Received: 6 June 2023 | Revised: 17 January 2024 | Accepted: 5 March 2024

DOI: 10.1002/btpr.3456

BIOTECHNOLOGY PROGRESS

RESEARCH ARTICLE

Bioseparations and Downstream Processing

A modular and multi-functional purification strategy that enables a common framework for manufacturing scale integrated and continuous biomanufacturing

Leon P. Pybus[1] | Charles Heise[1] | Tibor Nagy[1] | Carmen Heeran[1] | Terri Dover[1] | John Raven[1] | Junichi Kori[2] | Graeme Burton[1] | Hiroshi Sakuyama[2] | Benjamin Hastings[1] | Michelle Lyons[1] | Shinichi Nakai[2] | Jonathan Haigh[1]

[1]Process Development, FUJIFILM Diosynth Biotechnologies, Billingham, UK

[2]Bio Science & Engineering Laboratories, FUJIFILM Corporation, Kaisei, Japan

Correspondence
Leon P. Pybus, Process Development, FUJIFILM Diosynth Biotechnologies, Billingham, UK.
Email: leon.pybus@fujifilm.com

Abstract

Biopharmaceutical manufacture is transitioning from batch to integrated and continuous biomanufacturing (ICB). The common framework for most ICB, potentially enables a global biomanufacturing ecosystem utilizing modular and multi-function manufacturing equipment. Integrating unit operation hardware and software from multiple suppliers, complex supply chains enabled by multiple customized single-use flow paths, and large volume buffer production/storage make this ICB vision difficult to achieve with commercially available manufacturing equipment. Thus, we developed SymphonX™, a downstream processing skid with advanced buffer management capabilities, a single disposable generic flow path design that provides plug-and-play flexibility across all downstream unit operations and a single interface to reduce operational risk. Designed for multi-product and multi-process cGMP facilities, SymphonX™ can perform stand-alone batch processing or ICB. This study utilized an Apollo™ X CHO-DG44 mAb-expressing cell line in a steady-state perfusion bioreactor, harvesting product continuously with a cell retention device and connected SymphonX™ purification skids. The downstream process used the same chemistry (resins, buffer composition, membrane composition) as our historical batch processing platform, with SymphonX™ in-line conditioning and buffer concentrates. We used surge vessels between unit operations, single-column chromatography (protein A, cation and anion exchange) and two-tank batch virus inactivation. After the first polishing step (cation exchange), we continuously pooled product for 6 days. These 6 day pools were processed in batch-mode from anion exchange to bulk drug substance. This

Leon P. Pybus and Charles Heise contributed equally to this study.

manufacturing scale proof-of-concept ICB produced 0.54 kg/day of drug substance with consistent product quality attributes and demonstrated successful bioburden control for unit-operations undergoing continuous operation.

KEYWORDS
Chinese hamster ovary, downstream processing, integrated and continuous biomanufacturing, mAb bioprocessing, perfusion, upstream processing

1 | INTRODUCTION

The current *modus operandi* for biopharmaceutical production using mammalian cell lines is to build biomanufacturing facilities with fixed capacity and a focus on fed-batch bioreactor production with independent, batch downstream unit operations.[1] Recombinant monoclonal antibodies (mAbs) produced by Chinese Hamster Ovary (CHO) cell lines continue to dominate biopharmaceutical approvals.[2] The established low risk profile of mAbs generated by CHO cell lines provides a foundation for a rapid development approach that relies on platform development, manufacturing technology, and infrastructure.[3] However, biopharmaceutical development pipelines are diversifying[2] with current and next-generation products requiring different manufacturing requirements to meet high, low or uncertain market demand.[4–6] Furthermore, novel modalities may present processing challenges, stretching the mAb platform paradigm and potentially necessitating custom development, manufacturing technology, and infrastructure.[7–10]

There is also a drive to reduce cost-of-goods[11] and the environmental impact[12] to manufacture biologic drugs. Cost-of-goods is directly related to manufacturing and product demand, necessitating modular plug-and-play manufacturing solutions that can respond quickly to market and product processing requirements.[6] Designing of next generation manufacturing facilities has therefore focused on incorporation of single-use, flexible and modular concepts into facility design that gives advantages of faster speed to market, capital investment deferment and better cost predictability.[6,13,14]

A key enabler is the move from fed-batch bioreactor production with independent, batch downstream unit operations toward single-use integrated and continuous bioprocessing (ICB), utilizing continuous perfusion bioreactors with a linked and continuous downstream.[15,16] ICB has well documented advantages for clinical and in some cases commercial manufacturing. These include: reduced capital and operating costs[6,15,17–24]; increased productivity and reduced facility size through process intensification[6,15,17,18,20]; facilitation of multi-product "ballroom" facilities[25,26]; improved flexibility to respond to changing demand[6,18,26]; increased process sustainability through a reduction in process mass intensity (PMI)[15,22,24]; and improved product quality consistency.[10,27]

A common framework is emerging within the industry for companies implementing ICB.[15,16] This common framework employs many of the previously used and familiar batch processing operations, provides backwards compatibility with legacy batch processes without regulatory license changes and is adaptable to demand, capable of supporting pre-clinical and commercial production product quantities.[15,16]

Lab/pilot scale ICBs have been published within the literature.[28–36] However, the literature lacks explicit manufacturing scale ICB demonstrations and widespread implementation is limited by a lack of commercially available flexible plug-and-play ICB equipment.[15,16,26] For example, even in a traditional manufacturing facility each unit operation required customized and purpose-built process equipment with a specific flow path, operating software and procedures. This in particular hinders ICB operation because equipment communication, compatibility, and connectivity limit automation or necessitate additional manufacturing facility orchestration.[37] Buffer production, storage, and utilization also disrupt process production scheduling and operations.[38] Furthermore, although the use of disposable single-use technology has enabled multi-product facilities it has also created complex consumable supply chains due to the demand for and diversity of flow paths required.[39]

We developed SymphonX™, a downstream processing skid with a single disposable, generic flow path design to address the need for plug-and-play flexibility across all downstream unit operations and a single interface to reduce the risk of complex connected bioprocessing operations.[26] The equipment also has advanced buffer management (in-line conditioning and dilution) within the proprietary flow path, making it stand-alone or integrated connected equipment for batch and continuous processes with rapid facility turnaround, which is essential for multi-product cGMP facility operations. SymphonX™ can support flow rates ranging from 0.1 to 12.3 L/min and thus could support a common ICB framework manufacturing facility capable of producing batches as small as 0.5 kg or as large as 500 kg.[15,16] This single-use ICB manufacturing plant's annual output could reach 8 tonnes.[15]

Traditionally, transitioning from batch to ICB was viewed as an "all or nothing" approach that necessitated end-to-end continuous flow. While end-to-end continuous manufacturing may be seen as an ideal future state, the actual requirement may lie somewhere between an end-to-end batch process and a fully continuous process.[15,40] Furthermore, a variety of terms are used to describe ICB processes (e.g., continuous, end-to-end, integrated, connected, closed, and hybrid). To provide a multitiered classification for ICB, Crowley et al.[40] have described a well-defined structure with four levels of complexity. The classification begins with level 0, standard batch with standalone unit operations, and progresses via a series of incremental developments to level 3.1, a completely flow-through continuous process

with complete steady state flow and all bind elute steps being replaced with flow-through mode. Within levels, 1–3 are varying levels of process intensification. Level 1 represents an intensified standalone intensified unit operation. Level 2 denoted a connected process that has at least two unit operations running simultaneously. Level 3 describes a continuous process in which all unit operations are connected with steady state flow via small intermediate tanks, software orchestration, lengthy run times and closed processing.

Most clinical and commercial ICB processes described today are 'level 2',[15,40] hence we demonstrate the use of SymphonX™ in a manufacturing scale exemplar ICB which could support most biopharmaceutical ICB and legacy batch bioprocesses. Using a Apollo™ X CHO-DG44 cell line expressing a recombinant monoclonal antibody (mAb) as a model protein, we integrated all typical mAb drug substance unit operations.[1] A steady-state perfusion bioreactor, harvesting product continuously with a cell retention device was connected to seven unit operations utilizing SymphonX™ purification skids to perform advanced buffer management and the following processing steps: (i) single pass tangential flow filtration (SPTFF), (ii) Protein A capture chromatography, (iii) low pH viral inactivation, (iv) cation exchange (CIEX), (v) anion exchange (AIEX), (vi) virus filtration (VF), and (vii) ultrafiltration and diafiltration (UFDF) (Figure 1a).

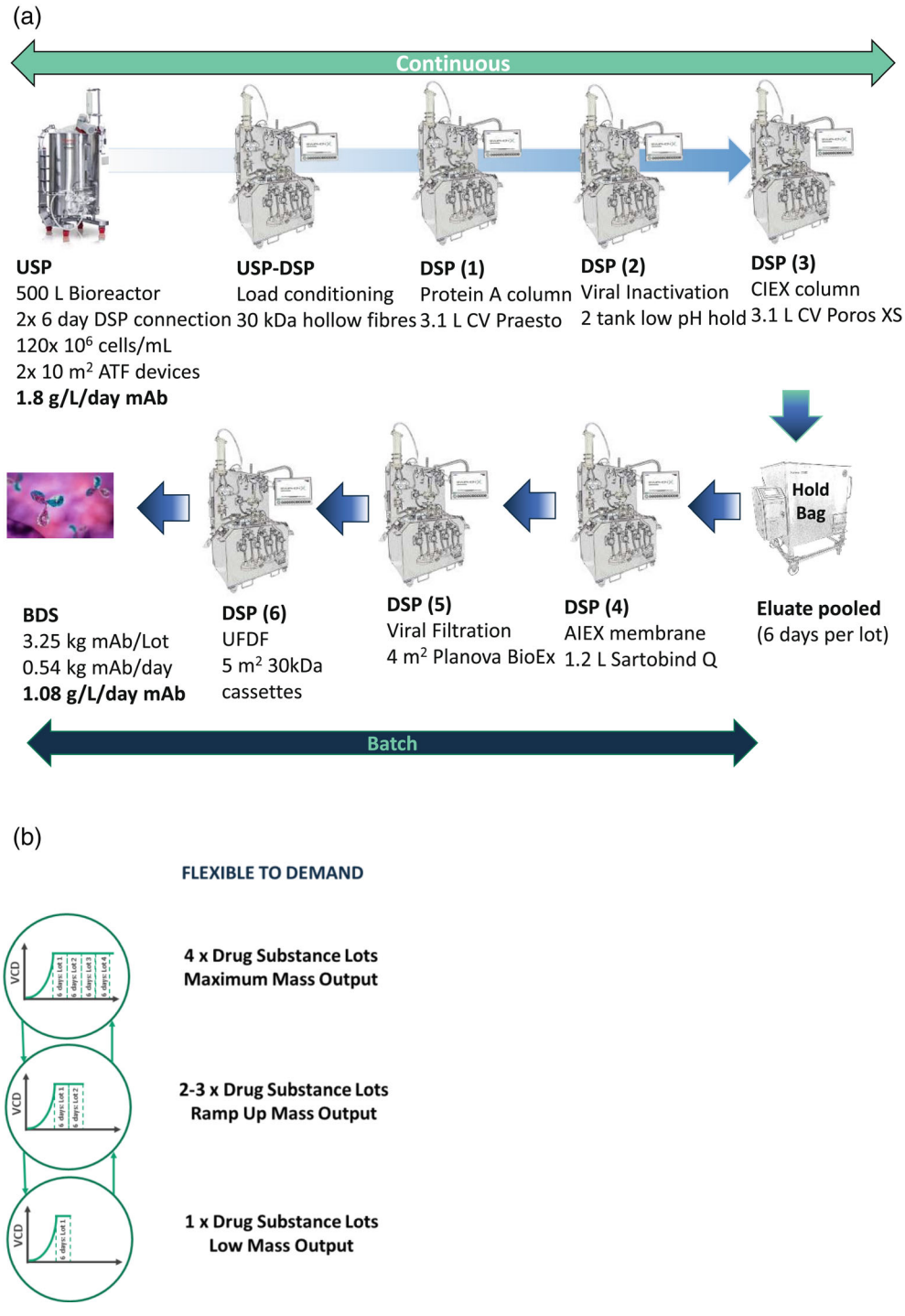

FIGURE 1 Schematic of the end-to-end integrated and continuous biomanufacturing (ICB) process. (a) Each unit operation was physically integrated and product flowed continuously from the bioreactor to the cation exchange (CIEX) column for 6 days before batch processing of this intermediate pool from the anion exchange (AIEX) column to bulk drug substance (BDS). ATF, alternating tangential flow; DSP, downstream process; mAb, monoclonal antibody; UFDF, ultrafiltration and diafiltration; USP, upstream process. (b) This ICB approach is flexible-to-demand. "Ramp up" mass output was simulated in this study but the process can be run for a shorter length of time for "low mass output" and a longer length of time for "maximum mass output".

