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Continuous Bioprocessing Yields Major Gains in Biopharmaceutical Manufacturing

Will Miller

Biopharmaceutical manufacturers increasingly are turning to *continuous bioprocessing* — running production as an uninterrupted flow rather than in discrete batches — to improve productivity, lower cost of goods (CoG), and reduce facility footprints. Unlike traditional fed-batch processing, in which discrete cell-culture runs are followed by harvest, cleaning, and turnaround, a continuous process integrates upstream cell culture with one or more connected downstream operations so that material flows from step to step with minimal hold-up. Such approaches increase occupancy of capital-intensive equipment and improve use of culture media, process buffers, and chromatography resins **(1–3)**.

The commercial momentum behind continuous bioprocessing has accelerated. The global market for continuous biomanufacturing reached an estimated US$218 million in 2023 and is projected to reach $599 million by 2028 at a compound annual growth rate (CAGR) of ≈22% **(3).** Drivers include rising biologics demand, biosimilar competition, and the search for capital-efficient manufacturing models **(3)**. The International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q13 document, which was endorsed at Step 4 in November 2022 and now is being implemented across ICH regions, provides a global framework for continuous manufacturing of drug substances and drug products, including therapeutic proteins (see Annex III) **(4).** Q13 reduces — but does not eliminate — regulatory uncertainty for sponsors planning continuous biologics processes.

A key enabler in upstream processing is the perfusion bioreactor, in which fresh media are added continuously and a permeate stream containing product (and waste metabolites) is continuously withdrawn through a cell-retention device such as an alternating–tangential-flow (ATF) or tangential-flow filtration (TFF) skid. Perfusion sustains high viable cell densities (VCDs), typically 50–100 × 10^6 cells/mL, as opposed to 15–25 × 10^6 cells/mL in fed-batch mode. Thus, perfusion has correspondingly higher volumetric productivity: ≈0.5–2.0 g/L/day versus ≈0.2–0.5 g/L/day **(5, 6)**. Downstream, multicolumn periodic counter-current (PCC) chromatography systems — or, in the small-scale work discussed below, one-column continuous (OCC) chromatography — enable antibody capture to occur in step with the perfusion harvest, so that one column volume of resin is loaded, washed, eluted, and regenerated while another receives feed **(1, 7)**.

It is worth noting at the outset that *connected, integrated continuous*, *and end-to-end continuous* are not synonymous, although the literature uses them loosely. A fully end-to-end process with a single, uninterrupted material flow from bioreactor to drug substance remains rare in biologics manufacturing. Most reported implementations couple a perfusion bioreactor to a continuous capture step and then return to batch-mode polishing, viral inactivation, and final filtration, often with intermediate surge or hold tanks. Therefore, the economic and quality gains attributed to "continuous" processing are most often gains from a subset of connected unit operations, not from a fully end-to-end system **(7)**.

Through 2026, end-to-end continuous monoclonal antibody (mAb) drug-substance manufacturing has been demonstrated at pilot scale **(8–11)**. Clinical or commercial deployment is documented in only three programs or platforms: the process

for BiosanaPharma's BP001 (omalizumab) biosimilar, the WuXi Biologics WuXiUP platform, and the Enzene Biosciences EnzeneX platform. Of those, only the WuXi Biologics platform has supporting peer-reviewed, primary-process literature **(12).**

Case Studies

Bench-Scale Integration of ATF Perfusion with OCC Chromatography: A widely cited proof of concept for integrating upstream perfusion with continuous capture is a 2018 *Preparative Biochemistry & Biotechnology* article by Kamga, Cattaneo, and Yoon, who performed experimental work at Biovolutions Inc. (Woburn, MA) in collaboration with the Department of Chemical Engineering at the University of Massachusetts Lowell **(1)**. The authors used a Chinese hamster ovary (CHO) cell line expressing an immunoglobulin G (IgG) mAb. Cells were cultured in a bench scale (2-L working volume) bioreactor coupled to an XCell ATF2 cell-retention device (Repligen). The perfusion train was integrated directly with an OCC chromatography system for an antibody-capture step using a MabSelect protein A column. The writers describe their work as the first reported use of single-column continuous chromatography integrated with ATF perfusion for therapeutic-antibody capture. They intended to explore the configuration as a cost-effective alternative to multicolumn PCC systems for development-scale and small-volume commercial production **(1).**

Two process modifications were central to the reported gains. First, media optimization helped the team to reduce the upstream perfusion rate to one vessel volume per day (1 vvd), which increased antibody titer leaving the bioreactor, reduced media use, and reduced total perfusate volume. In turn, the writers were able to

- match the perfusion rate to the purification rate
- use a small affinity column (<1% of reactor volume) for capture
- perform the capture step at low superficial velocities
- achieve high residence times.

Those downstream operating conditions optimized protein A use, resulting in optimal dynamic binding capacities (DBCs) and leading to nearly 100% resin-capacity use.

The second modification involved creation of an integrative on-line method for monitoring column performance, which served to trigger column switching for the OCC step. Using real-time analysis of elution peak length at half the peak height, column failure could be predicted within three cycles based on decrease in cycle yield, elution peak height, and peak broadening.

Together, those changes increased productivity by ≈80% compared with the authors' own previously reported integrated process. Note that the comparator was an earlier perfusion–OCC configuration, not a traditional fed-batch process. This distinction is important: The 80% gain is a within-platform improvement, not a head-to-head comparison against legacy fed-batch processing. With regards to productivity, the authors reported average cell-culture titers of 2.5 g/L, affinity-resin productivity of 34 mg mAb/mL resin per hour (increased from 19.7 mg mAb/mL resin per hour), and an average of 1.25 g/L/day of purified antibody **(1).**

Although the authors did not assess their ATF-perfusion–OCC platform's impact on further downstream processing steps, it was shown to be effective in reducing host cell protein (HCP) levels in purified IgG to 8.5 ppm, a level much lower than the acceptable HCP limits in drug products **(1).**

The work was a bench-scale proof of concept rather than a good manufacturing practice (GMP) manufacturing campaign, and values for product output and purity should be interpreted in that context. Teams adopting such an approach must invest in a stably expressing cell line that maintains productivity and product quality throughout a perfusion campaign (typically two to eight weeks), a media formulation engineered for high cell densities at low perfusion rates, ATF or equivalent cell-retention hardware, and process analytical technology (PAT) that can trigger column switching in real time **(1, 6)**.

Roche Network Optimization Enabled by Upstream Intensification: A frequently cited industry example of how upstream intensification can reshape a manufacturing network is Roche's 2024 divestment of its Vacaville, CA, facility to Lonza for $1.2 billion. Housing about 330,000 L of bioreactor capacity, the Vacaville site is one of the largest biologics manufacturing facilities in the world. It has produced legacy blockbusters including Herceptin (trastuzumab), Avastin (bevacizumab), and Rituxan (rituximab) **(5, 13)**.

In commenting on the sale, Roche chief executive officer Thomas Schinecker attributed the divestment to "ongoing efficiency gains for biologics manufacturing," specifically referencing average titer improvements from 1.5 g/L (before 2015) to a projected 4.8 g/L. That increase of more than threefold was achieved through a combination of cell-line improvements, media development, fed-batch process intensification, and perfusion for selected products **(5, 13)**. Roche characterized the move as part of a broader geographic optimization, positioning manufacturing closer to US, European, and Asian end markets while supporting a shift toward smaller-volume, higher-potency new molecular entities. After the divestment, Roche retained over 530,000 L of biologics manufacturing capacity across 11 sites, which Schinecker described as the industry's largest amount of manufacturing capacity outside of contract development and manufacturing

organizations (CDMOs) **(13).**

The Roche example illustrates how upstream intensification — perfusion among other strategies — can translate sustained productivity gains into reductions in required physical capacity. It is not, however, a case of replacing fed-batch with end-to-end continuous processing across a company's commercial portfolio. The lesson for other manufacturers is that intensification compounds over time: Higher titers from any source (cell-line engineering, media development, or perfusion) can eventually make legacy large-scale, stainless-steel capacity uneconomical relative to smaller, more flexible footprints. Roche's legacy Vacaville site was designed for an era of 1–2 g/L fed-batch titers; at 4.8 g/L, the same product demand can be met with roughly one-third of the volumetric capacity **(13).**

Sanofi's Economic Modeling of an Integrated Continuous Biomanufacturing Platform: In one of the most thorough peer-reviewed industrial assessments to date, Walther and colleagues at Sanofi (Framingham, MA) used process-economic modeling, Monte Carlo simulations, and risk-based net present value (NPV) analysis to better understand the financial impact of migrating biomanufacturing operations toward continuous bioprocessing **(2)**. To achieve that, the writers estimated facility capital expenditure (CapEx) and operating expenses (OpEx) for single-use integrated continuous biomanufacturing (ICB) platforms as compared with conventional stainless-steel batch and fed-batch platforms. The team simulated costs over 10 years for a multiproduct portfolio that included both a high-demand, stable mAb (target output ≈ 200 kg/year) and a lower-demand, more-labile fusion protein (≈20 kg/year) **(2).**

The ICB mAb platform was modeled with a 500-L single-use perfusion bioreactor as the primary production vessel. That was integrated with a fully continuous downstream process comprising affinity capture using multicolumn PCC chromatography, viral inactivation, polishing chromatography with continuous multicycle membrane adsorbers, ultrafiltration/diafiltration (UF/DF), and final viral/sterile filtration. The conventional mAb comparator used stainless-steel fed-batch bioreactors at conventional scale (10,000 L) with equivalent downstream-process unit operations in batch mode. Non-mAb ICB and conventional platforms were modeled at the same scales as those for the mAb processes, but with larger polish columns **(2).**

The writers estimated that the ICB platform would reduce average cost by ≈55% ($335 million) across the 10-year portfolio relative to the conventional platform. The largest projected savings arose from reduced CapEx (smaller bioreactors, less stainless steel, and a smaller building footprint) and from improved consumables and resin use. Monte Carlo analysis indicated that those savings could increase by an additional ≈25% in higher-than-expected demand scenarios because continuous platforms scale primarily by extending campaign duration rather than by adding stainless-steel capacity **(2).**

ICB OpEx was lower for both the mAb and non-mAb facilities compared with OpEx for the corresponding conventional platforms. However, the breakdown of savings differed greatly across the two classes of molecules. For mAb production in the ICB platform, increased costs for upstream filters and media were primarily due to higher media consumption in a perfusion system relative to fed-batch processing. However, that increase was easily offset by several advantages with the ICB platform: Resin costs were significantly reduced due to the higher efficiency of continuous chromatography and the use of multicycle membrane adsorbers. Less upstream labor was necessary because there were fewer reactor turnarounds. And the continuous process did not require any standalone clarification operations **(2).**

On the other hand, for non-mAb production, OpEx savings were present in every category assessed. The major drivers included elimination of clarification operations, reduction in downstream labor requirements, reduced media consumption, and resin savings due to the efficiency of continuous capture chromatography **(2).**

Critically, the same analysis identified the principal OpEx driver as cell-culture media consumption. Perfusion processes consume substantially more media per kilogram of product than fed-batch processes do unless dedicated perfusion-medium development is performed. Moreover, media cost is the dominant lever that determines whether projected economic advantages materialize in practice **(2, 6)**. Teams transitioning from batch to continuous typically must invest in

- a media development program tuned to high–cell-density perfusion (often including spent-media analysis and feed reformulation)
- single-use bioreactors with perfusion capability
- skids for ATF or TFF cell retention
- continuous chromatography systems (single- or multicolumn)
- in-line and on-line PAT — e.g., for breakthrough monitoring based on Raman or ultraviolet-light (UV) spectroscopy
- the automation infrastructure required to operate connected steps without manual sample-and-test handoffs **(2, 7, 14).**

Comparative Benefits: A Balanced View

Productivity and Cost Reductions: Partopour and Pollard's 2025 technoeconomic and life-cycle analysis in *Trends in Biotechnology* compared an end-to-end continuous mAb process (3–5 g/L/day and 75% downstream recovery) against an intensified and optimized fed-batch (IO-FB) process (15 g/L titer fed-batch coupled to multicolumn capture chromatography and 70% downstream recovery) rather than a legacy stainless-steel batch process that generated titers of 1–3 g/L **(15)**. Setting the comparator at the best available fed-batch is a more conservative test than comparing against legacy plants, yielding more credible gain estimates.

Within that comparison, continuous processing reduced total annual production cost by up to 23%, facility footprint by up to 51%, plastic waste by up to 57%, and CO_2 emissions by up to 54% in a multiproduct clinical-and-commercial facility **(15, 16)**. Uncertainty analysis indicated that these advantages widened under demand fluctuations because continuous platforms accommodate variable output by adjusting campaign length rather than by reconfiguring fixed equipment.

Facility Throughput and Footprint: The facility-level advantages of continuous bioprocessing are the most consistently documented across independent analyses. Because perfusion bioreactors operate continuously rather than producing discrete batches separated by cleaning and turnaround time, equipment occupancy rises and surge-capacity requirements fall. Modeled comparisons show facility footprint reductions of 30–51% relative to optimized fed-batch processing **(2, 15)**. The combination of higher volumetric productivity from perfusion and continuous downstream operation means that a 2000-L single-use perfusion bioreactor can produce annual outputs that historically would have required 12,000–25,000 L of fed-batch capacity. Genentech has publicly cited that factor in describing the rationale for its small-batch, single-use clinical-supply center in South San Francisco and its next-generation manufacturing campus in Oceanside, CA **(17)**.

Quality, Consistency, and Real-Time Control: Continuous operation at steady state can reduce batch-to-batch variability and shorten the residence time of product in a bioreactor, which is especially beneficial for acid- or shear-sensitive molecules that degrade in extended fed-batch harvest pools **(6, 14)**. In-line and on-line PATs — such as tools based on capacitance sensors and Raman, near-infrared (NIR), and UV spectroscopy, among others — have been implemented in continuous platforms for real-time monitoring of critical process parameters (CPP's) that impact critical quality attributes (CQAs). For complete process integration, global control strategies should be implemented to assess the impact of upstream process conditions on downstream productivity, requiring elimination of the dichotomy between upstream and downstream processes **(7)**. It should be noted, however, that PAT and real-time monitoring are not exclusive to continuous processing; they are increasingly standard in modern fed-batch operations as well. The quality benefits attributed to continuous processing arise not only from PATs coupled with robust control systems, but also from steady-state operation and shortened product-residence times, which together constrain the operating window within which CQAs can drift **(6, 7)**.

Sustainability: Sustainability gains from continuous bioprocessing are real but conditional. The Partopour and Pollard analysis attributes the majority of CO_2 and plastic-waste reductions to a continuous process's smaller facility footprint and its use of smaller and fewer single-use bag assemblies relative to large-scale fed-batch processing **(15)**. By contrast, a 2016 life-cycle analysis by Bunnak and colleagues at University College London found that a standard perfusion process actually consumed ≈35% more water, demanded ≈17% more energy, and emitted ≈17% more CO_2 than a comparable fed-batch process unless pooling duration was extended to about eight days **(18)**. The lesson is that environmental gains are not automatic; they depend on specific process design choices, particularly media efficiency, perfusion rate, and implementation of single-use rather than stainless-steel equipment.

Limitations and What Teams Need To Change

A balanced account of continuous bioprocessing must acknowledge that the gains described above come with new operational, technical, and regulatory burdens. The published literature and industry experience converge on the following challenges.

Media consumption is a dominant OpEx driver. Perfusion at 1–2 vvd can require substantially more media per kilogram of product than would fed-batch processing. Realizing the economic case modeled by Walther et al. and by Partopour and Pollard therefore depends on a successful, dedicated perfusion-medium development program — typically including spent-media analysis, feed reformulation, and, in some cases, permeate-recycling strategies **(2, 6, 14)**.

Cell lines must remain stable over extended campaigns. Perfusion runs of two to eight weeks (and, in some cases, several months) require cell lines that maintain productivity and product quality attributes — e.g., glycosylation, charge-variant, and aggregation profiles — over a full campaign duration. Lines selected for high peak titer in a 14-day fed-batch process do not always remain stable in steady-state perfusion, and a perfusion-specific protocol for clone screening and stability is generally required **(6)**.

