



Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Continuous Bioprocessing Needs Control Architectures That Link Upstream Variability to Final Product Quality

Noah Williams^{1*}, Grace Collins¹, Ethan Brooks², Daniel Green³

¹Department of Continuous Bioprocessing and Process Control, Faculty of Pharmacy, University of Sydney, Sydney, Australia.

²Department of Upstream Variability and Product Quality, Faculty of Pharmacy, University of Queensland, Brisbane, Australia.

³Department of Manufacturing Process Integration, Faculty of Pharmacy, University of New South Wales, Sydney, Australia.

*Email: noah.williams@sydney.edu.au

ABSTRACT

Continuous bioprocessing is often described as a sequence of intensified unit operations connected by material flow. That description is operationally useful but analytically incomplete because it does not explain how disturbances originating in cell culture are transformed, attenuated, amplified, or obscured before they appear as final product-quality variation. This Systems Perspective argues that continuous biomanufacturing should be controlled as a coupled, multiscale system in which observable process variables, latent cellular states, intermediate product states, downstream transformation states, and critical quality attributes remain analytically distinct. The central proposal is a quality-first control architecture that links upstream disturbance detection to state estimation, cross-unit propagation logic, quality-relevant observation, and recovery decisions without assuming that any single surrogate variable is sufficient. Evidence from integrated continuous antibody manufacturing, perfusion culture, downstream intensification, online quality monitoring, soft sensing, and model-based control shows that many components of such an architecture are technically feasible, but not yet validated as one unified control system. The proposed framework therefore emphasizes evidence boundaries, applicability domains, model uncertainty, and the need to distinguish process recovery from product-quality recovery. Continuous manufacturing can become more robust not merely by increasing automation, but by organizing automation around the causal and inferential distance between upstream variability and final product quality.

Keywords: Continuous bioprocessing, Process analytical technology, Critical quality attributes, Perfusion culture, Digital twins, Bioprocess control

Received: 24 September 2025; **Revised:** 14 December 2025; **Accepted:** 18 December 2025

INTRODUCTION

Continuous bioprocessing has moved from an aspirational manufacturing concept toward technically credible implementation, yet the central control problem is still often framed too narrowly. Integrated manufacturing can reduce intermediate hold steps, enable smaller equipment footprints, and support intensified operation, but these benefits depend on how well the connected process is characterized and controlled across unit boundaries [1]. Pilot-scale end-to-end demonstrations show that continuous upstream and downstream operations can be physically integrated into a functioning manufacturing train [2]. The unresolved systems question is therefore no longer whether continuity is possible, but whether the state of the product can be followed reliably as biological variability is transmitted through successive transformations.

This distinction matters because downstream continuity is not achieved simply by placing continuous versions of familiar unit operations in sequence. Reviews of monoclonal-antibody purification have emphasized that intensification upstream changes loading patterns, equipment demands, residence-time behavior, and control requirements downstream [3]. Contemporary analyses of continuous downstream processing likewise identify automation, monitoring, synchronization, and regulatory qualification as integral parts of the manufacturing problem rather than peripheral engineering details [4]. A quality-first architecture must therefore connect upstream biological variation to downstream process states and final critical quality attributes (CQAs), while preserving uncertainty about links that are only predicted, indirectly observed, or product-specific.

Continuous bioprocessing as an interconnected system

An integrated continuous train behaves as a coupled system because the output of one operation becomes the time-varying input of the next. Pilot-scale work with acid-sensitive monoclonal antibodies has shown that connected upstream and downstream processing can be operated while maintaining product attributes within the tested process configuration [5]. Process-wide automation studies similarly demonstrate that multiple continuous operations can be coordinated through supervisory control rather than treated as isolated local loops [6]. These demonstrations support the feasibility of integrated operation, but they do not establish that a single system-wide control strategy will be transferable across molecules, cell lines, scales, or facility designs.

The coupling becomes especially important when a batch-derived platform is converted to continuous operation. Process characterization has been used to redesign downstream trains so that loading, viral inactivation, and polishing operate under new flow and residence-time conditions [7]. Earlier integrated antibody-production studies also showed that perfusion output and continuous capture can be coordinated through control loops while process performance and product quality are monitored [8]. Together, these observations motivate a distinction between physical continuity and quality-state continuity. The former is achieved when material moves reliably across connected operations. The latter requires evidence that biologically relevant changes entering the train can be detected, interpreted, and either corrected or shown not to threaten the final product.