To streamline compatibility to our legacy batch bioprocesses and reduce potential regulatory licensing concerns we used the same chemistry (resins, buffer composition and membrane composition) as our historical batch processing platform and singe-column chromatography (protein A, CIEX and AIEX). To further reduce the perceived "cost-of-quality" for ICB processes[18] we pooled product for 6 days after the first polishing step (CIEX) and the CIEX pool was processed in a connected batch from CIEX pool to bulk drug substance to form the lot.

As demonstrated in this work, ICB has progressed from proof-of-concept studies at the lab or pilot scale to manufacturing scale demonstrations. Furthermore, the development of modular plug-and-play equipment such as SymphonX™ enables ICB to be routinely operated at manufacturing scale.

2 | MATERIALS AND METHODS

2.1 | Integration and closure

All unit operations were physically integrated into a closed system, and surge vessels were placed between unit operations to smooth flow perturbations and give time to respond to process disruptions. Figure 2 depicts the bioburden control strategy. Before installation, all product contact materials were sterilized (gamma irradiation or autoclaving) or sanitized (0.5 M NaOH solution). Sterilizing grade filters (Sartorius Stedim) were used for sterile filtration of buffers and media, and to maintain a sterile barrier between the bioreactor and the SPTFF unit operation. ReadyMate™ single-use connectors (Cytiva), AseptiQuick™ single-use connectors (CPC Worldwide), or BioWelders™ (Sartorius Stedim) were used to make sterile connections to the perfusion bioreactor and SPTFF unit operation. Downstream unit operations and surge vessels were connected with sanitary tri-clamp fittings and 70% IPA spray. Resins and consumables that were not pre-irradiated were sanitized with 0.5 M NaOH solution. Pre-packed Protein A and CIEX columns were pre-sanitized and subsequently cleaned every three cycles (~12 h) using a 0.5 M NaOH solution for 15 min. This cleaning cadence was based on resin regeneration requirements and a pragmatic approach to bioburden control. Ultimately, column chromatography does not require sterility; it requires the demonstration of a sanitary state for connected operation with a bioreactor.[15] The UFDF membranes and their holder were sanitized using a 1 h contact time.

Disposable single-use consumables allowed a complete flow path change between manufacturing campaigns (defined by USP batch length) and DSP flow paths were changed between lots (6 days). Each continuously running or batched unit operation was bioburden monitored daily or every cycle, whichever was more frequent. Sampling bags or intermediate vessel sample ports were used for aseptic sampling.

2.2 | Process control and monitoring

Each unit operation was controlled independently based on process parameters determined by a scientific risk analysis and empirical data from small scale models of batch operated unit operations. The process was monitored using integrated sensors typical of bioreactors and DSP bioprocess systems as well as any associated load cells and balances. The SymphonX™ systems monitored and controlled load cells and impellers in the integrated surge and pooling vessels, with fill level set points determining when the preceding or following unit operation paused or resumed for continual processing. Any material held in a surge or pooling tank was constantly agitated at 100 rpm provided the manufacturer's minimum fill volume was exceeded. Process monitoring and review required 1 Hz data acquisition and local and central server storage.

2.3 | Bioreactor cell culture

A 500 L perfusion bioreactor (ThermoFisher Scientific) produced a recombinant monoclonal antibody (mAb) from a FUJIFILM Diosynth

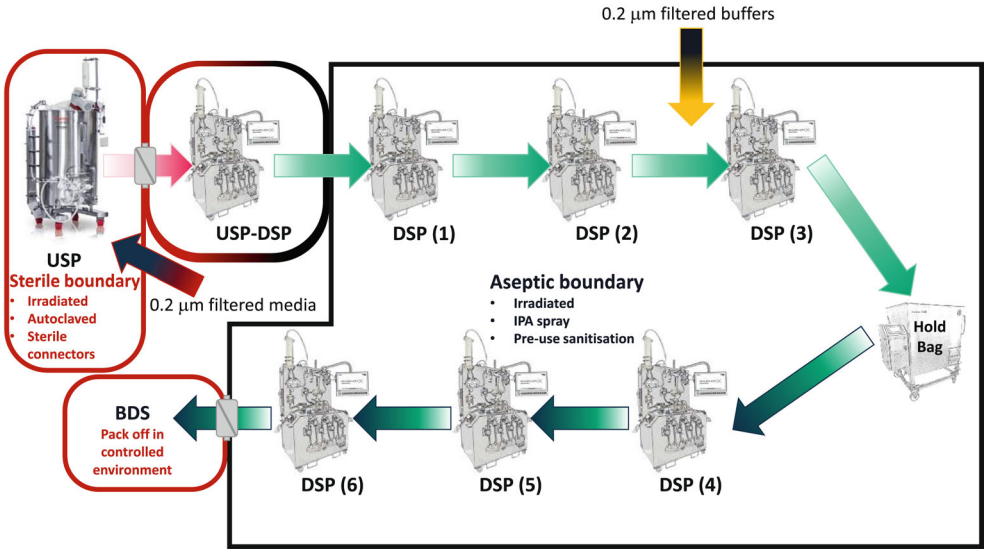

FIGURE 2 Schematic of the bioburden control strategy utilized in the integrated and continuous biomanufacturing (ICB) process. The maintenance of the closed system, ongoing sanitization procedures, the location of sterile filters and the frequency and location of bioburden samples are important consideration in ICB.

Biotechnologies Apollo™ X Chinese Hamster Ovary (CHO) DG44 cell line. 2 × ATF10 (Repligen) were integrated with the perfusion bioreactor and an integrated surge vessel collected the clarified, product-containing harvest before SPTFF. A capacitance probe measuring biomass (Aber Instruments) was used to remove excess cells into a waste bag to maintain 120×10^6 cells/mL. A load cell and pump-controlled perfusion media (FUJIFILM Irvine Scientific) addition and bioreactor volume. Combined permeate flux from the ATF filters was set at 1.2 vessel volumes per day using peristaltic pumps and monitored using clamp-on BioProTT™ Flow Track plus flow meters (em-tech).

2.4 | Single pass tangential flow filtration

Oscillating SPTFF with in-line perfusate conditioning using a 4 M NaCl stock solution was utilized to adjust the ionic strength and manage volume before the primary capture column. The SymphonX™ purification system fed 27.5 L/h of bioreactor perfusate conditioned with 1:10 salt solution into a 4.1 m^2 30 kDa cut off mPES hollow fiber (Repligen). After 1128 s, the retentate valve was opened to flush 1 L of conditioned, non-concentrated perfusate through the fiber. The fiber was replaced every 72 h. The Protein A capture column was loaded with retentate from an integrated pooling vessel.

2.5 | Chromatography

Using a high cycling strategy, a single pre-packed 3.1 L Praesto A50 jetted Protein A column (Purolite/Repligen) captured the SPTFF conditioned product. The column was loaded on a 4 h cycle to target 53 ± 5 g mAb/L resin per cycle and to ensure the empirically determined 65 g mAb/L resin binding capacity after 36 cycles was not exceeded (data not shown). The column was then washed with 20 mM sodium phosphate, 0.5 M sodium chloride at pH 7.4, and 50 mM sodium phosphate at pH 6.0, before product elution with 50 mM sodium acetate at pH 4.0. Before viral inactivation, the eluate was collected in an integrated surge vessel for pooling. Every three cycles, 0.5 M NaOH solution was used for 15 min to clean the column. All buffers were diluted 5-fold on the SymphonX™ purification system at point-of-use from 5× concentrate solutions.

After virus inactivation, a single pre-packed 3.1 L POROS XS (ThermoFisher/Repligen) was used for intermediate polishing chromatography in bind and elute mode. Each aliquot of viral inactivated feed was loaded onto the CIEX column in three cycles, at 33 ± 9 g mAb/L resin per cycle, which was less than the 61 g mAb/L resin binding capacity determined after a 76 cycles re-use study (data not shown), washed with 50 mM sodium acetate pH 5.0, and eluted at pH 6.0 in a 68 to 452 mM gradient of sodium acetate using 1× working buffer stocks. The CIEX eluate was collected in an integrated surge vessel and held for 6 days before anion exchange polishing. Every three cycles (~12 h), 0.5 M NaOH solution was used for 15 min to clean the column. This cleaning strategy was based on resin regeneration requirements, a pragmatic approach to bioburden control and alignment with the protein A cleaning cadence. The SymphonX™ purification system diluted all non-gradient buffers to 1× working solutions from 5-fold concentrates at point-of-use.

After pooling 6 days of CIEX eluate, the feed was pH adjusted with 1 M Tris solution to pH 7.3 using the SymphonX™ system before being diluted 1:4.9:4.1 in-line with 100 mM Tris acetate (pH 7.3 on dilution) and water. The conditioned feed was filtered through an AIEX 1.2 L Sartobind Q membrane filter (Sartorius Stedim) and collected in an integrated surge vessel prior to viral filtration.

2.6 | Virus inactivation

Two protein A elutions were pooled into the viral inactivation tank before adjustment to pH 3.5 with 1 M acetic acid, after 1 h incubation, a back titration to pH 5.0 with 1 M Tris solution was performed. Before loading on the CIEX column, all neutralized viral inactivated protein A eluates in each lot were filtered through a sterile filter (Sartorius Stedim) into a second integrated surge vessel. Adoption of a regulatory compliant approach to viral inactivation through inter-cycle cleans, bag replacements and transfer between the 1 h incubation and back titration was not implemented in this initial proof-of-concept.

2.7 | Virus filtration

The anion exchange flow-through was filtered as a single batch using a 4 m^2 Planova 20 N (Asahi Kasei) virus filter into an integrated surge vessel at 2.5 bar pressure.

2.8 | Ultrafiltration and diafiltration

The viral filtered anion exchange flowthrough was processed through the UFDF step in three 365 L sub-batches using 2×2.5 m^2 30 kDa cut-off SUIS cassettes (Repligen). The retentate volume was reduced to 80 L and buffer exchanged using 7 × diavolumes of 80 mM sodium phosphate and 30% sucrose, diluted 4-fold at point-of-use to give a working pH 6.0, at a feed pressure of 1.4 bar, and a TMP of 1.1 bar. The buffer exchanged mAb was concentrated to 50 ± 5 g/L and transferred to an integrated final drug substance vessel. UF/DF feed and retentate was processed at room temperature for the 36 h duration of the unit operation.

