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PROCESS INTENSIFICATION

DRIVING EFFICIENCIES ACROSS THE SPECTRUM OF BIOMANUFACTURING

Cheryl Scott, Himanshu Gadgil, and Josh Abbott

APRIL 2024

Process Intensification

Driving Efficiencies Across the Biomanufacturing Spectrum

by Cheryl Scott, Himanshu Gadgil, and Josh Abbott

Process intensification (PI) improves manufacturing processes at unit, operational, and functional levels to increase productivity — often reducing environmental footprints as well. Continuous biomanufacturing plays a key role in speeding biopharmaceutical products to market. As legacy processes and facilities built around batch processing age out, they are giving way to modern alternatives based on single-use technology, closed operations in open "ballroom" environments, automation, and continuous processing for overall intensified biomanufacturing.

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Introduction

Cheryl Scott

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Process intensification (PI) improves manufacturing processes at unit, operational, and functional levels, often by integrating unit operations to increase productivity — and often reducing environmental footprints as well (**1**). The COVID-19 pandemic accelerated an industry-wide trend toward increasing efficiencies to speed biopharmaceutical products through clinical testing and on to marketing. Continuous biomanufacturing plays a key role in doing so, thus making process intensification part of many future-oriented biomanufacturing strategies. As legacy processes and facilities built around batch processing age out, they give way to modern alternatives based on single-use technology, closed operations in open "ballroom" environments, automation, and continuous processing for intensified biomanufacturing.

In a 2020 *mAbs* journal article, Bristol Myers Squibb (BMS) authors reported on their development of intensified monoclonal antibody (mAb) processes at 1,000-L and 2,000-L scales (**2**). On the upstream side, a Chinese hamster ovary (CHO) cell line went through an intensified seed train to increase cell densities with enriched media and perfusion-mode cultures. "The increased final cell densities at the $N - 1$ step allowed for much higher inoculation densities in the production bioreactor operated in fed-batch mode and substantially increased titers by fourfold," the authors state, "while maintaining comparable final product quality."

The development team made multiple changes to intensify the corresponding downstream process to accommodate those increased titers: "New high-capacity resins were implemented for the protein A and anion-exchange chromatography (AEX) steps, and the cation-exchange chromatography (CEX) step was changed from bind–elute to flow-through mode" (**2**). BMS used a multicolumn chromatography system for the affinity capture step and semicontinuous, integrated polishing without pooling, increasing overall productivity while "reducing resin requirements, buffer consumption, and processing times." The overall cost of goods (CoG) was reduced substantially as well. The authors state that their hybrid intensified process "lays a good foundation to develop fully continuous manufacturing with even higher productivity in the future."

More recently, authors from AstraZeneca and University College London have published a "decisional tool that encompasses mass balance and design equations, process economics, stochastic simulation, and multicriteria decision-making" that assists companies evaluating different batch and continuous modes for mAb manufacturing (**3**). The collaborators compared traditional

batch processes with end-to-end continuous operations using protein A affinity chromatography or column-free capture (based on aqueous two-phase extraction or precipitation) with economics, environmental sustainability, and process robustness in mind. CoG analysis predicted that the continuous option offers substantial cost savings (20–40%) for products with low and medium annual commercial demands (100–500 kg). The authors found, however, that either comparable (with protein A and precipitation) or higher (two-phase extraction) costs resulted for high-demand products.

"The analysis of overall process mass intensities accounting for water and consumables suggested that the continuous flowsheet with protein A would result in the lowest environmental burden," they concluded. "When the economic, environmental, and operational criteria were reconciled using multicriteria decision-making (MCDM) analysis, the continuous protein A–based flowsheet was found to be the most favorable." The final analysis highlighted a need for process improvements in certain areas to reduce the manufacturing costs associated with column-free options for continuous capture: volumetric productivity of perfusion cultures, harvested cell-culture fluid percentages, and step yields based on implementation of buffer concentrates (**3**).