Upstream sources of variability

Upstream variability is not a single category. It includes deliberately manipulated inputs, slowly drifting process conditions, abrupt disturbances, and biological state changes that are not fully captured by conventional set points. In CHO N-1 perfusion, cell-specific perfusion rate influences growth and metabolism even when alternative perfusion-control approaches produce broadly similar biological behavior within the tested operating range [9]. Medium-composition studies in high-density perfusion likewise show that formulation choices can alter culture performance and glycosylation-relevant product attributes, with the resulting models remaining dependent on the investigated culture conditions [10]. These findings indicate that upstream disturbances should be represented by both their directly measured process variables and their potential biological consequences.

A second class of variability is temporal and partly latent. Perfusion cultures can move through distinct metabolic states despite apparently stable continuous operation, and engineered CHO systems have shown phase-dependent metabolic reorganization associated with changing productivity [11]. Time-resolved studies have also demonstrated feed-induced cell-cycle oscillations that disappear under continuous feeding, showing that the same average nutrient supply can produce different temporal cellular behavior [12]. For control purposes, the relevant upstream state is therefore not always equivalent to the instantaneous value of pH, dissolved oxygen, feed rate, or viable cell density. Sampling frequency and control interval determine whether biologically important oscillations, transitions, or drifts are visible at all.

These distinctions are easier to compare in **Table 1** because the practical control consequence depends on whether variability is directly observed, inferred through a cellular state, or recognized only after product behavior changes.

Table 1. Distinguishing upstream variability by the evidence needed to connect process disturbance with a quality-relevant state

Upstream scenario	Directly observable process or cellular state	Inference that is justified	Competing explanation Discriminating evidence Consequence
-------------------	---	-----------------------------	---

Scientific anchor	Directly observable process or cellular state	Inference that is justified	Competing explanation Discriminating evidence Consequence
Shift in cell-specific perfusion rate	Perfusion rate, viable-cell behavior, nutrient and metabolite trends	Cellular state may change with altered medium renewal	Competing explanation requiring discrimination Similar behavior may arise from clone-specific metabolism or another operating variable Discriminating evidence Matched metabolic and growth trajectories across the operating transition Consequence for control architecture Treat perfusion rate as a disturbance input, not as a complete biological-state descriptor
Change in medium composition	Defined media inputs, growth response, measured product-attribute change	Media chemistry can alter culture state and selected product attributes	Competing explanation requiring discrimination Apparent quality change may depend on the specific clone, formulation, or operating window Discriminating evidence Cross-condition culture and product-attribute measurements Consequence for control architecture Preserve a separate medium-to-cell-state link before quality inference
Metabolic phase transition during perfusion	Productivity trend and measurable metabolic markers	Continuous operation may contain distinct biological states	Competing explanation requiring discrimination The transition may reflect engineered genotype, adaptation, or process history Discriminating evidence Time-resolved metabolic and process data Consequence for control architecture Maintain a latent-state estimator rather than assuming stationarity
Feed-induced oscillatory behavior	Feed timing, online cell-cycle or population-state signals	Periodic operation can generate temporal cellular structure	Competing explanation requiring discrimination Equivalent average feed may mask different temporal dynamics Discriminating evidence Sampling fast enough to resolve the oscillation Consequence for control architecture Match sensing and control bandwidth to biological dynamics
Stable conventional set points with changing product behavior	Nominal pH, dissolved oxygen, temperature, flow, and density remain in range	An unmeasured biological state is plausible	Competing explanation requiring discrimination Downstream transformation or analytical drift may instead explain the change Discriminating evidence Orthogonal upstream-state and downstream-quality evidence Consequence for control architecture Do not escalate an upstream causal interpretation until alternative sources are separated

Intermediate product states

The product leaving the bioreactor is not merely a concentration value entering purification; it is an evolving molecular population whose composition can reflect upstream history. Intensified perfusion has been associated with changes in monoclonal-antibody homogeneity, including glycosylation-related distributions, under specific process conditions [13]. Such findings do not imply that intensification is intrinsically detrimental. They show instead that productivity and volumetric throughput cannot be used as sufficient proxies for product state. The control architecture must preserve the possibility that two cultures with similar bulk output differ in quality-relevant molecular composition.