2.9 | Drug substance formulation and final filtration

The three UFDF sub-batches were mixed to homogeneity in the drug substance vessel before adding polysorbate 20 to give a final formulation at 40 g/L mAb in 20 mM sodium phosphate, 7.5% sucrose and 0.01% (v/v) polysorbate 20. After filtration through a 0.2 μm sterilizing grade filter (Opticap XL, MerckMillipore), the drug was packaged in 1.8 L aliquots.

2.10 | Downstream buffer preparation

Merck Millipore supplied 5× or 4× concentrated buffer stocks and 1× working buffer stocks (gradient) as 0.2 μm filtered solutions, which were diluted at the point of use by the SymphonX™ system. To control bioburden, the final formulation 4× stock buffer was prepared and 0.2 μm filtered just before use.

2.11 | Analytical methods

Product concentration, host cell proteins (HCPs), DNA, high molecular weight species (HMWs), residual protein A, purity, charge isoform distribution, and mAb N-glycans were quantified using in-house assays. SoloVPE absorbance at 280 nm measured product concentration during the downstream process (Repligen). Commercial kits quantified rProtA and HCPs (Cygnus Technologies). UPLC-SEC was used to measure HMWS levels. In-house CE-SDS under non-reducing (NR) conditions measured purity. An in-house imaged capillary isoelectric focusing (icIEF) system determined charge isoforms. A Waters GlycoWorks RapiFluor MS kit measured mAb N-glycan percentage. Bioburden was measured using compendial methods (USP <61>).

3 | RESULTS AND DISCUSSION

3.1 | A manufacturing scale integrated and continuous biomanufacturing approach that adheres to a common industry framework

Several ICB are being implemented in clinical or commercial manufacturing.[15] These processes run between 14 and 30 days and employ N-stage perfusion and single or multi-column chromatography with linked processing, either through end-to-end product flow from bioreactor to BDS or, more commonly, by pooling the product in-process to define a lot.[15,16] A common ICB framework that fits these processes is possible because each ICB was derived from a common batch mAb platform process.[1,3,15,16]

In this common ICB framework, the bioreactor runs continuously, harvesting the product via a cell retention filtration step. The bioreactor is linked to an integrated downstream that employs batch single/dual column chromatography processing and chemistry (resins, buffer composition, and membrane composition) similar to historical mAb batch platforms.[15] Large product pools used in batch platforms are replaced by smaller surge tanks, and processes can pool to define a lot after virus activation, after the first polishing step, or just before virus filtration.[15] This common framework also supports fed-batch bioreactor processes with a filtration harvest step.[15]

In-process stability is a key consideration for ICB and may be the most important regulatory consideration.[15] The process should be integrated and continuous until a stable process intermediate is obtained. At this stable intermediate, a pool of the entire bioreactor run can be made and held. This pool is defined as the lot. Typically, manufacturing scale ICB pool anywhere from after the virus inactivation step to after the CIEX step.[15]

Process intermediate hold data for the mAb used in this study showed stability for 5 days at 20°C for most process intermediate steps and up to 8 days at 20°C for the CIEX pool (data not shown). Therefore, the product was pooled for 6 days at room temperature following CIEX in this study. Less stable products would necessitate the pooling of product in a more stable part of the process, the product pool/surge tank could be chilled, or smaller surge tanks could be used in proportion to the validated hold time/conditions. In-process pooling allows the combination of pre-validated viral clearance and material traceability from traditional batch processing with the productivity improvement of continuous operation.

This study demonstrates an exemplar manufacturing scale ICB process that adheres to a common industry framework and utilizes a modular and multifunctional downstream purification skid (SymphonX™) to streamline downstream ICB operation. Each unit operation was physically integrated, with product-containing material continuously processed through the first five linked unit operations (bioreactor, SPTFF, Protein A capture chromatography, VI and CIEX), the product was pooled for 6 days after the CIEX step before the intermediate pool was processed as a single connected lot through the final three steps to BDS (AIEX polishing, VF and UF/DF) (Figure 1a). The end-to-end integrated process from bioreactor to drug substance was ran for 12 days, with two six day DSP lots: the first lot ran the first 4 connected steps to optimize the integrated continuous section of the process (data not shown), and the second lot ran both the integrated continuous and batch downstream steps to BDS. This method is adaptable to demand and could be used for a single lot (6 days or less) or multiple lots (4 × 6 days – 24 days total) to maximize mass output (Figure 1b).

Figure 3 summarizes start-up, processing, and integration times of the ICB. After 10 days of bioreactor operation the production phase conditions were met ($120 \pm 10 \times 10^6$ cells/mL and >1.3 g/L product in the permeate) and the first downstream lot was initiated. The SPTFF step took ~3 h to generate sufficient material to start Protein A chromatography, after 8 h sufficient product was available for VI and after 10 h the first CIEX cycle began. After 6 days of operation flow paths and filters/columns were replaced (~2 h per unit operation), product from the bioreactor was diverted to waste during the time it took to change over the SPTFF flow path and the CIEX pool was disposed of. The second DSP lot was then initiated with the same cadence as the first lot. After a further 6 days of operation the CIEX pool was processed through batch processing through AIE, VF and UFDF to BDS.

A load cell or balance was used to detect when a sufficient amount of material had been accumulated in the surge vessel before a unit operation cycle began. Figure 4 shows the second lot (Protein A to BDS) downstream unit operation volume changes. The SPTFF settings were optimized to achieve an 80 kg maximum fill in the Protein A feed tank over one chromatography cycle, resulting in the feed tank oscillating between 10 and 45 kg to process all perfusate into DSP over the lot. Two protein A eluates were quickly transferred into

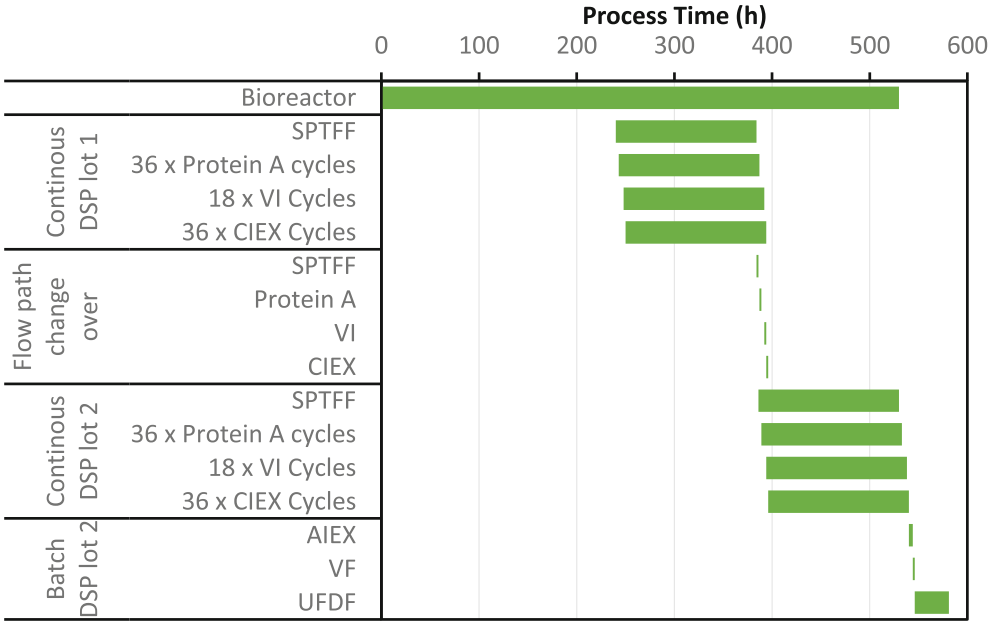

FIGURE 3 Summary of process integration and unit operation timings. AIEX, anion exchange chromatography; CIEX, cation exchange chromatography; UFDF, ultrafiltration and diafiltration; VI, viral inactivation; VF, viral filtration.

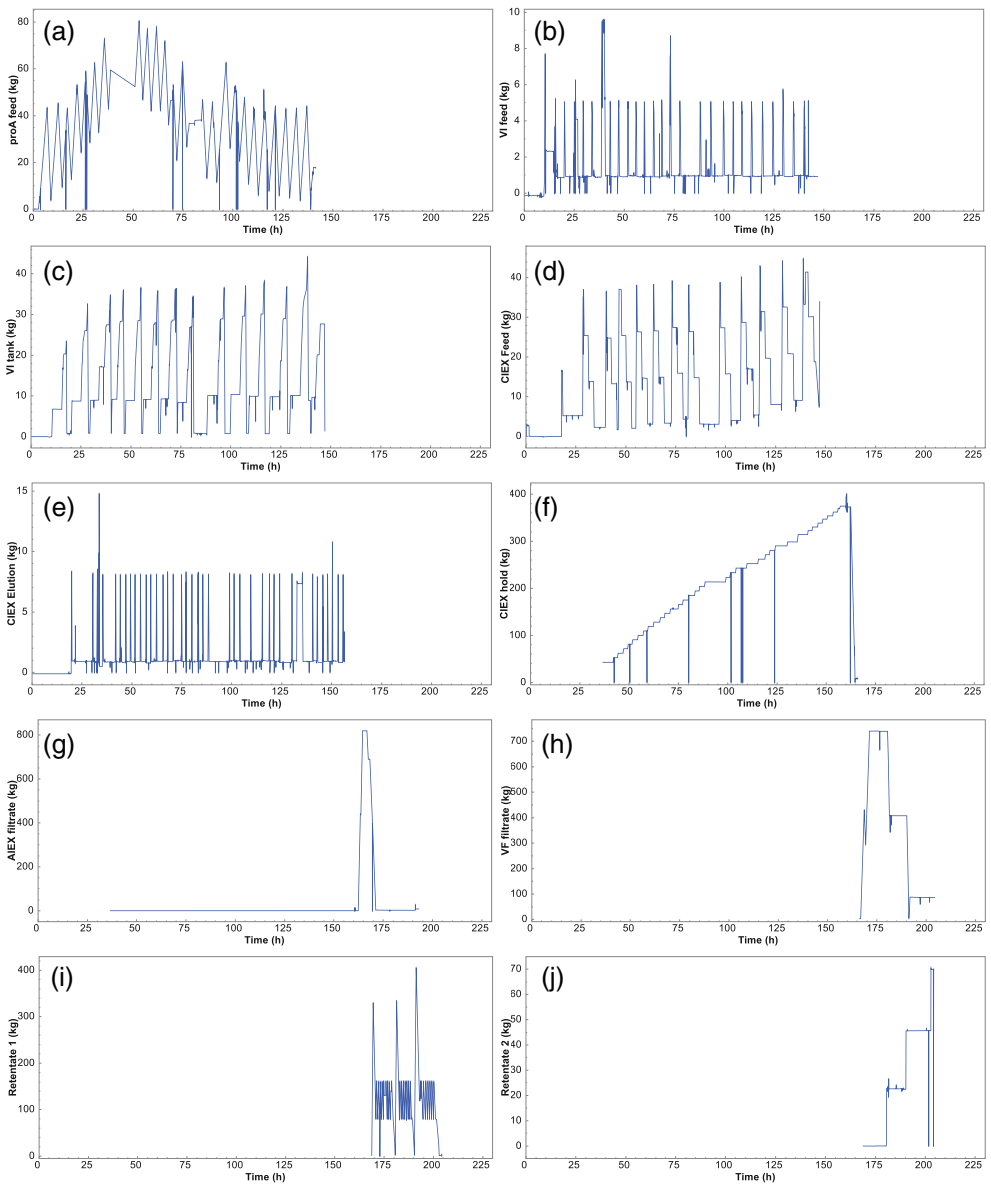

FIGURE 4 Volume changes in the intermediate bags/tanks placed between unit operations in the downstream process of the ICB process. Data from DSP batch 2 (Days 17 to Day 24 of operation) is displayed. Product flowed continuously from the bioreactor to the CIEX column (a–f) for 6 days before batch processing of this intermediate pool from the AIEX column to BDS (g–j). (a) Protein A load; (b) VI feed; (c) VI) tank; (d) CIEX feed; (e) CIEX elution; (f) CIEX hold; (g) AIEX feed; (h) VF filtrate; (i) UFDF retentate 1; (j) UFDF retentate 2.

the VI tank and pH titrated to 35 kg. The inactivated material was transferred as three CIEX loadings, decreasing the VI filtrate mass over time. The AIEX, VF, and UFDF were processed as a single connected batch after 6 days of pooling. After concentration, the three UFDF cycles with 7 DV buffer additions to Retentate 1 fed the Retentate 2 vessel, producing 82 L of BDS for pack off.