Contamination risk increases over long campaigns. A bioreactor run lasting six to eight weeks has a longer integrated exposure to contamination risk than does a 14-day fed batch. Sterility breaches in continuous campaigns can have larger consequences because more material is at risk and turnaround to restart is nontrivial. Single-use technology and improved cell-retention hardware mitigate but do not eliminate such contamination risks **(6, 7)**.

Continuous processing entails capital investment and operational complexity. Although continuous processes ultimately reduce facility CapEx through use of smaller bioreactors and footprints, the initial transition requires investment in ATF or TFF cell-retention skids, systems for single- or multicolumn continuous chromatography, in-line buffer-formulation skids, advanced PAT, and a higher level of automation than are necessary for typical batch installations. Such equipment requirements come from the need for global on-line monitoring and control of simultaneous operation of multiple integrated systems. Operator and engineer training is nontrivial as well; continuous processes are less forgiving of manual interventions and require new approaches to deviation management **(7, 16)**.

Regulatory and validation burdens increase. ICH Q13, including Annex III on therapeutic proteins, formally provides a globally harmonized framework as of November 2022, but jurisdictional implementation, expectations for residence-time distribution (RTD) modeling, strategies for real-time release, and approaches to handling out-of-specification (OoS) material diverted during a continuous run are still maturing. Sponsors transitioning approved batch products to continuous processes must seek explicit regulatory approval and demonstrate product comparability under ICH Q5E **(4, 7)**.

Genuine internal disagreement remains in the field. Khanal and Lenhoff (2021) note that, despite the approach's documented economic and environmental advantages, there is still meaningful disagreement within the bioprocessing community as to whether end-to-end continuous biomanufacturing is the appropriate future direction for all biologics. That is especially the case for established blockbuster drugs, for which modeled gains from continuous processing must be weighed against the very low operational risk of mature fed-batch platforms **(7)**. For many companies, the practical answer has been to introduce continuous unit operations selectively — e.g., *perfusion N – 1* seed expansion, continuous capture downstream of fed-batch production, or perfusion for sensitive molecules — rather than rebuilding a full platform from scratch.

Reflections and Future Outlook

Whether implemented as upstream perfusion alone, perfusion plus continuous capture, or as a more fully integrated end-to-end platform, continuous bioprocessing has produced measurable, peer-reviewed gains in productivity, CoG, facility footprint, and sustainability. The bench-scale work of Kamga and colleagues (2018) demonstrates the technical feasibility of single-column continuous capture integrated with ATF perfusion at process-development scale **(1)**. The Sanofi modeling work of Walther and colleagues (2015) quantifies the industrial business case, predicting ≈55% CoG reduction relative to conventional batch processing **(2)**. Partopour and Pollard (2025) update that picture against an optimized fed-batch benchmark, finding more modest but still meaningful gains of ≈23% in cost, ≈51% in footprint, and ≈54% in CO_2 emissions **(15)**. And Roche's Vacaville divestiture shows how cumulative upstream intensification can reshape an established manufacturing network at the corporate scale **(13)**.

Such gains are not free. They require investment in perfusion-tuned media, stable high-density cell lines, ATF or TFF hardware, continuous-chromatography systems, PAT, automation, and a regulatory submission strategy aligned with ICH Q13 and Q5E. Teams that succeed with continuous bioprocessing tend to do so because they treat it as a platform decision — applied to clinical-stage molecules for which a platform can be designed in from the outset — rather than as a retrofit to a single approved product.

The continued release of regulatory guidance, the maturation of hardware for single-use perfusion and continuous chromatography, and the entry of additional CDMOs offering continuous platforms suggest that the share of the biologics pipeline produced continuously will grow. For most companies, the practical question is no longer whether to use continuous bioprocessing at all, but where in the portfolio and at which stages of development the operational complexity is justified by the productivity, cost, and footprint advantages documented above.

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Equipment-Level Digital Twins for Bioprocessing

Practical Models for Upstream, Downstream, Fill–Finish, and Life-Cycle Controls

Bhasker Sambar

Biopharmaceutical manufacturing depends on complex biological and separation processes that must operate consistently under strict quality expectations. Upstream operations such as seed train expansion, fed-batch cell culture, perfusion culture, and microbial fermentation influence product titer, impurity burden, glycosylation, charge variants, aggregation risk, and harvest timing. Downstream operations such as capture and polishing chromatography, viral inactivation and filtration, and ultrafiltration/diafiltration (UF/DF) affect yield, purity, impurity clearance, concentration, buffer exchange, and process robustness. Fill–finish operations then introduce sterile manufacturing expectations, line performance requirements, and container–closure considerations.

Traditional bioprocess control remains essential. Recipes, set points, alarm limits, batch records, in-process testing, laboratory assays, preventive maintenance, deviation systems, and process validation cannot be replaced by models. However, traditional controls may not always reveal early trajectory drift, predict when a culture or filtration step will reach an endpoint, warn that a column is approaching a breakthrough limit, or show when equipment behavior differs from that recorded in historical batch data. A digital twin adds a model-based layer that uses current or recent process data to improve interpretation, forecasting, diagnosis, and decision support **(1–6)**.

Examples are direct and practical. A bioreactor twin may forecast viable cell density (VCD), glucose demand, lactate accumulation, oxygen uptake, or harvest timing. A chromatography twin may predict breakthrough, pooling windows, impurity clearance, or resin-aging effects. A tangential-flow filtration (TFF) twin may detect flux decline, fouling, concentration endpoint, or DF completion. A fill–finish twin may monitor fill-volume drift, stopper or seal trends, reject patterns, or equipment-related interruptions. Such uses support process understanding, quality risk management, operational efficiency, and continued process verification **(7–10)**.

Rather than replacing process scientists, operators, engineers, and quality systems, digital twins are designed to increase the visibility of current process states and to forecast likely behavior before the next sample, alarm, or deviation occurs. We might expect broad enterprise programs to be the most useful early implementations of digital twins. In practice, the best early use cases are focused unit-operation pilots that answer one high-value question reliably.

What Is an Equipment-Level Bioprocess Digital Twin?

An *equipment-level bioprocess digital twin* is a connected model of a specific unit operation or equipment item. It can represent a stirred-tank bioreactor, perfusion bioreactor, microbial fermentor, chromatography skid, TFF system, viral-filtration setup, single-use mixing system, lyophilizer, or filling line. The twin receives data from a physical operation, applies a model, and produces outputs such as a predicted endpoint, deviation warning, recommended operating window, process-health score, or maintenance alert.

The key difference between a digital twin and an ordinary model is the connection to operating data. An off-line simulation may be used during process development to explore scenarios. A dashboard may display current values. A digital twin, however, links data and a model so that the displayed information reflects the actual process state and can support prediction or diagnosis (Table 1). Figure 1 depicts the general flow of information within a digital twin.

Tool	Purpose	Description
Dashboard	Display data	Shows values for pH, dissolved oxygen, pressure, ultraviolet absorbance, flow rate, viable cell density, and fill weight; does not necessarily predict behavior
Off-line simulation	Study scenarios	Runs a model to study what may happen under selected conditions; may not be connected to routine operating data
Digital model	Represent behavior	Represents the behavior of a process, unit operation, or piece of equipment; may be static or updated only periodically
Digital twin	Mirror and predict process behavior	Connects contextualized operating data with a model so that users can monitor, predict, diagnose, and recommend actions

Table 1: Practical distinctions among dashboards, simulations, digital models, and digital twins

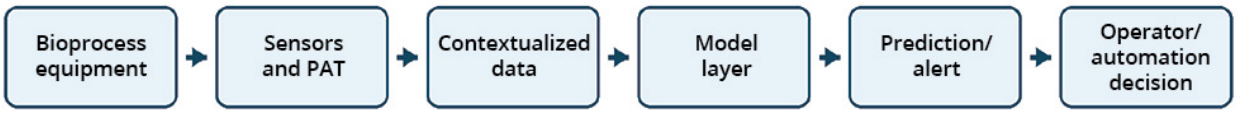


Figure 1: Basic digital twin information flow; PAT = process analytical technology

Digital Twin Architecture: Five Building Blocks

A useful equipment-level digital twin usually has five building blocks: the physical process, the data layer, the model layer, the integration/control layer, and the application layer (Table 2). These blocks do not need to be complex at the start. Many successful implementations begin with one unit operation, a few reliable signals, and a model that supports one defined decision.

Layer	What It Includes	Bioprocess Example	Why It Matters
Physical	Equipment, sensors, actuators, single-use assemblies, skids, and PAT probes	Fed-batch bioreactor with pH, DO, temperature, gas-flow, off-gas, feed-pump, capacitance, and Raman signals	Provides the real-world signals that keep the twin connected to the process
Data	Data acquisition, historian tags, contextualization, batch alignment, calibration status, and storage	Historian and LIMS data linked to batch identification, campaign, cell line, media lot, and instrument status	Turns raw signals and delayed lab results into traceable information
Model	Mechanistic, machine-learning, statistical, or hybrid models	Growth and metabolite model combined with a Raman soft sensor and anomaly detection	Converts data into state estimates, predictions, or recommendations
Integration/ control	Interfaces with PLC, DCS, SCADA, MES, LIMS, EBRs, and MPC	Read-only OPC–UA connection for early decision support; later integrated with supervisory control if justified	Allows controlled communication among the twin and plant systems
Application	Dashboards, alerts, workflows, reports, and user decision points	Batch-trajectory screen showing forecasts for glucose, lactate, VCD, titer, and deviation risk	Can be used for routine manufacturing decisions

Table 2: Five building blocks of an equipment-level bioprocess digital twin; DCS = developability classification system, DO = dissolved oxygen, EBR = electronic batch record, LIMS = laboratory information management system, MES = manufacturing execution system, MPC = model predictive control, PAT = process analytical technology, PLC = programmable logic controller, SCADA = supervisory control and data acquisition, OPC–UA = open platform communication–unified architecture, VCD = viable cell density

Digital twins can also be described at different levels. A component twin represents one equipment item, such as a pump, sensor, column, or bioreactor vessel. A *unit-operation twin* represents a bioreactor run, chromatography step, or TFF operation. A *process twin* links multiple unit operations, and a site or fleet twin compares similar assets across a facility or network. For most biomanufacturing sites, a practical starting point for implementation is a component or unit-operation twin because scope, data quality, ownership, and validation are easier to define compared with other attributes.

Choosing the Right Model Type

The model is a part of a digital twin that transforms data into a state estimate, forecast, diagnosis, or recommendation. The best model depends on the bioprocess question, available data, biological and physical understanding, expected prediction speed, and regulatory traceability required for the intended use. Three broad model families are common: mechanistic, machine learning (ML), and hybrid models (Table 3).

Model Type	What It Means	Uses	Drawbacks
Mechanistic	Uses equations based on biological, chemical, transport, or equipment understanding	Scale-up, mass balances, oxygen transfer, chromatography transport, filtration resistance, root-cause analysis	Can require difficult parameters and may be too slow for real-time use unless simplified
Machine learning	Learns patterns from historical, experimental, or campaign data	Soft sensors, anomaly detection, endpoint prediction, fault classification, and fast forecasting	Needs representative data and clear controls for extrapolation, bias, drift, and interpretability
Hybrid	Combines mechanistic structure with data-driven correction or estimation	Bioreactor trajectory prediction, Raman soft sensors with process constraints, chromatography pooling, and model-based control	Requires process knowledge, data-science skill, and life-cycle governance for both model components

Table 3: Common model types used in bioprocess digital twins

Practical Model-Selection Guidance: Use a mechanistic model when process physics or biology is reasonably understood and your team needs interpretability, extrapolation, or scale-up insight. Use ML when representative data exist and the goal is fast prediction, pattern recognition, or abnormal-condition detection. Use a hybrid model when mechanistic understanding matters but a purely mechanistic model is too slow, incomplete, or difficult to maintain. Avoid overcomplication. A simple model that is validated for one decision is more valuable than a sophisticated model that users do not trust or maintain.

Applications Across Bioprocessing Unit Operations

Digital twins are most useful when three conditions are present: data are available and reliable, a problem is important enough to justify governance, and a model output is connected to a practical decision. Table 4 provides examples of digital-twin uses in biomanufacturing unit operations.

Unit Operation	Twin Can Predict or Detect	Useful Data Sources	Benefits
Mammalian cell-culture bioreactor	VCD, viability, glucose demand, lactate trend, ammonia, osmolality, oxygen demand, titer trajectory, harvest timing, abnormal batch drift	pH, DO, temperature, agitation, gas flows, off-gas O_2/CO_2, feeds, base addition, capacitance, Raman/NIR spectroscopy, VCD, metabolites, titer	Better process understanding, earlier intervention, improved feeding strategy, and stronger continued process verification
Microbial fermentation	Substrate limitation, oxygen limitation, heat generation, induction response, productivity trend, foam, pH instability	DO, pH, airflow, agitation, OUR, CER, substrate feed, temperature, off-gas, biomass, product assay	Improved productivity, scale-up understanding, and reduced deviation risk
Perfusion culture	Steady-state drift, cell-retention performance, bleed/harvest balance, membrane or filter fouling	VCD, viability, perfusion rate, cell-specific perfusion rate, bleed rate, metabolites, product titer, pressure, turbidity	More stable long-duration operation and improved response to slowly developing failures
Protein A or affinity chromatography	Breakthrough, loading limit, pooling window, resin-aging trend, pressure rise, cleaning performance	UV, conductivity, pH, flow, pressure, column volume, cycle count, HCP, hcDNA, titer, product quality data	Higher yield, better resin-use strategy, and more robust capture operation
Polishing chromatography	Impurity clearance, charge-variant pooling, aggregate removal, conductivity or pH sensitivity	UV, pH, conductivity, flow, pressure, on-line/off-line HPLC, impurity assays, product-quality data	More consistent product-quality profile and stronger control-strategy understanding
TFF or UF/DF	Flux decline, fouling, concentration endpoint, DF completion, membrane performance drift	TMP, feed/retentate/permeate pressure, flow, conductivity, UV absorbance, concentration, temperature, volume, mass	Reduced fouling, better recovery, fewer endpoint misses, and improved scale-up
Viral filtration	Pressure rise, throughput limit, fouling, flow decay, risk of filter blockage	Pressure, flow, volume processed, protein concentration, turbidity, prefilter performance	Lower failure risk and better batch-interruption prevention
Fill–finish line	Fill-volume drift, reject trends, line stoppage patterns, stopper/seal defects, equipment misalignment	Checkweigher, flow meter, pump signals, machine vision, pressure, event logs, environmental and intervention records	Fewer rejects, earlier detection of drift, and better aseptic process monitoring

Table 4: Examples of digital-twin applications across bioprocessing; CER = carbon-dioxide evolution rate, DO = dissolved oxygen, hcDNA = host-cell DNA, HCP = host-cell protein, HPLC = high-performance liquid chromatography, NIR = near infrared, OUR = oxygen-uptake rate, TFF = tangential-flow filtration, TMP = transmembrane pressure, UF/DF = ultrafiltration/diafiltration, UV = ultraviolet light, VCD = viable cell density

Focused Example: Mammalian Cell-Culture Bioreactor

A mammalian cell-culture bioreactor is a strong candidate for a first bioprocess digital-twin pilot because it is data-rich, biologically important, and directly connected to product yield and downstream burden. In addition to determining whether pH, dissolved oxygen (DO), and temperature are within set points, the twin will need to assess whether batch trajectory is consistent with the expected process state and whether future behavior creates a quality or operational risk.

Practical Objective: The objective of a first bioreactor twin may be to forecast VCD, metabolite trajectories, oxygen demand, feed needs, harvest timing, or deviation risk. The twin does not need to control the bioreactor automatically at first. A valuable preliminary version can provide decision support to an operator, process engineer, or manufacturing science and technology (MSAT) scientist: for example, whether a batch is following the expected golden-batch envelope, whether glucose is likely to fall below a target before the next scheduled sample, or whether lactate reversal will be delayed.