Some informative states may also be intracellular rather than directly visible in the harvested product stream. In CHO cells producing a bispecific antibody, elevated endoplasmic-reticulum pH was associated with high growth and increased aggregation behavior [14]. Because the evidence is associative, ER pH should not be treated as a proven causal control target. Its broader significance is methodological: latent cellular conditions can carry information about product formation that conventional process variables may miss. A systems architecture therefore needs a place for intermediate biological and product states that are estimated from multiple measurements rather than equated with any single sensor output.

Downstream propagation

Downstream processing adds new state transitions rather than simply revealing the quality state created upstream. Continuous membrane capture can develop time-dependent pressure and fouling behavior that changes the operating condition of the separation step [15]. Continuous precipitation-filtration creates additional particulate and washing states that can be monitored during capture [16]. These operations therefore introduce their own sources of variability, and an observed final-product deviation cannot automatically be traced backward to the bioreactor.

Coupling between adjacent downstream operations further complicates attribution. Integration of single-pass tangential-flow filtration with high-performance countercurrent membrane purification demonstrates that upstream concentration and preconditioning can alter the load presented to the following purification step [17]. Mechanistic analysis of single-pass tangential-flow filtration also shows that axial concentration, viscosity, and osmotic-pressure effects can create internal transport states that are not represented by inlet and outlet values alone [18]. In a continuous train, propagation should therefore be represented as a sequence of state transformations, each with its own uncertainty. The practical question is not simply whether a disturbance moves downstream, but whether the downstream operation attenuates it, amplifies it, converts it into another quality-relevant state, or makes the original source no longer identifiable.

Critical quality attributes

CQAs provide the point at which process control is expected to reconnect with product-quality knowledge, but analytical observability is uneven. A digital twin developed for continuous chromatography has shown that model-based control can use online analytical information to support quality-oriented pooling and operation of an antibody purification step [19]. Real-time detection of monoclonal-antibody aggregates has also been demonstrated within an integrated downstream process [20]. These studies establish that selected quality-relevant states can be monitored or estimated during manufacturing rather than waiting exclusively for final offline testing. The limitation is that a measurable CQA is not equivalent to complete knowledge of product quality. Rapid process analytical methods have been implemented for selected attributes of co-formulated monoclonal-antibody products, including concentration- and ratio-related measurements, but the analytical scope remains attribute- and product-specific [21]. A control architecture should therefore distinguish four questions: whether an attribute can be measured; whether the measurement is sufficiently reliable for the intended decision; whether the attribute meaningfully reflects a propagated process state; and whether that relationship has been qualified for the product and operating domain. **Table 2** reconciles these evidence states without assuming that analytical availability alone establishes regulatory or clinical significance.

Table 2. Reconciling observable quality signals with the inference required for continuous-process decisions

Quality-state case	Context	Interpretation	Validation	Decision
Scientific anchor	Evidence sources creating the state	Inference justified Inference not yet justified	Qualification test needed	Manufacturing decision endpoint
Online aggregate signal changes during downstream processing	Real-time analytical signal plus contemporaneous process state	Inference justified from current evidence Aggregate-related product state has changed within the measurement domain Inference not yet justified The upstream cause or clinical consequence is known	Orthogonal aggregate assay, process-state alignment, product-specific acceptance logic	Escalate investigation or control action according to qualified process knowledge
Digital-twin estimate crosses a quality-oriented operating boundary	Model state, online analytical update, chromatography context	Inference justified from current evidence The modeled purification state may require a pooling or operating adjustment Inference not yet justified Final product quality is guaranteed by the model	Prospective model verification, residual analysis, continued analytical confirmation	Permit model-assisted control only inside the validated domain

Rapid concentration or component-ratio measurement remains stable	Qualified rapid analytical method and product-specific assay context	Inference justified from current evidence Selected measured CQAs remain stable Inference not yet justified Unmeasured CQAs or biological activity are necessarily unchanged	Orthogonal CQA panel and product-specific method qualification	Maintain operation while preserving broader quality surveillance
Upstream process state changes but measured CQA remains stable	Upstream-state evidence plus downstream CQA observation	Inference justified from current evidence The monitored attribute has not moved detectably Inference not yet justified The disturbance had no product consequence	Adequate detection sensitivity, residence-time alignment, additional relevant CQAs	Continue observation until the affected material window has cleared
CQA change appears without a corresponding upstream signal	Product-quality observation with no matched upstream deviation	Inference justified from current evidence A quality-relevant change exists Inference not yet justified The bioreactor is the source	Downstream-state, analytical-system, and material-history discrimination	Localize the source before assigning corrective action

Because the central problem is the inferential distance between upstream variability and final quality, **Figure 1** organizes the evidence into a cross-scale updating architecture rather than representing the process as a direct upstream-to-CQA arrow.