3.2 | Process closure and bioburden control

The existing model of biopharmaceutical manufacturing in classified cleanrooms is being reconsidered as the biopharmaceutical industry seeks to improve access to products by lowering costs while maintaining product quality and patient safety assurances and reducing environmental impact.[15,41]

Whereas the upstream process must be carried out in an aseptic environment, the majority of downstream operations are not considered aseptic and operate under "low bioburden" or "bioburden-controlled" conditions.[42] Bioburden excursions during cell culture are not acceptable and if confirmed would result in batch rejection, however, alert/action levels are commonly set at 1–10 CFU/mL for downstream unit operations.[42] As downstream operations are frequently attached to the bioreactor during ICB then the bioburden control strategy should be more stringent than in typical batch processing and should take into account the initial state of the fluid path (e.g., gamma irradiation), ongoing sanitization procedures, the location of sterile filters and the frequency and localization of bioburden sampling.[15]

Closed processing is the concept that product and material flow paths can be operated as a closed system, reducing or eliminating the need for a costly controlled cleanroom environment to prevent environmental contamination.[41] Another important concept is closed connected processing, which reduces the need for an expensive controlled cleanroom environment to prevent environmental contamination.[15,41] Closed processing also allows for "ballroom" operation, which is defined as the production of multiple biopharmaceutical products in the same open facility.[25,26] A ballroom style facility utilizing closed systems can be therefore be an economically attractive facility design.

As shown in Figure 2, each unit operation in the ICB demonstrated in this study was designed for closed processing, with sterile or sanitized components and aseptic connections. This ICB was operated in a laboratory as a "worst case environment" rather than in a classified cleanroom. Bioburden samples were taken from the bioreactor daily and samples were collected after every cycle (~3–4 h) during continuous DSP and after each unit operation had finished during batch operation. All bioburden samples up to the AIEX step had bioburden measurements <1 CFU/10 mL, confirming the connected, continuously operating steps operated as a closed system (Figure 5). However, bioburden was detected at in the post-VF and UF/DF product pool (Figure 5). The VF, which had been connected to the closed flow path for 6 days before use, did not lead to bioburden ingress into the upstream AIEX step, indicating unidirectional flow within the batched steps. Bioburden could not be detected in either

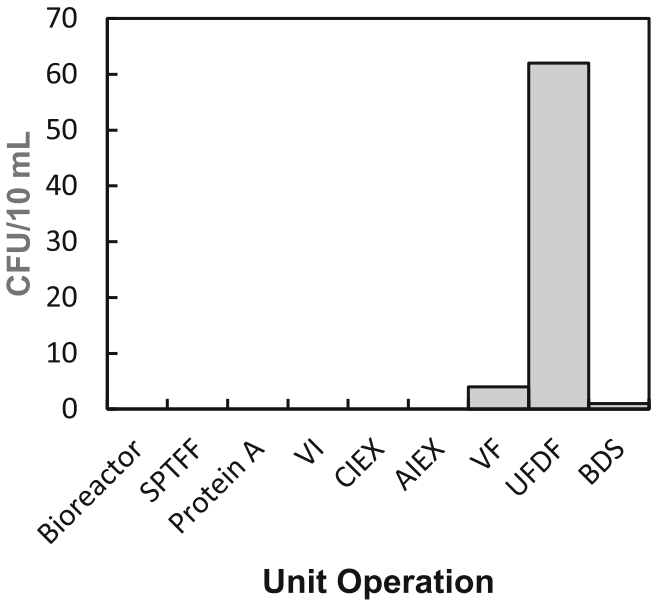

FIGURE 5 Bioburden colony forming unit (CFU) per 10 mL across the ICB process. Bioburden was measured using a compendial method (USP <61>). Sterile samples were taken from the bioreactor, SPTFF, VI, CIEX, AIEX, VF, UFDF and BDS.

product or process streams entering the VF and a route-cause-analysis (data not shown) identified that the VF was not sanitized prior to use and was the most likely cause of bioburden ingress into the process. The final sterilizing grade 0.2 μm filter after the UF/DF step removed the bioburden, leaving the BDS with <1 CFU/10 mL.

Bioburden excursion in a downstream process that meet or exceed an action level do not necessarily indicate that product quality has been compromised, but they do indicate the need for further investigation.[42] A key consideration is the assessment and identification of any microbial bioproducts (e.g., exotoxins, endotoxin, flagellin, microbial DNA and cell wall polysaccharides) that can have adverse effects on patient safety.[42] The development of rapid adventitious agent testing remains a pressing need.[43–45]

3.3 | Process performance of each unit operation in an exemplar manufacturing scale integrated and continuous biomanufacturing process

3.3.1 | N-stage bioreactor cell culture

Integrated and continuous biomanufacturing utilizes perfusion bioreactors to achieve high specific productivity in either steady state or dynamic perfusion.[15,46] Over the last 25 years, over 17 commercially launched biologics have used perfusion processing with volumes of up to 4000 L and a variety of cell retention devices such as gravitational settlers, ATF and TFF.[15,46,47]

A 500 L single-use bioreactor with ATF cell retention was used in this ICB demonstration. A mAb producing Apollo™X CHO-DG44 cell

line was inoculated at a target cell concentration of 0.5×10^6 viable cells/mL. Over 10 days of bioreactor operation the perfusion rate was ramped up to 1.2 vessel volumes per day and production phase conditions were met ($120 \pm 10 \times 10^6$ cells/mL and >1.3 g/L product in the permeate) and the first downstream lot was initiated. The perfusion rate was maintained throughout the production period (day 10 to day 22 of culture). The bioreactor run duration was set to achieve 2×6 day downstream lots (ramp-up mass output: Figure 1b) while maintaining consistent cell density (120×10^6 viable cells/mL), viability (>90%), titre (2 g/L/day), and product quality (Figure 6). To demonstrate the "flexible-to-demand" ICB paradigm (Figure 1b) we also ran a second cell line producing a different mAb for 40 days to simulate maximum mass output from the bioreactor (Figure 7). Using the same platform approach to the upstream process as the previous perfusion bioreactor run we obtained similar and consistent cell density (~120×10^6 viable cells/mL), viability (>90%), titre (~2 g/L/day) and product quality (Figure 7). The second bioreactor run was not connected to a downstream process.

Scaling-out 4×500 L bioreactors (~4 kg/day bioreactor mass output) or scaling-up single or multiple (4×) 2000 L (~4–16 kg/day bioreactor mass output) would result in additional productivity gains. Further increases in mass output/L would necessitate an increase in cell density[48] and/or cell specific production rate[49] as well as an increase in cell bleed processing[50,51] (or a reduction in cell bleed rate[52]). A bioreactor productivity of 4.8 g/L/day remains possible and 4×2000 L bioreactors would deliver 46 kg/day bioreactor mass output.[15]

A single perfusion chemically-defined and protein free media formulation was used in this study which was prepared from a bulk powder. At increased scale (or parallelization) of operation media preparation becomes an increasing concern and may necessitate the introduction of 3–5 fold media concentrates and a limited perfusion volume of 1.5 v.v.d.[15]

3.3.2 | Cell retention

Perfusion bioreactors commonly employ either an alternating tangential flow (ATF) or tangential flow filtration (TFF) system as a cell retention device.[15,46,47] These systems enable the achievement of high cell concentrations exceeding 100×10^6 cells/mL and have successfully

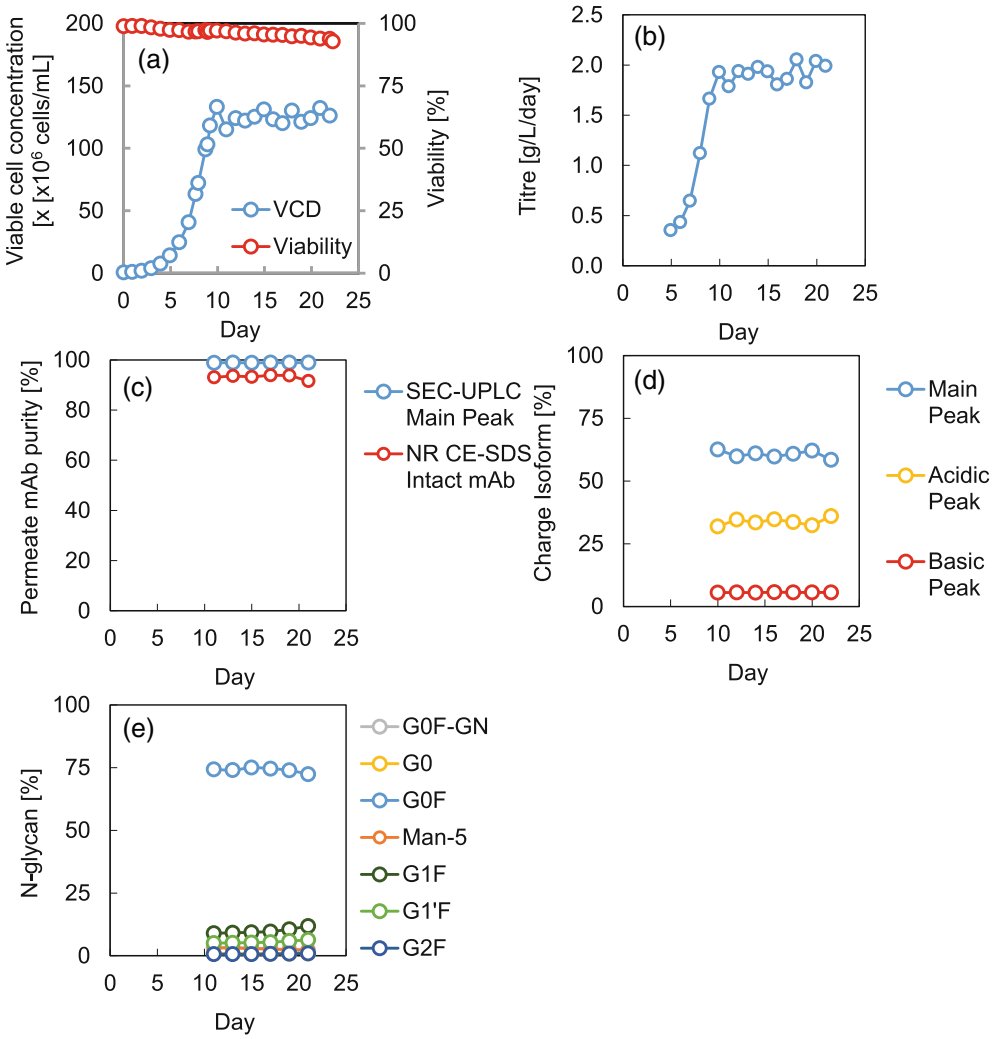

FIGURE 6 Cell growth (a), productivity (b) and mAb product quality (c–e) from the upstream perfusion bioreactor integrated to the downstream process. Viable cell concentration and viability (a) was measured using a ViCell XR (Beckman Coulter). Protein concentration (b), purity (c), charge isoforms (d), and mAb N-glycans (e) were quantified using in house assays.