Data and Sensors: Data sources for a bioreactor digital twin can include

- primary process data — pH, DO, temperature, agitation, gas flows, overlay/sparge settings, backpressure, feed rates, base addition, antifoam addition, and batch time
- cell-culture data — VCD, viability, total cell density, capacitance, osmolality, glucose, lactate, glutamine, ammonia, amino acids, titer, and product-quality attributes (PQAs)
- PAT data — Raman, near infrared (NIR), and dielectric spectroscopy; off-gas O_2/CO_2, mass spectrometry, and other in-line/on-line measurements. Raman and NIR spectroscopy have been demonstrated for simultaneous monitoring of multiple Chinese hamster ovary (CHO) cell-culture variables, with recent Raman studies reporting accurate prediction of metabolites and immunoglobulin G (IgG) titers in 10-L bioreactors **(11, 12)**
- context data — cell line, clone, seed train, media and feed lots, bioreactor scale, single-use assembly, calibration status, operator interventions, campaign number, and deviation history.

Table 5 lists model options and their benefits for use in a bioreactor digital twin.

Model Option	How It Helps	When To Use
Batch-trajectory model	Compares current batch behavior with historical batches and expected operating envelopes	Early decision-support pilot and continued process verification
Soft-sensor model	Estimates variables that are not measured continuously, such as glucose, lactate, titer, VCD, and amino acids	When laboratory measurements are delayed and PAT or high-frequency process data are available
Mechanistic growth and metabolism model	Represents biomass growth, nutrient consumption, metabolite formation, oxygen demand, and feeding responses	When process understanding, extrapolation, and scale-up justification are important
Hybrid model	Combines process equations with PAT, historical data, and machine-learning correction	Routine forecasting when interpretability and prediction speed are needed
Anomaly-detection model	Identifies unusual trajectory patterns, sensor issues, and developing process deviations	Batch monitoring, troubleshooting, and early warning systems

Table 5: Model options for a mammalian cell-culture bioreactor twin; PAT = process analytical technology, VCD = viable cell density

Below is a step-by-step pilot workflow for implementing such a digital twin (Figure 2).

1. Define the decision. For example, forecast whether glucose will remain within a target range until the next scheduled sample.
2. Define the user and action.
3. Map data sources.
4. Assess data quality.
5. Build the simplest useful model first. Add mechanistic detail, ML features, or hybrid structure only when needed for the decision.
6. Validate a model using independent batches or campaigns that were not used for training or calibration.
7. Deploy the twin as a decision support before using it for automated control or decisions that are critical for good manufacturing practice (GMP) activities.
8. Monitor life-cycle performance and manage recalibration, retraining, or software updates through approved change control.

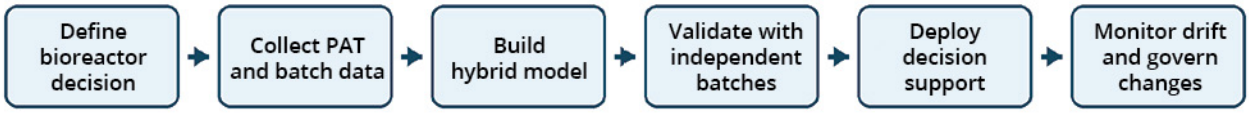


Figure 2: Example bioreactor twin workflow; PAT = process analytical technology

Downstream and Fill–Finish Examples

Chromatography Skids and Columns: Chromatography is a strong downstream candidate for digital-twin implementation because it has rich equipment signals, clear process phases, and direct connection to yield and purity. A protein A capture twin can estimate breakthrough risk, pressure rise, binding capacity, column-cycle variability, cleaning effectiveness, or resin-aging trend. A polishing chromatography twin may support pooling decisions, impurity clearance, charge-variant control, and aggregate removal. Digital twins for continuous chromatography have been demonstrated with on-line high-performance liquid chromatography (HPLC) PAT data and empirical or mechanistic models to support real-time pooling decisions for monoclonal antibody purification **(13)**. Connected mechanistic models also have been used to represent chromatography and adjustment steps across a biopharmaceutical downstream process, supporting prediction of manufacturing variability and process robustness **(14)**.

TFF, UF/DF, and Viral Filtration: Filtration operations are practical candidates because pressure, flow, volume, conductivity, and concentration data are usually available at a useful frequency. A TFF twin may estimate flux decline, membrane resistance, concentration endpoint, DF completion, and risk of excessive product exposure. A viral-filtration twin may detect pressure rise, prefilter overload, flow decay, or approaching throughput limits. Such applications are often useful as decision supports because they can prevent batch interruptions or endpoint misses without changing the validated process recipe.

Fill–Finish Equipment: A fill–finish twin may monitor fill-volume accuracy, reject patterns, line stoppages, stopper or seal defects, equipment drift, and leak-detection trends. For sterile products, digital twins should be introduced carefully because aseptic operations depend on a validated contamination-control strategy, environmental monitoring, intervention control, and equipment qualification. A fill–finish twin should strengthen monitoring and process understanding without weakening established aseptic controls **(15)**.

Predictive Maintenance and Equipment Health

Predictive maintenance is one of the most practical early uses of digital twins because it connects equipment-health signals with planned interventions. Instead of maintaining equipment only on a calendar schedule or after failure, predictive maintenance uses data to identify developing degradation. Signals may include vibration, motor current, torque, pressure, valve response, pump speed, flow stability, temperature, cycle count, alarm history, cleaning trends, and reject trends (Table 6).

Signal	Possible Meaning	Example Action
Increasing vibration	Bearing wear, imbalance, or looseness	Inspect during planned downtime and trend against maintenance history.
Rising pressure or TMP	Filter fouling, column blockage, tubing restriction, or viscosity change	Review process conditions, prefilter performance, and equipment condition.
Rising torque or power draw	Agitator wear, mixing-load change, fouling, or material behavior shift	Compare with batch context and determine whether the trend persists.
More frequent alarms	Control instability, sensor issue, component deterioration, or operator-workflow issue	Review alarm history and determine whether maintenance or process action is needed.
Increasing reject rate	Line drift, equipment misalignment, vision-system issue, or component variability	Investigate product, process, and equipment causes.
Longer cycle time	Reduced equipment efficiency, cleaning delay, sensor lag, or process variability	Review root causes and adjust maintenance or operating plan if justified.

Table 6: Examples of equipment-health signals used in predictive maintenance; TMP = transmembrane pressure

In a regulated environment, predictive-maintenance outputs must be linked to a company's quality system when they affect maintenance timing, batch disposition, validated state, or product-quality decisions. A vibration model used only for engineering awareness may require less formal control than a model that automatically changes a maintenance interval or supports release-impact decisions. The intended use should determine the required level of validation and governance.

Validation, Regulatory Expectations, and Life-Cycle Governance

A bioprocess digital twin should be validated according to its intended use and risk. A twin used only for process-development learning may require less formal control than a twin used for GMP decision support, automated process control, deviation investigation, real-time release support, or batch disposition. Guidances from the US Food and Drug Administration (FDA) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) support science-based, risk-based, life-cycle approaches when models, data, and decisions are justified and controlled **(7–10)**.

Because biologics processes are sensitive to raw-material variability, cell-line behavior, scale, equipment configuration, sampling frequency, and analytical-method variability, the validated operating space of a digital twin should be defined carefully. The model should be challenged with independent batches or campaigns representing expected variability. Teams should define what a model can and cannot predict, how uncertainty is displayed, and when users should fall back to established procedures rather than trusting the model output.

Computerized-system expectations also matter. If a twin is deployed in a good practice (GxP) environment, then software, interfaces, data-integrity controls, access management, audit trails, backup/recovery, and change-control procedures should be proportionate to risk. The International Society for Pharmaceutical Engineering's fifth-iteration good automated-manufacturing practice (GAMP 5) guidance provides a widely used risk-based framework for compliant GxP computerized systems **(16)**. When PAT or spectroscopic models are part of the control strategy, then analytical-procedure validation and life-cycle considerations should also be aligned with ICH Q2(R2) and ICH Q14, as appropriate **(17, 18)**. Table 7 lists examples of questions to ask during digital-twin validation.

Validation Question	Why It Matters
What decision will the twin support?	Validation depends on intended use and patient/product risk.
Who will use the output and what action may follow?	The workflow matters as much as model accuracy does.
What data are used?	Data must be accurate, representative, traceable, contextualized, and protected.
What model is used and why?	Model structure should be scientifically justified and appropriate for the question at hand.
How was the model trained or calibrated?	Training and calibration records support traceability and repeatability.
How was the model tested?	Independent data show whether the model generalizes beyond training batches.
What are the model limits?	Users need to know when predictions are unreliable or outside the validated domain.
How is uncertainty communicated?	Confidence intervals, warning bands, and prediction-quality indicators prevent false precision.
How is model drift monitored?	Performance can change as cells, media, resin, membranes, sensors, or equipment change.
How are updates controlled?	Retraining, recalibration, software patches, and data-pipeline changes should follow change control.

Table 7: Practical validation questions for bioprocess digital twins

Implementation Roadmap for Bioprocess Teams

A digital-twin program should start with a focused bioprocess use case rather than a broad technology initiative. The first project should solve a real problem, use accessible data, fit into routine workflow, and produce a measurable benefit. A practical roadmap is listed below, and Table 8 summarizes common challenges for implementation:

1. Select a high-value use case, such as bioreactor trajectory prediction, chromatography pooling support, TFF endpoint prediction, viral-filtration risk monitoring, fill-volume drift detection, or predictive maintenance.
2. Define what decision the twin will support and who will use the output: operators, MSAT scientists, automation engineers, maintenance engineers, quality reviewers, or site leaders.
3. Map available data sources and confirm data quality, frequency, ownership, calibration status, and traceability.
4. Choose the simplest model that can support the decision with acceptable accuracy, robustness, and explanation.
5. Validate the model against independent data and document assumptions, limits, acceptance criteria, and performance.
6. Deploy first as decision support before considering automated control.
7. Train operators, engineers, MSAT, quality, maintenance, validation, and data-science teams on how to interpret and challenge outputs.
8. Monitor model performance and manage changes through life-cycle governance.

Challenge	Mitigation
Poor data quality	Start with data mapping, sensor verification, calibration review, historian review, and clear data ownership.
Weak batch context	Link historian data to batch identification, product, cell line, media lot, equipment configuration, scale, campaign, and sampling events.
Too broad a scope	Begin with one unit operation and one decision. Expand only after the first workflow is trusted.
Model too complex	Use the simplest model that meets the intended use. Add complexity only when doing so improves decisions.
Weak user adoption	Include operators, MSAT, automation, maintenance, and quality users during design and testing.
Validation uncertainty	Define intended use, risk classification, acceptance criteria, and life-cycle plan early.
Model drift	Set performance-monitoring rules, alert thresholds, and retraining triggers.
Integration difficulty	Start with read-only integration where possible and involve automation and IT/OT security early.
Cybersecurity and data integrity	Apply access controls, audit trails, network segmentation, backup/recovery, and change control based on risk.

Table 8: Common implementation challenges and practical mitigations; IT = information technology, MSAT = manufacturing science and technology, OT = operational technology

Future Directions

Digital twins are expected to become more useful as biopharmaceutical sites improve data infrastructure, PAT adoption, automation integration, model governance, and cross-functional digital literacy. Future developments may include stronger hybrid models, physics-informed ML, automated model calibration, multisite equipment twins, federated learning across fleets, augmented-reality operator support, and human–machine collaborative intelligence **(5, 6, 19)**.

The industry needs to move beyond better algorithms, too. Bioprocessing teams need better data context, clearer model ownership, stronger validation strategies, and user interfaces that help operators and scientists understand why a model is making a recommendation. A digital twin is successful when it improves a bioprocess decision in a controlled, explainable, and sustainable way.

Equipment-level digital twins can help biopharmaceutical manufacturers improve process understanding, batch trajectory monitoring, endpoint prediction, deviation detection, predictive maintenance, and life-cycle process verification. The most practical starting point is not a broad digital-transformation program, but a focused use case such as bioreactor trajectory prediction, chromatography pooling support, filtration endpoint prediction, fill-volume drift detection, or equipment-health monitoring.

For biologics manufacturing, digital twins should be developed with quality and validation expectations in mind from the beginning. A model should be appropriate for its intended use, resulting data should be traceable and representative, user workflows should be clear, and changes should be governed throughout a model's life cycle. When implemented carefully, digital twins can become a practical bridge for bioprocess data, process knowledge, and more reliable manufacturing performance.

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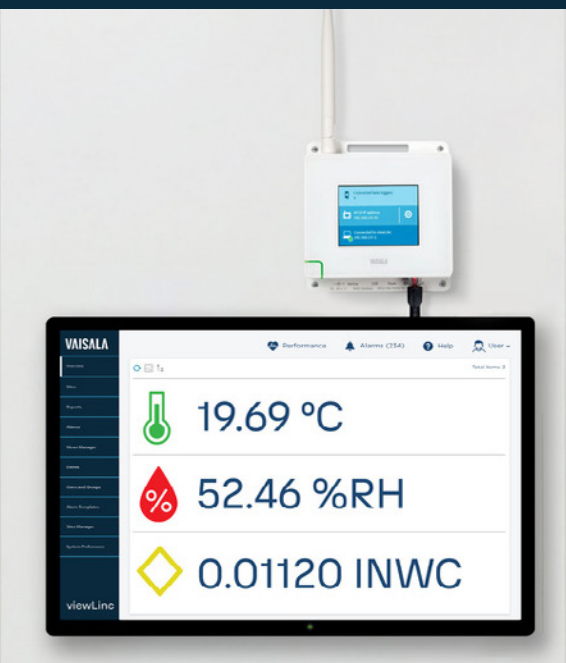
Bhasker Sambar *is senior manager of external R&D and technical services at Alvogen Inc., Morristown, NJ, with more than 15 years of experience developing and commercializing sterile injectable and drug–device combination products.* *This article was originally published on 10 September 2026 at https://www.bioprocessintl.com/pat/equipment-level-digital-twins-for-bioprocessing-practical-models-for-upstream-downstream-fill-finish-and-life-cycle-controls.*

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Thinking Beyond Individual Processes and Technologies

A Conversation on Contract Manufacturing, Intensified Processes, and Operational Excellence

Brian Gazaille with Mateusz Ludwisiak and Piotr Sabiło



Mateusz Ludwisiak



Piotr Sabiło

Process intensification has become a common refrain in biopharmaceutical manufacturing. Companies have clear business reasons to increase process productivity while decreasing resource requirements, equipment footprints, and production costs. Intensified processes have an ethical dimension, too, holding promise for improving scalability and decreasing costs in ways that increase drug accessibility. Recent technical advances are beginning to make such processes — perfusion and high-density cell culture, multicolumn chromatography, fully continuous manufacturing methodologies, and more — viable for commercial operations. Thus, industry discussions now turn quickly from technical capability to adoption: What form(s) of intensification might be most effective for my company, and how should my facility go about implementation?

In September 2026, the *BioProcess International* team had the chance to learn about future-forward biomanufacturing facilities from Mateusz Ludwisiak (service competitiveness director) and Piotr Sabiło (project management and operational excellence head) of Rezon Bio, an integrated biologics contract development and manufacturing organization (CDMO) based in Poland. Ludwisiak and Sabiło agreed that process intensification (PI) can bring significant benefits for process economics. However, they emphasized that PI should be just one consideration in a holistic discussion about flexibility and operational excellence in a company's manufacturing system. Below is our correspondence, with Ludwisiak and Sabiło's responses combined.

Foundations for Operational Excellence

BPI: How would you describe Rezon Bio's facilities and their core capabilities?

Ludwisiak and Sabiło: Rezon Bio is built around two complementary sites in Poland that together support biologics programs from early development through clinical and commercial supply. Our Gdańsk site serves as the development and early-stage clinical manufacturing hub. It brings together process, analytical, and drug-product development with flexible, phase-appropriate good manufacturing practice (GMP) capabilities, enabling programs to progress from process development and scale-up to clinical supply and technology transfer.

Our Warsaw-Duchnice site provides large-scale clinical and commercial manufacturing. It features two fully single-use manufacturing lines centered on 2000-L bioreactors, with further expansion underway, as well as smaller (50–500 L) single-use bioreactors that provide additional flexibility. The site is designed for high-volume commercial production of monoclonal antibodies (mAbs) and other complex biologics, with built-in space for additional lines as demand grows.

The strength of this two-site model lies in how seamlessly the facilities work together. Programs need to move from development into GMP manufacturing without unnecessary handovers, repeated work, and loss of process knowledge. We therefore focus on integrated governance, common quality expectations, and cross-functional planning across different teams.

