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Tutorial

Hybrid System Aims to Streamline Cell Culture

Bioreactor Combines Single-Use Vessel with Stirred-Tank Design

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During the past decade, single-use bioreactors have become widely accepted and often preferred in development and manufacturing processes. In particular, single-use vessels have proven perfectly suitable for the cultivation of low-oxygen-demanding cell types such as mammalian and insect cells.

These bioreactors can offer significant advantages over their reusable glass and stainless steel counterparts, including labor and cost savings, rapid turnaround between runs, and flexibility. This article focuses on single-use bioreactor design advancements introduced in the New Brunswick CelliGen® BLU benchtop stirred-tank bioreactor (*Figure*) from Eppendorf.

Non-Invasive Design

Control of pH and dissolved oxygen (DO) is critical in cell culture; use of traditional pH and DO probes, however, requires significant time (*Table 1*) and can introduce contaminants.

The CelliGen BLU system features a novel non-invasive pH and DO port and sleeve design, enabling accurate readings without either probe coming in direct contact with the culture.

The DO sleeve allows gas to readily diffuse across a permeable membrane into the sleeve where it is sensed by the probe. This design has several advantages over the current methodology. It eliminates contamination risks, as there is no contact between the vessel interior and the DO sleeve space where the probe is inserted. Vessel setup time is reduced because the reusable DO probe does not have to be sterilized and re-polarized for each run—a procedure that ordinarily takes about 6–8 hours. It also allows a nonsterile probe to be used, as the sleeve is a barrier to any contaminants, eliminating autoclaving and thereby extending probe life.

The CelliGen BLU also uses a non-invasive, optical pH probe that doesn't need to be autoclaved (it is not a traditional gel-filled pH probe).

Stirred-Tank Advantages

Stirred-tank bioreactors are commonly used for the culture of suspension cells, mainly because of the broad experience obtained in microbial fermentation using the same type of culture vessels. Basic design criteria have been derived from those of microbial cultures and modified to meet the requirements of mammalian cells.

Specifically, the shear sensitivity of animal cells requires consideration in the design of impellers, aspect ratio, and oxygenation. Scale-up principles for these reactors are generally better characterized than those of other bioreactor types such as rocker-style bags.

The New Brunswick CelliGen® BLU stirred-tank bioreactor has been engineered for high-density animal cell culture in research and production using interchangeable, single-use, stirred-tank vessels in 5.0 and 14.0 L total volume capacities. A compact controller enables advanced process management for research through cGMP-compliant manufacturing. Vessels come presterilized, prevalidated, and preassembled. Shown with 5 L vessel and optional scale.

Scalability. One of the essential elements in manufacturing processes is scalability. Developers of industrial cell culture methods for the production of recombinant biologics seek high efficiency, reproducibility, and predictability. Typically the time allotted for process development is short for defining culture conditions and scale-up protocols in order to maximize cell productivity and yield yet minimize process

Table 1. Time Study for Setup and Breakdown of Various Bioreactor Systems

Process Steps	Autoclavable Vessels (in hours)	Single-Use with Autoclaved Probes (in hours)	CelliGen BLU (in hours)
Autoclaving	2	2	0.0
Setup time before run and after autoclaving to assemble and connect tubing to vessel ports; calibrate pH; add and remove PBS; equilibrate; polarize DO probe; calibrate DO; add and inoculate medium	6	6	0.75
Shutdown time after run to remove, dismantle, sanitize, and clean vessel	2	0.25	0.25
Total time	10	8.25	1

Table 2. CelliGen BLU Design Parameters

	Vessel Height: Vessel Diameter
5 L	1.5:1
14 L	2:1
	Vessel Diameter: Impeller Diameter
5 L	1.7
14 L	2.1
Overlay Gas Flow Range 0.060–3.000 SLPM	
Sparger Gas Flow Range 0.002–0.100 SLPM	

scope and overall cost. For this reason, having identically designed bioreactors throughout the complete manufacturing process significantly facilitates scale-up.