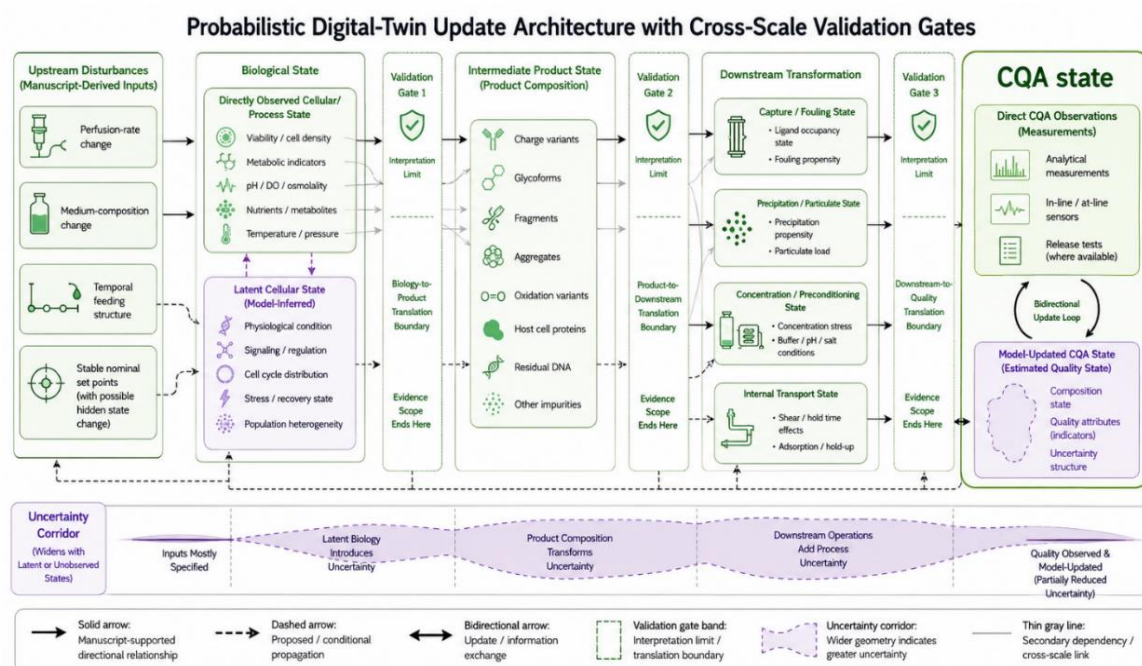


Figure 1. Cross-scale quality-state updating with validation gates between biological disturbance, process transformation, and CQA inference

Process analytical technology

Process analytical technology becomes valuable when it reduces the uncertainty between a changing process and a quality-relevant state, not simply when it increases the number of available signals. Raman spectroscopy combined with convolutional neural networks has been used to predict cell-culture product-quality attributes across tested operating conditions, illustrating how multivariate spectra can act as soft sensors when direct online measurement is difficult [22]. In perfusion cultivation, inline dielectric spectroscopy with an adaptive partial-least-squares model has also supported real-time viable-cell-density control [23]. These examples demonstrate two distinct PAT functions: estimating a difficult-to-measure state and using that estimate as part of a closed control loop.

A broader PAT architecture must preserve the difference between observation and authority to act. Continuous IgG monitoring with immobilized fluorescent reporters demonstrates that product concentration can be sensed in flowing process streams [24]. By contrast, PAT implemented around single-pass tangential-flow ultrafiltration has been coupled directly to feedback control to maintain output concentration during defined feed disturbances [25]. The first case improves observability; the second combines observation with a qualified control action. For continuous bioprocessing, this distinction should be explicit because a sensor may be analytically useful while still lacking the validation, timing, robustness, or mechanistic specificity required to drive an automated intervention.