That operating model is supported by a strong execution track record: more than 15 technology transfers, more than 35 customer and regulatory audits, and biologics that have been supplied commercially across multiple markets. Our facilities have received approval from the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) and are regularly inspected by major global regulatory authorities. This combination of development capability, commercial-scale infrastructure, and regulatory experience enables us to support both emerging biotechnology companies and established biopharmaceutical organizations.

BPI: Considering growing demand for diverse biologics, what capabilities must CDMOs have in place to keep pace with customer needs?

Ludwisiak: For CDMOs, flexibility is becoming just as important as installed capacity. Customer pipelines evolve, programs progress at different speeds, and facilities must accommodate

client needs without creating excessive idle capacity or adding unnecessary cost.

That requires flexibility across the entire operating model. Single-use platforms, rapid changeovers, and multiproduct operations can improve asset utilization, while flexible staffing and multidisciplinary teams help align resources with changing demand. Digital capacity modeling and digital-twin approaches can also support fast, data-driven decisions and identify constraints before they become operational problems.

The most effective CDMO facilities combine adaptable infrastructure with strong execution and can introduce intensified processes such as perfusion, high-density fed-batch cell culture, and continuous chromatography without requiring fundamental redesigns each time a portfolio changes.

The Role of Intensification

BPI: What role might PI play in helping CDMOs to optimize processes, operations, and resource use?

Ludwisiak: PI is an important lever for improving how effectively we use our manufacturing assets, people, and time. Heightened process productivity can reduce the number of batches required to deliver a given volume of product. That improves asset utilization and can create capacity for additional customer programs without immediately requiring further capital investment. It can also reduce cycle times and improve overall manufacturing economics.

However, PI only creates value if the broader operation is designed to capture those benefits. We therefore look beyond individual process performance and evaluate PI's impact on the entire manufacturing flow, including equipment utilization, staffing, throughput, cycle time, cost of goods, and delivery reliability.

For us, it is all about using the right process and operating model to achieve greater productivity and flexibility while maintaining robust, predictable, and compliant manufacturing.

BPI: How would you describe CDMO uptake of PI? Are certain processes and technologies being leveraged or underused more than others?

Sabiło: Adoption of PI is progressing, although not uniformly across the industry. Single-use bioreactors and fluid-management systems are already well established, whereas high–cell-density processes, intensified fed-batch, and perfusion are becoming increasingly relevant. We also see growing interest in approaches that increase throughput and equipment utilization, such as multicolumn chromatography.

Where we see the greatest opportunity is not simply adopting individual technologies, but integrating them effectively into the overall manufacturing system. A more productive upstream process, for example, can shift constraints downstream or into scheduling, staffing, and support functions. The benefit therefore needs to be assessed across the entire value stream. PI should go hand in hand with flexible facility design, effective capacity planning, workforce flexibility, and data-driven operations. A productive process on its own does not necessarily translate into a productive facility.

The next stage of PI will therefore be about optimizing the manufacturing system as a whole, connecting process technology with people, assets, and operating models to improve throughput, flexibility and predictable delivery.

BPI: What difficulties might arise when a site begins to implement intensified processes? And what strategies might alleviate such problems?

Ludwisiak: The challenges usually extend well beyond the process itself. From a technical perspective, higher cell densities and longer or continuous operations can place additional demands on media preparation, harvest, clarification, and chromatography. Process control also becomes more important, and in many cases, process analytical technology (PAT) requirements become more sophisticated. If those dependencies are not considered early, improving one process step can simply shift the bottleneck elsewhere.

Operationally, established procedures may no longer fit a new process. Standard operating procedures (SOPs), training models, shift structures, cleaning approaches, and changeovers may all need to be reconsidered. There is also a financial dimension: Intensification can require investment in equipment and automation, but technology transfer and validation can temporarily consume capacity before PI's benefits are fully realized.

The human factor is equally important. Teams that have operated traditional fed-batch processes for many years need time to build confidence in new ways of working. We have found that the best approach is phased implementation: starting with clearly defined pilots, establishing measurable key performance indicators (KPIs), and involving manufacturing, manufacturing science and technology (MSAT), quality, and operational excellence functions from the beginning.

Capacity modeling and digital simulation can help identify constraints before physical changes are made. At the same time, training, flexible staffing, knowledge management, and standardization need to develop in parallel, allowing lessons

learned in one area or at one site to be transferred efficiently across the organization.

Aligning People, Processes, and Functions

BPI: Have you had a particularly memorable experience with PI or new-technology adoption? What did you learn from that experience?

Sabiło: A memorable experience was an operational optimization project for a commercial manufacturing program. By improving process execution and driving cross-functional collaboration, we doubled annual production between 2023 and 2024 without compromising product quality, GMP compliance, or documentation standards. The project enabled us to respond to increasing demand using existing assets, and it reinforced that increasing manufacturing productivity is not just about introducing new technology. The greatest gains come from aligning people, processes, and functions around a shared objective.

BPI: Intensification aside, what facilities and operational concerns are most pressing for CDMOs?

Sabiło: One of the biggest challenges for CDMOs is maintaining high utilization while supporting an increasingly diverse customer portfolio. Programs have different timelines, technical requirements, and levels of maturity, which creates constant pressure on capacity, scheduling, and workforce planning.

Access to experienced biomanufacturing talent is equally important. Beyond recruitment, CDMOs need to build broad skills and capabilities as well as flexible operating models that allow adaptation as demand changes. Supply-chain resilience, particularly for single-use components and critical raw materials, also remains essential.

At the same time, CDMOs need to remain cost-competitive while consistently meeting global quality and regulatory expectations. Our response is a strong operating system: standardized work, visual management, structured problem solving, continuous capability development, flexible staffing, and data-driven capacity planning. Together, these elements significantly increase the reliability and efficiency of our facility.

Future Facilities — and the People Who Will Make Them Work

BPI: As we think about 2030 and beyond, what do you believe will be the most transformative changes in bioproduction facility design and operation?

Ludwisiak: We expect bioproduction facilities to become more modular and digitally connected, with a stronger focus on end-to-end product flow rather than individual manufacturing operations. The ability to move between traditional batch, intensified, and continuous or semicontinuous processes with limited downtime will become increasingly valuable, particularly for CDMOs managing complex portfolios.

Digitalization will also move beyond reporting systems and user dashboards. Digital twins, advanced process control, and sophisticated scheduling tools supported by artificial intelligence (AI) will increasingly support real-time decisions around both process performance and resource allocation.

We also expect organizational models to change. Fixed departmental structures will gradually give way to more flexible, cross-functional teams with broader skill sets and a greater ability to move between activities. Technically, we will continue to see closer integration between upstream and downstream operations and a gradual move toward increasingly connected manufacturing trains.

Sustainability will also become a more integral part of facility design and operation. Energy and water consumption, as well as material and plastic waste, will increasingly need to be considered alongside productivity and cost rather than separate priorities. CDMOs that successfully combine process technology, digital capabilities, organizational flexibility, and sustainability will be well positioned to meet the growing expectations for speed, capacity, cost-efficiency and reliable supply.

BPI: What other concerns merit attention?

Ludwisiak: We want to draw attention to the human side of operational excellence. The industry spends a lot of time discussing equipment, automation, and new process technologies, but ultimately the performance of a facility depends on the people who operate it every day. The real challenge is creating an environment in which operators and supervisors do not simply execute a process, but also understand performance, identify losses, and take ownership of continuous improvement and cost-out opportunities. In our experience, technology and facility design deliver their full potential only when different parts of an organization develop at the same pace in terms of skills, ownership, accountability, and the ability to adapt.

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When the Lights Start Going Out

Photo Credit: Adobe Stock

Leading Quality Through Site Closure in Cell and Gene Therapy Manufacturing

Gurinderpal Singh

The good manufacturing practice (GMP) obligations of a licensed biomanufacturing site do not ease when the closure announcement is made. If anything, they intensify yet must be met by a team whose institutional future has just been put in question. That is the central challenge of quality leadership through site closure, and it is one that the biopharmaceutical industry is confronting with increasing frequency. I have led a cell and gene therapy (CGT) manufacturing site through this process, and what follows is what I have learned. This discussion is not a compliance checklist, but rather an account of judgment under pressure: the decisions that matter, the failures that surprise you, and the obligations that do not end when a building goes dark.

The Investment Boom That Preceded the Closures

Between 2020 and 2023, three forces converged to drive extraordinary capital investment into US biomanufacturing. COVID-19 exposed the fragility of offshore supply chains and created political momentum for domestic production capacity. Interest rates at near historic lows made borrowing cheap. And the clinical pipeline for CGTs was generating results that seemed to justify major infrastructure bets.

Companies that had previously relied on contract development and manufacturing organizations (CDMOs) began building or acquiring their own dedicated sites. The logic was defensible: Owning your manufacturing capacity meant owning your timeline, quality systems, and intellectual property (IP). Many of those facilities were purpose-built for viral-vector or cell-therapy manufacturing, featuring the biosafety containment, cleanroom architecture, and environmental-monitoring infrastructure that those modalities require. Some were built in established biotechnology clusters; others were the largest employers in small communities, representing significant local economic investment.

Within a few years, a different reality emerged. Sites built for anticipated commercial demand were producing only small phase 2 and 3 clinical-material batches and finding their manufacturing calendars far emptier than projected. The pipelines that justified the capital had not matured fast enough to fill the capacity.

A Reckoning: White Space, Failed Programs, and the Cost of Not Yet Being Commercial

The financial model of the CGT industry is unforgiving in a way that is easy to underestimate until you are inside it. Companies generate no meaningful revenue from a program until it successfully completes process performance qualification (PPQ), receive health-agency approval under an applicable regulatory pathway, and enters commercial distribution. Everything before that latter milestone — clinical trials, site infrastructure, quality-management system (QMS) maintenance, regulatory submissions, staff, and utilities — represents operating costs with no offsetting revenue.

Industry data from this period are sobering. The biopharmaceutical sector recorded 187 layoff rounds in 2023 alone — a 57% increase over 2022 — with at least 9,150 positions eliminated from companies that publicly reported numbers **(1)**. That same year, 27 biotech companies shut down entirely, representing the highest closure count since 2010 and, according to S&P Global Market Intelligence, the greatest number of bankruptcies in a given year since 2013 **(2)**. In 2024, 22 more companies closed **(3)**. By the first quarter of 2025, CGT companies accounted for nearly one-third of the 63 layoff rounds reported across the biopharmaceutical industry **(4)**.

Regulatory outcomes compounded the financial pressure. Between 2020 and 2024, the US Food and Drug Administration (FDA) issued over 200 complete response letters (CRLs). An analysis of those letters found that 74% cited quality or manufacturing deficiencies gaps in process control, insufficient

stability data, unvalidated analytical methods, or unresolved findings from good manufacturing practice (GMP) inspections **(5)**. For CGT specifically, July 2025 brought high-profile CRLs for both Capricor's CAP-1002 allogeneic cell therapy for Duchenne muscular dystrophy and Ultragenyx's UX111 adenoassociated virus (AAV)–based gene therapy for Sanfilippo syndrome type A, both citing manufacturing data gaps and facility readiness concerns **(6)**. About 40% of investigational new drug (IND) applications are stopped or not accepted due to chemistry, manufacturing, and controls (CMC) issues **(6)**, meaning that quality failures are shaping clinical futures long before a site closure is contemplated.

The practical consequence for many organizations was consolidation: Focus resources on the most commercially proximate asset, deprioritize the rest, and close sites that no longer fit the revised strategy. Site closures became, and remain, an industry-wide phenomenon.

The QMS Does Not Wind Down — It Closes Properly

The first thing a quality leader must internalize when a closure is announced is that GMP requirements remain fully in force until the last regulated activity is complete — and in important respects, well beyond that. The regulatory framework does not recognize "transition mode" as a basis for reduced scrutiny.

The starting point is a comprehensive inventory: What products were manufactured at this site, at what clinical phase, under which IND numbers, and to what clinical sites? Products distributed outside the United States to support clinical trials carry data retention and availability obligations under the International Council for Harmonisation (ICH) Q10 guidance document that must be mapped and honored regardless of the site's operational status **(7)**. Released batches in active distribution must remain fully supported. Any product not yet released at closure must have dispositioning planned: The product either must be completed and shipped before the site closes, or it must be formally rejected and destroyed per 21 *US Code of Federal Regulations* (CFR) Part 211 Subpart K **(8)**.

Early in the closure process at the site I led, we discovered several batches in various stages of disposition review that had not been formally tracked as open items. Identifying and working through each of those, with a team that was simultaneously managing their own professional transitions, required deliberate triage and daily prioritization that the closure plan had not originally accounted for. Build this discovery phase into your plan from the start; do not assume that your disposition picture is clean.

The level of decommissioning required is determined by the site's GMP history — e.g., whether it produced phase 2–3 trial material only or also commercial products, whether it was inspected by regulatory agencies, and so on. Sites where drug-product filling was performed must complete end-of-life aseptic process simulations (media fills) in accordance with FDA guidance on sterile drug manufacturing **(9)**. Sites where viral-vector manufacturing occurred must implement specialized cleaning protocols, including vapor hydrogen peroxide (VHP) treatment and validated sporicidal application, before decommissioning can proceed. Events — e.g., when equipment fails end-of-life calibration — must be managed through a company's QMS deviation system, just as it would have been during active operations. This is not a technicality; it is the same quality discipline, applied to a different operational phase.

Electronic System Decommissioning: The Governance Problem That Most Teams Miss

In my experience, electronic-system decommissioning is the area in which closure teams are most likely to underestimate both the complexity and the stakes. It deserves dedicated planning well ahead of any shutdown timeline.

Laboratory information management systems (LIMS), electronic QMS platforms, manufacturing execution systems (MES), and instrument-connected data acquisition systems all hold records subject to 21 CFR Part 11 requirements for electronic records and electronic signatures **(10)**. The retention obligations under 21 CFR Part 211.180 do not expire when a system goes offline. Batch records must be retained for at least one year beyond the product expiration date or one year after the date of approval, whichever is longer. For investigational products, records must be retained for two years after approval or two years after the investigation is discontinued.

The critical issue that most teams encounter too late is data format. When an electronic system is decommissioned, the underlying data frequently does not export into the formats that users have worked with throughout the system's life. What appeared as a formatted electronic document during operations may be stored as metadata that is only meaningfully readable within the original software application. Once the application license lapses, that data may be effectively inaccessible, not lost, but practically unreadable without the original environment.

At the site I led, we encountered this specifically with our analytical data management system. The vendor's end-of-life export process produced files in a proprietary format that required a reader application neither our archive partner nor our parent organization

had licensed. Resolving this added weeks to the decommissioning schedule and required negotiating a time-limited license extension we had not budgeted for. The lesson: involve your electronic system vendors in decommissioning planning at the earliest possible stage, and explicitly discuss data format, retrieval process, and long-term accessibility before you begin.

Identify a named *final responsible person* for each critical system — someone who understands the data architecture, the retrieval process post-decommissioning, and the chain of custody for access. This is a governance problem with a technical dimension. It requires a named owner, not a shared assumption.

Closing Records: The Longest and Most Underestimated Task

The formal closing and archival of all GMP records are consistently the longest and most underestimated tasks in a site closure. Under 21 CFR Part 211.180 and related provisions, no regulated data may be destroyed until the applicable retention period is met. For a site with a multiyear manufacturing history spanning several products, clinical phases, and customers, the volume of records requiring formal closure review is enormous.

The most common complication is open records — deviation investigations without final closure, change controls without final approval, and batch records with outstanding items — where the person responsible for the open action has already left the organization. In some cases, records opened years earlier by staff who departed long before the closure are discovered only when the archival inventory is conducted. Resolving such actions requires formal reassignment, documented review, and in some cases escalation to quality leadership for a risk-based disposition decision.

Plan for this inventory process to take longer than your initial estimate and start it earlier than feels necessary. In my experience, even well-maintained QMS environments surface unexpected open items when subjected to a true closure audit.

Materials Management, Vendor Obligations, and the Hidden Costs of Closure

The GMP warehouse at a closing site contains far more complexity than its square footage suggests. The disposition of its contents is one of the most logistically demanding and financially consequential aspects of site closure. Failing to plan out that process systematically creates both regulatory exposure and significant financial waste.