Eppendorf developed the CelliGen BLU single-use, rigid-walled, stirred-tank bioreactor system, currently available in interchangeable 5- and 14-liter vessels, with these scale-up concerns in mind. In developing this bioreactor, the focus was to improve comparability of single-use and classic processes by adopting stirred-tank design. To assure this symmetry, several critical design criteria were taken into account.

Vessel Design. As shown in *Table 2*, the height:diameter ratio of CelliGen BLU vessels is within the limits of standard cell culture vessels (between 1:1–2.3:1). This allows improved gas transfer rates for medium oxygenation to take place both through the efficiency of gas bubbles via the porous microsparger and through the headspace ("Overlay Gas Flow Range" in *Table 2*) due to the relatively high surface-to-volume ratio.

In contrast to many single-use bag type bioreactors, the CelliGen BLU vessel has no need for an outer container support because it is free-standing and constructed from rigid plastic. The rigid CelliGen BLU vessel design also eliminates the risk of folding stress likely to be present in the plastic film of disposable bag designs which, under internal medium pressure, may crack and leak.

Agitation. Agitation in stirred-tank bioreactors is intended to provide a homogeneous chemical (nutrients and waste products), physical (pH, O_2, and CO_2), and thermal environment within the vessel and a uniform suspension of cells. The CelliGen BLU vessel uses a low shear pitched blade impeller, which provides both axial and radial mixing. The blades are pitched at 45° with a classic impeller-to-vessel diameter ratio of approximately 3 to 5. The impeller is propelled by a magnetic drive.

Aeration and Gas Control Loops: Oxygen is critical to cellular metabolism. Even though the oxygen demands of animal cell cultures are several orders of magnitude lower than those of microbial cultures, oxygenation is often the primary challenge in cell culture scale-up. The importance of aeration increases with bioreactor volume and cell concentration. Just as low oxygen concentration affects cell growth and yield, excessive oxygen tensions can also be toxic to cells.

The classic, widely used aeration method in bioreactor systems is direct sparging, bubbling gas within the culture medium. Proper sparger selection is important for increasing aeration and minimizing cell damage and foaming.

A variety of gases, including air, O_2, CO_2 and occasionally N_2, is sparged into animal cell cultures with ring spargers similarly designed to those used in microbial fermentors or with microspargers made of sintered metal or polymeric materials. Microspargers provide significantly higher gas-medium interfaces and thereby higher O_2 mass-transfer coefficients (kLa) with lower gas flow rates. A porous polymeric microsparger is standard in CelliGen BLU systems, installed below the impeller for efficient gas transfer.

CelliGen BLU controllers can optionally be configured with overlay gas flow control, a highly useful method for managing the headspace environment and thereby an additional means to increase the overall kLa and additionally remove CO_2.

To meet higher demand for oxygen, one option is to increase gas flow via the sparger. If gas flow is too high, though, bubbles may coalesce, decreasing the gas/liquid interface and increasing cell shear. To prevent this, the CelliGen BLU system is equipped with two independent, completely flexible, gas control systems that function automatically to maintain DO and pH setpoints. The first is for direct aeration through the sparger and the second for flushing the headspace via overlay.

In both systems, up to four gases (air, O_2, N_2, and CO_2) can be automatically and correctly proportioned according to the feedback from the DO and pH sensors. The gas mixture then passes through a thermal mass flow controller (TMFC) to the sparger and/or the headspace.

The TMFC controls the overall flow rate of the gas mixture set by the user. For animal cell cultivation, low aeration rates, below 0.1 vvm, are generally preferred to minimize the potential for foam

and shear effects from bubbles. The CelliGen BLU can be configured with 0, 1, 3, or 4 TMFCs meeting the requirements of most cell culture customers.

Conclusion

Engineering design, field testing, and industry use have shown that the CelliGen BLU single-use stirred-tank, rigid-wall bioreactor performs as well as traditional autoclavable bioreactors and outperforms bag-and-rocker-style single-use systems.

With the added advantages of a non-invasive design for pH and DO sensors that reduces contamination risk, speeds setup, extends sensor life, and eliminates the need for a biosafety hood, coupled with advanced aeration, and gas control capability and the scalability of a traditional stirred-tank design, the CelliGen BLU provides a new alternative to bioreactor technologies currently on the market. GEN

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