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Sartorius Takes a Single-Use Approach to Derive Value from Biomanufacturing

By K. John Morrow Jr., PhD

Some of the key challenges facing the biotech industry were examined recently at a Sartorius Stedim Biotech (SSB) Conference in San Francisco. Miriam Monge, head of the mAb and recombinant protein market segment at SSB, and chair of the conference, emphasized four critical issues: a pressing need for cost reduction; the importance of flexibility in the design of facilities; meeting the demands of speed to the clinic through development and tech transfer; and precise measurement of product quality in order to meet the demands of the FDA and other agencies.

Weichang Zhou, PhD, senior vp at WuXi Biologics, gave the keynote address and discussed his company's development of an accelerated timeline, down to eight months from 25, for producing biologics in high quantities. By employing 2,000 L disposable bioreactors they achieved productivity that was comparable to 20,000 L stainless steel bioreactors, resulting in significant reductions in cost of goods. The platform is based on continuous cell culture and continuous direct product capture and can be scaled up to generate clinical levels of product. According to Zhou, "Scale out strategies provide an effective solution to meet product demands during product life cycles and eliminate cell culture scale up risks."

Process intensification

Gerben Zijlstra, PhD, global tech expert at SSB, noted that "process intensification enables the use of flexible and low-cost single-use facilities for medium to high output commercial manufacturing." To optimize cell output, the Sartorius Cellca Cell Line Generation team employs ambr® systems, and has found them to be quite useful, saving time and resources in large scale screening and optimization protocols. This strategy allows selection of the optimal clone for process intensification at an early stage. In addition, the use of 100 mL cryobags as process intermediates to reduce the seed train was demonstrated successfully.

To evaluate the range of intensified cell culture approaches, including N-1 and main bioreactor perfusion, the team set up modeling experiments employing ambr 15 screening workstations fitted with centrifuge inserts. To intensify the upstream expression in the main bioreactor, high volume, high inoculation protocols from N-1 perfusion were chosen. This auspicious choice yielded a 2-fold productivity increase on a standard fed batch protocol when selecting the appropriate clone (from 4 g/L in 14 days to over 6 g/L in 10 days).

The team also adopted an intensified fed batch approach, with one volume per day of media exchange using the centrifugal containers that are available with the ambr system. This protocol was determined to be the most productive, with a high level of production of >13 g/L in

12 days (from 3 g/L in 12 days). And by identifying the best clones, cumulative titers of up to 20 g/L were achieved at 1VVD medium exchange and only around 30 million cells/mL.

Using the ambr 250 platform, both internal SSB teams as well as large biopharma customers were able to ramp up their cell numbers to over 100 x 106 cells/mL. Also, extended cultivation lengths of 30 days have been demonstrated and customers have been able to show comparable product quality results to existing small scale models (benchtop bioreactors).

"Therefore, we have found that when using this new ambr perfusion toolbox in cell line and process development, there is no longer a fundamental roadblock for efficient development of intensified, perfusion-based process platforms, in similar time frames as current fed batch process platforms," stated Zijlstra.

The overall improvements in upstream productivity were achieved by use of Sartorius tool sets for the optimization of various aspects of the intensified biomanufacturing process; the ambr development platforms, the RM and STR perfusion enabled manufacturing platforms, and simultaneous media and process enhancements. "With consistent effective titers of 10 g/L and beyond, annual outputs of greater than 1,500 kg from intensified single-use facilities can be realized," Zijlstra concluded.

Scalable single-use platforms

"The huge gains in cell culture productivity drive efforts to catch up performance at the downstream end; this is a constant, ongoing process," said Thomas Erdenberger, global tech consultant at SSB. "Today we find ourselves behind the eight ball again, so at this point we employ the tools worked out by our company to advance the speed and efficiency of the downstream performance."

The success at producing high cell densities at the upstream end means that the biomass must be removed without liberating host cell proteins into the culture medium. Erdenberger described the kSEP platform, a fully single-use, closed system with gamma irradiated consumables for all product contact surfaces. It is capable of clarifying high cell density mammalian cell cultures with low shear, minimizing cell lysis and release of host cell proteins. It is fully automated and can perform as a continuous operation during the primary recovery step. In model experiments, Erdenberger and his colleagues demonstrated cell high density clarification (108 cells/mL) with full product recovery (>95%) and no significant viability loss or cell lysis.

Erdenberger discussed the issue of viral clearance and advances made in this area, including the application of a simplified virus filtration unit with a single-use transfer assembly. This unit is pre-assembled, pre-sterilized, ready-to-use (so called "plug-and-play") and is designed for application in high intensity transfer situations.

