

Feature Articles : Apr 1, 2016 (Vol. 36, No. 7)

Continuity Promotes Bioprocessing Intensity

Moving From Batch Mode to Continuous Mode Concentrates Bioprocessing

Angelo DePalma, Ph.D. **Wonderfully**

Just five or six years ago, bioprocessors viewed continuous bioprocessing skeptically. Now, seemingly, almost everyone is interested.

For example, fully continuous bioprocessing has become a top priority at Pall, which maintains a continuous processing laboratory in Westborough, MA. The company has also demonstrated its commitment to continuous bioprocessing through significant acquisitions of critical technology.

To enhance its downstream processing capabilities, the company has acquired Tarpon Biosystems' BioSMB (simulated moving bed) multi-column chromatography system. For upstream processing, the company has purchased the LifeSciences business of ATMI, which includes the iCELLis[®] Bioreactor for adherent cell culture.

To bridge the gap between the bioreactor and downstream processing, Pall has purchased an exclusive license to acoustic wave separation (AWS) technology from FloDesign Sonics. AWS, which uses acoustics to trap and collect cells as they pass through a flow channel, complements Pall's own line of depth filters.

Continuous bioprocessing capabilities may be introduced along segments of processing lines, resulting in hybrid systems that accommodate continuous and batch processing. In hybrid systems, however, it is still necessary to harmonize upstream and downstream operations. For example, in a process architecture that combines batch upstream and continuous downstream operations, it is important to match mass capacities between harvest and subsequent steps. Within continuous processing segments and in fully continuous processes, aligning volumes is more critical.

Consider the integration of perfusion cell culture, which generally involves dilute feed streams, which means high buffer volumes—lots of media. High buffer volumes tax purification trains and counter the philosophy of continuous processing. "Buffers are the biggest thing moving into and out of continuous processes," says Michael Egholm, Ph.D., Pall's president of biopharmaceuticals.

To solve the problem of dilute feed streams, Pall uses an inline concentrator—a simplified version of tangential

flow filtration—which provides two- to fourfold concentration. Buffer usage also drops, but by how much depends on eventual optimization. Bottom line: By the time a batch reaches the capture column, the volume of a 25 L bioreactor is reduced to 5 to 10 L.

Small process volumes are tailor-made for continuous chromatography. Whereas conventional columns are sized according to the anticipated mass or yield, resin volumes in multi-column formats (as in BioSMB-simulated moving bed chromatography) are based on process volumes. Providing volumes remain constant, no surprises will derive from any titer increases that should occur.

Resins are underutilized in standard chromatography, limited to occasional (on the time scale of a purification) binding and elution. “In continuous mode, every grain of resin is occupied for the entire run, which makes possible scaling down and easier linking of unit operations,” Dr. Egholm explains. “In continuous mode, balancing the production line becomes critical.”

Specifically, volumes for column elution, virus inactivation, polishing, diafiltration, virus filtration, sterile filtration, etc. must be comparable. Balancing is invaluable for connecting unit operations, which is a cornerstone of continuous processing.

Regulatory Issues

Regulatory concerns will be different, but not necessarily more onerous, for each unit operation in continuous mode. Yet companies must think more deeply about bioburden, which causes filters to fail. They must also assure that product on day 27 is the same as on day 17, which is not an issue in batch mode. Industry-standard thinking regarding the very definition of “batch” must change as well. In many instances, bioreactors and separations are no longer discrete events.

“We believe these issues are solvable,” Dr. Egholm declares. “Regulations are a barrier, but they will be overcome as well. We have engaged the FDA and our industry in a very transparent manner.”

The history of disposable bioprocessing suggests this will take time, which is okay since adoption of continuous bioprocessing will also take time, and is more likely to be evolutionary than revolutionary. “But we’re already quite far on our journey,” asserts Dr. Egholm.

How continuous processing will alter the industry’s view of process analytical technology (PAT), quality by design (QbD), and quality in general is what Dr. Egholm calls “the 64 million dollar question.”

