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# Markets Expand for Single-Use Bioreactors

## Biobags Offer Advantages Over Fixed-Tank Systems

*Angelo DePalma, Ph.D.*

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Sartorius Stedim Biotech's Biostat STR bioreactors are scalable single-use instruments that provide working volumes from 12.5 to 2,000 L. This family of bioreactors is based on conventional stirred-tank design.

Single-use bioreactors are steadily encroaching on territory where previously only fixed-tank processes dared tread. Such incursions, along with consolidations of existing strongholds, help explain why single-use bioreactors are gaining ground so quickly.

According to Transparency Market Research, the global market for single-use bioreactors will increase 11% per year between 2015 to 2024, from \$2.4 billion to \$5.9 billion. This estimate comes from a study (Single-Use Bioprocessing Systems Market) that predicts North America and Europe will continue to dominate usage markets through a combination of geography and numbers. Demand for disposable bioprocessing in Asia will grow faster than the global average, however, due to the emerging nature of those markets.

Notable trends driving anticipated growth include the need for flexibility and multiproduct facilities; the development of biosimilars and highly potent biologic active pharmaceutical ingredients; the trend

toward high cell densities and product titers; the demand for personalized medicine; and the interest in continuous upstream processing.

This is what experts, consultants, and report authors keep saying. But remember that the top 10 biotech drugs enjoy combined sales of about \$80 billion. Rising titers notwithstanding, all such products are clearly blockbusters, and their descendents are likely to follow "obsolete" economic models.

Large suppliers continue to dominate single-use bioreactors in terms of sales and process volumes. These suppliers are in the ascendancy thanks to their ongoing development efforts. A few examples:

- Pall continues to invest in continuous biomanufacturing through the launch of continuous chromatography products, single-use stirred tank bioreactors, and single-use sterile genderless connectors.
- MilliporeSigma recently expanded its single-use cGMP facility with the addition of the Mobius 2,000 L single-use bioreactor, enabling the company to provide upstream development and manufacturing

services using its own equipment through its Provantage End-to-End Services offering.

- Sartorius Stedim Biotech has developed a fully integrated technology platform combining a royalty-free expression system, the automated ambr15 micro bioreactor for development and scaledown work, and Biostat® STR single-use bioreactors at GMP production scales.

### Novel Mixing Technology

PBS Biotech specializes in single-use culture systems for therapy-worthy cells, both in suspension and cultivated on microcarriers.

“Culturing these cells remains challenging because the cells are not as well characterized as, say, the suspension CHO cells that are used to produce therapeutic proteins,” says Joe Petrosky, the company’s vp of global sales. In terms of industrialization, this segment of the cell culture business is at the same stage as therapeutic production was 30 years ago.

Scale-up is an issue, as it has often been with mammalian cell culture. Protein bioprocessors have solved most production-relevant issues, especially for well-trodden expression systems such as CHO. “For therapeutic cells,” Petrosky notes, “we’re still figuring out how to scale from dozens to thousands to potentially millions of doses.”

Cell viability is a major concern for CHO-based biomanufacturing, but losses are considered an occupational hazard. Bioprocess designers nevertheless try to minimize cell losses that are due to shear from agitation systems. Where cells themselves are the product, additional care is warranted, particularly for microcarrier-based cultures. Even cells that survive are susceptible to reduced post-transplantation vitality and efficacy.

PBS Biotech’s claim to fame is its Vertical-Wheel™ Bioreactor, which the company touts as “the first truly novel and innovative approach to bioreactor design since the ‘single-use’ concept began.”

Competitors in the single-use bioreactor space may dispute this claim, but Vertical-Wheel technology does represent a stark departure from horizontally oriented impeller mixing. Resembling a mill wheel, Vertical-Wheel is pneumatically or magnetically driven, providing vertical agitation that the company says provides scalable and homogenous mixing, reduced cell-damaging shear, improved mass transfer, and uniform particle suspension. One model, AirDRIVE, spins through the action of rising gas bubbles. MagDRIVE operates through magnetic coupling.

