500 bottle. In this study, the applications of BelloCell-500 for growth of suspend CHO cells and production of human IgG with different operation setting is illustrated. 1×10^8 CHO cells were seeded in each BelloCell-500 unit. A final cell population from 1.7×10^9 to 4.0×10^9 in one BelloCell -500 unit was obtained. For the protein production, a total of 250 mg to 475 mg IgG protein in different setting was harvested within 20 days culture by consuming 6.5 L culture medium. At the later stage of culture, approximately 50 mg IgG could be produced in 500 ml culture medium everyday. This study shows that BelloCell can control cell behavior by switching between growth and production by simply adjust the bottom holding time (B_H). Compared with other culture system, it may require sophisticate feeding control in order to achieve the goal. Reduce the bottom holding time will promote cell growth, while increase the bottom holding time will restrict the nutrient and promote production. This is a standard example for non-growth associated production in many cases such as CHO and hybridoma. This technical sheet provides a general protocol for users to start up their culture. However, the optimum condition of each cell culture for each case may require users to determine.

Material

Device	Cell Line/Product	Medium	Seed
BelloCell-500	CHO/IgG	HyQ-PF-CHO (Hyclone)	1.0×10^8 cells/bottle

Protocol **Detail protocol is in General Instruction Manual*

Inoculum preparation Prepare one 1000 ml spinner flasks and inoculate 1.8×10^5 suspend cells/ml in 500 ml HyQ-PF-CHO culture medium. Culture at 50 rpm, 37℃ for 3 days. After cell density reaches above 1×10^6 cells/ml and viability remain above 95%, it is ready for the preparation of inoculation. Collect 5.0×10^8 suspend cells from the spinner flask by centrifugation and separate into five 50 ml centrifuge tubes with each 50 ml fresh media.

Preparation before cell seeding Place five BelloStage-3000 controllers in 37℃ incubators. Set up the inoculation parameters (See below). Warm up HyQ-PF-CHO medium in

37℃ water bath. Take out five BelloCell-500 bottles aseptically and place them in a biosafety cabinet. Open the cap and add each bottle with 450 ml fresh culture medium in the bottle. **Inoculation** Open the cap and dispense 50 ml media containing 1.0×10^8 suspend cells that has been prepared previously on top of the matrix of BelloCell-500. Bring the bottle and lock up on the BelloStage-3000 controller in incubator at 37℃ immediately. Press "START" button to start the run. **Culture** After 3 to 4 hours, reset the parameters for culture condition. Usually, above 90% cells will be immobilized in the matrices within 4 hours. The inoculation parameters are set as below:

Rising rate	Top holding time	Down rate	Bottom holding time
2.0 mm/s	20 sec	2.0 mm/s	0 sec

The culture parameters for each bottle are set as below:

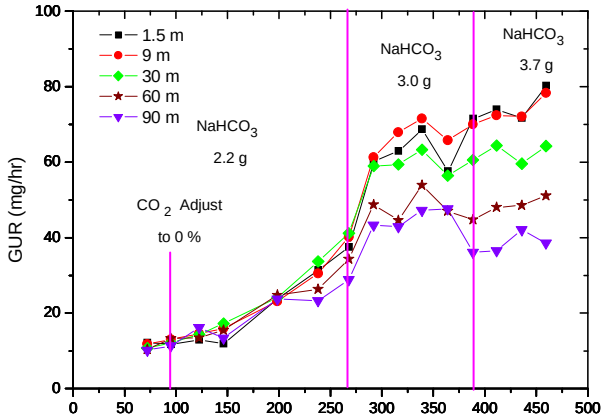
Rising rate	Top holding time (T_H)	Down rate	Bottom holding time (B_H)
1.0 mm/s	0 sec	1.0 mm/s	1.5 mins
1.0 mm/s	0 sec	1.0 mm/s	9 mins
1.0 mm/s	0 sec	1.0 mm/s	30 mins
1.0 mm/s	0 sec	1.0 mm/s	60 mins
1.0 mm/s	0 sec	1.0 mm/s	90 mins

During culture, monitor the pH, residual glucose concentration and other metabolic in order to predict the time for medium replenishment. Below are the tips:

When pH drop below 7.0 during culture, adjust CO2 concentration inside incubator to 3% and then 0%. We strongly suggest to add HEPES in culture medium to stabilize the pH during culture. If pH continues go below 7.0 when CO2 concentration is zero, increase NaHCO3 concentration from 2.2 g/L to 3.7 g/L. Keep initial glucose concentration at 3 g/L and glutamine at 4 mM. Exchange culture medium by day 3rd. Replenish culture medium once a day from the day 5rd of culture.