Figure 2 therefore treats PAT as a quality-first observer-and-control stack with an uncertainty corridor rather than as a collection of interchangeable sensors.

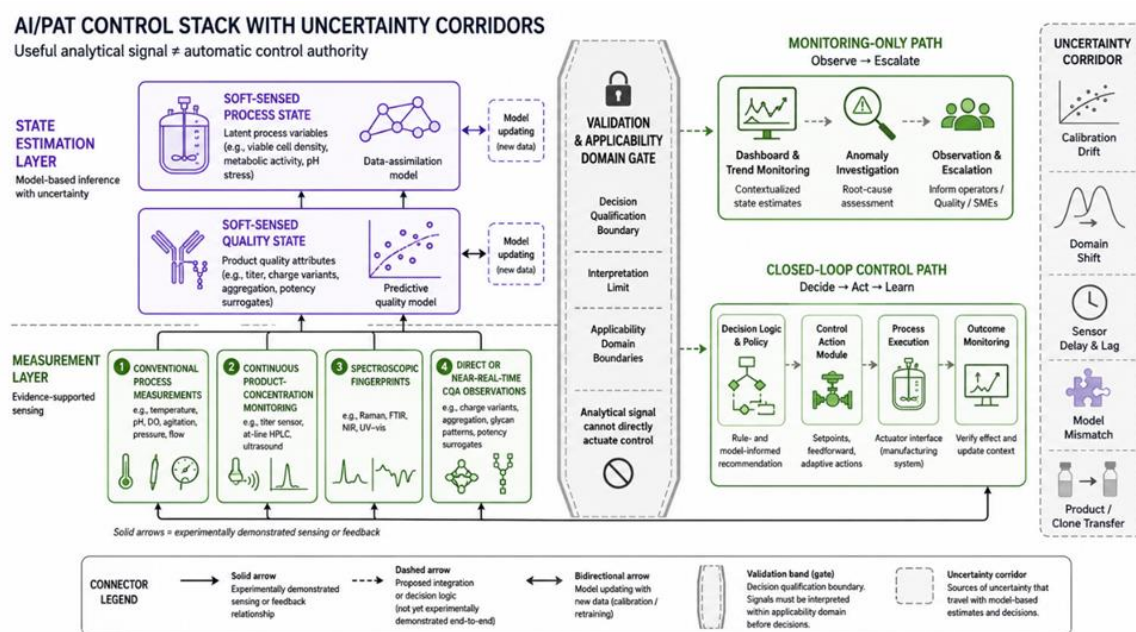


Figure 2. PAT decision stack separating sensing, state estimation, control authority, and quality qualification under model uncertainty

Feedback and feedforward control

Continuous bioprocessing requires different control functions to be separated rather than collapsed into a single automated loop. Hybrid models can combine mechanistic structure with data-driven estimation, allowing unmeasured or slowly measured process states to be reconstructed while retaining some physical interpretability [26]. Their value, however, depends on model structure, data quality, training domain, and the stability of the relationship between measured variables and the state being estimated. Adaptive hybrid approaches can additionally represent time-varying or history-dependent process kinetics [27], but successful estimation does not by itself establish that the estimated state is mechanistically correct or suitable for autonomous manufacturing decisions.

Within the proposed architecture, feedforward control anticipates downstream consequences of an upstream or intermediate-state change, whereas feedback responds to a measured deviation after it becomes observable. These functions operate on different time scales and evidence requirements. A feedforward action based on an inferred biological state should therefore carry greater uncertainty than a feedback action triggered by a directly qualified analytical measurement. The objective is not to automate every detected deviation, but to assign sensing, estimation, prediction, and actuation distinct roles according to how securely each state is known.

An upstream-to-quality control architecture

Molecular process-control concepts provide a precedent for linking cellular or molecular regulation with macroscopic manufacturing behavior rather than optimizing only bulk process variables [28]. Building on that principle, this article proposes an upstream-to-quality architecture in which the manufacturing train is represented through separable layers: observable upstream variables; latent cellular states; intermediate product states; downstream transformation states; directly measured CQAs; and model-estimated quality states. No layer is assumed to be an error-free surrogate for another.

