



## Upstream Processing: CHO DP12 Critical Parameter Monitoring Data from Spinner Flask and Bioreactor Cultures

The upstream process was started with the inoculation of a spinner flask containing 100ml of culture media with CHO-DP12 cells secreting anti IL-8 mAb (ATCC CRL-12444). The spinner flask culture was used to seed a 1 liter bioreactor.

### Documents used for cell culture and analysis:

- SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO-DP12 Cells
- SOP: Labconco Purifier Class 2 Biological safety Cabinet (BSC) Operation
- SOP: Bellco Spinner Flask (100ml) Cleaning and Autoclaving
- SOP: Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter
- SOP: Trypan Blue Assay
- SOP: Glucose Determination Assay
- SOP: Lactate Determination Assay
- Batch Record: Anti-IL8 Monoclonal Antibody Production From CHO-DP12 Upstream process

These SOP's and other resources can be found at NBC2 website at the link below:

<http://biomanufacturing.org/curriculum-resources/program-units/upstream-processing>

### Spinner Flask Culture:

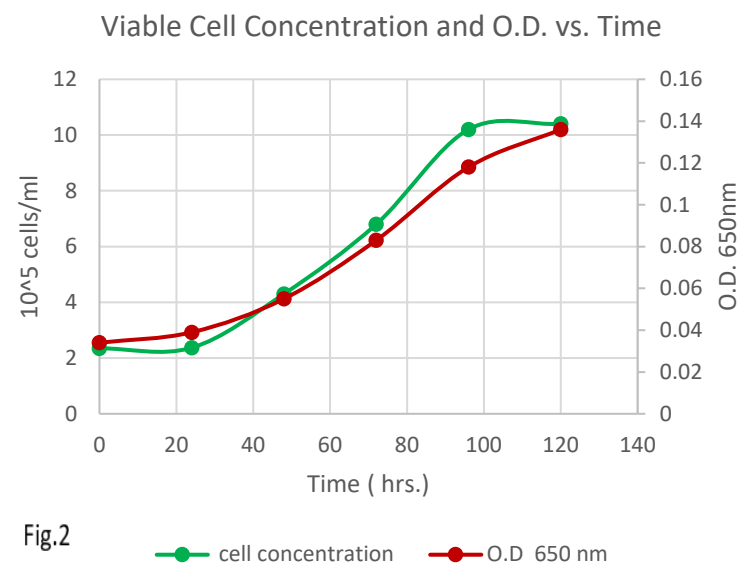
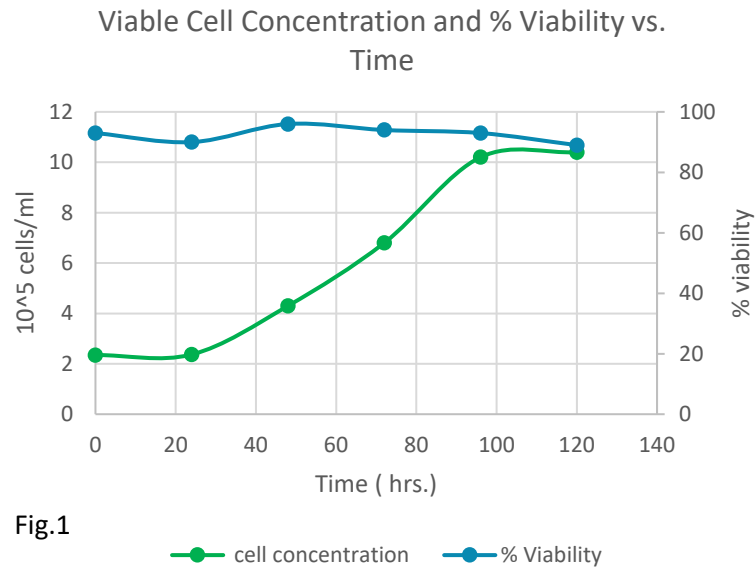
The 100 ml spinner flask was inoculated with  $2.2 \times 10^7$  cells on day 0. The students collected samples from day 0 to day 5 and performed analytical tests for optical density, pH, viable cell concentration, percent viability, glucose and lactate levels.