Harking back to the seminal "A-Mab" case study in bioprocess development published over 20 years ago, the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) recently published its N-mAb case study "to accelerate adoption of advanced manufacturing process technologies such as integrated continuous bioprocesses for mAbs" (**4**). This represents the evolution of integrated control strategies from production of early clinical-study materials through process validation and commercial manufacturing, with the N-mAb case focusing on elements that are unique to integrated continuous bioprocesses. Intended to serve as a "teaching document," it demonstrates the biopharmaceutical industry's increasing movement toward design, development, and characterization of integrated continuous bioprocessing.

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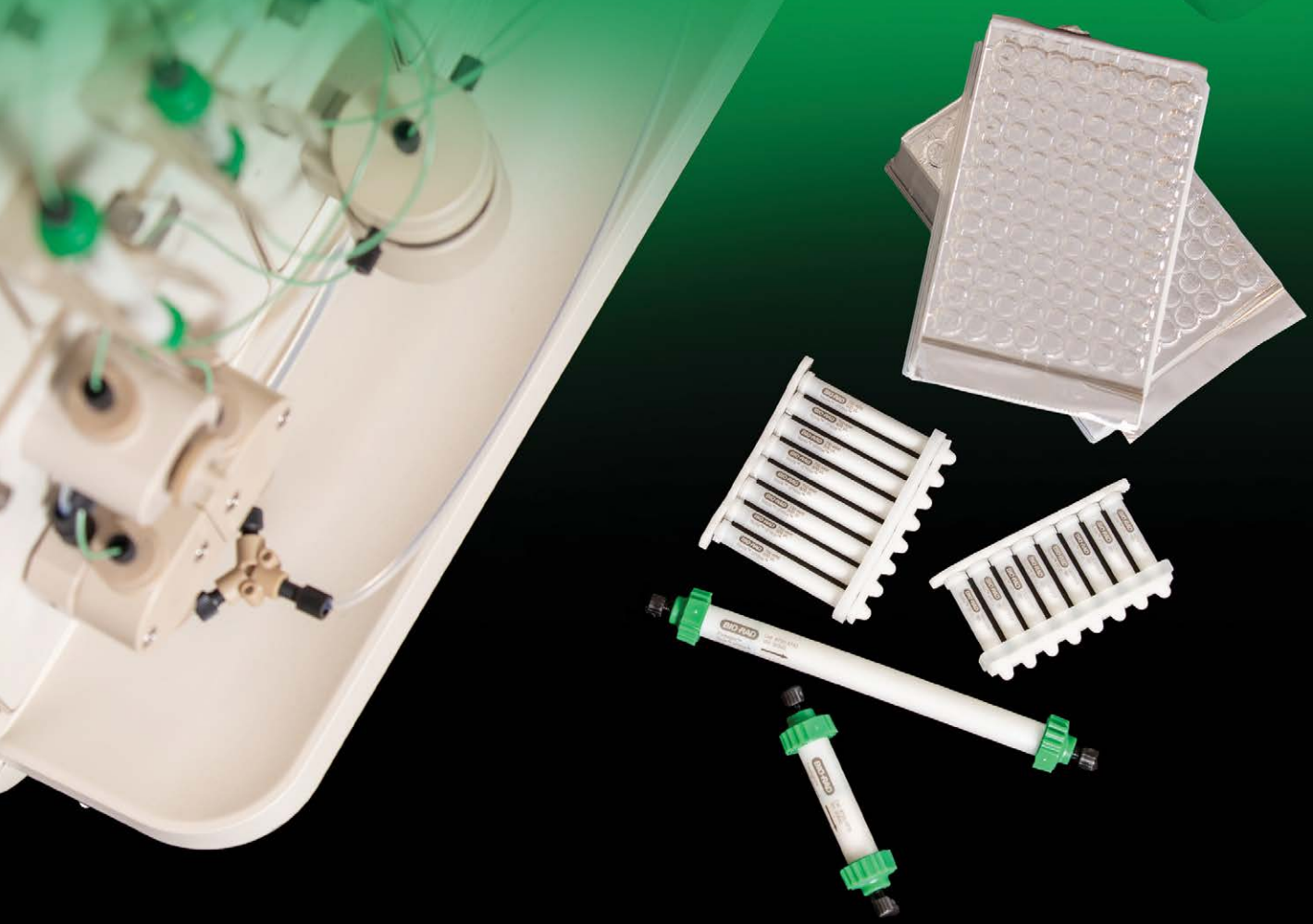