The proposed architecture also separates evidence from decision authority. PAT supplies observations; state estimators reconstruct variables that cannot be measured directly; propagation models forecast how those states may influence later operations; feedback and feedforward controllers propose corrective actions; and qualification boundaries determine whether a proposed action is permitted. This structure intentionally prevents a soft sensor, digital-twin output, viable-cell-density estimate, or local set-point deviation from being interpreted automatically as a final product-quality conclusion. The architecture is a synthesis of component-level evidence and therefore remains a proposed systems construct rather than an empirically validated end-to-end control model.

Disturbance detection and recovery

Disturbance recovery is particularly important in continuous manufacture because material generated during a deviation continues to move through the train. Surge tanks can partially decouple connected operations and have been modeled as active elements for buffering mismatched flow, interruptions, and selected failures in continuous monoclonal-antibody manufacturing [29]. Such buffering can prevent an upstream disturbance from immediately forcing downstream shutdown, but its protective value depends on capacity, residence time, disturbance duration, and the ability to identify the affected material window.

Recovery should therefore be defined more rigorously than return of a local process variable to its nominal set point. A fouling event may leave a downstream separation state altered after inlet conditions normalize; a delayed sensor may identify a deviation only after affected material has propagated; and restoration of viable cell density does not establish that product formed during the disturbance has the same quality state as subsequent material. The proposed sequence-aware logic in **Table 3** distinguishes detection, propagation, containment, correction, and quality-state recovery.

Table 3. Sequence-aware disturbance and recovery states for quality-linked continuous bioprocess control

Disturbance emergence Hidden-state transition Material-window propagation Downstream transformation Process-variable recovery					
Sequence stage	Disturbance emergence	Hidden-state transition	Material-window propagation	Downstream transformation	Process-variable recovery
Precursor state	Qualified upstream operating state	Process variable remains apparently acceptable	Altered biological or intermediate-product state exists	Disturbed material enters capture, filtration, chromatography, or another operation	Local disturbance has been corrected
Transition trigger	Change in feed, perfusion, cellular behavior, or local unit condition	Biological adaptation, temporal oscillation, transport shift, or unmeasured unit evolution	Continuous transfer into the next operation	Interaction with unit-specific transport, fouling, loading, or separation state	Set point or controller output returns to target
Observable readout	Direct process signal or abnormal trend	Soft-sensor estimate, orthogonal measurement, or delayed product signal	Residence-time estimate, inventory history, downstream sensor response	Pressure, concentration, analytical, or CQA-related signal	Conventional process variables normalize
Propagation consequence	Potential shift in biological or unit-operation state	Source attribution becomes uncertain	Affected material occupies a defined or uncertain portion of the train	Original disturbance may be amplified, attenuated, or converted	Product formed earlier may still remain within the train

Condition that interrupts or reverses the sequence	Disturbance removed before material state changes	Independent state measurement excludes a meaningful transition	Diversion, buffering, isolation, or demonstrated attenuation	Qualified operation demonstrates removal or containment of the relevant state	Material-history analysis confirms affected window has cleared
--	--	--	--	--	---

Validation and regulatory considerations

Model-based bioprocess development increasingly integrates quality-by-design principles, soft sensors, advanced control, and digital-twin concepts, but implementation depends on model verification, validation, data integrity, applicability domains, and lifecycle maintenance [30]. These requirements become more stringent when a model is moved from descriptive use to decision support and more stringent again when it is granted autonomous control authority. A model that predicts well under one clone, scale, chromatography configuration, or sensor package should not be assumed to retain the same performance after a material process change.

For the proposed architecture, validation must therefore occur at several levels. Sensors require analytical qualification; soft sensors require prospective assessment inside defined operating domains; propagation models require confirmation that material history and residence-time assumptions remain valid; control laws require disturbance testing; and the integrated architecture requires evidence that its interventions preserve relevant CQAs across representative disturbances. Regulatory acceptability cannot be inferred from successful implementation of any single PAT tool, controller, or digital twin. The evidence base currently supports many component technologies, but not the prospective validation of the complete quality-linked architecture described here.

CONCLUSION

Continuous bioprocessing changes the control problem because biological variation, material transformation, measurement uncertainty, and product-quality inference become coupled across time and unit operations. The proposed architecture responds by keeping observable variables, latent states, intermediate product states, downstream transformations, CQAs, model estimates, and control authority analytically separate. Its central principle is that process recovery is not necessarily product-quality recovery, and that automation should advance only as far as the available evidence justifies. Future progress therefore depends less on adding isolated sensors or algorithms than on validating the links that connect upstream disturbance to final quality.