## CHO-DP12 Upstream Processing Data from Spinner Flask and Bioreactor Cultures

### Spinner flask raw data:

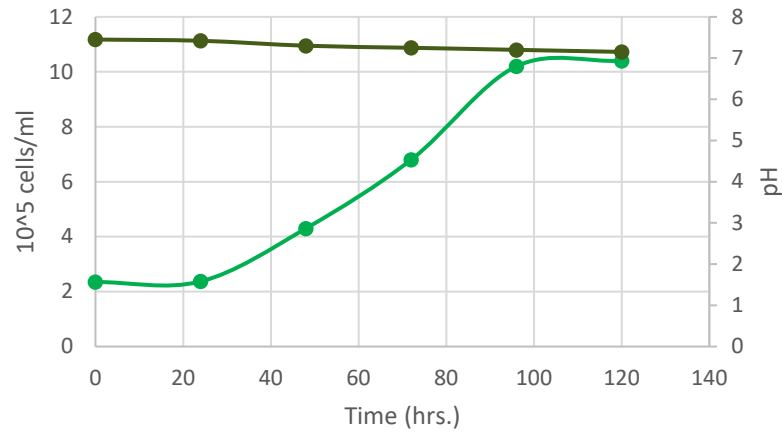
Table1

Time (hours)	OD 650nm	pH	Viable cells/ml	Percent Viability	Glucose (mg/dl)	Lactate (mmol/L)
0	0.034	7.45	2.35 X 10 <sup>5</sup>	93	384	2.48
24	0.039	7.42	2.37 X 10 <sup>5</sup>	90	370	6.71
48	0.055	7.30	4.30 X 10 <sup>5</sup>	96	281	13.55
72	0.083	7.25	6.80 X 10 <sup>5</sup>	94	207	20.94
96	0.118	7.20	10.20 X 10 <sup>5</sup>	93	110	27.86
120	0.136	7.15	10.40 X 10 <sup>5</sup>	89	85	28.97



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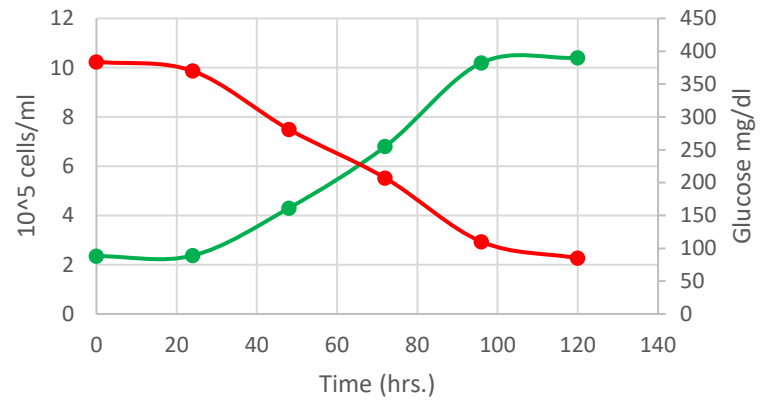
**Viable Cell Concentration and pH vs. Time**



**Fig.3**

● Cell concentration ● pH

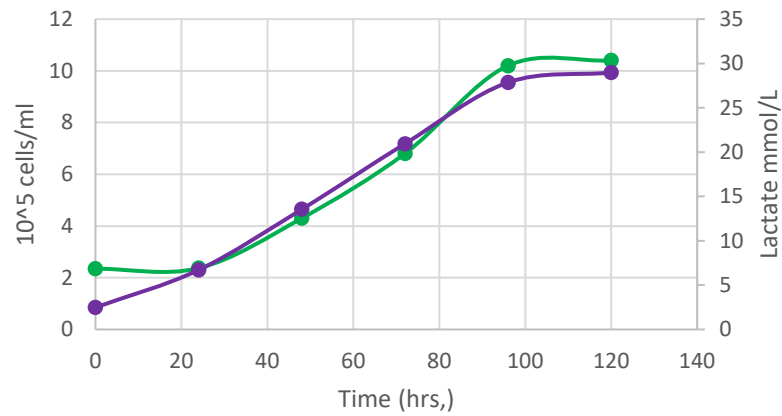
**Viable Cell Concentration and Glucose Conc. vs. Time**