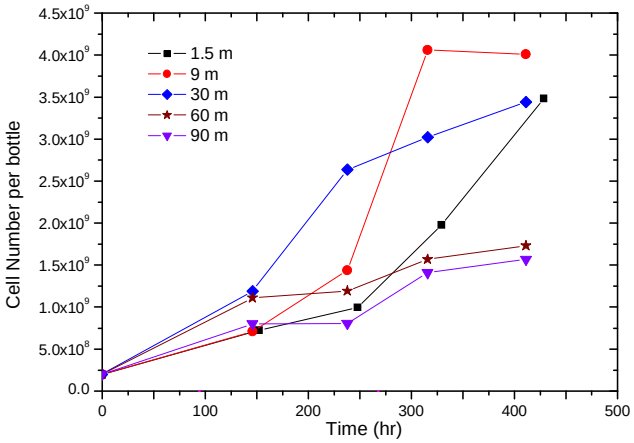
Results

1. Glucose uptake rate (mg/hr) and glutamine uptake rate (umol/hr)



Lower bottom holding time (B_H) got higher glucose uptake rate. Bottom holding time at below 9 mins did not show any sign of nutrient restriction. B_H at 30 mins shows nutrient restriction when GUR reach 60 mg/hr; B_H at 60 and 90 mins show nutrient restriction when GUR reaches around 30 mg/hr.

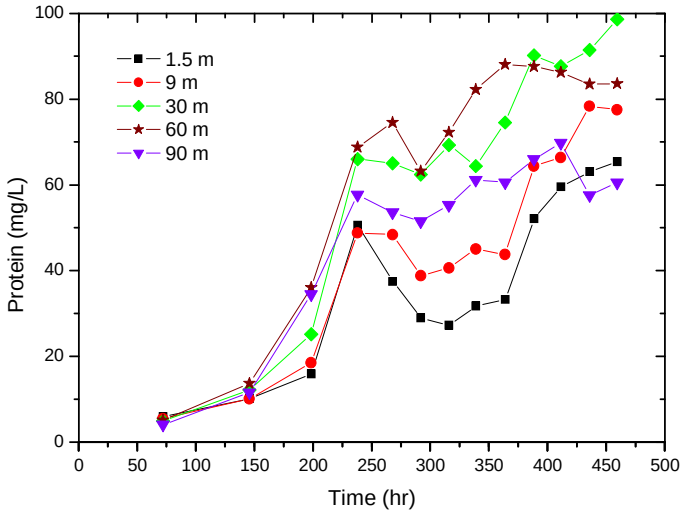
2. Cell growth curve



B_H at 30 mins and 60 mins shows a better growth by day 3rd, even the GUR is similar in

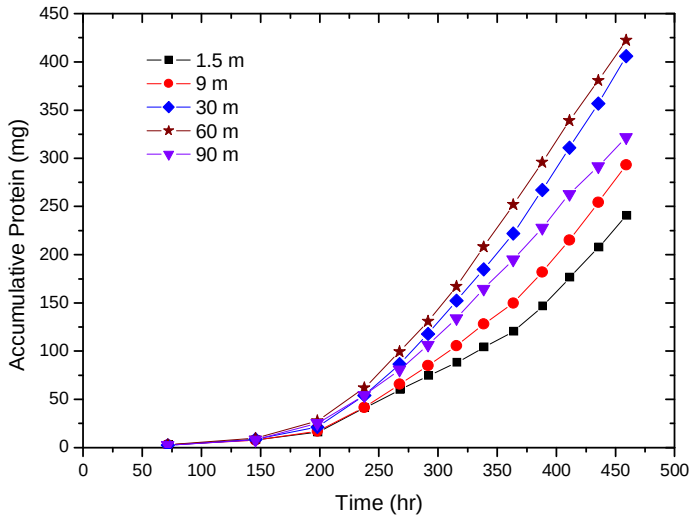
all testing group before day 3rd. It indicates other factors involved into the cell propagation. B_H at 30 mins has best growth rate during early culture period but got similar GUR compared with B_H at 9 mins and 1.5 mins. It indicates a better utilization of glucose in 30 mins' setting.

