Trends in Perfusion Bioreactors:

Will Perfusion Be the Next Revolution in Bioprocessing?

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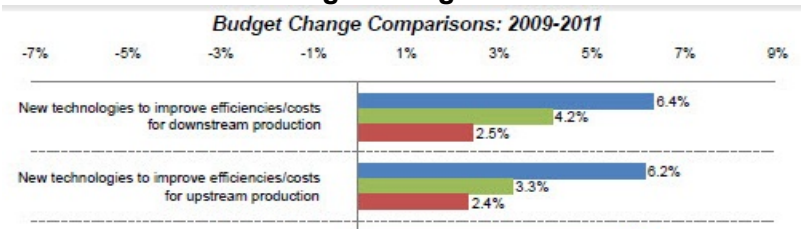
Single-use/disposable bioprocessing equipment has come to thoroughly dominate pre-commercial biopharmaceutical production in just a decade. Yet, even with these breakthroughs, performance and cost pressures on biopharmaceutical facilities continue to grow. Demands for greater productivity, more efficiency, and lower costs are resulting in unrelenting push for upstream improvements. Some in the industry are predicting that perfusion bioreactor technologies may be the next revolution in bioprocessing. Perfusion may possibly become a dominant single-use bioreactor technology, with fed-batch systems taking the position as a well-proven but legacy technology. As the technology evolves, researchers are finding perfusion may offer a variety of cost and performance advantages.

Data from the 2011 8th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production reports results from 352 bioprocessing professionals, and shows that biopharmaceutical companies have uniformly increased their budgets in essentially all areas related to bioprocessing (1). Survey data are also indicating that industry professionals are becoming impatient with a seeming relative lack of innovation in bioprocessing equipment, notably bioreactors, and that much of the industry remains aware of recent advances in perfusion bioreactors. The study shows the industry is both seeking improvements in productivity, and aggressively increasing budgets to invest in, and adopt new technologies, particularly in the area of up- and downstream bioprocessing (see Fig 1).

Figure 1: Selected data: Average Change in Biomanufacturers' Budgets



LEGEND
Avg Budget Change 2011 (BLUE)
Avg Budget Change 2010 (GREEN)
Avg Budget Change 2009 (RED)

Significant advances in clinical- and commercial-scale bioreactors in recent years, have taken place, especially in single-use/disposable bioreactor-based systems. Nearly all of these single-use innovations have involved fed-batch bioreactors, with most involving plastic bags/liners being dropped within a conventional fed-batch stainless steel bioreactor. Most larger bioreactor manufacturers have made substantial investments in this 1st-generation bag-in-a-bioreactor approach, including constructing new bag-making facilities. While the approach works, some critics perceive a lack of motivation to develop novel alternative technologies.

Our annual study data indicate that the industry wants more innovations in bioreactors. Single-use bioreactors ranked along with purification as the areas where bioprocessing innovations were reported as most needed, with 29.2% of respondents citing bioreactors. Bioreactors were also reported as taking up the single largest portion of companies' average ~$1 million 2011 bioprocessing budgets. This includes an average of $168,000/year for purchase of single-use bioreactors, with CMOs reporting spending an average of $260,000 and product developers reporting $147,000. Interest in new and improved bioreactors also differed between regions, with the highest interest, 32.2%, in Europe vs. 26.9% for the U.S. and 31% for rest-of-the-world respondents. Among separately surveyed suppliers/vendors, single-use bioreactors were the area where the largest portion, 40.5%, reported working on new technologies and products. Thus, innovations in bioreactors can be expected sooner or later. Although most major players do not discuss their R&D, it can be presumed that most established bioreactor manufacturers are at least considering, if not actively working on perfusion bioreactors.