**Fig. 4**

● Cell concentration ● Glucose mg/dl

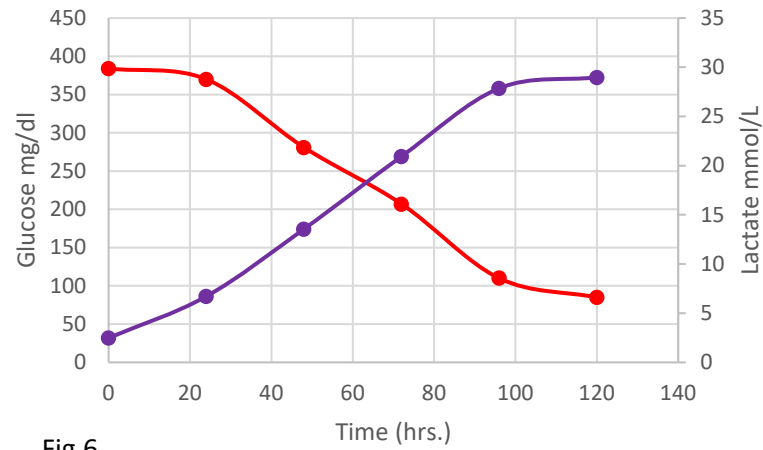
**Viable Cell Concentration and Lactate Conc. vs. Time**



**Fig.5**

● cell concentration ● Lactate mmol/L

**Glucose and Lactate Conc. vs. Time**



**Fig.6**

● Glucose mg/dl ● Lactate mmol/L

- Figure 1: A typical growth curve showing lag phase, exponential phase and stationary phase. The culture was in lag phase for 24 hours and before entering exponential phase. After 96 hours of cultivation it entered stationary phase after reaching the cell concentration of  $1.04 \times 10^6$  cells per ml. The percent viability remained high, above 90% throughout the growth curve indicating the culture was grown in optimal conditions.
- Figure 2: The optical density curve closely follows the cell growth curve. The turbidity of the cell suspension increases with the increase in the cell concentration.
- Figure 3: The pH remained in the expected range and dipped slightly as maximum cell concentration was reached.
- Figure 4: The glucose concentration decreased as cell concentration increased. Cells metabolize glucose to derive energy and produce lactate as a byproduct.
- Figure 5: The lactate concentration increased with the increase in cell concentration.
- Figure 6: The lactate concentration has an inverse relationship with glucose concentration. In exponential phase the cells maximally use glucose and produce lactate.

### **Bioreactor Culture:**

The bioreactor with 1100 ml of media was seeded with 82ml of cell suspension from the spinner flask at a cell concentration of  $1.04 \times 10^7$  cells/ml at day 5 (120 hours) of the spinner flask culture. The students collected samples from day 0 to day 6 and performed analytical tests for optical density, pH, viable cell concentration, percent viability, glucose and lactate levels. The bioreactor process controller measures and controls process parameter pH, temperature and dissolved oxygen with inline probes. The bioreactor was harvested at day 6 (144 hrs.) after seeding.

Documents used for cell culture and analysis:

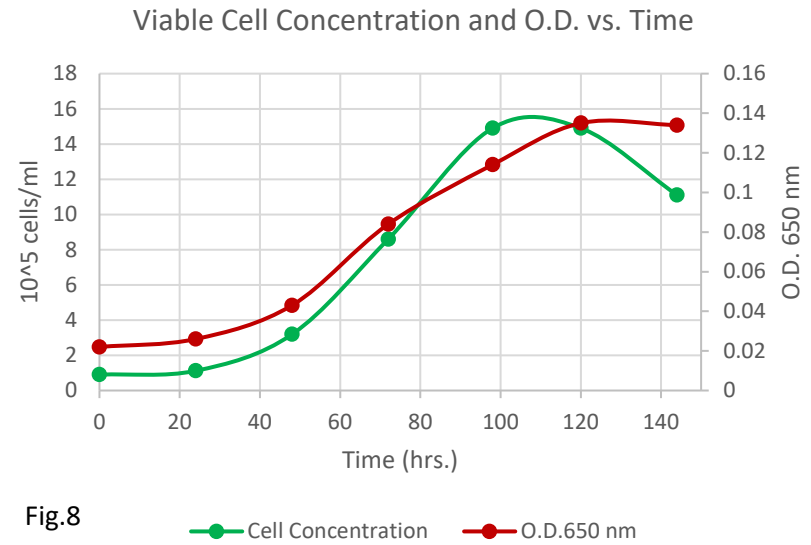
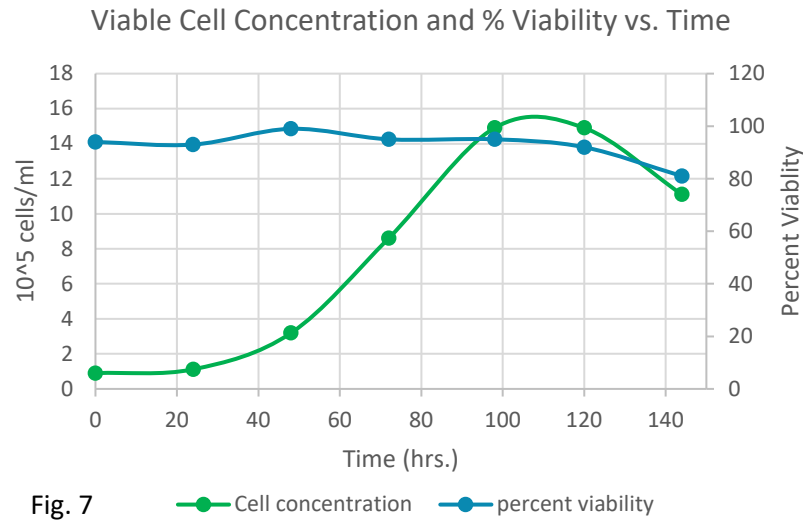
- SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO-DP12 Cells
- SOP: Applikon ez-Control Bioreactor Controller Operation
- SOP: Trypan Blue Assay
- SOP: Glucose Determination Assay
- SOP: Lactate Determination Assay
- Batch Record: Anti IL-8 Monoclonal Antibody Production From CHO-DP12 Upstream process

