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A Comparison of Performance Using Three Bioreactor Systems

CelliGen BLU Single-Use Bioreactor vs. An Autoclavable Bioreactor and Rocker/Bag Bioreactor

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Abstract

New Brunswick Scientific (NBS) has introduced a new benchtop cell culture bioreactor that combines the benefits of single-use technology with the trusted performance and true scalability of a stirred-tank design. CelliGen™ BLU eliminates the need for autoclaving and cleaning, reduces validation requirements, provides rapid turn-around between runs, minimizes startup costs, and significantly reduces the risk of contamination because the culture vessel is only used once and then discarded. A compact controller enables advanced process management for research or cGMP manufacturing. This paper compares performance of the CelliGen BLU vs. New Brunswick's autoclavable CelliGen 310 and a competitor's rocker and bag-style bioreactor.

Single-Use, Stirred-Tank Vessels

CelliGen BLU features interchangeable, single-use, rigid-wall, stirred-tank vessels in 5.0 and 14.0 L total volume capacities. Vessels are delivered pre-assembled with pitched-blade impeller, porous microsparge, and all the necessary tubing, filters, and connectors; and come pre-sterilized, ready for use right out of the box. All components in product contact are made of materials that meet USP Class VI standards and have been tested for leachables and extractables, making these vessels appropriate for cGMP environments.

Headplate penetrations are provided for RTD temperature sensor, pH and DO probes, three addition ports, harvest, exhaust, sample, overlay, and gas sparge. A reusable heat blanket with window cut-out for viewing your cultures is provided for maintaining temperature in the culture vessel. DO is monitored via a non-invasive, reusable polarographic DO probe. pH is monitored via a non-invasive optical pH probe and disposable fluorescence sensor.

Advanced Process Control

CelliGen BLU's control station includes NBS' powerful Reactor Process Controller (RPC) firmware to provide advanced process management and monitoring capability. An adjustable-position, 15" industrial color touchscreen monitor makes it easy to set and monitor up to 32 process parameters, store up to 10 recipes, as well as view up to 8 parameters simultaneously on a user-customizable trend graph. Built-in security features include variable levels of control functionality for operators, supervisors and administrators, and passwords to prevent unauthorized users from making any changes to the system.

The compact control station includes three high-performance, assignable, fixed-speed pumps for additions and harvesting. Built-in controls are also provided for temperature, pH, dissolved oxygen (DO) and 3- or 4-gas mixing. Up to four Thermal Mass Flow Controllers (TMFC) for fully independent gas control are available for sparging, and a gas overlay with TMFC or Rotameter is available for independent control of gas entering the vessel headspace. An optional weight scale for measuring additions and level, as well as optional gas analyzers, BioCommand® supervisory control and data acquisition (SCADA) software, validation packages and more, enable customization to your needs.

Comparison to Similar Culture Systems

To prove the CelliGen BLU's ability to control growth conditions at setpoint as well as to produce high cell densities, researchers at New Brunswick Scientific's in-house laboratory compared growth of a Chinese Hamster Ovary (CHO) cell line using three bioreactor systems:

- The CelliGen BLU benchtop bioreactor with 5 liter pre-sterilized single-use vessels
- The CelliGen 310 benchtop bioreactor with 5 liter autoclavable vessels (NBS)
- A bag and rocker style culture system with 10 liter single-use bags.

The CHO line (ATCC, Manassas VA) was pre-adapted to use in a serum-free medium, and a CD CHO serum-free medium (Invitrogen Cat # 12490-025) was used.

Control parameters for all three systems, shown below, were set as identically as possible.

CelliGen BLU Setpoints:

- Temperature ..... 37°C
- pH set point 7.0
- Dead-band 0.10
- DO 40%
- Agitation ..... 70-80 rpm
- Gas overlay at 0.2 - 0.3 standard liters per minute (SLPM) via 4-Gas mode for the entire run
- Gas sparger using 4-Gas mode at 0-20 CCM via Cascade control from the Dissolved Oxygen (DO) to Gasflo, after 2 days of the run.

CelliGen 310 Setpoints were identical, except:

- Agitation ..... 80-100 rpm
- Gas overlay at 0.2 - 0.3 SLPM using 4-Gas mode for the first 2 days
- Gas sparger at 0.1 - 0.20 SLPM by 4-Gas mode after 2 days of the run

Bag & Rocker Bioreactor Setpoints are thought to be nearly equivalent to those used in the stirred-tank reactors:

- Temperature ..... 37°C
- pH ..... about 7.0
- DO ..... about 40%
- Rocking speed ..... 18 rpm
- Rocking angle ..... 8°
- Gas overlay at 0.1 - 0.3 SLPM by air with 0 - 5% CO_2 based on the pH

Each of the batch runs lasted 7 days. Daily off-line measurements of glucose and lactate concentration were read using a YSI 2700 analyzer (YSI Inc., Yellow Springs OH), and cell density and cell viability were measured using a NucleoCounter (NBS).