Pall’s development strategy involves addressing less-risky downstream processing first. “We obviously must work with the FDA to determine everything that can possibly go wrong, and verify that they’re not going wrong,” states Dr. Egholm. “So most of the emphasis should probably be placed on QbD since fully characterizing antibodies as they’re produced is impractical and not very meaningful. A greater need for PAT may be needed as well, particularly for bioburden monitoring and control.”

Flexibility and Facilities

Continuous processing, process intensification, and process miniaturization go hand in hand in hand, and their interplay will affect how tomorrow’s biomanufacturing facilities are built. As processes become smaller and more

efficient, facility planners must rethink the traditional, says Maik Jornitz, president of G-CON Manufacturing. G-CON specializes in PODs, which are self-contained, autonomous, and mobile cleanroom units, each with a separate HVAC system.

“Single-use process technology is excellently flexible, but its benefits are thwarted by existing cleanroom infrastructure,” Jornitz says. Using disposable manufacturing involves moving a lot of equipment around. PODs are designed to facilitate moving from one operation to the next, and bringing equipment in and out or transferring product via aseptic connection from one POD to the next.

“Autonomous” relates to the cleanroom’s HVAC system itself and says nothing about the potential connectivity among unit operations. The value of self-containment becomes obvious when one considers recent contamination issues at large bioprocessing facilities.

“If the PODs’ air-handling capabilities were connected and contamination occurred in one cleanroom, other cleanroom areas are immediately affected. ‘Autonomous’ means you can continue working if one POD needs to be shut down,” Jornitz explains. “This POD may be cleaned and sanitized with vaporized hydrogen peroxide while the other POD units continue to function. PODs are essentially walk-in isolators.”

Jornitz predicts that with the adoption of PODs, autonomous cleanroom clusters will spring up in shell buildings that will be capable of producing multiple products. “That’s possible,” Jornitz asserts, “because the cleanroom infrastructures are not connected.” Were they connected, he argues, serious contamination would potentially force the entire facility to shut down. Cleaning large, complex ductwork is almost always impossible without a general facility shutdown.

The future evolution of bioprocessing and multi-product facilities will cause their paths to cross at what Jornitz calls “the crossroads of processing and facilities,” whereby process equipment become less multiple-use and facilities more so. “Multiple product facilities are the wave of the future,” Jornitz predicts, “even for large biomanufacturers.”

Building a single-product facility in a relatively small country makes no sense based on capital expenditures and ongoing operational costs. “But constructing a flexible, multi-product plant in such a location makes perfect sense,” Jornitz insists.

Process Intensification

A key benefit of perfusion cell culture is process intensification, which plays deeper than simply running continuously or eliminating steps: It encompasses intensifying bioreactor performance itself, a consequence of large increases in cell density.

This conclusion was reached, for example, by Genentech, which has published information about about large-volume, high-density cell banks that had been produced by growing cells with the help of a cell-retention device. Perfusion-produced seed banks take a bit longer to grow than conventional inoculant, but because of the cell density fewer bioreactors are required in the seed train process. Additionally, the production bioreactor begins at much higher cell density.

“It’s possible to go directly from a 20 L seed reactor to a production-scale vessel,” says John Bonham-Carter, sales director at Repligen. This idea applies to seed trains for fed-batch processes as well. “It can provide a significant boost in output for an existing facility with very low capital investment.”

Inoculating at higher cell density translates to shorter production times and more slots from that bioreactor per year, hence greater utilization of capital equipment. The final process from that day forward is identical, harvest occurs at exactly the same point, and product quality is the same, but the final production process is shorter. “You might expect a boost of 20 to 40% for an existing facility,” Bonham-Carter adds. “It’s perfusion, but not as you know it.”