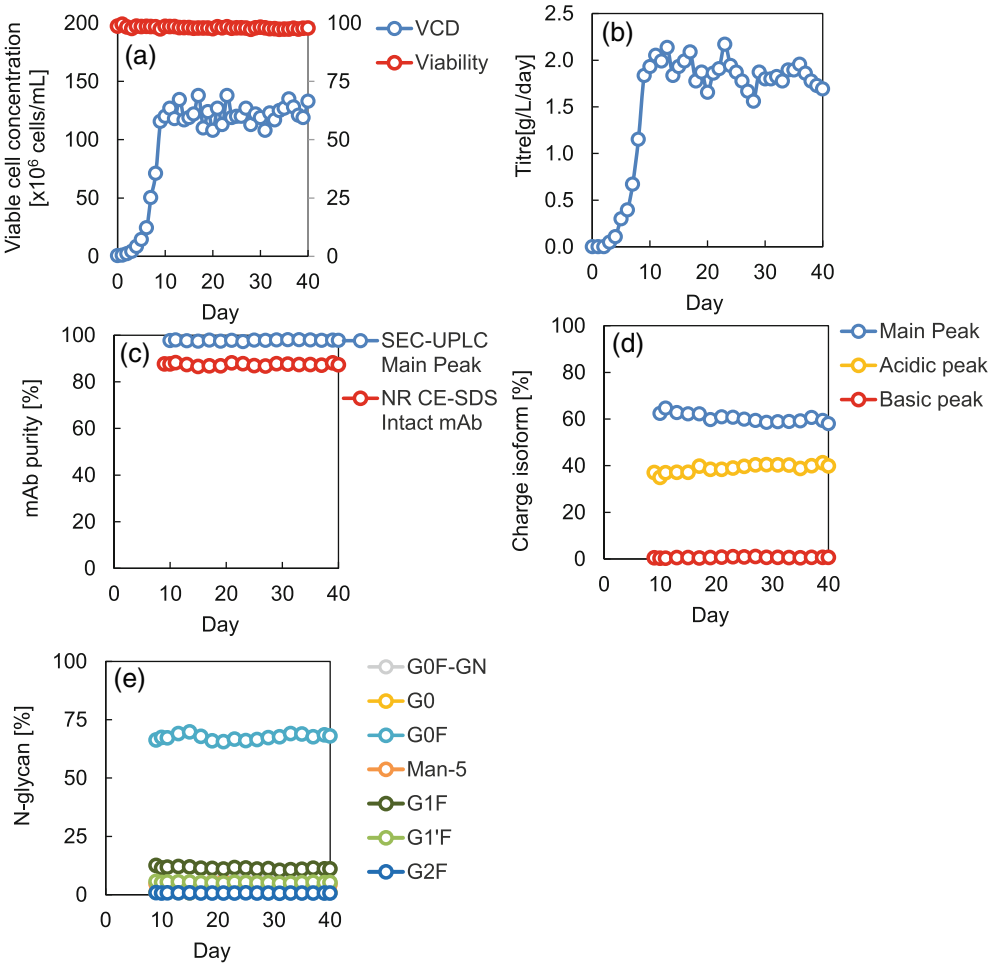

FIGURE 7 Cell growth (a), productivity (b) and mAb product quality (c–e) from a stand-alone 500 L perfusion bioreactor with a different cell line and mAb. Viable cell concentration and viability (a) was measured using a ViCell XR (Beckman Coulter). Protein concentration (b), purity (c), charge isoforms (d), and mAb N-glycans (e) were quantified using in house assays.

been scaled up to a volume of 2000 L.[15] High cell densities have been observed to accelerate the rate of membrane fouling.[53,54] This phenomenon can lead to a reduction in the duration of manufacturing campaigns and necessitate inconvenient switching of cell retention systems during a production run.[55] There remains a significant knowledge gap in the understanding the underlying mechanisms of membrane fouling, particularly in relation to unwanted product retention and membrane fouling. Based on mechanistic studies, it has been observed that biological material adheres to the microfiltration membrane, resulting in the formation of a cake that obstructs the pores and leads to sieving of the desired product.[54,56,57]

The performance of ATF surpasses that of TFF in terms of the rate at which sieving decay occurs. This superiority may be attributed to the bidirectional flow characteristic of ATF, which mitigated the accumulation of biomaterial on the hollow fiber mebrane.[58,59] Operational instability has been associated with the ATF at elevated cell densities.[53,29] The implementation of backflushing techniques using perfusate or fresh media, along with the utilization of diverse membrane chemistries and pore size structures, has demonstrated promising results in prolonging the lifespan of cell retention filters.[53,54,60] Nonetheless, this research successfully attained a duration of operation ranging from 21 to 40 days, while maintaining high cell densities of up to 120×10^6 viable cells/mL. Moreover, this study achieved these extended durations with high permeability levels exceeding 90%, all without necessitating the replacement of ATF filters.

In this study, two ATF10 systems were used to support a 500 L bioreactor, assuming linear scaling that would be eight ATF10 systems to support a 2000 L bioreactor. These would occupy considerable floor space, must be immediately adjacent to the bioreactor and may impact cost-of-goods. Examples of up to four ATF10 systems to support a 2000 L bioreactor have been reported.[15] However, the parallelization of 3 or more ATFs still remains an operation and potential cost-of-goods burden. As ATFs require less development time than TFF systems they may be best utilized in early phase clinical trials or commercial products that do not have high material needs.[15] Ultimately, TFF systems may be preferrable for high material needs[15] and it is clear that opportunities remain to improve the understanding of filter fouling and for the pursuit of non-membrane approaches.[61,62]

3.3.3 | Single pass tangential flow filtration and Protein A capture

Optimization of the protein A capture step receives significant attention as it utilizes the costliest raw material for typical mAb processes.[63] Multicolumn chromatography has been proposed to

improve productivity, resin utilization and buffer consumption relative to batch chromatography.[64–67] However, system complexity and in some scenarios lower resin utilization outweighs pseudo continuous processing.[67–71]

SPTFF has been evaluated for use in fed-batch[72,73] and perfusion[68] processes to de-bottleneck the harvest step, improve Protein A capture[74,75] as well as to achieve high concentration drug substance formulation.[76] In this study, SPTFF was used to reduce the volume of the product pool prior to the Protein A step and accelerate the load phase of protein A chromatography, which is the rate-limiting step at feed concentrations of <2 g/L (which are typically achieved in perfusion processes).[68] After a steep increase in productivity, productivity plateaus at higher concentrations of 6–12 g/L, and time spent in load phases becomes rate limiting.[68]

During the 6 day lot, 3350 L perfusate was conditioned by a constant salt addition and 1.9-fold volume reduction into the Protein A feed tank, reaching equilibrium within 8 h. Each concentration cycle increased the feed pressure to 0.7 ± 0.2 bar and returned to 0 bar on each 1 L flush. As the SPTFF system reached equilibrium, the maximum feed pressure increased from 0.5 to 0.9 bar. The 2 mS/cm conductivity difference between the perfusate feed (∼48 mS/cm) and the retentate (∼46 mS/cm) was consistent with the Donnan effect asymmetrically distributing the buffer salt ions across the SPTFF membrane in response to concentration of the product.[77]

A fixed volume of 57.5 L conditioned perfusate was loaded onto the Protein A column for each cycle to ensure that the column's maximum binding capacity of 65 g/L was not exceeded. This value was calculated based on the minimum dynamic binding capacity obtained from columns cycled 36 times (data not shown), which exceeds the study's requirements.

The 6 day lot ran 27 Protein A cycles with consistent elution profiles (Figure 8a; mean volume equivalent to 3.1 CV) for cycles 2 to 27. Cycle 1 included SPTFF pre-steady state material, which resulted in an earlier and smaller (1.2 CV) elution volume. The material was processed and pooled with the rest of the lot before the anion exchange step. Cycle 1 eluate analysis showed no significant product quality difference (data not shown). The cleaning strategy on every third cycle-maintained flow rates across each stage of the chromatography run, resulting in an average run time of 270 min without cleans and 295 min with cleans.

Using the same SymphonX™ DSP skid, the SPTFF and single column Protein A capture step described in this study would allow purification of around 25 kg/day from a 43 L Protein A column. For purification up to 57.6 kg/day, two 25 L protein A chromatography columns operated in series may be required.[15,16]

3.3.4 | Low pH viral inactivation

The low pH inactivation unit operation typically follows Protein A capture chromatography in batch[1] and ICB processes.[15,16] This step can be operated in a continuous plug-flow reactor with in-line pH reduction, hold and neutralization or a multi-vessel stirred-tank reactor with in-situ pH reduction, hold and neutralization.[15,16,78–82] While the multi-vessel stirred-tank reactor approach generates a pause in the continuity of flow its advantages include that: (i) the approach is identical to the low pH inactivation step commonly employed for historical batch processes; (ii) collection of elution pools enables homogenization of the product pool and therefore eliminates any impact of protein concentration gradient on VI and simplifies viral clearance studies; (iii) the approach is transferable to other inactivation methods using solvent/detergent, and so forth, where the volume of the inactivation agent(s) is well characterized.[82]

To orchestrate fluid flow, the semi-continuous VI approach used in this study used two single-use mixers (SUMs) with closed-loop pH control and a central SymphonX™ control system. The two SUMs were used asynchronously and alternately. Two pooled Protein A elution's were transferred from a surge vessel and collected in one of the SUMs where acidification, hold and neutralization took place. While the inactivation process was occurring, the other SUM was receiving the next set of Protein A eluates. When the inactivation was complete in the first SUM, the processed pool was filtered through a sterile filter into a surge tank.

Off-line and on-line pH measurements after-neutralization were consistently 5.00 +/−0.2. Intermittent off-line pH measurements after acidification (data not shown) revealed that by cycle 12, the pH probe had drifted so that the incubation step was controlled at pH 3.2 rather than pH 3.5. This offset was reduced by calibration of the on-line probe, but the pH drifted again during the final two cycles. Product stability data for the mAb used in this study showed no impact on product quality attributes at an inactivation hold of pH 3.0 (data not shown), confirming processing was within the product's proven stability range.