3. IgG Production



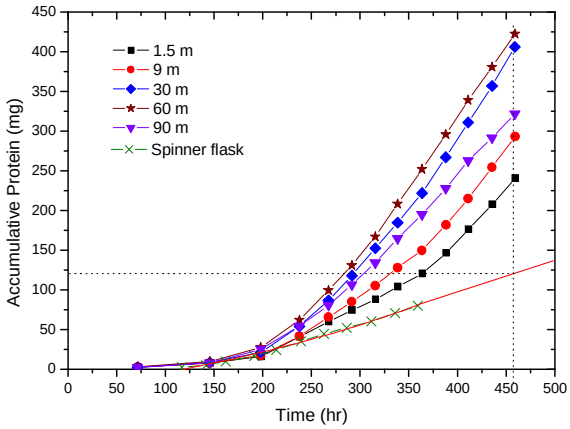
B_H setting with 60 mins and 90 mins turn into production phase earlier than other settings. B_H setting with 30 mins follows the 60 and 90 mins one. The IgG concentration lower down in 90 mins is due to lower cell density. B_H with 30 mins finally caught up the 60 mins one because of its higher cell density. However, 90 mins is the best one of specific production rate per cell, 60 mins ranking the second, while 30 mins ranking the third. This is purely a non-growth association example.

4. Accumulated IgG



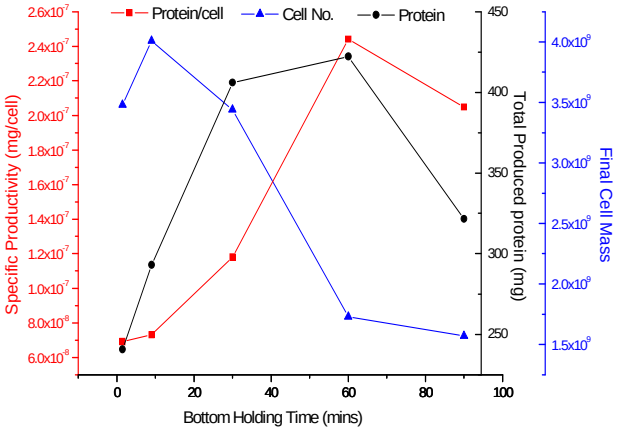
B_H setting with 60 mins got the best protein production among the other testing groups within 20 days culture. However, B_H with 30 mins will catch over the one with 60 mins after 20 days because its cell density is doubled than 60 mins, and its productivity will continue increase and predicted to reach around 160 mg/L/day. Therefore, for longer cell culture period, BH setting with 30 mins will be the best setting among the other testing groups. By calculation, we believe the optimum setting will locate between 30 mins to 60 mins and probably 50 mins will be the best one.

5. IgG production comparison



Total harvest IgG in BelloCell-500 in the highest one is 3.3 folds increased compared with a 500 ml spinner flask within 15 days culture period; 3.5 folds increase within 20 days culture and forecast to 4.5 folds increase within a month. With the same working volume, production in spinner flask with 500 ml working volume will require at least 48 days to approach the same IgG production as that in BelloCell in 20 days.

6. Effect of bottom holding time on cell growth and productivity



The figure shows that cell growth is reverse proportional to specific productivity. Higher growth rate results lower productivity. The best bottom holding time setting for the best outcome of IgG production will then locate between 30 mins to 60 mins.

Summary

The growth and production phase can be easily controlled by the setting of bottom holding time. Many of valuable pharmaceutical products are produced in the non-growth associated manner. That's why many processes are using fed-batch operation. In BelloCell system, nutrient restriction can be easily achieved by extending the bottom holding time and it is no need to worry the feeding of nutrient in the bulk medium solution. This could simplify and stabilize a complex process and benefit to set up a robust process.

Seed	**Inoculum volume**	**Medium volume**	**Medium**
1.0×10^{8} cells/total	50ml	500 ml	HyQ-PF-CHO
Total culture age	**Total medium consumed**	**Total medium replenish frequency**	**Total cell counted**
20 days	6500 ml	13 times	2×10^{9} ~ 4.5×10^{9} cells/total
Max. GUR	**Max. Total Protein produced**	**Max. Protein concentration**	**Multiplication of cells**
85.7 mg/hr	422.2 mg	98.64 mg/L	45 folds

Please contact Cesco Bioengineering Technical Support for any questions or comments

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