This is one reason the term “perfusion” may be misleading. Perfusion implies a product is emerging on the permeate side, whereas in the seed train the cells are the product. For seed-train improvement, Bonham-Carter therefore prefers “process intensification in the seed train using a cell retention device.”

Upstream-downstream connectivity presents additional opportunities for intensification. For example, perfusion bioreactors can generate a 0.2 μ filtered cell-free product harvest, so depth filtration or centrifugation are eliminated, and the process fluid is ready for the first chromatography column. The productivity figures previously mentioned— up to 3 g/L/day—are appropriate for columns without concentration. However, as a risk-mitigation option, bioprocessors can install a storage tank between bioreactor and column to handle surges or variations in flow.

Perfusion processes have been shown to be highly productive compared with tradition fed-batch processes, thereby driving down bioreactor volume requirements and enabling the use of single-use bioreactors that facilitate local manufacturing trends and the concept of “scaling-out” versus “scaling-up.”

The benefits of perfusion cultures are not limited to fully continuous operation. “Perfusion is applicable to high-volume/high-density cell-bank generation that can minimize seed-train time requirements, and modified inoculum trains that incorporate perfusion to further reduce train size and total upstream processing time,” observes Michael Phillips, Ph.D., director of bioprocess development at MilliporeSigma. The current weaknesses of perfusion typically revolve around the lack of a turnkey process that includes full control.

Downstream Operations

Downstream, the leading technologies supporting continuous operation include continuous multi-column chromatography, in-line viral inactivation (low pH and/or detergent), flow-through polishing trains, and single-pass filtration. These technologies are needed for intermediate concentration and buffer conditioning applications as well as final formulation applications.

“Since the above-mentioned approaches are typically engineering enhancements of existing technologies,” says Dr. Phillips, “many current process development and manufacturing personnel are comfortable with their adoption for solving existing facility-fit and process bottleneck issues encountered during batch processing.”

Additionally, the adoption of these technologies to support batch operation is expected to help drive the transition from batch to fully continuous operations, which Dr. Phillips calls “an evolutionary transition to a revolutionary

paradigm change.” However, improvements in system design, sensor technology, and process integration and control are gaps that must be addressed in order to achieve true continuous processing.

Other downstream operations ripe for operating continuously are tangential flow filtration for buffer exchange or concentration, and final virus reduction filtration via nanofiltration. Virus filtration filters are placed in-line, and when the first one plugs, the flow is directed through a second parallel filter.

But the so-called midstream operations involving primary recovery are still problematic, says Parrish Galliher, chief technical officer, upstream, GE Healthcare Life Sciences. “The ability to filter or centrifuge cell mass is not entirely predictable for today’s very high density cultures. That’s another example of the variability of biological systems—this time leading to midstream inconsistencies. That’s why this step is often done in batch mode.”

Clarification is not an issue downstream of perfusion reactors using filtration. The filtrate from the perfusion filter is cell free, and it can load continuously and directly onto a continuous chromatography system.

Boosting Monoclonal Antibody Productivity

According to Novasep, there is a growing interest in developing alternative processes that incorporate continuous manufacturing capabilities. Such processes can rapidly adjust production capacity, reduce the cost impacts associated with increased competition from biosimilar products, and integrate downstream processing operations with ongoing upstream improvements.

A Novasep official says the company’s BioSC Lab, a low-pressure continuous chromatography system, and a smart simulation software program called BioSC Predict were designed to meet these needs. Alain Lamproye, president of the biopharma business unit at Novasep, cited a study carried out by the company where a monoclonal antibody purification process based on two continuous chromatography steps (capture and intermediate ion exchange) was developed and optimized using batch data.

“Significant gains were observed on productivity, on buffer consumption, and on the volume of resin required,” pointed out Lamproye. “This process demonstrated significantly better performance compared to the batch equivalent while reaching the qualitative specifications. Specifically, the productivity was multiplied by four, and the chromatography step time was divided by four.”

He added that similar process platforms could also be used for vaccines and recombinant protein products.