ACKNOWLEDGMENTS: None

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

REFERENCES

1. Rathore AS, Thakur G, Kateja N. Continuous integrated manufacturing for biopharmaceuticals: A new paradigm or an empty promise? *Biotechnol Bioeng.* 2023;120(2):333-51. doi:10.1002/bit.28235
2. Coolbaugh MJ, Varner CT, Vetter TA, Davenport EK, Bouchard B, Fiadeiro M, et al. Pilot-scale demonstration of an end-to-end integrated and continuous biomanufacturing process. *Biotechnol Bioeng.* 2021;118(9):3287-301. doi:10.1002/bit.27670
3. Somasundaram B, Pleitt K, Shave E, Baker K, Lua LHL. Progression of continuous downstream processing of monoclonal antibodies: Current trends and challenges. *Biotechnol Bioeng.* 2018;115(12):2893-907. doi:10.1002/bit.26812
4. Jungbauer A, Satzer P, Duerauer A, Azevedo A, Aires-Barros R, Nilsson B, et al. Continuous downstream processing. *Sep Purif Technol.* 2024;338:126439. doi:10.1016/j.seppur.2024.126439
5. Schwarz H, Gomis-Fons J, Isaksson M, Scheffel J, Andersson N, Andersson A, et al. Integrated continuous biomanufacturing on pilot scale for acid-sensitive monoclonal antibodies. *Biotechnol Bioeng.* 2022;119(8):2152-66. doi:10.1002/bit.28120

6. Feidl F, Vogg S, Wolf M, Podobnik M, Ruggeri C, Ulmer N, et al. Process-wide control and automation of an integrated continuous manufacturing platform for antibodies. *Biotechnol Bioeng.* 2020;117(5):1367-80. doi:10.1002/bit.27296
7. Konoike F, Taniguchi M, Yamamoto S. Integrated continuous downstream process of monoclonal antibody developed by converting the batch platform process based on the process characterization. *Biotechnol Bioeng.* 2024;121(8):2269-77. doi:10.1002/bit.28537
8. Karst DJ, Steinebach F, Soos M, Morbidelli M. Process performance and product quality in an integrated continuous antibody production process. *Biotechnol Bioeng.* 2017;114(2):298-307. doi:10.1002/bit.26069
9. Walther J, Ribeiro da Costa T, Winkler L, Schaub J, Habicher T. Cell specific perfusion rates drive growth dynamics and metabolism in CHO N-1 perfusion processes independent of perfusion rate control method. *Front Bioeng Biotechnol.* 2025;13:1608889. doi:10.3389/fbioe.2025.1608889
10. Schwarz H, Lee K, Castan A, Chotteau V. Optimization of medium with perfusion microbioreactors for high density CHO cell cultures at very low renewal rate aided by design of experiments. *Biotechnol Bioeng.* 2023;120(9):2523-41. doi:10.1002/bit.28397
11. Nöbel M, Barry C, MacDonald MA, Baker K, Shave E, Mahler S, et al. Harnessing metabolic plasticity in CHO cells for enhanced perfusion cultivation. *Biotechnol Bioeng.* 2024;121(4):1370-82. doi:10.1002/bit.28613
12. Möller J, Bhat K, Riecken K, Pörtner R, Zeng AP, Jandt U. Process-induced cell cycle oscillations in CHO cultures: Online monitoring and model-based investigation. *Biotechnol Bioeng.* 2019;116(11):2931-43. doi:10.1002/bit.27124
13. Liang G, Madhavarao CN, Morris C, O'Connor T, Ashraf M, Yoon S. Effects of process intensification on homogeneity of an IgG1:κ monoclonal antibody during perfusion culture. *Appl Microbiol Biotechnol.* 2024;108(1):274. doi:10.1007/s00253-024-13110-9
14. McFarland KS, Hegadorn K, Betenbaugh MJ, Handlogten MW. Elevated endoplasmic reticulum pH is associated with high growth and bisAb aggregation in CHO cells. *Biotechnol Bioeng.* 2025;122(1):137-48. doi:10.1002/bit.28866
15. Schmitz F, Knöchelmann E, Kruse T, Minceva M, Kampmann M. Continuous multi-column capture of monoclonal antibodies with convective diffusive membrane adsorbers. *Biotechnol Bioeng.* 2024;121(6):1859-75. doi:10.1002/bit.28695
16. Minervini M, Mergy M, Zhu Y, Gutierrez Diaz MA, Pointer C, Shinkazh O, et al. Continuous precipitation-filtration process for initial capture of a monoclonal antibody product using a four-stage countercurrent hollow fiber membrane washing step. *Biotechnol Bioeng.* 2024;121(8):2258-68. doi:10.1002/bit.28525
17. Minervini M, Mohammadzadehmarandi A, Behboudi A, Oppenheim SF, Zydne AL. Integration of single pass tangential flow filtration and high performance countercurrent membrane purification for intensification of monoclonal antibody processing. *Biotechnol Bioeng.* 2025;122(9):2410-19. doi:10.1002/bit.29038
18. Jabra MG, Lipinski AM, Zydne AL. Single pass tangential flow filtration (SPTFF) of monoclonal antibodies: Experimental studies and theoretical analysis. *J Membr Sci.* 2021;637:119606. doi:10.1016/j.memsci.2021.119606
19. Tiwari A, Masampally VS, Agarwal A, Rathore AS. Digital twin of a continuous chromatography process for mAb purification: Design and model-based control. *Biotechnol Bioeng.* 2023;120(3):748-66. doi:10.1002/bit.28307
20. São Pedro MN, Isaksson M, Gomis-Fons J, Eppink MHM, Nilsson B, Ottens M. Real-time detection of mAb aggregates in an integrated downstream process. *Biotechnol Bioeng.* 2023;120(10):2989-3000. doi:10.1002/bit.28466
21. Godbole A, Chen L, Desai J, Raghava S, Ruzanski R, Patel B, et al. Implementation of innovative process analytical technologies to characterize critical quality attributes of co-formulated monoclonal antibody products. *Biotechnol Bioeng.* 2025;122(2):322-32. doi:10.1002/bit.28881
22. Khodabandehlou H, Rashedi M, Wang T, Tulsyan A, Schorner G, Garvin C, et al. Cell culture product quality attribute prediction using convolutional neural networks and Raman spectroscopy. *Biotechnol Bioeng.* 2024;121(4):1231-43. doi:10.1002/bit.28646
23. Sun Y, Zhang Q, He Y, Chen D, Wang Z, Zheng X, et al. Real-time auto controlling of viable cell density in perfusion cultivation aided by in-line dielectric spectroscopy with segmented adaptive PLS model. *Biotechnol Bioeng.* 2025;122(4):858-69. doi:10.1002/bit.28930