The use of flow-through polishing for impurity removal through a two-step purification process of mAbs was also covered in Erdenberger's presentation. A high level of removal of aggregates was achieved using Sartobind S cation exchange (CEX) in a high flow-through mode. In model experiments with Murine leukemia virus there was very good LRV and removal of impurities by AEX and salt-tolerant AEX membrane adsorbers, including host cell proteins (HCPs), and DNA.

Carrying forward the discussion of continuous bioprocessing, Anna Persson, principle data scientist at SSB, emphasized that "continuous and intensified bioprocessing is attracting much attention from biopharmaceutical companies seeking to maximize the efficiency of their manufacturing assets." Benefits of this approach include smaller facilities, reduced scale up risk, more consistent product quality, and higher throughputs.

To drive the data analytical process forward, Persson takes advantage of Umetrics Suite of Data Analytics Solutions, a common, large-scale, automated platform designed for handling large quantities of data. Through the mining and modeling process, predictive power is generated, making possible large improvements in real life situations. A challenge put forward by Lonza was a record of lower than expected average yield in their facility, which made it difficult to plan work and delivery to the end customers. Employing variability analysis allowing optimization of yield and time-based multivariate data analysis of real-time process parameters increased yield and decreased the cycle time, resulting in a significant increase of plant output.

Similar strategies were put to use in a project with Amgen, in which viable cell density was improved 23% and another effort with Novartis, analyzing Out of Control Incidents, allowing improvement of process consistency.

According to Persson, multivariate analysis is essential in value generation, and regulatory agencies are now encouraging use of tools such as the Umetrics Suite. "Looking to the future, we foresee the use of mechanistic modeling and deep learning, and an expansion into hybrid modeling," Persson stated.

Moving manufacturing productivity beyond the initial facilities' capabilities requires a fundamental understanding of the needs of large bioprocessing operations, explained Priyanka Gupta, bioprocessing modeling manager at

SSB. Gupta directs a team that analyzes production processes in order to define the most cost-effective framework. They employ Biosolve software as a modeling tool to select the most significant impact on overall process economics. The team examined three industrial scenarios for production of biologics: 1500, 500, and 150 kg/yr. The analysis identified those steps with the greatest impact on overall economics, allowing outcome optimization.

Future of bioprocessing

Biopharma and, more specifically, the field of bioprocessing are highly conservative industries due to the strong level of regulation which can result in long delays in introducing new technologies. Moreover, the inherent complexity of these activities increases the drag on the process of innovation. Dan Kopec, PAT technology expert at SSB, discussed approaches to expediting technological advances in bioprocessing.

The PAT (Process Analytical Technology) toolbox is, according to Kopec, "a system for designing, analyzing, and controlling bioprocess manufacturing. This is accomplished through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes. The overall goal is to minimize time to market and the cost and risk of failure, while at the same time, maximizing process productivity, efficiency, and flexibility."

Kopec stressed the fact that today, drug approvals move forward too slowly, and the rate of approval is quite low. According to a report by the Sloan School of Business Management, only 14% of drugs in clinical trials eventually win FDA approval. There is ample evidence that a more rapid and expeditious system is badly needed in the bioprocessing industry, where a faster time and a surer path to market can be achieved through better process understanding and automation.

The application of the PAT strategy can move bioprocessing from a rigid (fixed process) approach to one that maximizes cell specific productivity and product quality. An automated, flexible feed strategy, which would be based on a combination of real-time inputs from PAT sensors. "This enables consistent high product quality," Kopec stated.

Moreover, the integrated, dynamic control provided by PAT allows a fast, predictive, and automated response to changing conditions, also freeing up operator time. These conditions can be monitored by a combination of various forms of spectroscopy, including NIR (Near-infrared spectroscopy), 2D fluorescence, Raman. UV/Vis, scattering, and dedicated sensors like bio-capacitance, nutrient/metabolite and off-gas. The benefits of this approach include data and signal integration and real time control.

"Using real-time sensors allows a much clearer picture moving from research to development to manufacturing," said Kopec.

In looking to the future of next-generation manufacturing, Kopec stated that some bioprocesses are challenging to automate. These include existing pipelines and related processes, and critical parameters/quality attributes that lack available robust sensor technologies (e.g., differentiation between proteins). "Ten years from now we anticipate machine learning, fully automated processes and advanced simulation and prediction," Kopec predicted. GEN