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Efficient Cell Cultivation

Single-Use Bioreactor for Process Development Enables More Flexibility

Now, biotechnologists are not only able to choose between single-use and reusable bioreactors and fermentors in the production sector, but in process development as well, where single-use systems are making their mark with advantages like lower costs and faster run times.

During the production of biopharmaceutical products like monoclonal antibodies, enzymes or vaccines, the central process step is performed in bioreactors. In a bioreactor, cells are cultivated monoseptically under the most optimally controlled conditions (temperature, pH, dissolved oxygen concentration (DO) and culture media components).The most frequently used fermentors are called stirred tank bioreactors. Traditionally, they are made of glass (150-milliliter to 10-liter working volumes) for seed and small-scale cell cultivation and of stainless steel for larger applications (ten to several 10,000 liters).

Since the mid-90s, single-use bioreactors have been increasingly employed for cultivating mammalian cells from laboratory to production scale. The advantages of single-use systems are clear: Easy handling and no sterilization or cleaning times.

The first instrumented single-use bioreactor systems to become established were two-dimensional bioreactor bags (2D) like the Biostat CultiBag RM from Sartorius Stedim Biotech. These systems work with a platform that rocks back and forth; the wavelike, low-shear motion thoroughly mixes the liquid in the bioreactor bag. Due to their design, the working volume is limited to a maximum of several hundred liters. The considerable demand for single-use stirred tank bioreactors has inspired a variety of manufacturers to develop equivalent systems.

These systems employ three-dimensional (3D) stirred tank bioreactor bags. A bag container made of stainless steel not only guarantees stability, but also controls the temperature. Systems are supplied with working volumes ranging from 50 l to a maximum of 2,000 l. Ideally, these 3D stirred tank bioreactor bags essentially feature the same design criteria as do conventional stainless steel bioreactors.

Beyond this, it is practical to equip these 3D stirred tank bioreactor bags with single-use sensors for pH and DO measurement to fully exploit all advantages of single-use systems.

Single-use Stirred Tank Bioreactors

The latest evolution in single-use stirred tank bioreactors are vessels for the process-scale development, providing an alternative to conventional glass bioreactors. This vessel category is used in the biopharmaceutical industry mainly for developing and optimizing cell lines and processes, as well as for scale-up and scale-down experiments. That is why it is of significant importance that these vessels replicate the design criteria of production-scale stirred tank bioreactors for both reusable and single-use applications. Stirred tank bioreactors with 2-liter working volumes have proven to be particularly well-suited and deliver high performance. These models feature reactor geometries comparable to those of the production vessels; their volume capacity is both affordable and sufficiently large for taking analytical samples, and above all, they are easy to handle.

The advantages that single-use culture vessels offer on this scale are remarkable. Beyond the criteria mentioned above, autoclaving relatively cumbersome glass vessels is eliminated as are costs for their servicing and maintenance.

Moreover, single-use culture vessels can also be used interchangeably with glass vessels so that during capacity peaks or maintenance of bioreactors, for instance, reusable vessels can be easily exchanged for single-use bioreactors in the interim. This enables downtimes in bioreactor process control to be reduced or even eliminated. For such uninterrupted cultivation, the only other alternative is to purchase additional, fully equipped glass bioreactor vessels, which entails high capital costs.

The UniVessel SU is a single-use culture vessel that meets the design criteria of conventional glass and production-scale stirred tank bioreactors. The body of the culture vessel is made of polycarbonate and contains graduation marks. The bottom conforms to the torospherical base design of stainless steel stirred tank bioreactors. To ensure a stable upright position during operation, the vessel is placed in a holder. Like on glass stirred tank bioreactors, all vessel inlet ports are located on the lid of the device. The lid is firmly assembled onto the body of the vessel and equipped with a number of ports. All inlet and harvesting ports are supplied with thermoweldable tubing and feature MPC or Luer connectors. A needle-free septum port is installed for sampling. The culture vessel is drained completely through the harvesting tube that is channeled to the center of the culture vessel base.

Aeration can take place either in a submerged configuration and/or through the headspace; both air inlet and exhaust channels are equipped with sterilizing-grade filters. Submerged aeration takes place through an L-sparger with tiny holes. Moreover, the device features three threaded ports (steel conduit thread PG 13.5) for attaching standard 12 mm sensors. The stirrer shaft has two ball bearings that ensure stability and a lip seal that maintains sterility. Thorough, low-shear mixing of the cell culture medium is accomplished by two 3-blade segment impellers (down-flow).

Additionally, the device includes non-invasive, optical, single-use pH and DO sensors. These sensor patches contain special dyes that are excited to fluorescence when exposed to light emitted at a characteristic wavelength through the culture vessel wall.