Perfusion Technology Advantages

In contrast with fed-batch bioreactors, where cells are cultured in media-filled bioreactors and harvested in batches, e.g., after 8-21 days, perfusion bioreactors involve continuous culture and feeding and withdrawal (harvesting) of spent media generally for much longer periods, even months. Cells are held within these bioreactors by either 1) being bound and growing on capillary fibers or other membranes or 2) retained in the bioreactor though use of special filtration systems, such as Xcellerex's centrifugation or Refine Technology's filtration units that can be added to most stirred-tank bioreactors (2).

Unlike batch fed systems, with perfusion there is no accumulation of waste products. Expressed proteins are rapidly removed and available for purification; a significant advantage with proteins prone to instability. The cost of a batch failure with perfusion also tends to be much lower. If contamination is found, earlier unaffected harvests are still usable; and if contamination occurs early, then relatively small amounts of media and effort are wasted. Conversely, the whole batch must be discarded with a fed-batch process. Once established, bioprocessing in perfusion bioreactors can in many cases, be simpler and experience fewer failures. Also, in perfusion systems with cells bound to a solid substrate, cells grow more naturally and with less traumatic mixing/agitation and shear. This allows perfusion to provide recombinant proteins/antibodies that are purer, more like native proteins and more consistent in their biological activities than fed-batch bioreactors, such as having fewer variations in glycosylation.

Perfusion bioreactors are not new

Companies such as Endotronics, Cellex, and Biosyn manufactured these back in 1970s and 80s, particularly for hybridoma-based monoclonal antibody manufacture. However, advances in recombinant monoclonal antibody technology and its predominant use with fed-batch bioreactors led to the demise of these companies. The few perfusion bioreactors available in recent decades continued to be used mostly for specialized purposes, such as culture of stem cells and as feeder bioreactors. But in recent years, interest in perfusion has increased, with an increasing number of perfusion bioreactors being marketed, including for large-scale commercial product manufacture.

Among the major advantages of perfusion over fed-batch bioreactors is their smaller scale/size due to their culturing cells at 10x-30x concentrations compared with fed-batch bioreactors, with some operating at 100x or even higher. This size reduction makes perfusion more amenable to single-use applications. Thus, a disposable 50 L perfusion bioreactor could produce the same amount of product as a 1,000 fed-batch bioreactor. With perfusion, less equipment supporting space, utilities, and labor are generally needed compared with fed-batch bioreactors; thus, perfusion can provide considerable cost savings. Also, culture in perfusion bioreactors can be more precisely controlled, which can provide further advantages. Large-scale perfusion bioreactor systems can be expected to involve less upfront and operating costs than comparable output conventional batch-fed bioreactors. While most companies with biopharmaceuticals

currently in development are likely planning for commercial manufacture using batch-fed bioreactors, recent innovations in perfusion could well result in increased adoption of perfusion bioreactors for commercial product manufacture for products in the pipeline.

Perfusion in the Marketplace

New perfusion bioreactors and technologies are being rapidly introduced. Perfusion bioreactors are now available from companies including FiberCell, ZellWerk (Glen Mills in the U.S.), Biovest, ATMI, PBS/Refine, AmProtein, Xcellerex and Wave Biotech. Some systems involve filtration to retain suspended cells in the bioreactor, while others are more like original perfusion bioreactors and involve cells bound to capillary fibers or membranes. These solid substrate systems can offer advantages over filtration systems added to conventional bioreactors, including higher cell densities, lower or zero shear, lower apoptosis rates and contaminating cell degradation products. Further, as with the FiberCell system, hollow fibers can selectively remove and concentrate the desired product. Companies such as Refine Technology offer filtration systems that can be added-on to many or most stirred-tank bioreactors, so even fixed stainless steel bioreactors can be adapted to perfusion.

Despite using a smaller bioreactor, perfusion generally provides quantities of product comparable to batch fed manufacture. For example, CMC ICOS Biologics (Bothell, WA), a CMO offering both perfusion and batch-fed manufacture, has reported studies comparing the two methods, with these clearly favoring perfusion in many respects (3). However, despite its advantages, perfusion has yet to capture significant market share and cut into the market for fed-batch bioreactors.