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### Bioreactor raw data:

Table 2

Time (hrs.)	OD 650 nm	pH	Viable cells/ml	Percent Viability	Glucose (mg/dl)	Lactate (mmol/L)
0	0.022	7.28	$0.9 \times 10^5$	94	330	5.49
24	0.026	7.26	$1.12 \times 10^5$	93	300	7.21
48	0.043	7.17	$3.19 \times 10^5$	99	286	9.87
72	0.084	7.11	$8.60 \times 10^5$	95	205	19.98
98	0.114	7.12	$14.90 \times 10^5$	95	122	25.69
120	0.135	7.10	$14.90 \times 10^5$	92	58	29.69
144	0.134	7.10	$11.10 \times 10^5$	81	19	31.33



## CHO-DP12 Upstream Processing Data from Spinner Flask and Bioreactor Cultures

Viable Cell Concentration and pH vs. Time

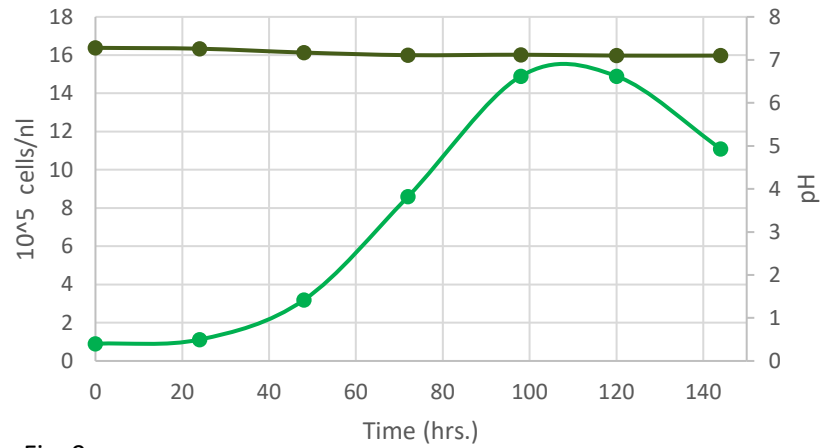


Fig. 9

● Cell Concentration    ● pH

Viable Cell Concentration and Glucose Conc. vs. Time

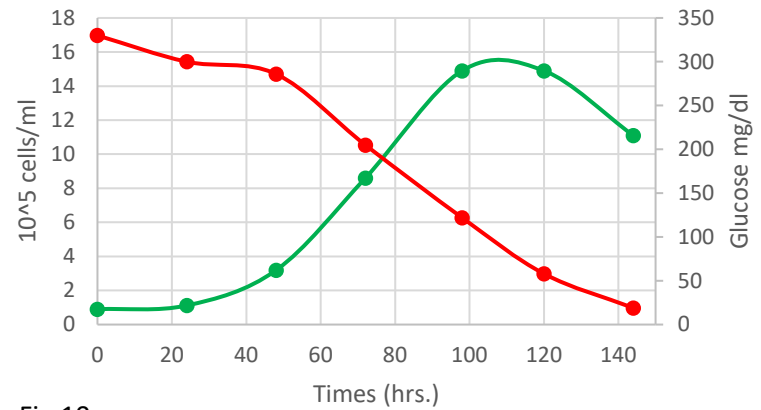


Fig.10

● Cell Concentration    ● Glucose mg/dl

Viable Cell Concentration and Lactate Conc. vs. Time

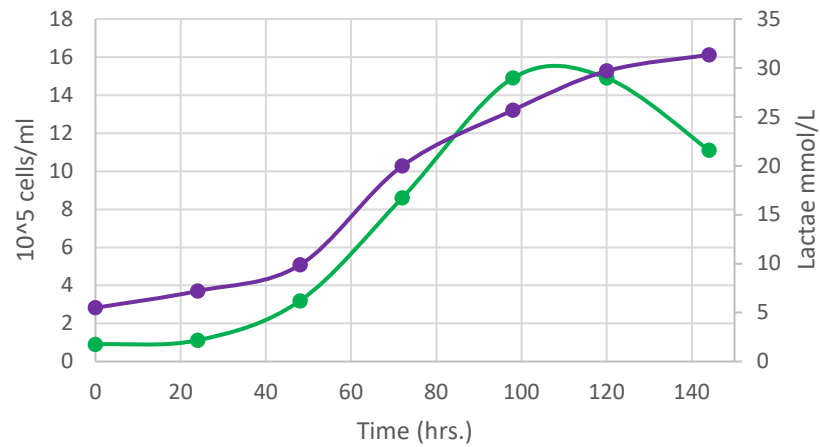


Fig. 11

● Cell concentration    ● Lactate mmol/L

Glucose and Lactate Conc. vs. Time

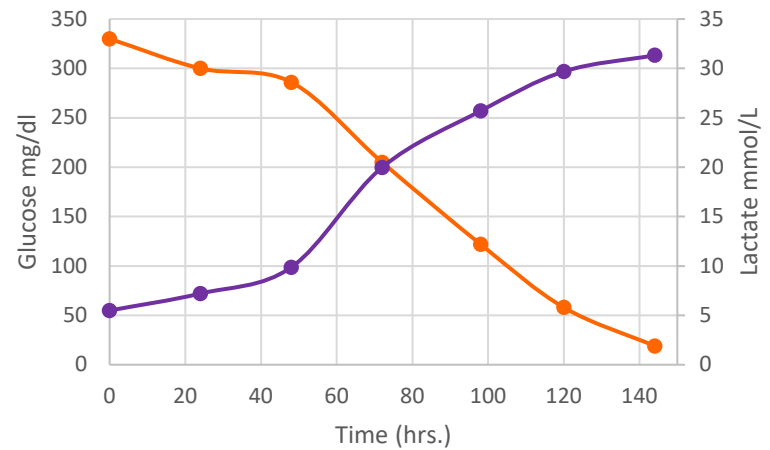


Fig. 12

● Glucose mg/dl    ● Lactate mmol/L

## CHO-DP12 Upstream Processing Data from Spinner Flask and Bioreactor Cultures

- Figure 7: A typical growth curve with lag, exponential, stationary and decline phases. The cells were in lag phase for initial 24 hours followed by an exponential phase from 24 hours to 100 hours. After 100 hrs. of cell growth the cell concentration plateaued. The cells reached the maximum cell concentration of  $1.49 \times 10^6$  cells/ml before entering the stationary phase. The percent viability was maintained above 92% and dropped in the decline phase
- Figure 8: The optical density followed the growth curve and increased with the increase in the cell concentration.
- Figure 9: The pH was maintained stable throughout the cultivation by the process controller of the bioreactor.
- Figure 10: Initially the glucose concentration was high then started to decline as the cells entered exponential phase.
- Figure 11: The lactate concentration followed the cell growth and increased with the increase in the cell concentration
- Figure 12: The glucose concentration decreased, and lactate concentration increased with the increase in the cell concentration. The cells metabolize glucose as they proliferate, and lactate is produced as a byproduct.

Experiments performed and recorded by Dr. Maggie Bryans, Hetal Doshi and Robin Zuck at Montgomery County Community College. Questions regarding data can be sent to [mbryans@mc3.edu](mailto:mbryans@mc3.edu). This work was funded by NSF ATE DUE 1501631, the Northeast Biomanufacturing Center and Collaborative