Not all materials are equal in terms of disposition pathway. Some items such as common laboratory consumables, single-use components, and standard packaging materials with no hazard classification and no regulatory restriction can be straightforwardly discarded through standard waste streams following site procedures. Others require formal, documented disposition through approved processes. Controlled substances must be handled under US Drug Enforcement Agency (DEA) authority. Biological materials, including cell banks, viral-vector stocks, primary cultures, and retained biological samples from viral-vector manufacturing programs, require containment, inactivation, or transfer procedures consistent with their biosafety-level classification and applicable US Centers for Disease Control and Prevention (CDC) and United States Department of Agriculture (USDA) regulations. Hazardous chemicals solvents, fixatives, and certain reagents used in molecular biology and analytical workflows must follow US Environmental Protection Agency (EPA) and local authority requirements for hazardous waste disposal. The site cannot simply leave any of that material behind; the obligation for proper disposition is explicit and enforceable.

A particularly frustrating and underappreciated financial reality emerges around materials with unexpired shelf lives. GMP warehouses typically carry significant inventory: raw materials ordered in bulk to support manufacturing campaigns, analytical reagents, reference standards, packaging components, and specialty single-use assemblies, just to name a few. When closure is announced, many such materials have months or years of remaining expiry. The instinct often is to donate or transfer those materials to another site in the network. In practice, doing that is harder than it seems. Every receiving site operates under its own quality system and is bound by its own approved supplier qualification program under 21 CFR Part 211 Subpart E. A material that was approved for use at the closing site is not automatically approved at the receiving site. The receiving site must either have the supplier already on its approved vendor list (AVL), initiate a supplier qualification process for the transfer, or formally accept the material through a documented quality agreement and risk assessment, none of which are fast or simple. For standalone sites with no sister facilities, finding an external recipient willing and able to absorb materials through a compliant transfer is even more difficult. Most of such inventory, despite representing real procurement dollars, ends up as waste.

Vendor and supplier contract management adds another layer of complexity that generates its own financial and administrative burden. When a site closure is announced, the downstream effect on the supply chain is immediate: Vendors begin receiving termination notices, purchase orders stop flowing, and outstanding orders must be assessed for cancellation or receipt. Many supplier agreements carry minimum purchase commitments, early termination fees, or auto-renewal clauses that the site may be locked into regardless of its operational

status. Starting the contract review process early (ideally within the first weeks of a closure announcement) is critical to minimizing such costs. The returns process generates its own administrative wave: return merchandise authorizations (RMAs), credit memos, shipping logistics, and vendor quality documentation requirements that, in aggregate, create weeks of work across the procurement and quality teams.

For sites that were working with a CDMO for any portion of their manufacturing or analytical process at the time of closure, an additional coordination layer is mandatory. The CDMO may be holding in-process materials, quality-control samples, retained samples, reference standards, or finished product on behalf of the closing site. These materials do not automatically transfer or dispose of themselves when the client site closes. The parent company must work directly with the CDMO through formal change notifications, disposition orders, and updated quality agreements to ensure that every item is accounted for, formally planned for dispositioning, and either returned, destroyed, or transferred to another qualified facility. Retained samples held at the CDMO must continue to meet retention period requirements regardless of where operational responsibility has shifted. That factor requires explicit contractual and quality documentation, and in many cases, renegotiation of the CDMO agreement to cover wind-down services that were not contemplated in the original scope of work.

Proprietary Information and Intellectual Property: What Closure Does Not End

The data generated at a manufacturing site over its operational life represent a substantial and often irreplaceable body of scientific and technical knowledge. Process development data, cell banking records, viral-vector production parameters, analytical-method development packages, comparability studies, stability datasets, and manufacturing history reports contain information that may have direct value for future programs whether at the same organization, through a licensing arrangement, or through a future partnership. In the CGT space specifically, where the manufacturing science is still maturing and validated processes are hard-won, such knowledge has real and lasting value. One clinical failure or site closure does not invalidate the underlying biology, process understanding, or analytical methods developed to characterize a product.

The regulatory framework reinforces that point. Proprietary manufacturing data, including batch production records, process validation packages, and analytical-method validation data, are explicitly protected under applicable trade secret law and, where applicable, data-exclusivity provisions under the Biologics Price Competition and Innovation Act (BPCIA) **(11)**. The closing of a site does not release such data into the public domain, nor do they become available to competitors or academic collaborators. The same confidentiality obligations that governed their use during manufacturing continue to govern their custody after operations cease.

In practice, this factor means that quality and regulatory leadership must identify a designated custodian for all proprietary technical data packages early in a closure-planning process. That person is distinct from the regulatory record-retention custodian responsible for GMP documentation. The IP custodian is responsible for ensuring that process knowledge, trade secrets, and proprietary analytical data are transferred to appropriate organizational stakeholders. In many cases, that transfer process requires coordination with legal counsel and, when applicable, with licensing partners or codevelopment collaborators who may have rights to certain data under existing agreements.

Electronic systems again create specific risks in that context. MES data, process-historian records, and analytical data repositories may contain proprietary process parameters and in-process control data that are not formally classified as trade secrets but still carry significant competitive value. When those systems are decommissioned, ensuring that such data are captured in a secure, accessible format and transferred to the correct organizational owner rather than simply being archived requires deliberate planning. The default behavior of many decommissioning processes is to archive data in a format optimized for regulatory retrievability, not for future technical use. These are different requirements that need separate planning tracks.

In CGT and viral-vector manufacturing, scientific and technical knowledge is often the most valuable asset that a site leaves behind. A clinical program that failed still generated process understanding that may inform a next-generation approach, a partnership, or a future IND. A site that closes because a company ran out of capital still has manufacturing knowledge that a successor could build upon. Treating the wind-down of that knowledge base with the same rigor as the wind-down of the physical facility is both a good practice and a form of stewardship for the science. In a field where the medicine still being developed could one day change the lives of patients with rare or life-threatening diseases, that stewardship matters.

Managing Surprises: What the Plan Never Anticipates

Every site closure that I am aware of encounters at least one significant unplanned event. At the site I led, our most acute moment came when a key validation engineer — the person most familiar with our advanced planning and scheduling (APS) qualification history and our end-of-life media-fill protocol —

accepted a position elsewhere four weeks before the media fill was scheduled to be executed. The media fill that was supposed to be a routine run turned very quickly into the critical path for our site closure timeline.

The media fill itself identified an excursion that required a full deviation investigation, impact assessment, and remediation cycle before decommissioning could proceed. None of that extra time was accounted for in the original closure plan. Having maintained the QMS with full rigor throughout the closure process rather than treating it as a formality once the shutdown was announced meant that we had the documentation infrastructure to manage the deviation properly and close it defensibly.

Other surprises to anticipate include electronic-system migrations that surface data-integrity gaps requiring retroactive remediation; decommissioning activities that encounter unexpected equipment conditions; and key subject-matter experts departing earlier than expected, consistently. Build explicit contingency reserves into the schedule for each of these. Map your single points of failure — the people and systems where departure or failure creates the greatest compliance risk — and establish redundancy before you need it.

What This Work Asks of Leaders — and What It Costs Communities

Leading quality through a site closure requires applying the full technical rigor of GMP compliance to a situation where every organizational incentive is working against you. Employees are managing their own futures. Systems are being systematically dismantled. And the institutional energy that once ran the facility is being redirected elsewhere.

Emotional intelligence in this context is not a secondary skill; it is what makes technical execution possible. The quality professionals, operators, and scientists who are being asked to close a site correctly are the same people whose careers, incomes, and professional identities are bound to that site as well. Many of those employees were involved when site was being built; some of them turned down other opportunities to be there. A site-closure announcement does not affect just the employees in the room, but also families, local suppliers, service businesses, and communities, particularly those in which the manufacturing facility was among the largest employers in the region. The human weight of that reality is something that quality leaders must hold genuinely. because the people they are leading feel it every day.

In my experience, the quality leaders who navigate closures most effectively are not the ones who compartmentalize the human reality and focus purely on process. Rather, they acknowledge the difficulties openly, communicate with transparency about what is known and what is not, and then, precisely because they have earned their team's trust, are able to ask people to bring their best professional judgment to an extraordinarily hard situation.

The obligation to patients who received product from a closing site does not end with the announcement. The obligation to data integrity does not end. The obligation to regulators who may conduct a follow-up inspection years after closure does not end. Quality leadership means honoring those obligations even when the environment makes it hard. The work of closing a site correctly is, in its own way, a form of patient care.

Looking Forward

The wave of site consolidations across the CGT space is not over. The industry added significant manufacturing capacity during an extraordinary period of capital availability, and the rationalization of that capacity will continue. More closures are coming, and the quality leaders who will navigate them are in many cases already working at sites that do not yet know their future.

And yet, as I have tried to argue throughout this article, the end of a site is not the end of the science. The process knowledge, analytical methods, and biological understanding that was generated within those walls have a future. Treating the wind-down of that knowledge base with the same discipline and intentionality as the wind-down of the physical facility is an obligation that the profession owes both to the patients that those programs were designed to serve and to the scientists who built the knowledge in the first place.

The regulatory framework will not change to accommodate the difficulty of this work. What can change is how prepared the profession is — how clearly the lessons of the first wave of closures are articulated, shared, and built into the plans of those who come next.

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Breaking the $50/g Barrier

From Aspiration to Industrial Reality

Jean-Marc Cappia, Jon Kruger, Gerben Zijlstra, Beatrice Claverie, Fabrizio Pelella, Vincent Lam, Sherwin Ting, Behnam Partopour, and Katy McLaughlin

Over the past three decades, monoclonal antibody (mAb) manufacturing has shifted from batch processes in large, stainless-steel equipment to smaller, more flexible operations based on single-use systems (SUS) and, increasingly, intensified and continuous processing. This evolution reflects several converging market and manufacturing trends:

- changing market economics — the mAb market continues to grow at a compound annual growth rate (CAGR) of about 10% **(1)**, but average peak sales per molecule have declined while development costs have increased
- increasing product diversity — pipelines are shifting toward biosimilars and complex modalities, including multispecifics, antibody–drug conjugates (ADCs), and fragment crystallizable (Fc)–fusion proteins, driving the need for rapid facility buildout, high asset use, and efficient multiproduct operations
- lower-volume demand — more than 80% of commercial biologics require production volumes of <500 kg/year, and almost 50% require <100 kg/year **(2)**
- evolving production models — regionalization and sustainability pressures are pushing manufacturers toward lean production models.

Such developments fundamentally challenge the economics of traditional, large-scale batch manufacturing and favor smaller, more agile, and more productive facilities enabled by process intensification (PI). In this environment, reducing manufacturing cost per gram of antibody product is increasingly important to commercial viability, particularly for therapies serving small patient populations, for which development costs must be recouped across relatively fewer doses.

Manufacturing Economics for mAbs: A Turning Point

Currently, the industrial cost of mAb manufacturing ranges from US$50–500/g of product, with an average of about $300/g depending on annual production volume, process efficiency, and facility footprint and utilization **(3)**.

Manufacturing cost distribution is broadly consistent across the industry. Upstream processing represents about 40% of total cost of goods (CoG), downstream processing about 50%, and formulation and fill–finish about 10%. Protein A chromatography is the single largest cost contributor (Figure 1).

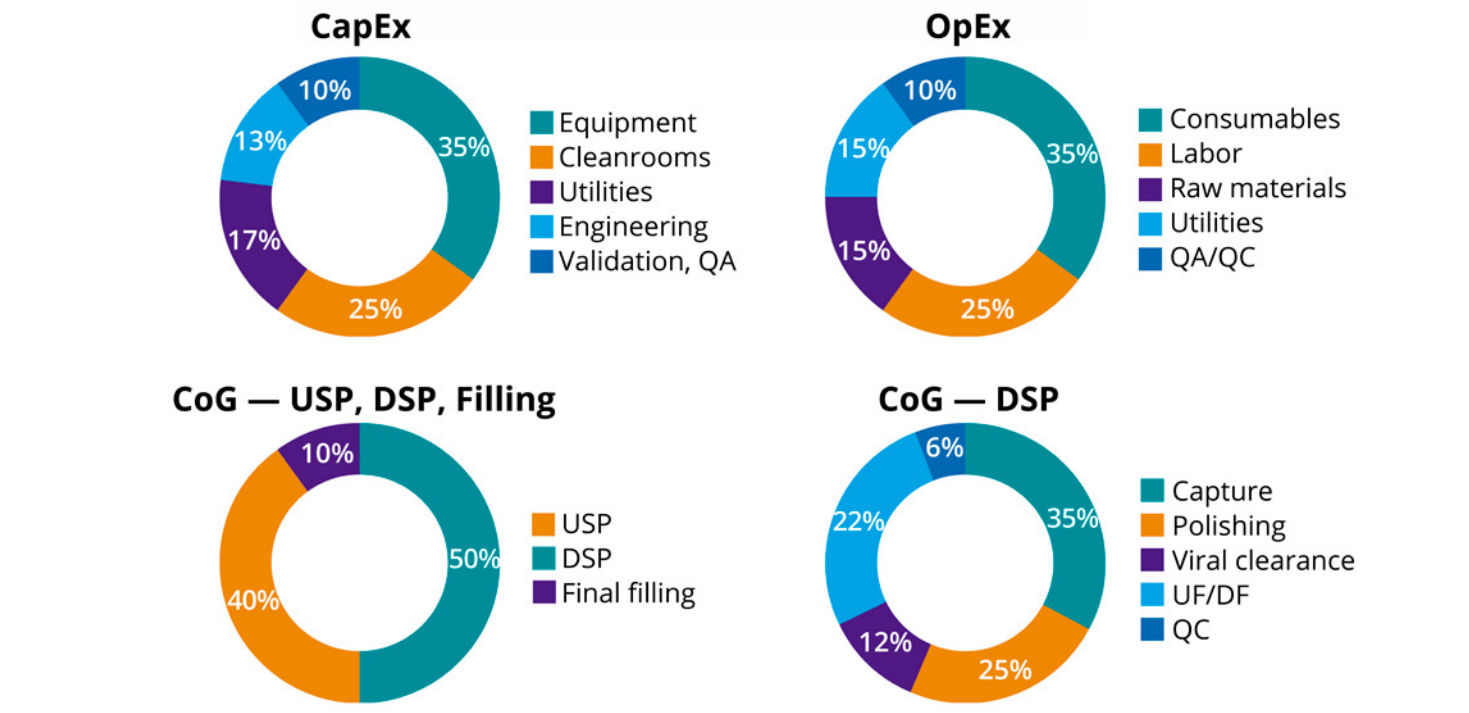


Figure 1: Cost breakdown for a typical monoclonal antibody (mAb) manufacturing process; CapEx = capital expenditure, CoG = cost of goods, DSP = downstream processing, OpEx = operating expense, QA = quality assurance, QC = quality control, UF/DF = ultrafiltration/diafiltration, USP = upstream processing

Production scale and annual volume have a strong influence on unit cost: Production volumes under 500 kg/year typically incur costs of $250–500/g, whereas production exceeding 2 tons/year can achieve costs close to $50/g.

Improvements in cell-culture productivity, platform processes, and SUS have already reduced costs for large-demand production. However, even at large scales, conventional batch processes involving protein A–based separation have a practical limit of $50/g **(4, 5)**.

The holistic application of PI now makes it possible to consistently reduce mAb manufacturing costs to below $50/g — a threshold that once was considered theoretical but now achieved by some innovative companies.

This progress has brought manufacturing economics into sharp focus. High-profile initiatives, most notably from the Gates Foundation, have established an aspirational target of $10/g for mAb production to broaden global access, particularly in low- and middle-income countries **(7)**. Such targets highlight both the feasibility of further cost reduction and the growing urgency to close the remaining gap.

A manufacturer's economic position shapes how readily lower costs can be achieved. New market entrants face the capital expenditure (CapEx) required to build greenfield facilities, but new builds also provide the opportunity to implement system-level transformation across process design, facility configuration, and digital operations from the outset, which will be critical to driving CoG below $50/g. Established manufacturers with brownfield infrastructure may already achieve costs well below that level because the CapEx associated with large, existing facilities has largely been amortized, with further reductions made possible by upgrading and repurposing current assets. Organizations with legacy operations and overseas facilities also benefit from supplier relationships, discounts, and relatively low labor costs, allowing them to reach CoG in the range of $20/g.

Across the industry, further cost reductions will rely on PI across upstream processing, downstream processing, facility design, and digital operations. The following sections examine how such approaches are being evaluated and implemented incrementally to support efficient, scalable mAb manufacturing below $50/g.