Industry Perspectives Remain Out-Dated

BioPlan survey data has shown that industry perceptions and attitudes towards perfusion remain dated and rooted in the past. Given a list of 17 problems commonly encountered in bioprocessing, respondents consistently rated all of these as significantly more serious concerns with perfusion vs. batch-fed systems, with this often contrary to the state-of-the-art. This includes concerns about:

- Process complexity (66.1% indicating greater concern involving perfusion vs. 4.9% for batch-fed)

- Contamination risks (63.9% perfusion vs. 3.8% batch fed)
- Ability to scale-up process (60.7% vs. 8.2%)
- Need for greater process control (56.3% vs. 3.3%)
- Cell density problems (31.7% vs. 21.9%)
- Accumulation of wastes (33.9% vs. 24%)
- Product quality (37.2% vs. 18%)
- Product concentration (38.3 vs. 18.3%).

However, current perfusion systems, incorporating decades of incremental improvements (culture media, genetic engineering, membranes and filters, methods for oxygenation, pumps, single-use equipment, process monitoring, etc.), have resolved many of perfusion's prior problems, including high failure rates. Industry professional attitudes towards perfusion will likely change as more vendors bring these up to world-class cGMP manufacturing scale and quality, and as more perfusion systems are adopted starting with smaller-scale manufacturing. Eventually, smaller, less expensive perfusion facilities involving much less space, utilities and labor may replace large-scale fed-batch bioreactors, both fixed stainless steel and single-use, for commercial product manufacture. Already, perfusion bioreactors can often meet or beat comparable fed-batch bioprocessing on the basis of productivity, capital requirements, speed of manufacture and other factors directly affecting the bottom line.

Today, marketed recombinant proteins/mAbs are reported as manufactured using perfusion bioreactors, including Kogenate (Factor VIII) from Bayer Schering; ReoPro (anti-platelet mAb and Remicade (tumor necrosis factor mAb) from Centocor/J&J; Campath (CD52 mAb) from Genzyme/Sanofi; and Xyntha/ReFacto (a modified Factor VIII) from Wyeth/Pfizer, with most of these approved in the 1990s (5). Thus, although some regulatory issues remain, adoption of more advanced perfusion technology should not involve serious regulatory issues. In the present environment, with regulators demanding increased assurance of safety, and companies perpetually seeking cost-savings, perfusion may eventually become the preferred technology for manufacture of biopharmaceuticals where productivity, consistency and costs are among the primary concerns.

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Survey Methodology: This eight in the series of annual evaluations by BioPlan Associates, Inc. yields a composite view and trend analysis from 352 responsible individuals at biopharmaceutical manufacturers and contract manufacturing organizations (CMOs) from 31 countries. The methodology also encompassed an additional 186 direct suppliers (vendors) of materials, services and equipment to this industry. This year's survey covers such issues as: current capacity, future capacity constraints, expansions, use of disposables, trends and budgets in disposables, trends in downstream purification, quality management and control, hiring issues, employment and training. The quantitative trend analysis provides details and comparisons by both biotherapeutic developers and CMOs. It also evaluates trends over time, and assesses differences in the world's major markets in the U.S. and Europe.

References:

1) Langer, E., 8th Annual *Report and Survey of Biopharmaceutical Manufacturing Capacity and Production: A Survey of Biotherapeutic Developers and Contract Manufacturing Organizations*, BioPlan Associates, April 2011, 490 pages.

2) Whitford, W.G. and Cadwell, J.J., "Interest in Hollow-Fiber Perfusion Bioreactors Is Growing," *BioProcess International*, Oct. 2009, p. 54-63.

3) Carsten, J.A., et al., "Perfusion: Jeopardy or the Ultimate Advantage," CMC Biologics, 2009, online at www.cmcbio.com/perfusion.pdf.

4) Rader, R.A., *BIOPHARMA: Biopharmaceutical Products in the U.S. and European Markets*, BioPlan Associates, database online at http://www.bioplanassociates.com/publications/pub_bpuseu.htm.