24. Goyal A, Vu B, Maranholkar V, Patil U, Kourentzi K, Willson RC. Continuous monitoring of IgG using immobilized fluorescent reporters. *Biotechnol Bioeng.* 2023;120(2):482-90. doi:10.1002/bit.28254
25. Thakur G, Thori S, Rathore AS. Implementing PAT for single-pass tangential flow ultrafiltration for continuous manufacturing of monoclonal antibodies. *J Membr Sci.* 2020;613:118492. doi:10.1016/j.memsci.2020.118492
26. Mahanty B. Hybrid modeling in bioprocess dynamics: Structural variabilities, implementation strategies, and practical challenges. *Biotechnol Bioeng.* 2023;120(8):2072-91. doi:10.1002/bit.28503
27. Mowbray MR, Wu C, Rogers AW, Del Rio-Chanona EA, Zhang D. A reinforcement learning-based hybrid modeling framework for bioprocess kinetics identification. *Biotechnol Bioeng.* 2023;120(1):154-68. doi:10.1002/bit.28262
28. Treinen C, Peternell C, Noll P, Magosch O, Hausmann R, Henkel M. Molecular process control for industrial biotechnology. *Trends Biotechnol.* 2025;43(9):2117-32. doi:10.1016/j.tibtech.2025.04.003
29. Thakur G, Saxena N, Tiwari A, Rathore AS. Control of surge tanks for continuous manufacturing of monoclonal antibodies. *Biotechnol Bioeng.* 2021;118(5):1913-31. doi:10.1002/bit.27706
30. Mu'azzam K, da Silva FVS, Murtagh J, Sousa Gallagher MJ. A roadmap for model-based bioprocess development. *Biotechnol Adv.* 2024;73:108378. doi:10.1016/j.biotechadv.2024.108378