The advent of more robust pH probes that do not need to be calibrated as frequently or can be calibrated automatically in closed systems would clearly be advantageous. A control strategy that incorporates additional process analytical technology, such as a UV sensor to calculate the linear relationship between the flow rate of acid, base, and water needed to titrate the product stream and/or gravimetric feed control, may also be advantageous.[15,16]

The use of a multi-vessel stirrer-tank reactor approach to VI replicated our defined and validated batch strategy used in fed-batch processes and was dependent on ensuring the Protein A elution cadence was greater than the duration of the VI step, but raises the possibility of untreated product coming into contact with inactivated product.[82] This could be accomplished through "hanging drops" and "hold ups." Low-point feed ports for eluate loading and sub-surface acid and base additions can be used to reduce the risk of hanging drops. To avoid hold ups, the mixer speed can be pre-set so that foaming does not occur at a given volume. A control strategy that adjusts mixing speed based on volume could also be developed. Another option is to switch between product contacting bags, flow paths and single-use bags between each VI step. However, this would impose a cost-of-goods and operational burden. Ultimately, further experiments are required

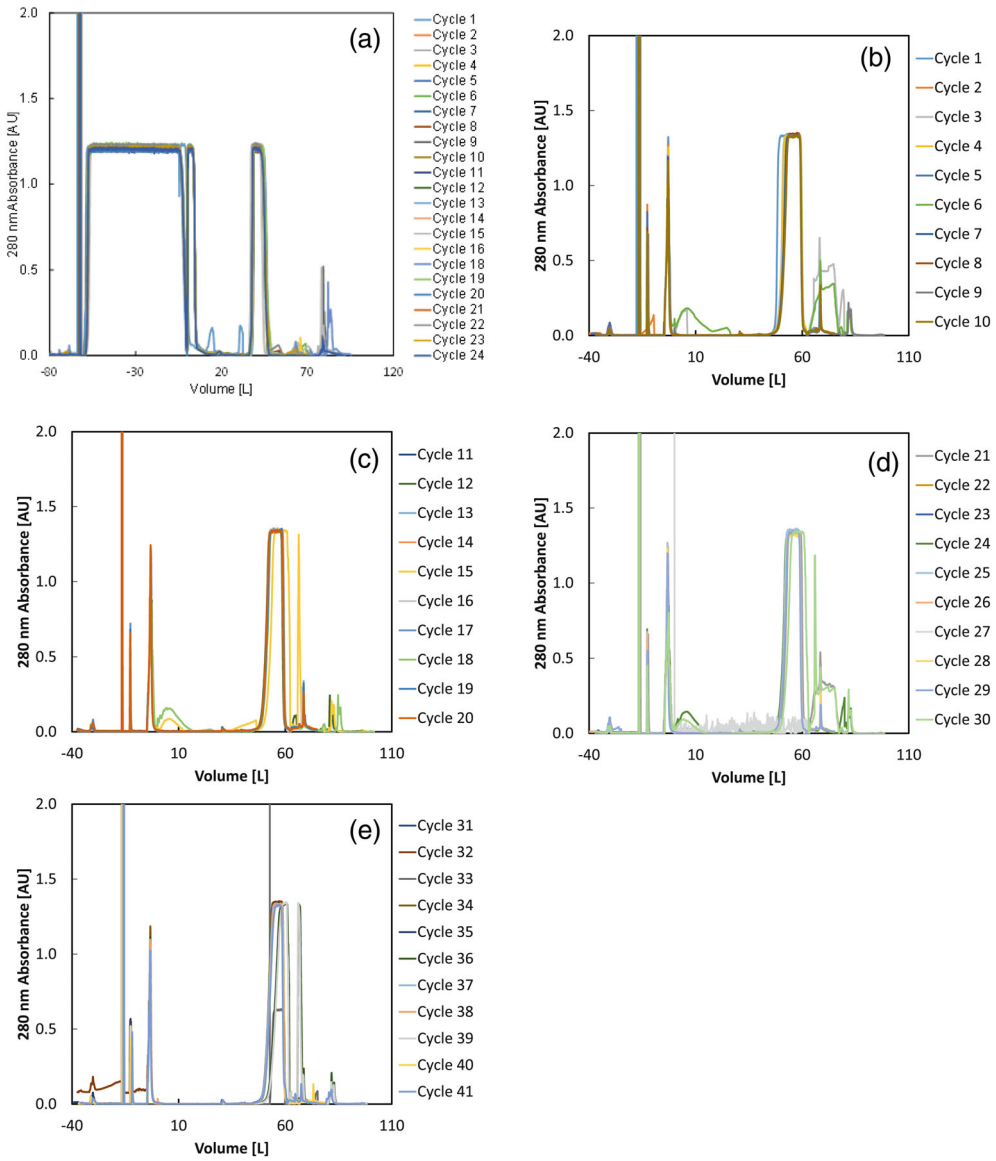

FIGURE 8 UV absorbance from the Protein A column and the CIEX column during lot 2 of the downstream process. (a) Protein A column, (b) CIEX cycles 1–10, (c) CIEX cycles 11–20, (d) CIEX cycles 21–30, (e) CIEX cycles 31–41.

to determine and potentially further mitigate against hold ups and hanging drops.[82] Riboflavin could be used as a visual colorimetric test for hold ups and hanging drops.[82] Furthermore, spiking with exemplar bacteria and viruses may also be required to simulate adventitious agent inactivation.[82] It is important to note that a similar multi-tank approach to VI has previously demonstrated undetectable untreated product contamination and effective adventitious agent inactivation over 24 h of operation.[82]

3.3.5 | Cation exchange polishing chromatography

Multicolumn approaches to bind elute polishing chromatography do not significantly increase column capacity,[15] therefore, they may only provide a marginal benefit in terms of pseudo continuous product flow verses increased operational complexity. As product was pooled post CIEX then continuous product flow was not required and a single column chromatography approach was utilized to reduce system complexity and align to our current mAb batch purification platform. CIEX was performed in bind-elute mode where the column is loaded and then eluted with a gradient in either salt concentration and or pH.[83,84]

To avoid surpassing the 65 g/L binding capability of the CIEX column, a constant load of 10.5 L of VI eluate was transferred each cycle. Due to pH drift-induced dilution of the mAb in the VI stage, forty one cation exchange cycles with varying loading capacities ranging from 24.5 to 41.4 g antibody/L resin were achieved across the 6 day lot. All cycles had an average elution volume of 3.0 CV and comparable chromatography profiles with and without the 0.5 M sodium hydroxide cleaning step (Figure 8b). The greater variance in the elution point was attributed to minor fluctuations in the specific conductivity at any given point in the elution gradient between cycles. These, along with

variation in the column loadings, were not found to impact product quality, nor were they indicative of column aging or loss of resin performance (data not shown). The cleaning strategy on every third cycle maintained flow rates across each stage of the chromatography run, resulting in an average run time of 140 min without cleans and 165 min with cleans. Over 6 days, the intermediate pooling bag collected 343 L of eluates from the cation exchange cycles.

Further process intensification of the CIEX unit operation may be possible by replacing bind-elute chromatography with frontal chromatography, in which the product binds to the resin (loadings can exceed 1000 g/L) but is displaced by aggregates and impurities that bind more tightly.[15,85,86]

3.3.6 | Anion exchange polishing chromatography

Flow-through chromatography is preferred for at least one of the polishing steps due to ease of integration, potential to enable continuous product flow and lower buffer/solution volume than bind/elute chromatography.[15,85–90] Typical flowthrough polishing steps include AIEX or mixed mode resins that capture impurities and aggregates as the product flows through.[15] Flow-through AIEX was deployed in this study to process the 6 day pooled CIEX eluate in a single batch. 860 L of the CIEX pool was filtered at two bar, and average flow rate of 500 L/h was achieved. The AIEX filter was then flushed with 200 L of equilibration buffer and pooled to give a final volume of 1060 L.

3.3.7 | Virus filtration

A complex interplay between product concentration, pH, ionic strength of the feed and fluctuations in operating pressure can impact the overall performance of the VF process.[91–99] Some processes are able to avoid flux decay and operate essentially continuously for long periods, enabling end-to-end continuous processing. Other processes require less than a day of operation in batch-mode where new filters are used between lots rather than cleaning.[15] In this study, we aligned our ICB VF approach with that of our historic batch platform to minimize regulatory impact.

The VF step began after 150 L AIEX filtrate was collected into a surge vessel and processed as a single batch through the viral filter at 240 L/h. The UFDF step received three ~400 L aliquots for a total of 1144 L viral filtrate.

3.3.8 | Ultrafiltration, diafiltration and formulation

Similar to most ICB we used the same UFDF set up as batch to minimize regulatory impact.[15] Three 385 ± 20 L feed material sub-batches were concentrated and formulated from viral filtrate to drug substance. 300 L of surge vessel viral filtrate was transferred to the first retentate vessel. The material was concentrated to 80 kg and sequentially diafiltered seven times with 80 kg of in-line diluted 4× formulation buffer stock at 1.15 bar and 84 LMH. Each cycle was concentrated to 26 kg and moved to the second retentate vessel to process more cycles. After all three cycles were completed, pooled, and assayed for product concentration, the retentate was formulated with 870 mL 20 mM sodium phosphate, 7.5% sucrose and 1% (v/v) polysorbate 20 and diluted to 82.3 L of BDS at 39.5 g/L. This was filtered into 1.8 L aliquots and stored at −65°C.

Continuous UFDF process typically utilize SPTFF to continuously concentrate the product through several stages of dilution and SPTFF modules.[15,17] Efficient operation typically utilizes counter-current mode.[34,100–103]

3.4 | Overall process performance of the manufacturing scale integrated and continuous biomanufacturing process

Product quality attributes (Table 1) and impurity clearance (Table 2) profiles were monitored to assess unit operation performance, it has been reported that a benefit of perfusion cell culture is that it generates superior quality product compared to fed-batch material.[27,10] Product quality characteristics were compared between fed-batch, and perfusion cell culture (Table 1). mAb produced in the perfusion process displayed comparable aggregation, reduced charge heterogeneity and increased mAb galactosylation compared to fed-batch production from the same cell line. The ICB process also cleared residual host cell protein, DNA and Protein A ligand in an acceptable manner (Table 2).

The 3350 L of generated perfusate yielded 3.25 kg purified product with an average titre of 1.59 g/L after 6 days of purification, yielding 61% and 0.54 kg/day of drug substance. A small-scale batch run yielded 68%. The 7% difference between the two runs was attributed to the CIEX loading strategy, where the load was directed through the bubble trap, and resulted in ~0.8 L load material not being processed each cycle (~7% loss). It is important to note that a platform downstream process was utilized in this study and that yields would likely increase following further process development.[104]

TABLE 1 Product quality attributes for mAb BDS produced either in an integrated and continuous biomanufacturing (ICB) process or a fed-batch upstream and batch downstream process.

	ICB process	Fed-batch USP and batch DSP
A280 conc (mg/L)	39.5	37.2
SEC-UPLC (% main peak)	98.4	97.3
NR-CE (% intact mAb)	91.1	90.6
icIEF (% main peak)	56.9	48.9
N-glycan (% Galactosylation)	20.9	7.9

	Sampling Point				
Residual	Protein A Feed	Protein A Elution	CIEX pool	AIEX pool	BDS
DNA [pg/mg]	1,212,990	299	6.4	<5.0	<0.2
HCP [ng/mg]	115,923	780	61.7	<20.0	0.9
Protein A [ng/mg]	0	16.0	6.2	4.5	4.0

Note: HCPs, DNA and residual Protein A were quantified using in-house assays. The sampling points from the continuous (Protein A Feed, Protein A Elution and CIEX pool) were at early, mid and late cycle numbers. The batch sampling point (AIEX pool) was sampled at early and late process times. BDS was sampled at the end of the process. Data is presented as an average of these samples.

TABLE 2 Residual clearance of host cell protein (HCP), Protein A (ProA) and DNA during lot 2 of the downstream process.