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The PBS 15 bioreactor system from PBS Biotech uses the company’s Vertical-Wheel mixing mechanism to achieve efficient mixing and homogenous particle suspension with low shear force. The system has a 9–15 L recommended working volume.

PBS Biotech sells complete systems ranging from 100 mL up to working volumes of 500 L. Bioreactor components include single-use bags, stainless-steel housings, and housing-embedded controllers.

Specialization of single-use bioreactors raises interesting questions of who “owns” the result, who is responsible for quality. “The advantages of single-use are well known,” states Petrosky. “You can’t beat the convenience and flexibility. The disadvantage is, you’re handing off some of the responsibility for quality to your suppliers.” He specifically mentions leachables and extractables, which as issues of material compatibility are well outside the expertise of process development and manufacturing engineers.

### Up from Standardization?

We hear a great deal in bioprocessing about standardization, a term that is often associated with large-scale production and attendant economies. That pull is particularly strong with respect to connectors and bag ports. Is it possible to exploit the advantages of standardization within the framework of customization?

Per Stobbe, CEO of Danish bioreactor manufacturer CerCell, thinks so. Rather than supplying standardized biobags in large quantities, CerCell focuses on customized single-use bioreactors ranging in size from 250 mL to 30 L. Users can custom-design their product using CerCell’s Configurator Tool, an online utility that allows selection of diameter, height, agitation mechanism, and third-party single-use sensors. About 15 million different design options are possible. The polycarbonate bioreactors are suitable for mammalian cell processes, microbial fermentations, and packed-bed cultures.

“We’re not in the same league as vendors who supply thousands of bags per year,” Stobbe admits. “Our products are obviously more expensive, but in the end, customers can get exactly what they need.”

Bags are also limited in size, but that doesn’t bother Stobbe, either. He believes that since “continuous processing is the future of bioprocessing,” single-use systems considered small today will be the production platform of the future.

CerCell’s online configuration tool makes it possible to specify any of more than 5,000 components and options. For example, options include 650 impellers and 150 turbines. Stobbe likens the design process to configuring a high-end automobile by starting with a base model and then selecting numerous add-ons.

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CerCell designed this highly customized 3 L single-use bioreactor for Merck MSD.

“Standardization may be the current trend for production bioreactors, but during research and development, customers aren’t necessarily thinking of standardization,” advises Stobbe. “They want a bioreactor that will perform their specific and advanced tasks.”

In many instances, CerCell is asked to advance the development of a customer process that has been performed only with stainless-steel equipment and brought to some degree of refinement. To minimize risk and preserve the process advantages accruing to that point, the customer will ask for customized single-use bioreactors that mimic the stainless-steel or glass tank in every physical dimension, using the same stirring mechanism, physical dimensions, and sensors.

“These customers want to continue benefiting from historical data they may have been accumulating for 20 years,” Stobbe tells GEN. “That is impossible to achieve using off-the-shelf bags or even single-use bioreactors of standard design.”

For customers who eventually do scale-up to much larger volumes, CerCell will create intermediate-sized bioreactors to ease the transition and facilitate seed-train expansion.

Customization also works in reverse, such as when scale-down versions of successful large-scale disposable bags or stainless-steel systems do not exist. Several large single-use vendors have asked CerCell to duplicate their production-scale bags for benchtop or intermediate volumes. “This approach,” Stobbe remarks, “allows customers to work in small volumes that simulate what they will eventually encounter at much larger scale.”

#### From Microorganisms to Cells

The CELL-tainer® 20 L system from Celltainer Biotech, a bioreactor for microbial fermentations and mammalian cell culture, uses a removable “expansion set” to provide very high turndown rate over a range of volumes.

According to Nico Oosterhuis, Ph.D., Celltainer’s director of technology, the expansion set makes it possible to expand cultures from 150 mL to 18 L, all in the same bag. “This capability,” Dr. Oosterhuis asserts, “saves handling, conserves bags, and reduces risk associated with cell loss and contamination.”