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Continuous Biomanufacturing

A Revolution in Efficiency

Himanshu Gadgil

Biologics can be revolutionary treatment options for many diseases, but affordability and accessibility challenges sometimes keep them from reaching all the patients who need them. Continuous manufacturing is a potential solution to drive efficiency in biologics production, providing improvements in cost-effectiveness that ultimately should increase accessibility. Below I explore trends that are bringing the field closer to realizing its goal of accessability and affordability through process improvements.

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Enzene operators set up a small-scale bioreactor.

Growth in the Biologics Market

Biologics have gained substantial interest and investment in recent years. Now making up a significant proportion of products in pharmaceutical development overall, biologics offer potentially life-changing treatments for many indications. About 70–80% of the top-selling drugs currently on the world market are biologics targeting diseases such as cancer, rheumatoid arthritis, and chronic inflammatory conditions (**1, 2**). Valued at US$348.03 billion in 2022, the global biopharmaceutical market is projected to grow at a compound annual growth rate (CAGR) of 6%, reaching $620.31 billion by 2032 (**3**).

Monoclonal antibodies (mAbs) such as cetuximab and bevacizumab are the leading class of biologics because they provide highly targeted treatments for cancer and other diseases. But the field of biologics has progressed with the advent of next-generation modalities such as antibody–drug conjugates (ADCs) and bispecific antibodies (bsAbs), both of which enhance the targeting capabilities of mAbs. ADCs incorporate cytotoxic payloads for highly targeted and potent treatments (**4**). Bispecifics bind to two distinct targets, which expands their clinical applications and versatility beyond those of single-target mAbs (**5**).

Affordability and Accessibility Challenges

Even as the integration of biopharmaceuticals into medical practice progresses, the expense of biologic products often represents an accessibility challenge to some patients. Biologics are complex molecules that require advanced, multistep development and manufacturing processes. That complexity makes production more challenging and expensive, increasing the costs of the final products. Biologic drug prices range from $10,000 to $500,000 per

year or even more (**6**). Such costs limit availability significantly, especially on a global scale. Healthcare systems and infrastructure differ from country to country, further complicating biopharmaceutical accessibility. Consequently, many patients are unable to receive the transformative benefits of biologic products (**7**).

This biomanufacturing suite at Enzene Biosciences integrates a single-use bioreactor system from Sartorius Stedim with continuous-chromatography equipment from Novasep.

Those affordability and accessibility challenges place biologics developers under increasing pressure to reduce the costs of their products. Meanwhile, demand for such products is rising, adding pressure to innovate. Thus, drug developers frequently partner with contract development and manufacturing organizations (CDMOs).

Innovative technologies also help to drive efficiency in biologics production, reducing the costs associated with biomanufacturing. Traditional fed-batch production is common, but its efficiency is limited, which contributes to the expense. Brocess engineers are increasingly interested in continuous culture methods as an alternative for bioproduction. Such technologies can reduce processing costs and increase productivity, thus helping to improve affordability for biologics development and manufacturing.

An Innovative Solution

Continuous biomanufacturing processes operate without breaks, automatically loading and processing materials to produce a final product (**8**). Industries producing chemicals, food, and other manufactured goods have adopted such technologies to enhance processing efficiency, minimize costs, and maintain high product quality (**8**). Recognizing the advantages of such technologies, the US Food and Drug Administration (FDA) now advocates for application of continuous manufacturing in the development and production of drug substances and finished products (**8**). However, the pharmaceutical industry has been cautious in adopting continuous manufacturing. It has yet to reach widespread use in biologics production, as many companies cite, for example, the expense and regulatory burden of making major changes to established facilities and processes.