Intensification Across the Manufacturing Train

Achieving costs below $50/g requires a systematic increase in productivity rather than further gains from scale alone. PI enables that shift by increasing output per unit volume, time, and footprint while reducing the capital, labor, and consumables required per gram of product. Intensification strategies continue to be implemented incrementally across the industry, progressing from conventional batch processes (Level 0) to intensified batch (Level 1), connected operations (Level 2), and fully continuous manufacturing (Level 3) **(8)**. Figure 2 illustrates that progression for downstream processing.

Efforts have focused primarily on adoption of SUS and upstream PI, featuring the development of increasingly productive cell lines and culture media combined with intensified fed-batch or perfusion technologies. However, as upstream yields improve, downstream processing efficiencies become the next challenge. Stepwise downstream intensification — ranging from intensified batch operations to fully continuous downstream workflows — can significantly reduce timelines, cut chromatography CoG, and reduce facility footprint while providing a pathway to end-to-end intensification for sustainable and cost-effective facilities.

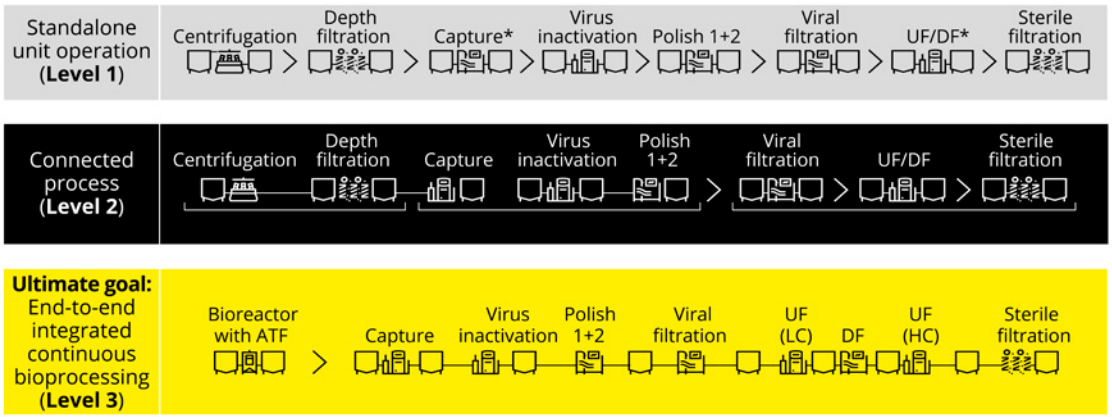


Figure 2: Levels of process intensification for monoclonal antibody (mAb) manufacturing, from intensification of standalone unit operations to fully integrated continuous bioprocessing; an asterisk (*) indicates use of an intensified consumable. (ATF = alternating tangential-flow filtration, DF = diafiltration, HC = high concentration, LC = low concentration, MCC = multicolumn chromatography, UF = ultrafiltration)

Upstream Productivity Gains: In upstream processing, innovation in cell-line engineering, omics-driven process understanding, advanced media and feed strategies, scalable single-use solutions, and real-time process analytical technology (PAT) have already dramatically improved cell-culture productivity. PI strategies are also well established in upstream operations, where intensified fed-batch and perfusion processes further increase productivity. Together, such developments have raised average titers from 0.5–1 g/L in the early 2000s to 5–10 g/L in 2026, with intensified processes exceeding 10 g/L. This increase in productivity has reduced the cost per gram of product by a factor of 2–5.

Upstream intensification progresses from *N* − 1 perfusion with high-inoculation fed-batch (Level 1), through concentrated fed-batch (CFB) processing (Level 2), to dynamic or continuous *N*-stage perfusion (Level 3). Each step increases productivity but also operational complexity. *N* − 1 perfusion can deliver ≈2× productivity gains with limited facility disruption. CFB can increase yield 3–5× and enable SUS to approach the output of large stainless-steel bioreactors, but it requires greater media, clarification, harvesting, and downstream capacity. *N*-stage perfusion can exceed 100 million cells/mL and reach 5 g/L/day with >10-fold yield gains for some molecules, but the technique demands substantially greater media use, advanced control, flow balancing, and downstream integration.

The Downstream Cost Challenge: Although upstream innovations and intensification strategies have contributed substantial cost reductions, significant gains come from downstream PI . Traditional downstream processing involves sequential, batchwise operations over three to six days, creating bottlenecks as upstream titers increase. Economically, downstream processing accounts for around 50% of total mAb CoG, with chromatography alone contributing 25–30% (Figure 1) **(5)**. Therefore, reducing downstream processing costs to below $25/g is critical to achieving an overall CoG below $50/g.

As in upstream processing, downstream intensification progresses through three levels, from intensified standalone operations, through connected processes, to fully continuous processing. Each step increases throughput and reduces idle time, hold volumes, equipment size, and buffer and resin requirements, but it also requires greater process coordination and automation.

Intensified batch operations can lower costs by up to 30% while shortening timelines by about 24 hours. At higher levels of intensification, parallelized and connected operations can reduce facility footprint by around 50% and increase throughput by ≈2×. Fully continuous downstream processing can reduce purification workflows from five to six days to 24–48 hours and can be coupled with either fed-batch or perfusion upstream processes **(9)**.

Chromatography as the Primary Cost-Reduction Lever: Chromatography is the major downstream cost driver, with protein A capture alone accounting for up to 35% of total downstream expenses (Figure 1). Breaking the $50/g barrier requires transformation of the chromatography train through innovations in resin or membrane use, process design, and technology integration.

Membrane-based rapid-cycling chromatography addresses limitations of traditional resin-based systems, including long cycle times and operational complexities associated with column packing and storage. Multiple short membrane cycles can substantially increase productivity while maintaining yields and product-quality levels comparable with resin-based capture. Membrane capacity can also be used fully within a limited number of production runs, making this approach particularly beneficial in clinical, low-volume, and multiproduct manufacturing, where resins are underused.

Fully membrane-based workflows, typically involving bind–elute capture followed by double–flow-through polishing, can reduce chromatography costs to below $10/g, creating the margins required to achieve overall CoG below $50/g.

Multicolumn chromatography (MCC) is an essential bridge toward continuous manufacturing. It increases effective binding capacity, reduces resin volumes and buffer consumption, and enables continuous or semicontinuous operation. MCC can be applied to both membrane- and resin-based systems, allowing small columns or devices to support high throughput.

Connected and Continuous Downstream Processing: At high levels of intensification, connecting and coordinating downstream unit operations can reduce the scale and cost of the purification train. In the modeled example of an upstream processing suite comprising six 2000-L fed-batch bioreactors operating at titers of 8 g/L, continuous downstream processing using MCC reduced total chromatography resin volume by 65% and buffer consumption by 26%. Those reductions lowered annual costs by $1.2 million (Figure 3).

	Column Size, Capture	Column Size, Polish 1	Column Size, Polish 2	Total Columns
Batch DSP	80 × 20 cm	80 × 20 cm	45 × 20 cm	3
Continuous DSP	40 × 8 cm (×3)	60 × 20 cm	40 × 20 cm	5

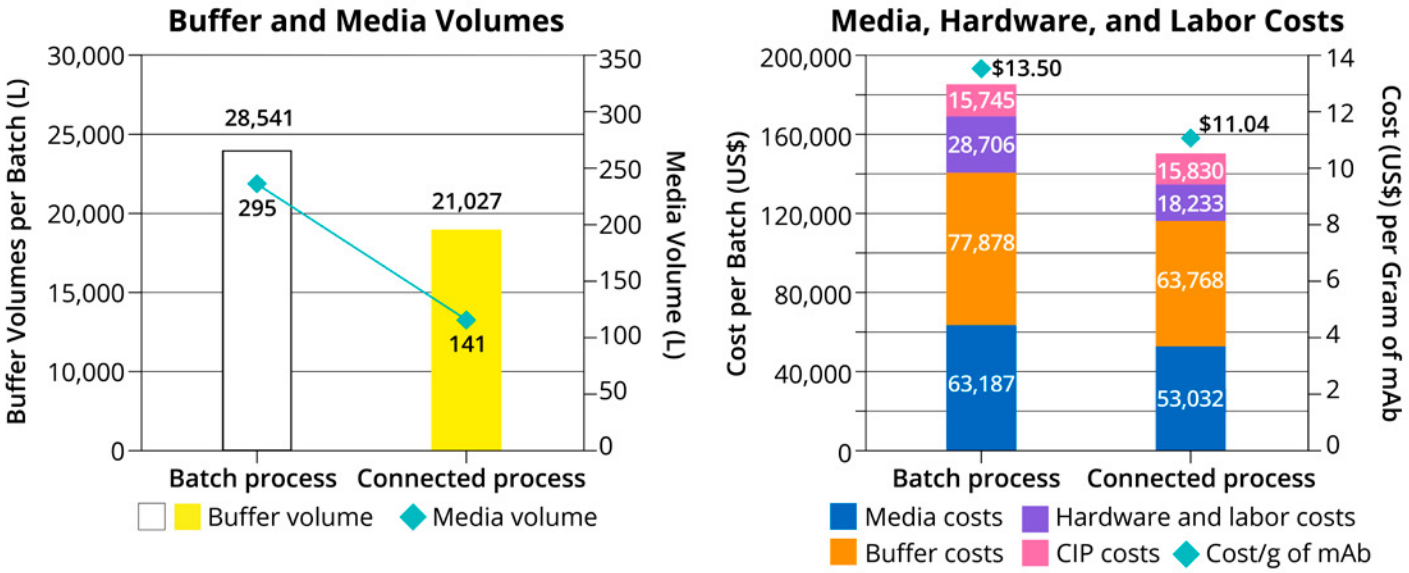


Figure 3: Continuous downstream processing (DSP) coupled with an upstream process based on six 2000-L fed-batch cell cultures significantly reduces chromatography column size, decreasing annual costs (by 19%), buffer consumption (26%), and footprint (40%). (CIP = clean in place, mAb = monoclonal antibody)

Enablers of Facility-Wide Process Intensification

Moving beyond intensification of individual unit operations requires enabling technologies and capabilities that support implementation across a facility. SUS provide a flexible manufacturing platform, a strong digital and data infrastructure enables proactive and coordinated operation, and collaborative partnerships and decision-support tools help manufacturers to select and implement the most appropriate strategies.

Single-Use Technologies: SUS are foundational elements of cost-efficient intensified manufacturing. They reduce both direct costs (e.g., for utilities) and indirect costs (due to downtime, lost capacity, and validation effort). When combined with intensified processes, SUS enable modular and adaptable facilities that are correctly sized and optimized for actual product demand rather than theoretical capacity. Single-use technologies also support efficient multiproduct operations, which is particularly relevant given that the majority of commercial mAb products are produced at annual volumes below 500 kg. In contrast, facilities with large stainless-steel equipment entail large upfront capital investment, chronic underuse in single-product facilities, and changeover inefficiencies in multiproduct operations.

Data Integration and Automation: As processes become more intensified and interconnected, manual operation becomes inefficient and unsustainable. A robust digital ecosystem is therefore essential for process intensification **(13)**. Capabilities might include real-time monitoring of critical process parameters and quality attributes with linked PAT and multivariate data analysis, predictive and model-based control, synchronization of parallel unit operations, automated deviation detection, and electronic batch records.

Automated control and flow balancing across perfusion rates, chromatography loading, buffer preparation, and virus inactivation can reduce labor requirements, minimize operator-induced variability, and improve right-first-time performance. Such capabilities support diminished CoG through improved effective capacity use and reduced product loss from deviations or failures.

Data analytics, cost-modeling tools, and process simulations also enable manufacturers to identify bottlenecks, predict the benefits of PI, and make data-driven decisions early in development. The Expert Chromatography Intensifier Tool (ExCIT), for example, enables comparison of batch, connected, and continuous downstream configurations; resin- and membrane-based workflows; and bind–elute and flow-through strategies. By predicting CoG, buffer requirements, throughput, and timelines, such tools can reduce risk, accelerate development, and support decision-making from early process development through commercial scale.

The Path Forward

There is no one-size-fits-all path or single PI format for all mAbs; a structured, stepwise approach enables manufacturers to secure early gains while building toward fully integrated, intensified operations. However, achieving the <$50/g benchmark will require manufacturers to change fundamentally how their processes are

designed and operated at scale rather than effecting incremental improvements to legacy systems and workflows.

Implementing upstream PI by combining highly productive engineered cell lines with CFB processes or continuous perfusion can reduce costs by enabling small, single-use setups to achieve outputs comparable with those of large, stainless-steel facilities. But such an approach is insufficient on its own. The largest PI gains come from downstream processing; approaches such as low-cost or alternative protein A resins, membrane chromatography, and continuous capture and polishing reduce costs by lowering chromatography media and buffer consumption.

Further gains are achieved through integrated continuous biomanufacturing, which reduces CapEx, OpEx, and cycle time while increasing asset utilization. Combined, those measures realistically can bring manufacturing costs down to $30–40/g for large production volumes, as demonstrated for greenfield investments in our previous report **(14)**.

Bringing CoG below $50/g requires overcoming the limitations of the traditional "protein A + batch" approach.

Moving beyond that threshold requires coordinated efforts across upstream processes, downstream processes, SUS, digital integration, and overall manufacturing strategy. Few organizations possess all of those capabilities internally. Partnerships with experienced technology and solution providers can provide access to technoeconomic models, integrated processing platforms, digital infrastructure, and the technical and validation support required for implementation.

Sartorius can support the development of your PI strategy through consulting services. Our bioprocess expertise and cost-modeling tools can help you to compare manufacturing scenarios, from fed-batch to continuous processes, and identify the optimal strategy for reducing CoG below $50/g.

Learn more about the economics of <$50/g mAb manufacturing in our custom report **(14)**. It's time to intensify.

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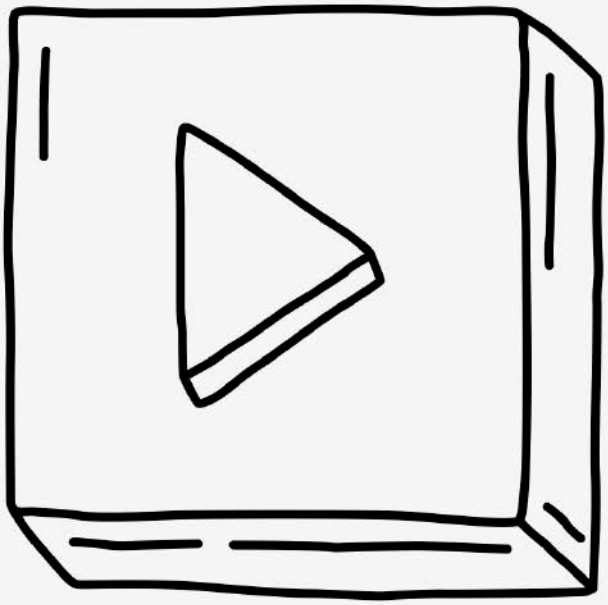
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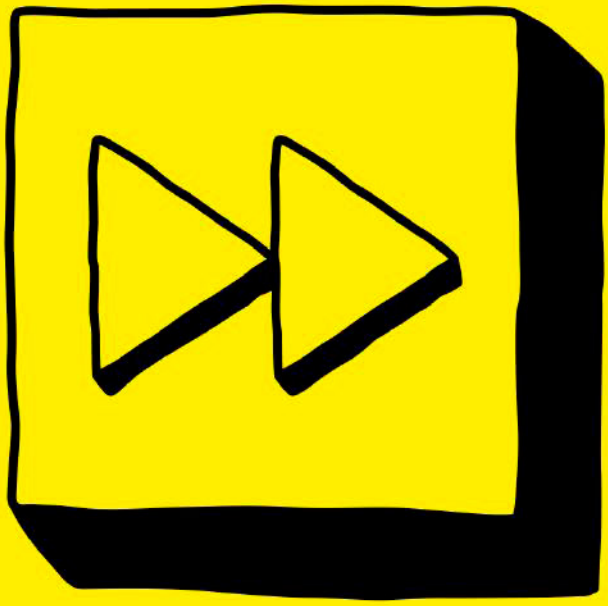
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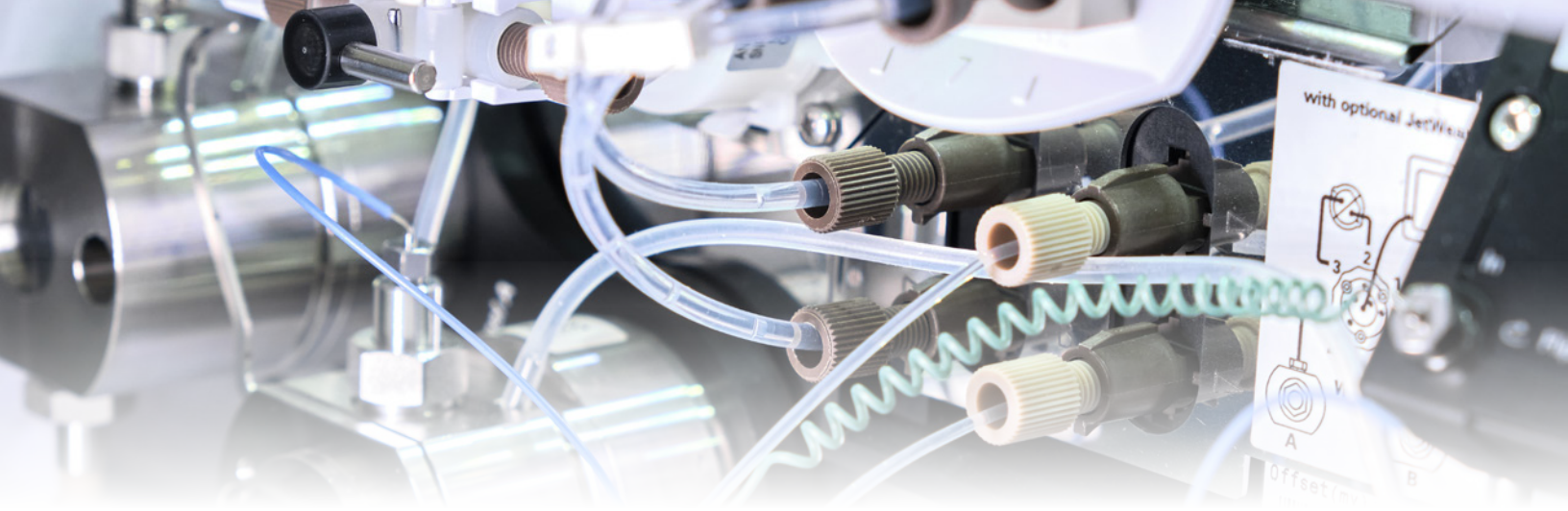