4 | CONCLUSION

The biopharmaceutical industry is transitioning from batch to ICB. A common framework is emerging within the industry for companies implementing ICB, which should improve patient access to life-saving biopharmaceuticals.[15,16]

Despite this momentum, there have been few explicit demonstrations of commercial scale ICBs in the literature, and those that have are limited in scale (e.g., from a 100 L perfusion bioreactor)[15,16,28–36] Manufacturing scale ICB demonstrations are currently hindered by a lack of commercially available flexible plug-and-play equipment.[15,16,26]

To address this need we developed SymphonX™ a multifunctional downstream processing skid with advanced buffer management capability and a single generic flow path for plug-and-play flexibility across all downstream unit operations. SymphonX™ supports flow rates ranging from 0.1 to 12.3 L/min, potentially enabling a common ICB framework manufacturing facility to flexibly produce batches based on commercial demand.[15,16] This facility's mass output could be as low as 0.5 kg/batch or as high as 8 tonnes/year.[15,16] In this study, we conducted a 500 L manufacturing scale ICB, which produced 0.54 kg/day of drug substance. The upstream process was operated between 22 and 40 days, the downstream process was operated for 12 days, consisting of 2 × 6 day lots of continuous processing from the bioreactor to CIEX pooling. The second lot was processed in a batch from CIEX pool to BDS. This demonstration aligns with an established industry framework for ICB[15,16] and identifies the critical measures to facilitate future commercial production and optimize mass production in a next-generation ICB enabled facility. Such a facility has the potential to accommodate a wide range of biopharmaceutical ICB and traditional batch bioprocesses. The exemplar manufacturing ICB documented in this study therefore represents a notable advancement toward the commercialization of ICB processes and the subsequent realization of substantial benefits stemming from this transition.

AUTHOR CONTRIBUTIONS

Leon P. Pybus: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Charles Heise:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Tibor Nagy:** Conceptualization; data curation; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – original draft; writing – review and editing. **Carmen Heeran:** Data curation; investigation; project administration; resources; validation; visualization; writing – original draft; writing – review and editing. **Terri Dover:** Data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **John Raven:** Data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **Junichi Kori:** Data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **Graeme Burton:** Data curation; formal analysis; methodology; validation; visualization; writing – original draft; writing – review and editing. **Hiroshi Sakuyama:** Data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **Benjamin Hastings:** Data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft; writing – review and editing. **Michelle Lyons:** Data curation; formal analysis; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Shinichi Nakai:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Jonathan Haigh:** Conceptualization; project administration; resources; supervision; writing – original draft; writing – review and editing.

ACKNOWLEDGMENTS

The authors would like to thank Isa Senica, Robyn Hoare, Kyle Montgomery, Luke Johnston and Zaynab Ibrahim for help in running the Upstream process, and Adebisi Ifeh, Atsushi Nakahodo, Andrew Binieda, Bertrand Delmas, Amelia Hide, Alexandra Keogh, Hasib Qureshi and Fransesc Gonzalez for running the Downstream process.

CONFLICT OF INTEREST STATEMENT

At the time of publication all authors are employed by FUJIFILM Diosynth Biotechnologies or their parent company FUJIFILM Corporation.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/btpr.3456.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Leon P. Pybus https://orcid.org/0000-0001-5614-8758

REFERENCES

1. Kelley B. Industrialization of mAb production technology: the bioprocessing industry at a crossroads. *MAbs*. 2009;1:443-452.
2. Walsh G, Walsh E. Biopharmaceutical benchmarks. *Nat Biotechnol*. 2022;40:1722-1760.
3. Kelley B. Developing therapeutic monoclonal antibodies at pandemic pace. *Nat Biotechnol*. 2020;38:540-545.
4. Cha M, Rifai B, Sarraf P. Pharmaceutical forecasting: throwing darts? *Nat Rev Drug Discov*. 2013;12:737-738.
5. Ecker DM, Seymour P. Supply and demand trends: mammalian biomanufacturing industry overview. *Bioprocess Int*. 2020;18:10-14.
6. Garcia FA, Gefroh E. Reducing biopharmaceutical manufacturing costs through continuous processing in a flexible J.POD® facility. *Drug Discov Today*. 2023;28:103619.
7. Chen SW, Zhang W. Current trends and challenges in the downstream purification of bispecific antibodies. *Antib Ther*. 2021;4:73-88.
8. Duivelshof BL, Murisier A, Camperi J, et al. Therapeutic Fc-fusion proteins: current analytical strategies. *J Sep Sci*. 2021;44:35-62.
9. Wang Y, Qiu H, Minshull J, et al. An innovative platform to improve asymmetric bispecific antibody assembly, purity and expression level. *Biochem Eng J*. 2022;188:108683.
10. Gomez N, Lull J, Yang X, et al. Improving product quality and productivity of bispecific molecules through the application of continuous perfusion principles. *Biotechnol Prog*. 2020;36:e2973.
11. Love JC, Love KR, Barone PW. Enabling global access to high quality biopharmaceuticals. *Curr Opin Chem Eng*. 2013;2:383-390.
12. Argoud S, Budzinski K, D'Aquila D, Madabhushi SR, Smith P. Green metrics for biologics. *Curr Opin Green Sustain Chem*. 2022;35:100614.
13. Hernandez R. Modular manufacturing platforms for biologics. *BioPharm Int*. 2015;18:18-25.
14. Jacquemart M, Vandersluis V, Zhao K, Sukhija N, Sidhu J, Stout A. A single-use strategy to enable manufacturing of affordable biologics. *Comput Struct BiotechnolJ*. 2016;14:309-318.
15. Coffman J, Brower M, Connel-Crowley L, et al. A common framework for integrated and continuous biomanufacturing. *Biotechnol Bioeng*. 2021;118:1721-1735.
16. Coffman J, Bibbo K, Brower M, et al. The design basis for the integrated and continuous biomanufacturing framework. *Biotechnol Bioeng*. 2021;118:3323-3333.
17. Arnold L, Lee K, Rucker-Pezzini J, Lee JH. Implementation of fully integrated continuous antibody processing: effects on productivity and COGm. *Biotechnol J*. 2019;14:e1800061.18.
18. Fisher AC, Kamga MH, Agarabi C, Brorson K, Lee SL, Yoon S. The current scientific and regulatory landscape in advancing integrated continuous biopharmaceutical manufacturing. *Trends Biotechnol*. 2019;37:253-267.
19. Hummel J, Pagkaliwangan M, Gjoka X, et al. Modeling the downstream processing of monoclonal antibodies reveals cost advantages for continuous methods for a broad range of manufacturing scales. *Biotechnol J*. 2019;14:e1700665.
20. Konstantinov KB, Cooney CL. White paper on continuous bioprocessing. May 20–21, 2014 Continuous Manufacturing Symposium. *J Pharm Sci*. 2015;104:813-820.
21. Klutz S, Holtmann L, Lobedann M, Schembecker G. Cost evaluation of antibody production processes in different operation modes. *Chem Eng Sci*. 2016;141:63-74.
22. Pollock J, Coffman J, Ho SV, Farid SS. Integrated continuous bioprocessing: economic, operational, and environmental feasibility for clinical and commercial antibody manufacture. *Biotechnol Prog*. 2017;33:854-866.
23. Walther J, Godawat R, Hwang C, Abe Y, Sinclair A, Konstantinov K. The business impact of an integrated continuous biomanufacturing platform for recombinant protein production. *J Biotechnol*. 2015;213:3-12.
24. Pollock J, Ho SV, Farid SS. Fed-batch and perfusion culture processes: economic, environmental and operational feasibility under uncertainty. *Biotechnol Bioeng*. 2013;110:206-219.
25. Kavara A, Sokolowski D, Collins M, Schofield M. Chapter 4: recent advances in continuous downstream processing of antibodies and related products. In: Matte A, ed. *Approaches to the Purification, Analysis and Characterization of Antibody-Based Therapeutics*. Elsevier; 2020:81-103.
26. Erickson J, Baker J, Barrett S, et al. End-to-end collaboration to transform biopharmaceutical development and manufacturing. *Biotechnol Bioeng*. 2021;118:3302-3312.
27. Walther J, Lu J, Hollenbach M, et al. Perfusion cell culture decreases process and product heterogeneity in a head-to-head comparison with fed batch. *Biotechnol J*. 2019;14:e1700733.
28. Coolbaugh MJ, Varner CT, Vetter TA, et al. Pilot-scale demonstration of an end-to-end integrated and continuous biomanufacturing process. *Biotechnol Bioeng*. 2021;118:3287-3301.
29. Feidl F, Vogg S, Wolf M, et al. Process wide control and automation of an integrated continuous manufacturing platform for antibodies. *Biotechnol Bioeng*. 2020;117:1367-1380.
30. Ferreira KB, Benlegrimet A, Diane G, et al. Transfer of continuous manufacturing process principles for mAb production in a GMP environment: a step in the transition from batch to continuous. *Biotechnol Prog*. 2022;38:e3259.
31. Godawat R, Konstantinov K, Rohani M, Warikoo V. End-to-end integrated fully continuous production of recombinant monoclonal antibodies. *J Biotechnol*. 2015;213:13-19.
32. Levison P. Moving one unit operation at a time toward continuous biomanufacturing: an overview of Pall solutions for integrated continuous biopharmaceutical production. *Bioprocess Int*. 2016;14:s6-s9.
33. Ramos I, Sharda N, Villafana R, et al. Fully integrated downstream process to enable next-generation manufacturing. *Biotechnol Bioeng*. 2023;120:1869-1881.
34. Rucker-Pezzini J, Arnold L, Hill-Byrne K, Sharp T, Avazhanskiy M, Forespring C. Single pass diafiltration integrated into a fully continuous mAb purification process. *Biotechnol Bioeng*. 2018;115:1949-1957.
35. Schwarz H, Gomis-Fons J, Isaksson M, et al. Integrated continuous biomanufacturing on pilot scale for acid-sensitive monoclonal antibodies. *Biotechnol Bioeng*. 2022;119:2152-2166.
36. Steinbach F, Ulmer N, Wolf M, et al. Design and operation of a continuous integrated monoclonal antibody production process. *Biotechnol Prog*. 2017;33:1303-1313.
37. Tung G, Morris K, Perrone P, Reinbigler R, Miller S, Lai C. The value of plug-and-play automation in single-use technology. *BioProcess Int*. 2019;17:12-19.
38. Isaksson M, Gomis-Fons J, Andersson N, Nilsson B. An automated buffer management system for small-scale continuous downstream bioprocessing. *J Chromatogr A*. 2023;1695:463942.