Hesitancy notwithstanding, an entire biomanufacturing process can be performed continuously. From upstream production to downstream processing and end-to-end integration, technologies have been developed to enable continuous biologics manufacturing:

Intensified Perfusion: Continuous upstream processes incorporate single-use bioreactors and perfusion methodologies. Cells can be cultured at higher densities with intensified perfusion, which feeds fresh cell-culture media into bioreactors and removes spent media containing expressed protein products at the same

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rate (**9**). Such technologies improve process consistency and increase cell productivity while reducing levels of metabolites and other impurities (**8**).

Multicolumn Chromatography: Continuous downstream processing of therapeutic proteins can be achieved using automated multicolumn chromatography (**10**). With multiple columns organized in line for automated loading and elution/regeneration phases, chromatography steps can run continually without manual loading, which further increases productivity (**8**).

End-to-End Integration: Biologics can be processed continually as concentrated liquids or lyophilized to achieve end-to-end integration (**8**). Increased productivity and reduced costs have been shown with end-to-end integrated processes that extend from bioreactor to final drug substance (**11**).

On-Line Quality Control: The FDA encourages implementation of process analytical technologies (PATs) to monitor biomanufacturing processes in progress. On-line PAT systems can analyze critical quality attributes (CQAs) and critical process parameters (CPPs) continually, which helps to maintain product quality throughout continuous manufacturing (**8**).

Driving Efficiency, Reducing Costs, and Increasing Accessibility

The increased productivity achievable with continuous biomanufacturing is illustrated by comparing production in fed-batch cultures with that of a continuous train for production of a typical mAb. A fed-batch culture typically would generate 0.6 kg of mAb over 15 days. Over 25 days in the same bioreactor setup, a continuous culture could produce about 7 kg of mAb. The continuous process can run at a smaller scale in single-use systems (SUS) compared with that of fed-batch production using large stainless-steel bioreactors. Continuous upstream and downstream processing both run in relatively small-scale equipment, which also further increases productivity by optimizing use of facilities and resources.

Single-Use Bioreactors: Partly because single-use bioreactors typically have substantially reduced capacity compared with that of stainless-steel bioreactors, SUS can increase efficiency and product quality for continuous manufacturing. Smaller bioreactor sizes do not reduce productivity when cultures are perfused, which helps with scaling up manufacturing. Additional single-use bioreactors can be used in a "scale-on" approach rather than traditional scale-up through increasing bioreactor size. Multiple bioreactor suites — even in multiple facilities and geographic locations — can be used after that in a "scale-out" strategy, with the resulting equipment footprint still smaller than would be required with stainless-steel bioreactors and fed-batch cultures.

Small-Scale Chromatography: Continuous manufacturing also enables the use of smaller-scale chromatography columns

An Enzene technician operates a multicolumn chromatography set-up.

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than the 1-m to 2-m column diameters that have been used in biomanufacturing based on fed-batch culture. The reduced equipment scale provides cost benefits. Downstream processing is an expensive part of biologics manufacturing — especially for mAb platform processes based on costly protein A affinity capture — but smaller chromatography columns can use such resins more efficiently. Such column sizes enable companies to get the maximum capacity out of those resins before they need to be changed out, which improves the overall cost-effectiveness of continuous downstream processing.

Although it has been demonstrated that continuous manufacturing can increase productivity and efficiency in biologics production, the approach has yet to find widespread use in the biopharmaceutical industry. Companies have raised questions about high costs and large quantities of required raw materials such as buffers and media. It's true that intensified perfusion requires larger volumes of cellOculture media, which brings additional costs. But the productivity that can be achieved with continuous culture should alleviate such concerns. High production yields can offset initial investments, leading to a reduction in overall cost of goods (CoG) and highlighting the potential of continuous manufacturing to improve affordability and accessibility of biologics.

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Driving the Future

Another reason for the biopharmaceuitcal industry's slow adoption of continuous manufacturing is the prevalence of legacy stainless steel bioreactors and other fed-batch equipment still in use at many CDMOs and other facilities. Significant investment would be required to replace such legacy equipment and optimize new technologies, so very few manufacturers can offer both capabilities or achieve the efficiency gains of continuous manufacturing.

