Causing a Stir

Advanced benchtop bioreactors can harmonise unit operations between development and production while supporting the aims of quality by design. With a wide variety to choose from, understanding the crucial factors in the choice is key to making the most of this technology.

Kathrin Schmale and Karl Rix at DASGIP

Biopharmaceutical markets have been growing steadily over the last two decades. Global sales are expected to increase to $167 billion by 2015; between 2009 and 2016, revenue growth is predicted to increase at a compound annual growth rate of 11.2 per cent (1).

Biologics are now poised to reach revenue parity with small-molecule drugs. In 2000 just two of the top 20 pharmaceutical products sold in the US were biologicals; today six protein drugs make the list. A study by EvaluatePharma predicts that by 2016, 11 of the 20 leading biologicals sold in the US will be biologics, including four of the top five. Within the leading five products, biologicals will comprise 81 per cent ($32.4 billion of $39.9 billion) of sales (2).

The high potency of biopharmaceuticals, and their potential for acting on disease targets beyond the reach of traditional small molecule drugs, gives the biotech industry huge scope for future growth (3). But to fulfil these expectations, the biotech industry must reduce development costs, minimise late-development failures, expedite the discovery of new targets and molecular classes, and accelerate process development.

The US Food and Drug Administration's (FDA) Quality by Design (QbD) initiative offers guidance for easing the regulatory burden associated with drug approvals by streamlining overall operations, shortening drug developmental times and optimising labour utilisation.

The term 'quality by design' was first coined by US management consultant Joseph Juran. The FDA's definition, derived from ICH-Q8, states that: "Quality by design means designing and developing a product and associated manufacturing processes that will be used during product development to ensure that the product consistently attains a predefined quality at the end of the manufacturing process" (4).

Although QbD's benefits are primarily realised during manufacturing, its impact reaches far back to the earliest stages of product development. Design of experiment, predictive scale-up models, and process analytics during development all fall under the QbD philosophy.

Bioprocess Development Tools

Initial bioprocess development involves cell line optimisation, clone selection, and screening for media, feed components and strategies, as well as other process conditions. Shake flasks, the most common vessels used in early cell and microorganism work, have served the biotech industry well over the decades, but their limitations for optimising cell culture or fermentation conditions are well known. Shake flasks allow control of temperature, ambient gas mix and agitation rate, but industry standard monitoring and controlling of critical process parameters such as pH, dissolved oxygen (DO) and feed schedules are beyond the capabilities of these vessels.
Yet these critical factors influence cell behaviour, viability and productivity, and ultimately product quality and stability.

By operating within such broad design space variability, process developers may easily miss cues suggesting the superiority of one clone over another, or the influences of media, feed and supplementation strategies – factors that have been instrumental in improving volumetric productivity over the last decade. Selecting suboptimal clones during early development when using shake flasks is not uncommon, and diminished cell productivity and product quality then persist through development and beyond.

Equipment used during screening should mimic the physical and mechanical characteristics of production-scale reactors to the greatest degree possible, to ensure consistency throughout development phases. Ideally these best practices will support the aims of QbD: that quality measures initiated during development carry forward and are manifested in product quality.

Advanced benchtop bioreactors have the potential to address process consistency and harmonise unit operations between development and production. Today's state-of-the-art benchtop systems use

Parallel benchtop bioreactor system for cell culture

Image: DASGIP AG

sensors and information technology control, monitor, and record critical process parameters such as temperature, pH, dissolved oxygen and agitation. As in production-scale bioreactors, gassing and feeding proceed according to defined settings. Some benchtop bioreactors even incorporate process analytics for monitoring off-gases, nutrients, biomass and other parameters in real time.

The good news is that several vendors now manufacture benchtop bioreactor systems (generally less than 10L) that are virtually indistinguishable, except for size, from industrial production-scale bioreactors. Identical aspect ratios, for example, allow for the calculation of hydrostatic pressure and oxygen solubility while similar agitation systems ensure comparable fluid dynamics, mass transfer and mixing. Development scientists can now predict the cell growth and product kinetics of 2,000-litre bioreactors from benchtop results.

The bad news is that, in practice, development projects involving clone selection and expansion, media/feed strategies, cell line improvement and microorganism screening impose a huge bioreactor burden: most benchtop bioreactors fail to provide the breadth of design space required for conducting multi-parameter experiments.

Bioreactor scale is critical for gleaning as much information as possible from development-stage bioreactors. Usually vessel size is defined as micro-scale (less than 1mL), mini-scale (1mL-500mL) and benchtop-scale (0.5L-10L). Pilot and manufacturing scales generally run at 10-100L and above 100L, respectively, although specific volumes may differ considerably depending on the product.

Benchtop bioreactors typically provide working volumes of two to three litres and occupy large benchtop areas. Space constraints limit using such bioreactors in highly parallel screening steps. Moreover, bioreactors of this size are sub-optimal for rapid clone and cell line screening, or even media development, due to their considerable consumption of valuable materials.

On the other end of the size spectrum are microtiter plates and sub-millilitre-sized parallel bioreactor systems, whose limitations are readily apparent. Micro scale systems enable a high degree of parallelism. Many experts believe that these systems are suitable for basic screening experiments, but not for serious process development work. Dr Frank Baganz at University College London has illustrated the direct relationship between bioreactor size and the quality of data they generate: as size diminishes, so does their ability to generate meaningful process data (5).

Merck Research Institutes has defined the optimal small-scale bioreactor system for process development as a parallel bioreactor system with a minimum of 24 individual bioreactors, each of about 100mL working volume, with dual capability for mammalian culture or microbial fermentation, and automated sampling (6). Only a few commercial systems meet these criteria for mini-scale bioreactor systems.