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Simulation-Aided Development of Multicolumn Chromatography Processes for Protein-Based Vaccine Production

Alan Deduyer, Eric Forma, Estelle Guiot, Lucrèce Nicoud, and David Pfister

Vaccines based on recombinant proteins represent a diverse class of immunological products, with antigenic proteins exhibiting significant variability depending on the target. Such proteins can differ substantially in terms of molecular weight, isoelectric point, tertiary structure, and glycosylation profiles. Furthermore, they are produced using various expression systems, including prokaryotic platforms such as *Escherichia coli* and eukaryotic systems such as Chinese hamster ovary (CHO) cells or *Spodoptera frugiperda* 9 (*Sf*9) insect cells **(1, 2)**. Such heterogeneity poses considerable challenges for downstream process development because purification strategies must be tailored to each product.

To address those challenges and accelerate vaccine availability, Sanofi is pursuing a broadly applicable, platform-based approach to process development. This strategy aims to reduce development timelines, facilitate scale-up, and streamline process transfer into manufacturing facilities. A key element of the platform is a capture step using affinity-tag–based chromatography, which offers high selectivity across diverse protein targets. However, affinity resins present significant limitations: They are relatively expensive and often exhibit limited dynamic binding capacity (DBC) **(3)**. Such constraints can necessitate large column geometries with substantial facility footprints, which may be incompatible with industrial-scale implementation, as well as considerable resin volumes that translate into prohibitive costs.

In recent years, *multicolumn chromatography (MCC) configurations*, in which several columns are interconnected and operated in a coordinated manner, have demonstrated significant potential for enhancing process productivity. Although the approach has been primarily adopted in continuous bioprocessing applications, it is equally relevant for recombinant-protein–based vaccine manufacturing. MCC can improve use of resin binding capacity, thereby reducing required resin volumes and associated buffer consumption (therefore contributing to process sustainability) while ensuring consistent product quality and facilitating scale-up.

MCC operation relies on semicontinuous or continuous purification based on cyclic chromatography, in which at least two columns are connected in series in the loading zone to efficiently capture breakthrough from the first loaded column. Meanwhile, other columns operate in parallel to perform the remaining chromatography phases: wash, elution, cleaning, conditioning, and so on **(4)**. Flow paths switch cyclically, such that each column progresses through the sequence of steps, ultimately leading to steady-state operation after an initial ramp-up phase. Figure 1 (next page) depicts the principle of MCC operation.

Despite such advantages, MCC development can be experimentally intensive, requiring numerous trials that consume substantial quantities of product to reach steady-state conditions and to identify optimal operating parameters. Those include the number of columns in the loading zone, the percentage of breakthrough at the first column in the loading zone before column switching (linked to the loading ratio), loading flow rate (i.e., residence time), column geometry, and the number of

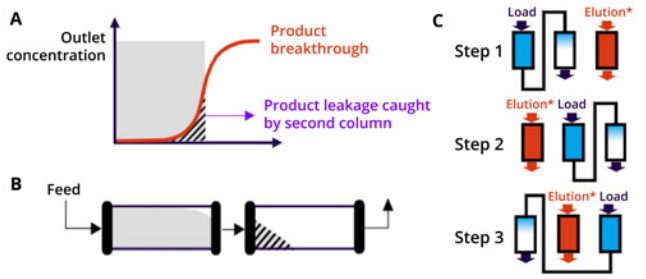


Figure 1: Principle of multicolumn affinity chromatography; (a) illustrative product breakthrough curve at the column outlet of the column; (b) illustrative profiles inside the columns; (c) example of implementation of a process with three columns, two in the loading zone and one in the elution zone; * = elution includes wash, regeneration, and reequilibration operations.

cycles required to process a defined amount of product, as well as classical chromatography parameters such as step volumes, buffer composition, and gradient or isocratic elution conditions.

Mechanistic modeling of chromatography steps offers an efficient solution to accelerate MCC process development while significantly reducing both the experimental burden and product requirements **(5, 6)**. By accurately predicting chromatographic behavior under a range of operating conditions, mechanistic models enable rapid in silico screening of process parameters, thereby streamlining the path from concept to implementation. In silico approaches enable selection of the most appropriate MCC parameters based on predefined objectives (e.g., yield, cost, and process duration) before performing confirmatory MCC experiments at laboratory or bench scale.

This study demonstrates the application of mechanistic modeling to support development of an MCC affinity capture step for recombinant-protein–based vaccine production, illustrating how such an approach can facilitate and derisk the implementation of a broadly applicable platform process-development strategy.

Materials and Methods

Materials: The target product is a multimeric recombinant protein expressed in CHO cells. The product is clarified before injection on a chromatography system.

Applied buffer solutions included

- 20 mM tris(hydroxymethyl)aminomethane (tris) (pH 7)
- 20 mM tris, 150 mM NaCl (pH 7)
- 20 mM tris, 1 M NaCl (pH7)
- 20 mM tris, 4 M NaCl (pH 7)
- 20 mM tris, 1.5 M $MgCl_2$ (pH 7)
- 20 mM tris, 2 M $MgCl_2$ (pH 7)
- 0.5 M citric acid
- 20% ethanol.

Single-column experiments were performed on an Äkta Pure 25 chromatography system from Cytiva, and a BioSMB PD system from Sartorius was used to perform MCC experiments. At laboratory scale, 5-mL prepacked columns (diameter = 0.8 cm, bed height = 10 cm) were used. Pilot-scale experiments leveraged 43.5-mL of resin packed in HiScale columns from Cytiva (diameter = 2.6 cm, bed height = 8.2 cm).

Linear-Gradient Elution (LGE) Experiments: These experiments used 5-mL prepacked columns. For each condition, 2 mL of purified product dialyzed against equilibration buffer was injected. Table 1 details operating conditions for the LGE experiments.

Experiment	Velocity (All Steps)	Gradient Solution	Gradient Length
LGE1	200 cm/h	A: 20 mM tris, pH 7 B: 20 mM tris, 2 M $MgCl_2$, pH 7	20 CV
LGE2	200 cm/h	A: 20 mM tris, pH 7 B: 20 mM tris, 2 M $MgCl_2$, pH 7	10 CV
LGE3	200 cm/h	A: 20 mM tris, pH 7 B: 20 mM tris, 2 M $MgCl_2$, pH 7	5 CV
LGE4	200 cm/h	A: 20 mM tris, pH 7 B: 20 mM tris, 4 M NaCl, pH 7	20 CV

Table 1: Operating conditions for the linear-gradient elution (LGE) experiments; CV = column volume

Breakthrough Experiment: The breakthrough experiment was performed using a 5-mL prepacked column. Clarified harvest was loaded at a flow rate of 100 cm/h. During injection, flowthrough was collected in 2-mL fractions. Product was loaded until the ultraviolet (UV) signal of the flowthrough was equal to that of the clarified harvest.

Single-Column Purification Experiments: Single-column experiments were performed using 5-mL prepacked columns. The optimal loading ratio was determined to be 4.8 column volumes (CV) of clarified harvest at a residence time of 5 minutes (120 cm/h). The single-column chromatography method was optimized thanks to the modeling of this step. The optimized method is described in Table 2 (next page).

Step	Buffer	CV	Velocity	Residence Time
Equilibration	20 mM tris, 150 mM NaCl, pH 7	5	300 cm/h	2 min
Injection	Clarified harvest	4.8	120 cm/h	5 min
Wash	20 mM tris, 1 M NaCl, pH 7	3	200 cm/h	3 min
Elution	20 mM tris, 1.5 M $MgCl_2$, pH 7	5	150 cm/h	4 min
Regeneration	0.5 citric acid	4	200 cm/h	3 min
Water rinse	H_2O	5	300 cm/h	2 min
Buffer rinse	20 mM tris, 150 mM NaCl, pH 7	5	300 cm/h	2 min
Storage	20% ethanol	5	300 cm/h	2 min

Table 2: Description of the optimized single-column process; CV = column volume

Multicolumn Purification Experiments: MCC experiments were performed using a BioSMB chromatography system from Sartorius. A three-column configuration, with two columns in the loading zone, was used for all experiments. Details of the method are provided in Table 3.

Step	Inlet	Volume (CV)	Velocity	Residence Time	Outlet
Loopback	Load/chase	12	Lab scale: 180 cm/h Scale-up (10 L): 148 cm/h	3.31 min	Flow through
Load	Clarified harvest	7	Lab scale: 180 cm/h Scale-up (10 L): 148 cm/h	3.31 min	Loopback
Chase	20 mM tris, 150 mM NaCl, pH 7	5	Lab scale: 180 cm/h Scale-up (10 L): 148 cm/h	3.31 min	Loopback
Wash	20 mM tris, 1 M NaCl, pH 7	5	Lab scale: 300 cm/h Scale-up (10 L): 246 cm/h	2 min	Wash
Elution	20 mM tris, 2M $MgCl_2$, pH 7	5	Lab scale: 300 cm/h Scale-up (10 L): 246 cm/h	2 min	Elution
Equilibration	20 mM tris, 150 mM NaCl, pH 7	10	Lab scale: 300 cm/h Scale-up (10 L): 246 cm/h	2 min	Drain

Table 3: Multicolumn-chromatography method; the scale-up volume listed (10 L) corresponds to the amount of crude solution processed. (CV = column volume)

Loading velocities at laboratory and pilot scales varied due to differences in column-bed heights between the two scales. To maintain identical residence time across all chromatography steps, and thus comparable step durations, velocities were adjusted accordingly.

At laboratory scale, the product feed concentration was 0.55 g/L, and three cycles were performed. An initial equilibration phase served to equilibrate all three columns simultaneously. Then, a shutdown phase was performed using a standard operation cycle in which the clarified harvest injection was replaced by a pause.

At pilot scale, the product feed concentration was 0.50 g/L, and 11 cycles were performed. An initial equilibration phase served to balance all three columns at the same time. A shutdown phase was performed using the same approach as at laboratory scale, consisting of a standard operation cycle in which the clarified harvest injection was replaced by a pause.

Mechanistic Model

Model Description: Mechanistic models for chromatography are built on three fundamental pillars **(7, 8)**:

- *thermodynamics,* which describes how species behave in a solution and how they interact with a chromatographic medium at equilibrium
- *hydrodynamics,* which describes how fluid flows through a column
- *kinetics,* which describes how rapidly species diffuse from a solution to a chromatographic medium and the other way around.

Thermodynamics acts as the "engine" driving the separation, whereas hydrodynamics and kinetics serve as two "brakes" that degrade separation performance due to peak broadening.

Solution Equilibria: Acid–base dissociation equilibria were accounted for to allow proper calculation of pH and conductivity signals. For simple molecules such as those found in buffer solutions (e.g., tris), each protonation state is considered as an individual species, and acid–base equilibria are described using their associated dissociation constant (pK_a) values. For instance, $tris^+ \rightleftarrows tris + H^+$ with a pK_a of 8.1. For complex molecules such as proteins, a single species is considered, with a net charge that varies as a function of pH. Dissociation equilibria are defined for each amino-acid residue together with their associated p*K*a values **(9)**. Based on that information, it is possible to calculate, at any given pH, the total number of negative charges, the total number of positive charges, and the net charge (i.e., the sum of negative and positive charges).

Adsorption Isotherm: Because this study focuses on highly specific affinity chromatography, it was assumed that only proteins bearing the affinity tag would interact with the chromatographic medium while all impurities would elute in the liquid phase without interacting with the solid phase. Based on those assumptions, a classical noncompetitive Langmuir adsorption isotherm was selected:

Equation 1:

$$\bar{C}_j = \bar{\lambda}_j C_j + \frac{\bar{N}\tilde{K}_j C_j}{1 + \tilde{K}_j C_j}$$

where $\bar{C}_j$ and C_j are the concentrations of protein j in the solid and liquid phases, respectively. The term $\bar{\lambda}_j$ represents the linear contribution of the isotherm, $\bar{N}$ is the adsorbent capacity, and $\tilde{K}_j$ is the affinity parameter, whose value depends on the magnesium chloride concentration [Mg^{2+}]. The following equation was used:

Equation 2:

$$\tilde{K}_j = \tilde{K}_{0,j}\left(1 + \frac{\tilde{K}_{0,j}}{\tilde{K}_{Mg2+}}[Mg^{2+}]^n\right)^{-1}$$

where *n*, $\tilde{K}_{Mg2+}$, and $\tilde{K}_{0,j}$ are model-fitting parameters.

Hydrodynamics: Column hydrodynamics were described using a cascade of mixing cells in series. The key model parameter is *J*, which represents the number of mixing cells.

Kinetics of Mass Transfer: Mass-transfer kinetics were described with the linear driving force approximation. For simple species, a constant characteristic internal mass transfer time (t_i) was used. Proteins required an expression depending on the retention strength:

Equation 3:

$$t_i = 1 + t_{i,0}\exp(-n[Mg^{2+}])$$

where $t_{i,0}$ is a fitting parameter and *n* is the parameter used in the adsorption isotherm.

External mass transfer (t_e) was described using the Wakao–Funazkri correlation:

Equation 4:

$$t_e = 1 + 2\frac{t_{e,0}}{2 + 1.1Re^{0.6}Sc^{0.33}}\exp(-n[Mg^{2+}])$$

where *Re* and *Sc* are the Reynolds and Schmitt numbers, respectively.

All simulations were performed with Ypso-Ionic software (Ypso-Facto).

Determination of Model Parameters: Model parameters were determined using a methodology described previously **(10, 11)**. Briefly, LGE experiments were performed to estimate the model parameters of the adsorption isotherm, with the exception of the adsorbent capacity, which was determined from a breakthrough curve. Parameters related to the kinetics of mass transfer were initially estimated from the breakthrough curve and subsequently refined using an MCC experiment conducted at 7 CV loading.

