• BelloCell-500 Technical Report VI •

Cultivation of suspend rCHO cells for protein secretion

Description BelloCell-500 provides a powerful tool to achieve high cell density and high productivity of target bioproducts in a cell culture because it has a 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 is illustrated. 7.9×10^7 CHO cells were seeded in one BelloCell-500 unit. A final 4.5×10^9 cell population in one BelloCell -500 unit was obtained. For the protein production, a total of 157.5 mg IgG protein was harvested in 5 L conditioned culture media. In comparison with 1.1 mg IgG protein in a 200 ml spinner flask. It is 5.7 folds productivity increase on an equal volume basis. 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 the users to determine.

Material

Device	Cell Line/Product	Medium	Seed
BelloCell-500	CHO/IgG	Ex-CELL 301 (JRH)	7.9×10^7 cells/bottle

Protocol **Detail protocol is in General Instruction Manual*

Inoculum preparation Prepare one 250 ml spinner flasks and inoculate 1.8 $\times10^5$ suspend cells/ml in 100 ml Ex-CELL 301 culture medium. Culture at 50 rpm, 37℃ for 3 days. After cell density reaches above 8×10^5 cells/ml and viability remain above 95%, it is ready for the preparation of inoculation. Collect 5×10^7 to 1.0×10^8 suspend cells from the spinner flask by centrifugation and collect in one 50 ml centrifuge tube with 50 ml fresh media.

Preparation before cell seeding Place BelloStage-3000 controller in a 37℃ incubator. Set up the inoculation parameters (See below). Warm up Ex-CELL 301 medium in 37℃ water bath. Take out one BelloCell-500 bottle aseptically and place it in a biosafety cabinet. Open the cap and add 450 ml fresh culture medium in the bottle.

Inoculation Open the cap and dispense 50 ml media containing 5×10^7 to 1.0×10^8 suspend cells on top of the matrix of BelloCell-500. Bring the bottle and lock up on the BelloStage-3000 controller in incubator at 37℃. Press “START” button to start the controller. Avoid swirling or shaking the bottle before start compression.

Culture The inoculation parameters were 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

After 2 to 3 hours, reset the parameters for culture condition. Usually, above 90% cells will be immobilized in the matrices within 2 hours. The culture parameters were set as below:

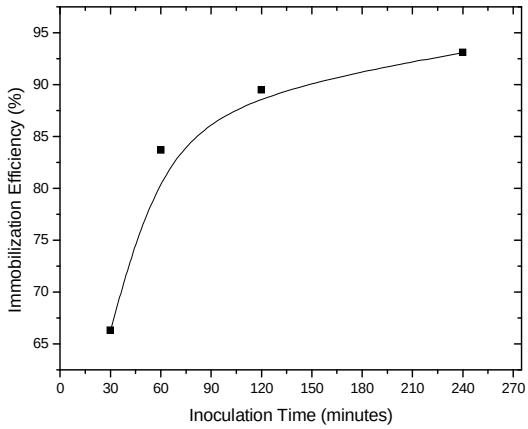
Rising rate	Top holding time	Down rate	Bottom holding time
1.5 mm/s	0 sec	1.5 mm/s	1 min

For optimal culture, monitor the pH, residual glucose concentration and other metabolic in order to predict the time for medium replenishment.

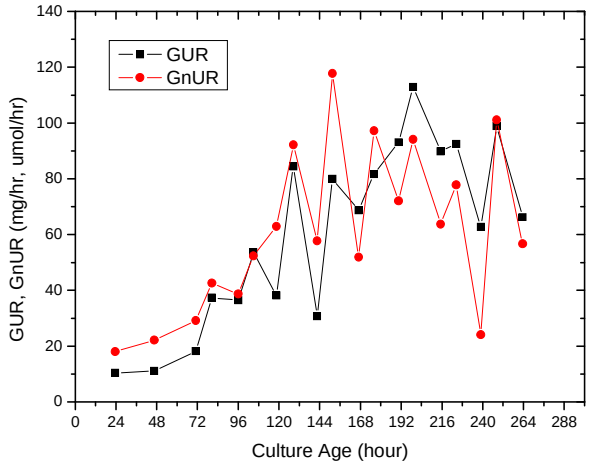
When pH drop below 7.0 during culture, add 5 ml 1M HEPES and/or 10 ml 7.5% $NaHCO_3$ to re-adjust the pH to above 7.5. When glucose concentration below 1.5 g/L, add 5 ml 18 w/w% glucose and 125 mM glutamine concentrate. Replenish culture medium once a day from the day 3^{rd} of culture. Add glucose, glutamine, HEPES and $NaHCO_3$ supplements once a day from the day 5^{th} of culture. We suggest that users can replenish culture medium in the afternoon, and add supplement the next day in the morning. For simpler operation, user can replenish medium twice a day in the morning and in the afternoon.