However, product developers need access to technologies that can drive the future of biomanufacturing. This is particularly the case as drug modalities increase in complexity, which further exacerbates the challenges of affordability and accessibility. Taking a proactive approach to development and manufacturing — such as by partnering with CDMOs that have invested in continuous manufacturing — can help biologics developers to ensure efficient, cost-effective production of their products both now and for the future.

Continuous manufacturing based on intensified perfusion and multicolumn chromatography provides a specific advantage for producing complex biologics, such as difficult-to-express proteins, human-engineered molecules, and bsAbs. Such molecules can be unstable and susceptible to degradation, which complicates manufacturing efforts to achieve high yields and maintain product quality when using fed-batch processes. By contrast, intensified perfusion is an optimal production method for biologics that are prone to degradation because it minimizes product contact with

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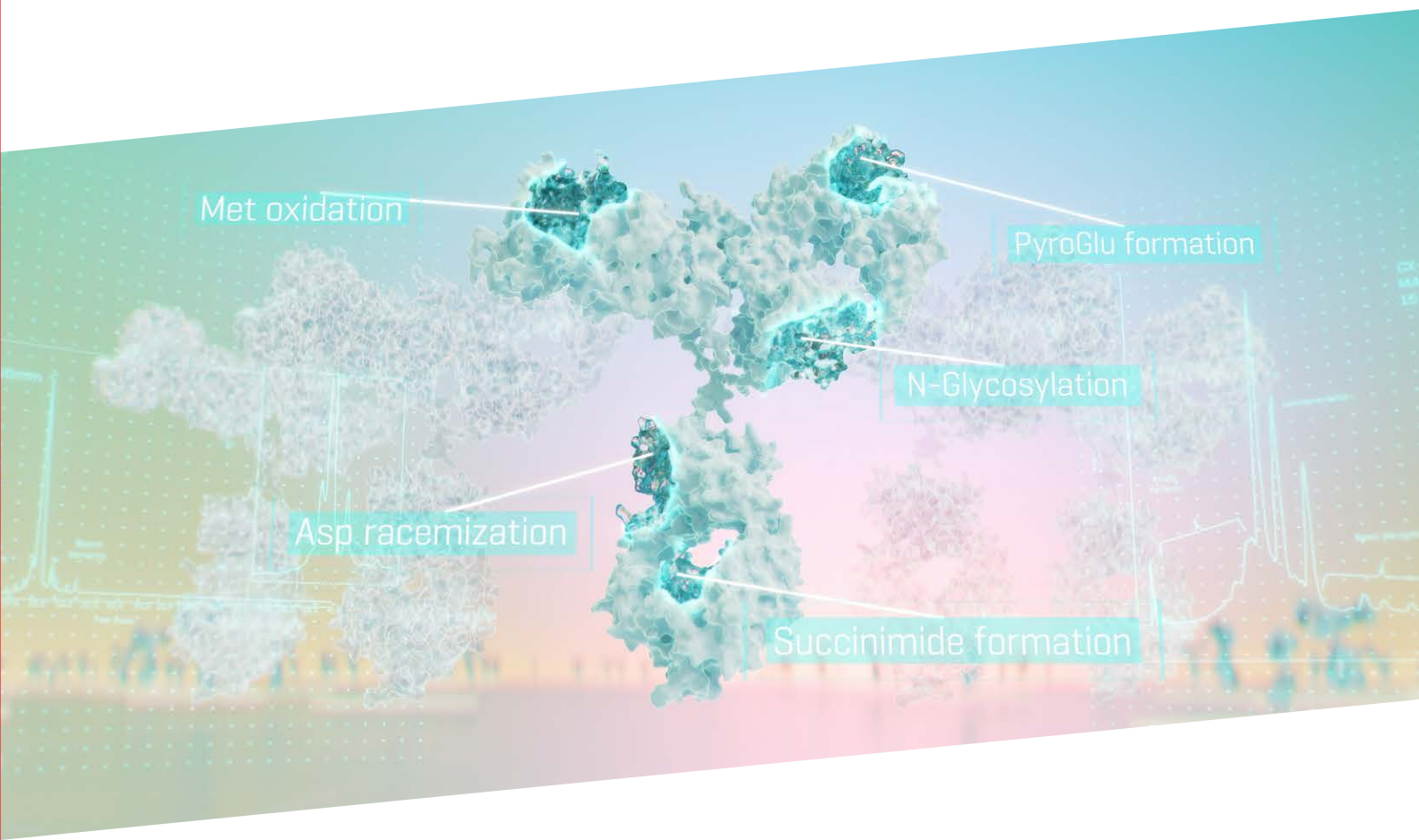