The properties of the longer wavelength fluorescent light emitted by the sensor patches are re-

	2L- reusable glass bioreactor *	UniVessel SU 2L	500 L reusable stainless steel bioreactor	CultiBag STR 200L
Stirred-tank bioreactor design / process parameters				
Working volume [L]	2	2	500	200
Stirrer speed [rpm]	200	212	65	100
Aeration rate [vvm]	0,1	0,1	0,16	0,1
Height-to-diameter ratio	1.5 :1	1:4 :1	1:1 1	1:3 : 1
Impeller design	3-blade segment impeller (standard stirrer)	3-blade segment impeller (standard stirrer)	Lightnin A320 (3-blade segment impeller)	3-blade segment impeller (standard stirrer)
Number of impellers	1	2	1	2
Sparger design	L-sparger with holes	L-sparger with holes	2-line aeration system/open pipe sparger with microsparger	Ring sparger
Process-related parameters				
Mixing time [s]	20	< 3	48	16
Specific power input [W/m³]	2.8	1,4	13,6	17.5
kLa [1/h]	10	6,9	8	6

* Data from A-mab: A Case Study in Bioprocess Development; CMC Biotech Working Group

Tab.1: Comparison of reusable vs. single-use bioreactors

spectively influenced by the parameters of pH or dissolved oxygen saturation of the culture medium in contact with the sensor patches. The Sensolux optoelectronic components built into the culture vessel holder are used both to excite fluorescence and to detect the fluorescent light emitted (see Fig. 2).

Scale-up and Comparability

The scale-up or scale-down of fermentation processes is not an exact science; rather it combines experience with the application of the equivalent process parameters and design criteria. The key scaling criteria include bioreactor design along with process-related parameters, such as mixing behavior and gas transfer. Design criteria essentially comprise the bioreactor's height-to-diameter ratio, impeller design, ratio of the impeller to bioreactor diameter as well as the construction of the sparger design.

With respect to mixing behavior, the mixing times and the specific power input are the parameters considered, while the volumetric oxygen transfer coefficient is important for gas transfer (see Table 1).

The single-use culture vessel can be easily integrated into both new as well as existing bioreactor control units. All that is needed for this purpose is a heating jacket for regulating the temperature. A reusable motor adapter enables connection of the device to the existing impeller motor; a variety of adapters are supplied for the various stirred tank bioreactors. The presterilized pH and DO sensors are attached to the vessel lid under a sterile workbench. The temperature sensor is simply inserted into the open port in the vessel lid. For enhanced time efficiency, a barcode scanner can be used to read in the calibration parameters of the precalibrated sensor patches. As a result, the labor-intensive steps involving autoclaving, calibrating and attaching the pH and DO sensors are eliminated, which means users can fully benefit from all the advantages of single-use vessels.

The UniVessel SU provides the flexibility required for connection to existing bioreactor control units. Further culture vessel components, such as an exhaust cooler, various impeller geometries, etc., are in the development pipeline and, moving forward, will allow microbial high cell-density cultivation with instrumented single-use culture vessels. Beyond all this, more vessel sizes will be added to the product family.

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Fig. 1: The UniVessel SU is a novel, single-use culture vessel specially designed for process development.

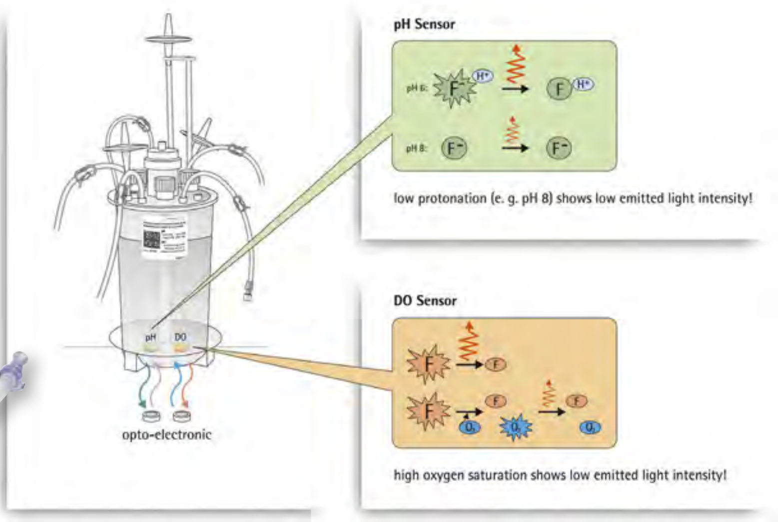

Fig. 2: Functional Diagram of the UniVessel's Optical pH and DO Sensors

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