Results

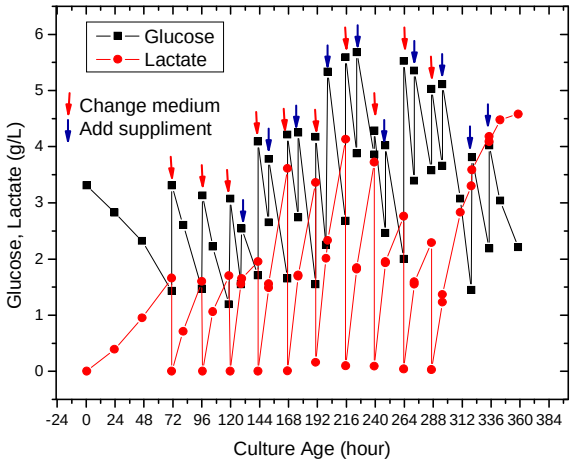
1. Immobilization Efficiency



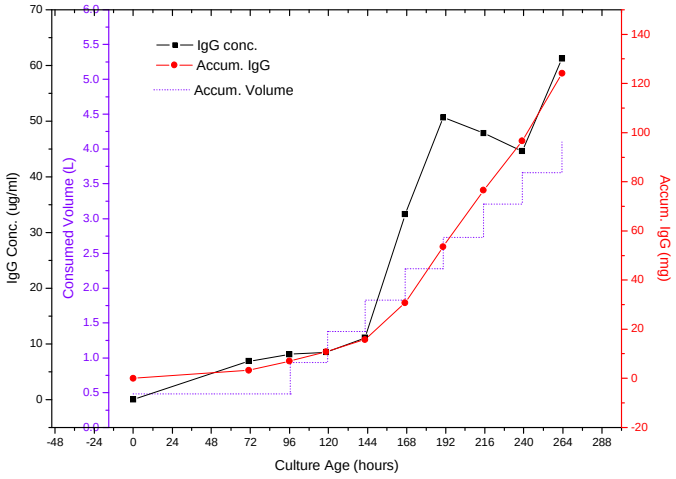
2. Glucose uptake rate (mg/hr) and glutamine uptake rate (umol/hr)