Results

Process Description: Switching from a batch process to an MCC approach required several adjustments. The first critical bottleneck identified was an imbalance between the duration of the nonloading steps and that for the loading step. Therefore, a study was conducted to evaluate the feasibility of eliminating resin cleaning between consecutive cycles. This study demonstrated that, even without resin regeneration between cycles, product quality still met specifications, and resin lifetime was not adversely affected.

To further improve the time balance between loading and nonloading steps, the flow rate and volume of each step were optimized. First, the mechanistic model was applied to predict optimal conditions; then, those conditions were confirmed experimentally.

The final step toward establishing a functional MCC method was determination of the loading volume. The availability of a second column in the loading zone enables higher loading volumes than

is possible in batch operation. To determine the optimal volume, model-based yield predictions were generated as a function of loading volume and flow rate. Based on those predictions, experimental trials were performed, confirming an optimal loading volume of 7 CV under the conditions investigated. Figure 2 presents a schematic representation of the selected MCC method.

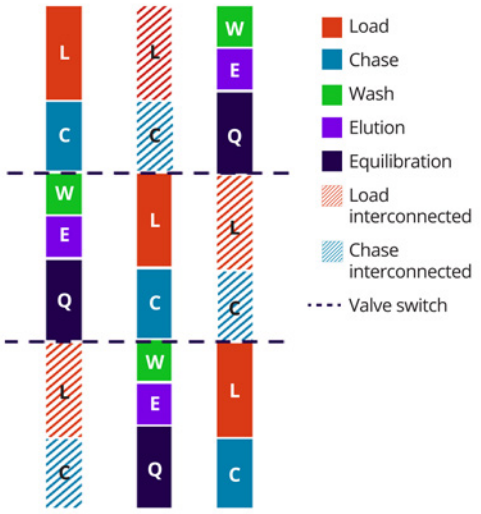


Figure 2: Selected sequence of operations for the three-column process

Process Understanding: Simulation provides access to information that is not easily obtainable experimentally. That is, for instance, the case for detailed outlet concentration profiles, which can be ascertained in silico at each column's outlet and inlet, within the collection tank, and even in the drain (e.g., waste tank). In contrast, experimental measurements are typically limited to ultraviolet (UV) signals recorded at a few specific locations.

Simulations also provide access to internal concentration profiles, the visualization of which enhances process understanding. One illustrative application is the study of system ramp-up, during which an MCC process progressively reaches a steady state. Figure 3a shows the product concentration at the outlet of the first column in the reference BioSMB run with three columns (7 CV loading) receiving identical injections. Figure 3b shows the corresponding internal concentration profiles. Note that the resin contains less bound product at the end of cycle 1 than at the end of subsequent cycles, indicating that a few cycles are required to reach steady state.

A strategy to accelerate the achievement of steady state and increase productivity consists of applying a power-feed approach, in which a larger amount of product is loaded during the first injection than during subsequent injections. The corresponding results are presented in Figures 3c and 3d, where the injection volume of the first cycle is doubled. Under those conditions, steady state is reached immediately.

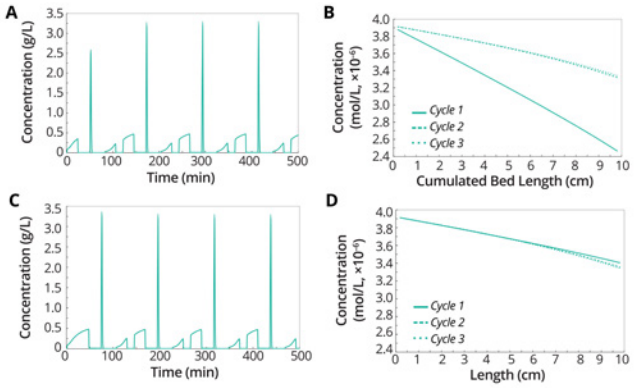


Figure 3: Simulated impact of the power-feed strategy to reach steady-state operation; (a) product concentration at the outlet of the first column in the reference case; (b) internal concentration profiles in the reference case; (c) product concentration at the outlet of the first column in the power-feed case; (d) internal concentration profiles in the power-feed case

Process Optimization: Simulation was used to predict the impact of two critical process parameters (CPPs) — namely, loading volume and loading velocity — on three key performance indicators (KPIs) — yield, productivity, and eluent consumption — for a three-column configuration at laboratory scale. KPIs were defined as follows:

Equation 5:

$$yield = \frac{m_{prod.eluate}}{m_{prod.feed}} = \frac{V_{eluate} \times C_{eluate}}{V_{feed} \times C_{feed}}$$

Equation 6:

$$productivity = \frac{m_{prod.eluate}}{V_{tot.col.} \times t_{run}} = \frac{V_{eluate} \times C_{eluate}}{n_{col.} \times V_{col.} \times t_{run}}$$

Equation 7:

$$eluent = \frac{V_{proc.water}}{m_{prod.feed}} = \frac{V_{proc.water}}{V_{feed} \times C_{feed}}$$

where C is the product concentration (in the eluate or feed), m is the mass (of product in the eluate or of product in the feed), $n_{col.}$ is the number of columns, t_{run} is the process run time, V is volume (of feed, eluate, or process water), $V_{col.}$ is the volume of one column, and $V_{tot.col.}$ is the total volume of the columns ($V_{col.} \times n_{col.}$).

Figure 4 (next page) presents simulation results with surfaces. Experimental data are shown as black dots, and the corresponding raw data are provided in Table 4. The simulation shows good agreement with the experimental results. Simulation can therefore be used to screen many operating conditions rapidly and to identify those with the most promising performance.

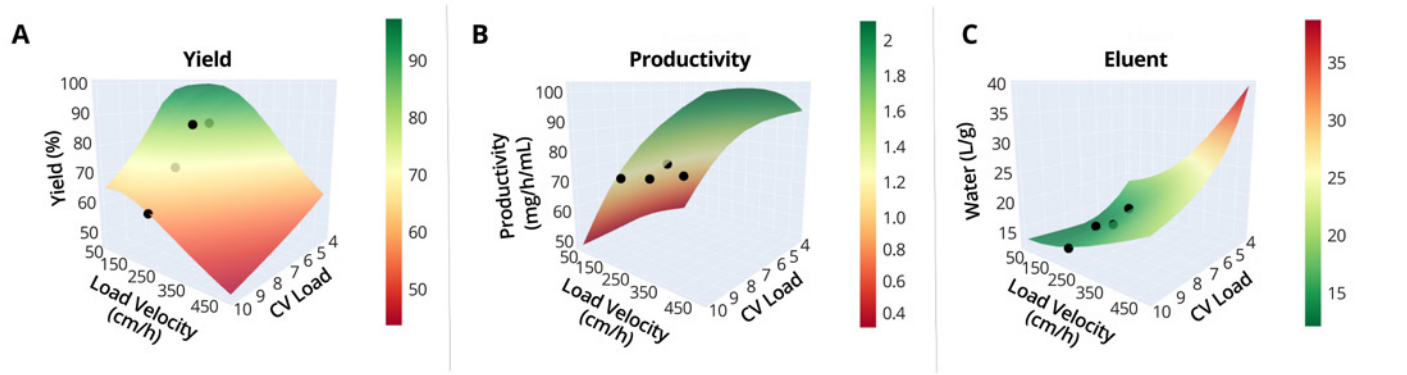


Figure 4: Impact of loading volume and velocity on yield, productivity, and eluent consumption; black dots correspond to experimental results. (CV = column volume)

Parameter	7 CV (reference)	6 CV	8 CV	10 CV
Load time (min)	23.47	20	26.67	26.316
Flow rate (mL/min)	1.5	1.5	1.5	1.9
Load linear velocity (cm/h)	180	180	180	227
Run time (min)	502	509	533	502
Eluate volume (mL)	290.5	291	290.2	291.5
Eluate concentration (g/L)	0.515	0.437	0.491	0.521
Mass entering process (mg)	174.26	148.50	198.02	247.50
Mass exiting process (mg)	149.61	127.17	142.49	151.87
Yield (%)	85.85	85.63	71.95	61.36
Productivity (g/L/h)	1.19	1.00	1.07	1.21
Eluent (L/g)	13.64	15.67	14.62	14.31

Table 4: Overview of experimental runs performed at laboratory scale with the BioSMB chromatography system; CV = column volume

The highest yields were obtained at low loading velocity and low loading volume because those conditions minimized product loss during the loading phase. However, low loading velocities are associated with reduced productivity. Therefore, a trade-off must be identified to achieve high yield alongside reasonable productivity. Eluent consumption increases with faster velocities due to greater product losses. It also decreases with higher loading volumes because a larger amount of product is processed per cycle, even though yield (as a percentage) may decrease.

Defining a cost function allows consideration of multiple KPIs at once and thus eases process selection. In this study, the following cost function was used:

Equation 8:

$$cost = \frac{a}{yield} + \frac{b}{productivity \times time} + c(eluent) + d(time)$$

where *time* refers to process time, *a* quantifies the feed cost (€/g of product), *b* represents the resin cost (€/L resin), *c* is the raw-material cost (€/L of eluent), and d equals the labor cost (€/hour). For those parameters, illustrative values were considered to demonstrate the impact of loading volume and velocity on cost.

Figure 5 shows the cost results as a function of the loading volume and loading velocity.

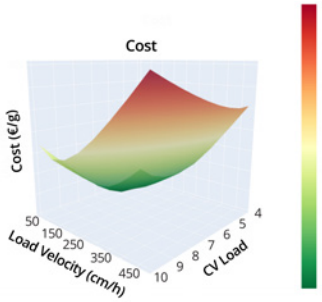


Figure 5: Impact of loading volume and velocity on cost; CV = column volume

Process Scale-Up: Once a mechanistic chromatographic model is developed at laboratory scale, it can be used to predict process performance at other scales. This capability can be useful, for instance, to

- verify in advance that an operating recipe considered for large-scale implementation is appropriate, thereby reducing the number of large-scale runs
- understand and interpret potential discrepancies between predicted and experimental results, thus supporting decision-making for corrective actions.

Such an approach is illustrated in Figure 6, where the BioSMB process operated at 7 CV loading was scaled up to process 10 L of clarified harvest. Three columns were used in the system. UV signals from the experiments nicely overlapped with the

simulated concentration profiles for both for the collection tanks (Figure 6a) and drain (Figure 6b). Because the UV signal measured in the drain was saturated, a detailed comparison between experiment and simulation was not possible for this stream. Experimental and simulated yields were 85.6% and 86.6%, respectively, demonstrating the good predictive capability of the model.

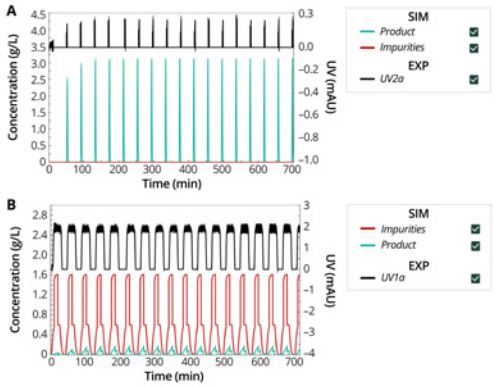


Figure 6: Experimental (EXP) and simulated (SIM) values for the three-column BioSMB run with 7 column volumes (CV) loading at the 10-L scale; (a) product and impurity concentrations at the inlet of the collection tank; (b) product and impurity concentrations in the drain. (UV = ultraviolet light)

Discussion

Interest of an MCC Approach: This section presents an application example with the following objectives:

- a yield above 85%
- 15 cycles per column, corresponding to the expected resin lifetime
- processing of 200 L of clarified harvest to generate drug substance for use in clinical trials.

According to Figure 4, the operating conditions that enable a yield above 85% while maximizing productivity (which is the main cost driver because it directly reflects the total CVs required) are a loading volume of 6.5 CV and a loading velocity of 180 cm/h.

The required total column volume ($V_{tot.col.}$) can be estimated as follows for both single-column and multicolumn systems:

Equation 9:

$$V_{\text{tot.col.}} = \frac{V_{\text{feed}} \times C_{\text{feed}}}{N_{\text{cycles}}} \times \frac{1}{capacity \times margin}$$

where V_{feed} is the volume of feed to be processed, C_{feed} is the product concentration in the feed, $N_{cycle}s$ is the number of cycles required to process the feed volume, *capacity* is the static capacity of the chromatographic medium, and *margin* corresponds to the fraction of the resin that is effectively used. The closer that value is to unity, the more efficiently a resin is being used. In a multicolumn system, columns connected in series during the loading phase allow capture of breakthrough product, resulting in a higher margin compared with a single-column system.

Table 5 presents results for both the single-column and MCC configurations, considering a C_{feed} value of 0.55 g of product/L of resin and a resin capacity of 3.4 g of product/L of column. For the single-column process, two scenarios were evaluated: a single injection of feed solution (single pass) and two consecutive injections of feed solutions (double pass). We found that the multicolumn process enables close to a 20× reduction in total column volume compared with the classical single-column, single-pass process. A 10-fold reduction in column volume was achieved relative to the double-pass single-column process.

	Single Column, Single Pass	Single Column, Double Pass	Multicolumn
Margin	60%	60%	95%
Number of cycles	1	2	15
Total column volume	53.92 L	26.96 L	3 × 0.76 = 2.27 L

Table 5: Column sizing for the single-column and multicolumn processes

Interest of Mechanistic Modeling Approach: MCC development requires substantial quantities of product as well as considerable development time. Indeed, for each experimental condition, several cycles must be performed to reach steady-state conditions. With a mechanistic modeling approach, computational experiments can be conducted, and only the most promising operating conditions need to be tested experimentally, substantially reducing laboratory effort and material consumption.

Selection of an optimal loading ratio and flow rate provides a clear illustration of the benefit. In the absence of a mechanistic model, a design of experiments (DoE) approach would typically be applied. Using a response-surface design with two center points (one of the simplest designs), 10 experiments would be required to explore the design space. In contrast, using a mechanistic model allows identification of three experiments that would be sufficient to select the appropriate operating parameters. In the data presented here, four experiments were conducted, with the additional condition (10-CV loading) used solely for model validation. Considering that ≈400 mL of product is required per experiment, a DoE-based approach would therefore require three times more material than would the mechanistic modeling strategy.

Beyond experimental efficiency, a mechanistic approach also represents a powerful tool for securing scale-up because it supports rational column-geometry selection and enables verification that selected flow rates will not exceed pressure-drop limitations.

Conclusion

This study demonstrates that mechanistic modeling represents a powerful, efficient, and reliable approach for MCC process development. Our findings also show that a mechanistic model for an MCC process can be readily derived from an existing batch chromatography model, providing a robust foundation for process understanding and optimization.

The established model proved to be accurate and predictive, as evidenced by the close agreement between experimental results and model simulations across different scales. That validation confirms the robustness and applicability of mechanistic modeling for MCC process development and scale-up.

Compared with conventional statistical DoE approaches, mechanistic modeling offers several distinct advantages for MCC development:

- enhanced process understanding — a mechanistic framework provides deeper insight into underlying physical and chemical phenomena, enabling more informed and rational decision-making
- improved process efficiency — for example, the model supports faster achievement of steady-state conditions and enables global process optimization across operating parameters
- system-independent process definition — process characterization can be performed independently of a specific chromatography system, requiring only limited adjustment of system specific parameters (e.g., dead volumes)
- optimal parameter determination — key operating parameters (e.g., loading volume and flow rate) can be optimized according to defined objectives (productivity, process duration, and yield), allowing rapid comparison of multiple scenarios to identify optimal trade-offs (e.g., yield versus productivity)
- significant reduction in experimental effort — a mechanistic approach requires approximately threefold fewer experiments than do standard DoE strategies, substantially reducing resource consumption and development timelines
- streamlined scale-up — the model accurately predicts process behavior across scales, reducing risk and experimental burden during scale-up activities.

Overall, implementation of mechanistic modeling for MCC process development provides capabilities that cannot be achieved by experimental approaches alone, including reduced experimental effort, enhanced process knowledge, and the ability to evaluate alternative process strategies in silico. Consequently, mechanistic modeling represents a key enabling element for the design of efficient, scalable, and robust MCC processes, supporting the successful achievement of development and manufacturing objectives in biopharmaceutical manufacturing.

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Disclosure of Interests

Eric Forma and Alan Deduyer are Sanofi employees and may hold shares and/or stock options in the company.

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