Celltainer also offers a 200 L version of the product, with claims of  $k_L a$  values comparable to the 20 L version, in excess of 400/ hr, at least 30 times higher than for conventional rocking-style and stirred single-use bioreactors. Optical densities for *Escherichia coli* have been reported to exceed 130 using just air as the oxygen supply.

Most Celltainer customers are involved in microbial fermentation, where mass transfer figures of the sort cited here, and published in respectable journals, are most applicable. “Some anecdotal evidence exists

as well for improved mass transfer in CHO cultures as well,” notes Dr. Oosterhuis. “This is believed to result from improved transfer of CO<sub>2</sub> between solution and gas phases.”

Celltainer attributes improved performance to its unique mixing/agitation mechanism. Where conventional rocker-type single-use bioreactors move the edges up and down along the y-axis, Celltainer adds back-and-forth motion along the x-axis as well. The resulting motion at the ends of the bag resembles an “infinity” sign. This motion entraps gases within the agitated liquid at higher rocking rates.

Dr. Oosterhuis indicates that single-use bioreactors would show even more promise if they were accompanied by two improvements: reliable sensors and continuous processing.

But for a few exceptions, single-use bioreactors employ reusable sensors, which require cleaning, calibration, validation, and a suitable means of introducing the probe into the reactor. Here the advantages of precalibrated, reliable, preinstalled single-use sensors are obvious. Celltainer is currently evaluating a disposable pH sensor from a Swiss manufacturer for use in CELL-tainer single-use bioreactors.

Disposable bioprocess equipment has spurred interest in continuous processing, and in that regard, Dr. Oosterhuis notes the difficulties in setting up and disposing of very large single-use bags. Also, the usual arguments in favor of using a 500 L single-use bioreactor to do the work of a 10,000 L fixed-tank bioreactor apply. He mentions a third factor: the production of biosimilars in far-flung global locations. This possibility has also earned a good deal of buzz among the biotech commentariat, but whether biosimilar production will ever be so widely distributed remains to be seen.

### Bigger and Bigger

One of the knocks on single-use bioprocessing is the difficulty that plastic equipment sometimes has in matching the performance that manufacturers have come to expect of stainless-steel equipment. This expectation gap extends even to new processes for which specific historical fixed-tank data does not exist. Thus, the market niche served by companies such as CerCell.

The performance gap between plastic and steel is perhaps best illustrated at large scale. Yet finding convenient points of comparison at this scale can be difficult. If you were to speak to vendors whose principal or only business is (or has ever been) disposable process equipment, you would think that leading biotech companies were melting their stainless-steel bioreactors down into spatulas.

I admit to some guilt in propagating the “small is all” myth, which may have strengthened through my tendency to emphasize rising protein titers. Although volumetric productivity has indeed risen—thus enabling cell culture processes to shrink—large volume bioreactors are not going away.

That was the basis of ABEC's decision to develop its 3,500 L Custom Single Run bioreactor, which the company released in late 2015. ABEC has seen "strong interest" in the product, which is nearly twice as large as the next-most-voluminous biobag.

"There's a sound argument for having single-use systems of that size, especially from the cost-of-goods sold perspective—getting more process volume from a single operation," insists Kevin Caffrey, strategic business development manager at ABEC. "Larger vessels allow you to enjoy economies of scale."

Issues around mixing and aeration have kept many end-users away from larger single-use systems, but Caffrey claims ABEC has resolved those issues by incorporating agitator and gassing systems similar to those used on ABEC's stainless-steel systems.

The high-productivity/small-volume argument hits at the core of ABEC's traditional market strategy. One might ask if the recent foray into plastic might undermine the company's stature as one of the world's largest suppliers of stainless-steel bioprocess equipment.

Caffrey brushes the question aside: "Despite our huge installed base of stainless bioreactors, we're offering single-use equipment because our goal is to provide customers with the best cell culture vessels for their goals, and doing so requires being uniquely agnostic with respect to materials of construction."