The CHO cells in all three bioreactor systems grew steadily through day 5 of the 7-day run (**Table 1**), coinciding with availability of nutrients in the medium. By day 5, a nutrient source, glucose, had become nearly depleted from the medium (**Fig. 2**). Cell viability in all three systems also remained high, at 96% or better, through day 5. The maximum viable cell count attained in each system was as follows: 5.55 x 10^6 cells mL^{-1} by the CelliGen BLU compared to 5.39 x 10^6 cells mL^{-1} for the autoclavable system, and 4.77 x 10^6 cells mL^{-1} for the bag-and-rocker system.

	CelliGen BLU bioreactor			CelliGen 310 bioreactor			Bag-and-rocker system		
Day	Total cells ($x10^6$ ml^{-1})	Viable cells ($x10^6$ ml^{-1})	Viability (%)	Total cells ($x10^6$ ml^{-1})	Viable cells ($x10^6$ ml^{-1})	Viability (%)	Total cells ($x10^6$ ml^{-1})	Viable cells ($x10^6$ ml^{-1})	Viability (%)
0	0.31	0.30	97.9	0.31	0.30	97.9	0.31	0.30	97.9
1	0.69	0.68	97.1	0.64	0.62	96.5	0.61	0.58	96.8
2	1.42	1.39	97.6	1.31	1.29	97.9	1.33	1.30	97.6
3	2.57	2.51	97.6	2.47	2.41	97.8	2.36	2.32	98.4
4	4.02	3.92	97.5	4.04	3.98	98.6	3.89	3.83	98.5
5	5.70	5.55	97.3	5.46	5.39	98.7	4.83	4.77	98.9
6	5.98	4.52	76.6	5.67	4.13	72.7	5.46	3.67	67.3
7	6.71	3.21	47.8	6.25	3.12	49.7	5.59	2.73	48.8

Table 1. *Comparison of CHO growth and viability achieved using three different bioreactor systems.*

Figure 1. *The new CelliGen BLU cell culture bioreactor features single-use, stirred-tank vessels and an advanced process controller for growth of animal cells in cGMP-compliant research and production environments.*

Conclusion

While no effort was made to optimize either the medium or the cell culture process control parameters in any study, this data demonstrates that the CelliGen BLU bioreactor is an efficient system for the culture of CHO cells, and can outperform bag-style systems. For a complete protocol on system setup, or for additional information on the CelliGen BLU, see www.nbsc.com/BLU.

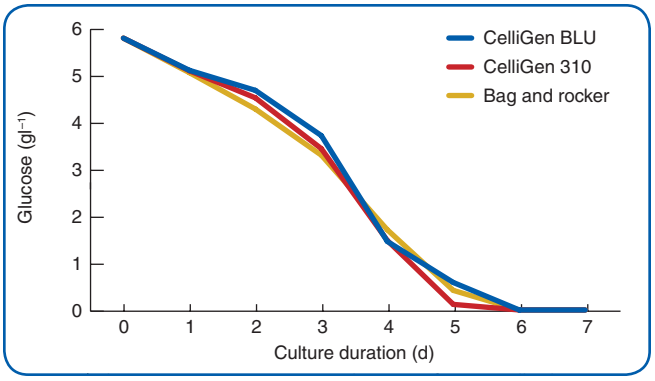

Figure 2. *Glucose concentration versus culture time for CHO cell cultures in three benchtop bioreactor systems.*

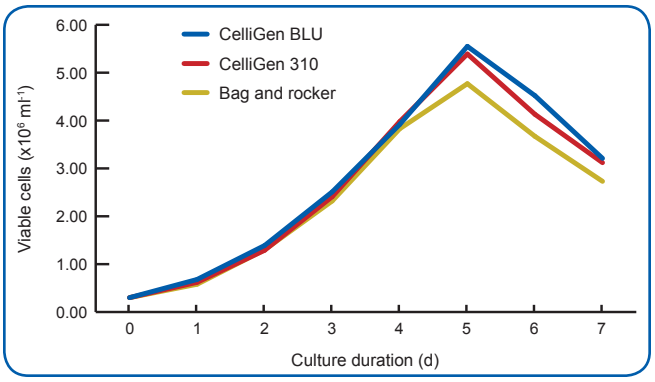

Figure 3. *Viable cell count peaked at 5.55 x 10^6 cells mL^{-1} in the CelliGen BLU, compared to 5.39 x 10^6 cells mL^{-1} in the CelliGen 310 autoclavable system and 4.77 x 10^6 cells mL^{-1} in the bag-and-rocker system.*

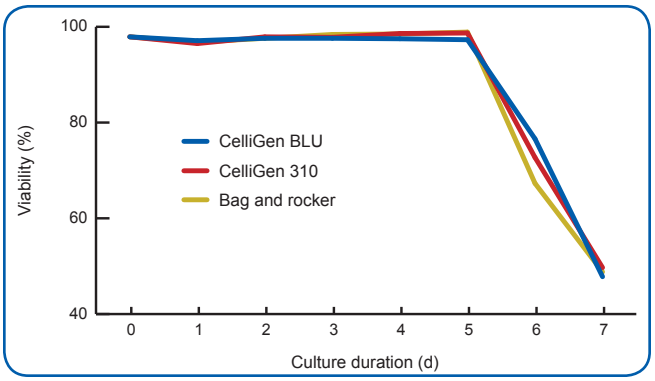

Figure 4. *Cell viability in all three systems was extremely high through day 5, until the nutrient source in the batch run became depleted.*