39. Lozano J, Lock A. Benefits of single-use standardization: adopting a standard design approach. *BioProcess Int*. 2021;19:34-40.
40. Crowley L, Cashen P, Noverraz M, Lobedann M, Nestola P. Reviewing the process intensification landscape through the introduction of a novel, multitiered classification for downstream processing. *Biotechnol Bioeng*. 2024;121:877-893.
41. Chalk S, Taber R, Probst S, et al. Challenging the cleanroom paradigm for biopharmaceutical manufacture of bulk drug substances. *BioPharm Int*. 2011;24:2-11.
42. Bain D, Hardy D, Bell BL, et al. Microbial monitoring for biological drug substance manufacturing: an industry perspective. *PDA J Pharm Sci Technol*. 2015;69:451-460.
43. Barone PW, Avgerinos S, Ballard R, et al. Biopharmaceutical industry approaches to facility segregation for viral safety: an effort from the consortium on adventitious agent contamination in biomanufacturing. *PDA J Pharm Sci Technol*. 2019;73:191-203.
44. Challener CA. Rapid adventitious agent testing remains a real need. *BioPharm Int*. 2018;31:34-37.
45. Sampath R, Blyn LB, Ecker DJ. Rapid molecular assays for microbial contaminant monitoring in the process industry. *PDA J Pharm Sci Technol*. 2010;64:458-468.
46. Bielser J-M, Wolf M, Souquet J, Broly H, Morbidelli M. Perfusion mammalian cell culture for recombinant protein manufacturing – A critical review. *Biotechnol Adv*. 2018;36:1328-1340.
47. Clincke MF, Molleryd C, Zhang Y, Lindskog E, Walsh K, Chotteau V. Very high cell density of CHO cells in perfusion by ATF or TFF in WAVE bioreactors. Part I. Effect of the cell density on the process. *Biotechnol Prog*. 2013;29:754-767.
48. Wolf MKF, Bielser JM, Morbidelli M. *Perfusion Cell Culture Process for Biopharmaceuticals*. Cambridge University Press; 2020.
49. Li F, Vijayasankaran N, Shen AY, Kiss R, Amanullah A. Cell culture processes for monoclonal antibody production. *mAbs*. 2010;2:466-479.
50. Bielser J-M, Aeby M, Caso S, Roulet A, Broly H, Souquet J. Continuous bleed recycling significantly increases recombinant production yield in perfusion cell cultures. *Biochem Eng J*. 2021;169:107966.
51. Romann P, Kolar J, Chappuis L, Herwig C, Villiger TK, Bielser J-M. Maximising yield of perfusion cell culture processes: evaluation and scale-up of continuous bleed recycling. *Biochem Eng J*. 2023;193:108873.
52. Donaldson JS, Dale MP, Rosser SJ. Decoupling growth and protein production in CHO cells: a targeted approach. *Front Bioeng Biotechnol*. 2021;9:658325.
53. Pinto NDS, Napoli WN, Brower N. Impact of micro and microporous TFF membranes on product sieving and chromatography loading for perfusion cell culture. *Biotechnol Bioeng*. 2020;117:117-124.
54. Wang SB, Godfrey S, Radonigi F, Lin H, Coffman J. Larger pore size hollow fiber membranes as a solution to the product retention issue in filtration-based perfusion bioreactors. *Biotechnol J*. 2019;14:e1800137.
55. van Reis R, Zydney A. Bioprocess membrane technology. *J Membr Sci*. 2007;297:16-50.
56. Bolton GR, Apostolidis AJ. Mechanistic modelling of the loss of protein sieving due to internal and external fouling of microfilters. *Biotechnol Prog*. 2017;33:1323-1333.
57. Kelly W, Scully J, Zhang D, et al. Understanding and modelling alternating tangential flow filtration for perfusion cell culture. *Biotechnol Prog*. 2014;30:1291-1300.
58. Radoniqi F, Zhang H, Bardliying CL, Shamlou P, Coffman J. Computational fluid dynamic modelling of alternating tangential flow filtration for perfusion cell culture. *Biotechnol Bioeng*. 2018;115:2751-2759.
59. Wang S, Godfrey S, Ravikrishnan J, Lin H, Vogel J, Coffman J. Shear contribution to cell culture performance and product recovery in ATF and TFF perfusion systems. *J Biotechnol*. 2017;246:52-60.
60. Pinto NDS, Brower M. Wide-surface pore microfiltration membrane drastically improves sieving decay in TFF-based perfusion cell culture and streamline chromatography integration for continuous bioprocessing. *Biotechnol Bioeng*. 2020;117:3336-3344.
61. Bettinardi IW, Castan A, Medronho RA, Castilho LR. Hydrocyclones as cell retention device for CHO perfusion processes in single-use bioreactors. *Biotechnol Bioeng*. 2020;117:1915-1928.
62. Kwon T, Prentice H, Oliveira J, et al. Microfluidic cell retention device for perfusion of mammalian suspension cultures. *Sci Rep*. 2017;7:6703.
63. Bansode V, Gupta P, Kateja N, Rathore AS. Contribution of protein A steps towards cost of goods for continuous production of monoclonal antibody therapeutics. *J Chem Technol Biot*. 2021;97:2420-2433.
64. Baur D, Angarita M, Muller-Spath T, Steinebach F, Morbidelli M. Comparison of batch and continuous multi-column protein A capture processes by optimal design. *Biotechnol J*. 2016;11:920-931.
65. Baur D, Angelo JM, Chollangi S, et al. Model assisted comparison of Protein A resins and multi-column chromatography for capture processes. *J Biotechnol*. 2018;285:64-73.
66. David L, Schwan P, Lobedann M, et al. Side-by-side comparability of batch and continuous downstream for the production of monoclonal antibodies. *Biotechnol Bioeng*. 2020;117:1024-1036.
67. Mahaja E, George A, Wolk B. Improving affinity chromatography resin efficiency using semi-continuous chromatography. *J Chromatogr A*. 2012;1227:154-162.
68. Brinkman A, Elouafiq S. Enhancing protein A productivity and resin utilization within integrated or intensified processes. *Biotechnol Bioeng*. 2021;118:3359-3366.
69. Ghose S, Nagrath D, Hubbard B, Brooks C, Cramer SM. Use and optimization of a dual-flowrate loading strategy to maximise throughput in protein-A affinity chromatography. *Biotechnol Prog*. 2004;20:830-840.
70. Guo J, Jin M, Kanani D. Optimization of single-column batch and multicolumn continous protein A chromatography. *Biotechnol J*. 2020;15:e2000192.
71. Kamga MH, Cattaneo M, Yoon S. Integrated continuous biomanufacturing platform with ATF perfusion and one column chromatography operation for optimum resin utilization and productivity. *Prep Biochem Biotechnol*. 2018;48:383-390.
72. Arunkumar A, Singh N, Peck M, Borys MC, Li ZJ. Investigation of single-pass tangential flow filtration (SPTFF) as an inline concentration step for cell culture harvest. *J Membr Sci*. 2017;524:20-32.
73. Brinkmann A, Elouafiq S, Pieracci J, Westoby M. Leveraging single-pass tangential flow filtration to enable decoupling of upstream and downstream monoclonal antibody processing. *Biotechnol Prog*. 2018;34:405-411.
74. Dizon-Maspat J, Bourret J, D'Agostini A, Li F. Single pass tangential flow filtration to debottleneck downstream processing for therapeutic antibody production. *Biotechnol Bioeng*. 2012;109:962-970.
75. Teske CA, Lebreton B, Reis RV. Inline ultrafiltration. *Biotechnol Prog*. 2010;26:1068-1072.
76. Casey C, Gallos T, Alekseev Y, Ayturk E, Pearl S. Protein concentration with single-pass tangential flow filtration (SPTFF). *J Membr Sci*. 2011;384:82-88.
77. Bolton GR, Boesch AW, Basa J, Lacasse DP, Kelley BD, Acharya H. Effect of protein and solution properties on the Donan effect during the ultrafiltration of proteins. *Biotechnol Prog*. 2011;27:140-152.
78. Gillespie C, Holstein M, Mullin L, et al. Continuous in-line virus inactivation for next generation bioprocessing. *Biotechnol J*. 2019;14:e1700718.
79. Klutz S, Lobedann M, Bramsiepe C, Schembecker G. Continuous viral inactivation at low pH value in antibody manufacturing. *Chem Eng Process*. 2016;102:88-101.

80. Martins DL, Sencar J, Hammerschmidt N, et al. Truly continuous low pH viral inactivation for biopharmaceutical process integration. *Biotechnol Bioeng*. 2020;117:1406-1417.
81. Parker SA, Amarikwa L, Vehar K, et al. Design of a novel continuous flow reactor for low pH viral inactivation. *Biotechnol Bioeng*. 2018; 115:606-616.
82. Schofield M, Jones K. Assessing viral inactivation for continuous processing. *BioPharm International*. 2018. Accessed October 8, 2023. https://www.biopharminternational.com/view/assessing-viral-inactivation-continuous-processing
83. Pabst TM, Suda EJ, Thomas KE, et al. Binding and elution behaviour of proteins on strong cation exchangers. *J Chromatogr A*. 2009; 1216:7950-7956.
84. Zhou JX, Dermawan S, Solamo F, et al. pH-conductivity hybrid gradient cation-exchange chromatography for process-scale monoclonal antibody purification. *J Chromatogr A*. 2007;1175:69-80.
85. Reck JM, Pabst TM, Hunter AK, Carta G. Separation of antibody monomer-dimer mixtures by frontal analysis. *J Chromatogr A*. 2017; 1500:96-104.
86. Vogg S, Pfeifer E, Ulmer N, Morbidelli M. Process intensification by frontal chromatography: performance comparison of resin and membrane adsorber for monovalent antibody aggregate removal. *Biotechnol Bioeng*. 2020;117:662-672.
87. Brown A, Bill J, Tully T, Radhamohan A, Dowd C. Overloading ion-exchange membranes as a purification step for monoclonal antibodies. *Biotechnol Appl Biochem*. 2010;56:59-70.
88. Ichihara T, Ito T, Kurisu Y, Galipeasu K, Gillespie C. Integrated flow-through purification for therapeutic monoclonal antibody processing. *MAbs*. 2018;10:325-334.
89. Ichihara T, Ito T, Gillespie C. Polishing approach with fully connected flow-through purification for therapeutic monoclonal antibody. *Eng Life Sci*. 2019;19:31-36.
90. Liu HF, McCooey B, Duarte T, et al. Exploration of overloaded cation exchange chromatography for monoclonal antibody purification. *J Chromatogr A*. 2011;1218:6943-6952.
91. Bakhshayeshi M, Zydney AL. Effect of solution pH on protein transmission and membrane capacity during virus filtration. *Biotechnol Bioeng*. 2008;100:108-117.
92. Bohonak D, Zydney A. Compaction and permeability effects with virus filtration membranes. *J Membr Sci*. 2005;254:71-79.
93. Bolton G, Cabatingan M, Rubino M, Lute S, Brorson K, Bailey M. Normal-flow virus filtration: detection and assessment of the endpoint in bioprocessing. *Biotechnol Appl Biochem*. 2005;42:133-142.
94. Ireland T, Bolton G, Noguchi M. Optimising virus filter performance with prefiltration. *Bioprocess Int*. 2005;3:44-47.
95. Rathore AS, Kumar V, Arora A, Lute S, Brorson K, Shukla A. Mechanistic modelling of viral filtration. *J Membr Sci*. 2014;468: 96-103.
96. Strauss D, Goldstein J, Hongo-Hirasaki T, et al. Characterizing the impact of pressure on virus filtration processes and establishing design spaces to ensure effective parovirus removal. *Biotechnol Prog*. 2017;33:1294-1302.
97. Syedain ZH, Bohonak DM, Zydney AL. Protein fouling of virus filtration membranes: effect of membrane orientation and operating conditions. *Biotechnol Prog*. 2006;22:1163-1169.
98. Tateishi J, Kitamoto T, Mohri S, et al. Scrapie removal using Planova virus removal filters. *Biologicals*. 2001;29:17-25.
99. Wickramasinghe SR, Stump ED, Grzenia DL, Husson SM, Pellegrino J. Understanding virus filtration membrane performance. *J Membr Sci*. 2010;365:160-169.
100. Jungbauer A. Continuous downstream processing of biopharmaceuticals. *Trends Biotechnol*. 2013;31:479-492.
101. Huter MJ, Strube J. Model-based design and process optimization of continuous single pass tangential flow filtration focusing on continuous processing. *Processes*. 2019;7:317.
102. Jabra MG, Yehl CJ, Zydney AL. Multistage continuous countercurrent diafiltration for formulation of monoclonal antibodies. *Biotechnol Prog*. 2019;35:e2810.
103. Nambier AMK, Li Y, Zydney AL. Countercurrent staged diafiltration for formulation of high value proteins. *Biotechnol Bioeng*. 2018;115: 139-144.
104. Baumann P, Hubbuch J. Downstream process development strategies for effective bioprocesses: trends, progress, and combinatorial approaches. *Eng Life Sci*. 2016;17:1142-1158.

How to cite this article: Pybus LP, Heise C, Nagy T, et al. A modular and multi-functional purification strategy that enables a common framework for manufacturing scale integrated and continuous biomanufacturing. *Biotechnol. Prog*. 2024;40(4): e3456. doi:10.1002/btpr.3456