Factors to Consider when Selecting a Bioreactor

Miniaturised benchtop bioreactors come in a variety of configurations and data-handling capability. The principal factor in evaluating these systems is the level of predictability between laboratory scale and pilot/product scale. The following specific factors should be considered when evaluating benchtop systems:

Size
Vessels range in size from tens of microlitres to several hundred millilitres. Dimensions must allow for mixing, gas exchange, sampling without depleting the vessel and control features. Aspect ratios should be similar to larger vessels to confer a high degree of predictability to larger-scale experiments.

Process Parameters
The monitoring of process parameters (PAT) under QbD-based process development should parallel anticipated later-stage in-process analytics. Developers must identify which parameters are useful *a priori* for quality monitoring, particularly those that are predictive for larger processes, and seek those capabilities in mini-scale systems. Users should be aware of the integration of lab devices – the capability of software to connect laboratory equipment, including bioreactor systems and analytics.

Autosampling
This ability frees experimenters from manual extraction of samples from the mini-scale bioreactor and injection into analytical instruments. Note that 'complete' automation may be undesirable since it does not duplicate real-world unit operations at larger scale.

Parallism
A system should permit testing of a reasonable number of critical parameters simultaneously, but not so many to necessitate over-miniaturisation, which results in data overload or

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incompatibility as vessel sizes become miniaturised to the extent that control of process parameters is inhibited, or is not predictive of larger-scale systems. Also, mini-bioreactors should be independently controlled, at least for critical parameters.

Single-Use Vessels
As in biomanufacturing, single-use bioreactor vessels incur an ongoing cost but reduce time between runs and eliminate cleaning, autoclaving, and associated validation.

Computerisation
This includes control over individual conditions (temperature, DO, pH and so on), as well as data acquisition, connectivity to the IT backbone (for example, supervisory control systems and data historians), report generation, data mining, and support for methods of storage and development. Related considerations are user interface and ease-of-use.

Direct Scale-Up Support
The major objective of 'scaledown' experiments is to predict behaviour at large scale from small-volume cultures. Systems that lack this predictive capability are in no way superior to shake flasks. Factors to consider are the mini-bioreactor's form factor (and whether it resembles that of a conventional bioreactor), stirring, feeding, monitoring and sampling mechanisms, and control features.

Footprint
Benchtop miniaturised bioreactors should occupy a footprint that is convenient in terms of maintenance, operation, servicing and workflow. Users should understand the trade-offs between individual reactor size, footprint, throughput and data quality.

Modularity
Software should enable the use of two or more mini-bioreactor systems, and provide seamless connectivity with respect to data collection, mining and transfer.

Upgrade Path
Users should be aware of potential improvements that might add monitoring and control functionality.

Direct Connectivity with Instrumentation
Real-time 'in-line' process monitoring by advanced instrumentation (LC, MS and so on) is still rare for large-scale bioprocessing. However, some benchtop systems are beginning to incorporate limited monitoring, for example of small-molecule products by mass spectrometry with integrated feedback control.

One other factor, mini-bioreactor control, merits a separate discussion. Today's manufacturing-scale bioreactors incorporate advanced controller functions (hardware and software). A few parallel benchtop bioreactor systems employ these control methodologies

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as well, both for generating high-quality data during development and for streamlining and compressing development workflows.

OLE for process control (OPC) communication is the buzzword within this context. OPC allows the seamless interaction of standalone laboratory devices such as analysers (HPLC, cell-counting, mass-spectrometry and nutrient analysis), auto-sampling and liquid handling.

Integration of proper software tools supports design of experiment (DoE) according to QbD standards and offers data mining for effective evaluation of process data and predictive exercises. Integration will conserve material and labour and consequently lower developmental costs. Interconnectivity to company-wide process control systems and corporate historians in turn provides documentation to support regulatory filings.

Conclusion

The biopharmaceutical market is steadily growing and is highly competitive. As patents expire, 'innovator' companies will experience competition, not only from their peers, but from biosimilars. Organisations that streamline drug development will be rewarded with lower development costs, shorter timelines, and perhaps lower cost of goods. These competitive advantages do not require discovery of new molecular targets or molecular classes, or the introduction of radically new process equipment or unit operations: these efficiencies already reside within companies with process development capabilities.

Advanced benchtop bioreactors with predictive capability with respect to scale-up have changed the way biomanufacturers standardise and streamline process development. These systems, which duplicate all aspects of large-scale fermentation and cell culture, offer comprehensive data- and information-management tools to support regulatory requirements for both filing support and QbD-driven process development.

Potential purchasers of miniaturised benchtop bioreactors would do well to create a check-list of factors related to their workflow, regulatory and scientific needs. This will help them assess, when viewing the product landscape, which factors specific systems are capable of satisfying. These features and requirements will differ according to the end-user's current and anticipated process development needs.

Leading suppliers of benchtop bioreactors are diligently improving the functionality and predictability of their miniaturised systems. Within the next few years we can expect a broader range of equipment designed for screening and early process development, particularly within the volume range between micro- and benchtop scales.

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About the authors

Kathrin Schmale has nine years of experience in biotechnology and joined DASGIP as Strategic Marketing Manager in 2010. Kathrin received her PhD in Biology at the Fraunhofer Institute for Molecular Biology and Applied Ecology, Aachen, Germany, where she focused on bioreactors and fermentation technology. Prior to joining DASGIP she worked in sales and global product management, the latter role at Sartorius Stedim Biotech.
Email: k.schmale@dasgip.de

Karl Rix is CEO at DASGIP BioTools, LLC in the USA and Director of North American Operations at DASGIP AG in Germany. Holding a doctoral degree in mechanical engineering Karl has more than 15 years of knowledge focusing on the application of advanced process technology. **Email: k.rix@dasgip.de**