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proteases and metabolites in cell-culture media. Thus, perfusion mode helps to ensure high-quality production.

Some commercialized biologics have been produced in fed-batch culture for decades also could be converted to continuous manufacturing. The resulting efficiency and cost-effectiveness of making such a change could provide sponsors with increased accessibility to new markets — ultimately even off-setting the regulatory burdens of making process changes.

Continuous manufacturing can drive efficiency in biologics production, which in turn can reduce the costs of biomanufacturing and provide more people with access to such therapies. Current biomanufacturing costs keep products from reaching some of the patients who need them. By improving efficiency with continuous manufacturing, biologics developers can reduce those costs and increase the affordability of their products, thus helping to expand access to biologic therapies for patients around the world.

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Applications of Process Intensification

Insights from BPI Europe 2024

Josh Abbott

In April 2024, experts representing biopharmaceutical companies, drug manufacturers, and academia gathered in Vienna, Austria, to speak about intensified and continuous processing among other biomanufacturing topics at BioProcess International (BPI) Europe. Speakers at a focused preconference workshop shared studies and discussed technologies that are bringing improved efficiency to bioprocessing.

Harris Makatsoris (professor of manufacturing systems in the Department of Engineering at King's College London) opened the workshop with a talk about new strategies for the rapid discovery, design, and continuous manufacture of biological drug products. Makatsoris has over 27 years of experience working in both academia and industry. During the COVID-19 pandemic in late 2020, his team at King's College London designed a miniature factory for vaccine manufacturing, capable of producing 30,000 doses of Pfizer/BioNTech's Comirnaty mRNA vaccine treatment per day (**1**).

Makatsoris described *process intensification* (PI) as the integration of multiple unit operations into a single operation capable of significantly improving resource efficiency to optimize critical process parameters (CPPs) and speed up the preparation of target material. Well-engineered PI measures give scientists precise control of factors that influence a given process, thus enabling repeatable and operator-independent production.

Information technology (IT) has helped drug manufacturers perform complex functions in process planning since the 1960s (**2**). Computer controls provide biomanufacturers a means for consistent maintenance of multiple processes. By precisely controlling the net flow of materials, companies can achieve reproducible residence times. Modern heat exchangers and IT enable efficient heat transfer for reliable control of vessel temperatures. Computer control of dispensing rates also enables precise management of mixture composition.

Makatsoris also discussed the value of developing synthetic RNA vaccines for worldwide distribution. He said that by developing such vaccines, organizations can save 8% on labor costs and ~1% on quality assurance/control (QA/QC) efforts. Such vaccines work well with modular manufacturing setups and leave a smaller environmental footprint than do biological vaccine processes. He cited Robin Shattock (head of mucosal infection and immunity in the department of medicine at Imperial College London), who writes

Produced by Informa Connect Life Sciences, the 2024 BPI Europe Conference took place at the Messe Wien Congress Centre in Vienna, Austria, on 9–12 April 2024. (HTTPS://WWW.MESSECONGRESS.AT)

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that such vaccines can be "made locally to defined specifications . . . without requiring cultured cells of infectious material" and "within a matter of weeks" (**3**).