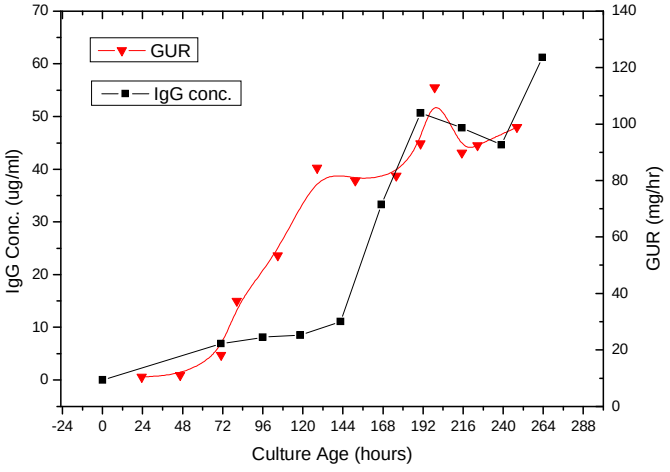
3. Glucose and Lactate concentration



4. IgG Production

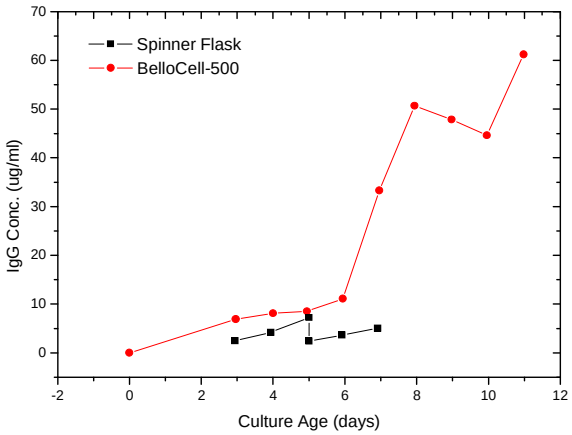


5. IgG and GUR



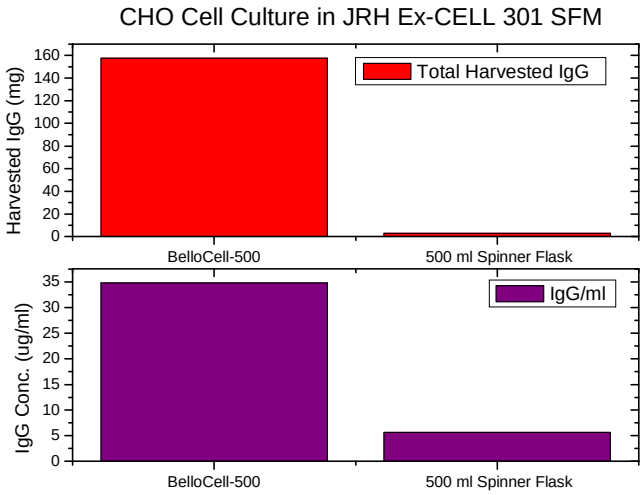
GUR shows a good correlation with IgG production.

6. IgG concentration between BelloCell-500 and Spinner flask



Max. IgG concentration in BelloCell-500 is about 6 folds compared with spinner flask

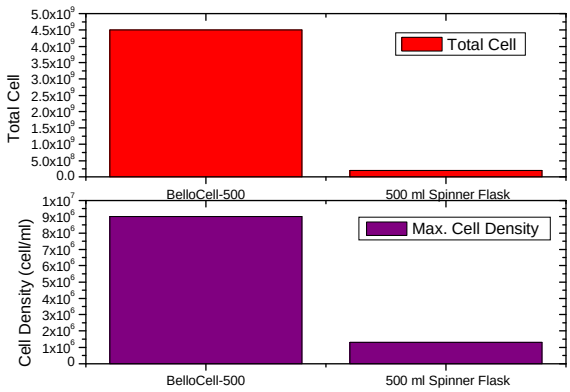
7. IgG production comparison with spinner flask



Total harvest IgG in BelloCell-500 is 71 folds compared with a 500 ml spinner flask

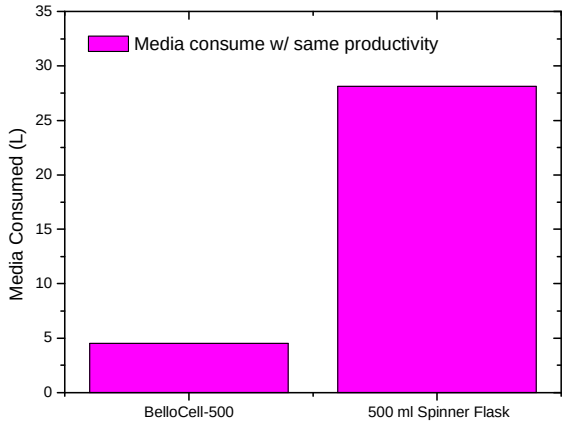
Average IgG concentration in BelloCell-500 is around 7 folds compared with a 500 ml spinner flask

8. Total cell population and cell density based on same volume



Total cell population in BelloCell-500 is 7.5 folds compared with a 500 ml spinner flask; cell density in BelloCell-500 is 8.2 folds compared with a 500 ml spinner flask.

9. Medium consumption based on same productivity



For the same production, it will consume 28 L culture medium in a 500 ml spinner to get the same productivity as one BelloCell-500 bottle.

Summary

Seed	Inoculum volume	Medium volume	Medium
7.9×10^7 cells/total	50ml	500 ml	Ex-CELL 301
Total culture age	**Total medium consumed**	**Total medium replenish frequency**	**Total cell counted**
15 days	5000 ml	9 times	4.5×10^9 cells/total
Max. GUR	**Total Protein produced**	**Max. Protein concentration**	**Multiplication of cells**
121.71 mg/hr	157.5 mg	61.2 ug/ml	57 folds

Please contact Cesco Bioengineering Technical Support for any questions or comments

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