The Right Feed

In a talk about developing perfusion processes, Veronique Chotteau (professor in mammalian-based bioprocessing at KTH Royal Institute of Technology in Stockholm, Sweden) said that the objectives of perfusion process optimization are to achieve high cell densities, high cell-specific productivity in expression of recombinant proteins, and well-tuned quality profiles. Process engineers also prioritize low perfusion rates while designing culture media that include feed concentrates and additives with a base medium. Perfusion operations should limit the production of by-products, of which lactate is particularly problematic in hindering the growth of healthy cells. Low cell-growth rates at the steady-state phase of culture also help to limit losses that can come from cell bleed during the filtration of supernatant.

Along with medium optimization, Chotteau suggested that developers implement targeted feeding (TAFE) of glucose, galactose, and mannose during perfusion processes. Her laboratory experimented with feeding each sugar at different combinations and concentrations (**4**). By default, cells favor glucose over galactose, which was a problem that required fine-tuning to overcome. Zhang et al. used the TAFE strategy to obtain different glycan profiles with 10 different feeding regimes (**4**). The published study applied a previously developed mathematical model called glycan residues balance analysis (GReBA), which can predict sugar consumption rates under 10 different perfusion conditions. Chotteau's laboratory used the same model to "design the feeding regime of a perfusion cell culture to obtain a desired glycosylation profile" (**4**).

In 2015, Chotteau et al. reported that high–cell-density perfusion can generate intensified processes if a steady-state culture is maintained at about 100 × 10^6 cells/mL (**5**). Results showed that perfusion provided significant stability increases in an extracellular metabolic profile when compared with fed-batch mode using high-resolution liquid chromatography–mass spectrometry (LC-MS). The authors noted "a strong correlation between the composition of the exometabolome and the viable cell density between 8 × 10^6 and 207 × 10^6 cells/mL" (**5**). And according to Schwarz et al. in 2022, cell density does not affect performance in a perfusion culture when multiple steady states operate at a constant cell-specific perfusion rate (CSPR) (**6**). However, "different CSPRs, indifferently obtained from variations of the cell density and/or perfusion rate, had a strong influence" on metabolism, recombinant-protein productivity, and product quality.

Improved Control with Modeling

Joseph Zeguer (senior scientist in synthetic and mammalian upstream production at CPI Biotech) presented research on end-to-

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end continuous antibody production. His company is a founding member of the UK government's High Value Manufacturing Catapult, which works as a catalyst to bring together experts from academia, business, and government to support innovation in a number of industries, including bioprocessing and biotechnology (**7**). The organization receives funding through Innovate UK and has a large platform of upstream and downstream technologies.

Zeguer described work by CPI's UK Continuous 2 Project, which seeks to accelerate delivery of cost-effective biotherapeutics for patients. The company uses a fully integrated platform that was developed primarily for monoclonal antibodies (mAbs) but can be adapted for other modalities. He presented a schematic of the continuous mAb process: It includes "a perfusion system with an upstream bioreactor centrifuged with alternating tangential-flow filtration (ATF) perfusion that flows through continuous protein A chromatography with viral activation, flowthrough, ion exchange, continuous cation exchange, and then formulation." For the capture step, the CPI team uses an ÄKTA pure protein-purification system (Cytiva) and protein A affinity chromatography. The technology enables continuous operation with simultaneous loading and elution.

In describing the corresponding upstream perfusion-development platform (UPDP), Zeguer detailed his role in "installing, functionally validating, and demonstrating routine operation of a continuous upstream perfusion rig with automation and advanced process control." His team used the UPDP to grow and hold cells for three weeks at 25 × 10^6 cells/mL, followed by another growth period and a second hold for two weeks at 50 × 10^6 cells/mL. Then the team investigated strategies to optimize gassing by observing its effects on titer and yield.

"One of the unique aspects of this experiment is what we call 'lights-out' operation," Zeguer said. "Essentially we were able to leave the system completely [unattended] for 10 days to take care of itself. We came back after that period, and the cells were exactly where we left them." He said that, thanks to previous system optimization efforts, his team had no need to make manual pH adjustments throughout the 55-day experiment.

For real-time glucose monitoring and control, Zeguer's team used midinfrared (MIR) spectroscopy to detect resonant frequencies of characteristic functional groups, with spectral absorbance at ~1,000 cm^{-1} correlated to glucose concentration. The researchers integrated their bioreactor with a Monipa MIR instrument (Irubis) through PharmaMV software (Perceptive Engineering). That combination feeds data into a calibration dashboard to maintain glucose concentration within the bioreactor. For cell density, the team uses a sensor probe to measure membrane capacitance for viable cells, which enables control of cell density within the reactor.

A key component of CPI's setup is an advanced algorithm that "learns" information from growth characteristics to improve growth-pattern predictions based on information that it receives.

Lights-Out Operation:
"Essentially, we were able to leave the system completely [unattended] for 10 days to take care of itself. We came back after that period, and the cells were exactly where we left them."
— Joseph Zeguer (CPI Biotech)

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Zeguer emphasized that such model predictive control (MPC) can replace and improve standard control measures.

Longtime BPI editorial advisor Alois Jungbauer (professor in the department of biotechnology at the University of Natural Resources and Life Sciences (BOKU) in Vienna, Austria) turned the discussion of on-line monitoring and control to continuous downstream processing. He shared an experiment that demonstrated how scaling large columns is easier than scaling very small columns.

Jungbauer emphasized the importance of tight control and monitoring for conductivity levels, which can affect binding capacity significantly. He outlined the complexity of describing physical processes with complete accuracy. Noting that mAbs alone have 285 million different potential variants, he said that it is impossible to grasp fully the physics of an entire mAb. Thus, models are important to enable researchers to understand related processes.

Jungbauer presented his laboratory's work in developing a model to predict downstream quantity, purity, and potency of recombinant proteins in real time about one second. It was designed in collaboration with Novartis and Boehringer Ingelheim to use commercially available sensors. After building the model, the BOKU team used process runs to train it and then verified it with independent operations. The model also can run off-line analyses of quality attributes such as product quantity, host-cell proteins (HCPs), endotoxins, aggregates, and double-stranded DNA (dsDNA).

Models for Characterization

Mark Duerkop (chief executive officer (CEO) of Novasign) said that intensified process design saves time. He also emphasized that complex bioprocesses require extensive experiments for sound optimization and characterization. During process characterization, organizations can choose among many possible CPPs to investigate. Researchers should define their goals and determine which CPPs might affect their target product profiles (TPPs). Process engineers can use risk assessment and smart workflow tools such as digital twins during research and development (R&D). Duerkop showed how digital twins enabled his team to simulate 1,000 experimental runs in 15 seconds. He also demonstrated sliding scales for simulating changes in temperature, feed rate, and induction strength in perfusion cultures to predict results from different combinations thereof.

Duerkop explored methods that developers can use to characterize their bioprocesses. The most basic workflow is to define CPPs, perform experiments, and then make simple surface models based on the identified endpoints to determine results attained from different input parameters. Such designs don't communicate much useful information, however.

Alternatively, process engineers can use data to model a regression that determines input parameters, then observe the connections between inputs and outputs. This strategy focuses mostly on the endpoint as a function of the starting conditions.

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Duerkop said that when scientists observe an entire bioprocess, they can notice deviations that intrinsically bring change and cause inconsistencies (e.g., in cell growth).

A mechanistic model enables researchers to apply process knowledge and equations in characterizing bioprocesses. According to Bayer et al., "such models are well suited for extrapolation, but due to their purely mechanistic nature and high biological complexity, inaccurate predictions frequently occur in case an important parameter was not considered, or the model is too simple" (**8**).

Hybrid models combine aspects of regression and mechanistic modeling to provide a viable option for process characterization. To optimize hybrid-model characterizations, scientists must choose appropriate CPPs to investigate within a design space. Developers must select meaningful experiments that enable models to link causes and effects. Those steps are crucial because "hybrid models learn more from data than their mechanistic counterparts" (**8**). Duerkop pointed to the value of using a combination of smart experimental workflows and hybrid modeling for viral-vector manufacturing, in particular.

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