

REVIEW

A common framework for integrated and continuous biomanufacturing

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Abstract

There is a growing application of integrated and continuous bioprocessing (ICB) for manufacturing recombinant protein therapeutics produced from mammalian cells. At first glance, the newly evolved ICB has created a vast diversity of platforms. A closer inspection reveals convergent evolution: nearly all of the major ICB methods have a common framework that could allow manufacturing across a global ecosystem of manufacturers using simple, yet effective, equipment designs. The framework is capable of supporting the manufacturing of most major biopharmaceutical ICB and legacy processes without major changes in the regulatory license. This article reviews the ICB that are being used, or are soon to be used, in a GMP manufacturing setting for recombinant protein production from mammalian cells. The adaptation of the various ICB modes to the common ICB framework will be discussed, along with the pros and cons of such adaptation. The equipment used in the common framework is generally described. This review is presented in sufficient detail to enable discussions of ICB implementation strategy in biopharmaceutical companies and contract manufacturers, and to provide a road map for vendors equipment design. An example plant built on the common framework will be discussed. The flexibility of the plant is demonstrated with batches as small as 0.5 kg or as large as 500 kg. The yearly output of the plant is as much as 8 tons.

KEYWORDS

biopharmaceutical, dual-column chromatography, integrated continuous bioprocessing, mammalian cells, perfusion, protein therapeutics

1 | INTRODUCTION

Many biopharmaceutical companies are considering switching from fed-batch bioreactors and independent, batch downstream unit operations to integrated and continuous bioprocessing (ICB), which uses a mammalian host in continuous perfusion bioreactors and a linked and continuous downstream. ICB has well-reviewed cost advantages for clinical and, for some scenarios, commercial manufacturing (Arnold et al., 2019; Hummel et al., 2019; Klutz et al., 2016a;

Levine et al., 2013; Pollock, Bolton, et al., 2013; Pollock et al., 2017; Walther et al., 2015).

The literature describes a dizzying array of options for ICB, so much so that one might think that biopharmaceutical companies would choose different and incompatible implementations. Here, we report and review many of the largest biopharmaceutical companies implementing ICB to show there exists a common denominator in the industry. We further demonstrate that the common framework uses many of the familiar batch processing operations, and allows

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backward compatibility with legacy batch processes. This review will enable the strategic discussion of IBC implementation in biopharmaceutical companies new to the area, as well as provide contract manufacturers a high-level blueprint for a manufacturing plant capable of running all known GMP integrated and continuous bioprocesses. It will also provide vendors a road map for developing plug-and-play ICB equipment.

The specific choices made for implementing ICB in GMP manufacturing are not often well described in the literature. Only a fraction of published and presented work is implemented. The common framework process is based on the authors' knowledge of what is being implemented in their companies, as well as interviews with other bioprocess engineers across the industry. This article will not disclose the details of individual companies' platforms except where a citation exists, or disclosure permission is given. Some features that build on top of the framework will remain proprietary and will not be described. All of the integrated and continuous bioprocesses can fit into a plant built on the common framework without significant regulatory changes and without large capital investments. The framework should not require new process characterization studies, nor should it require new virus removal or inactivation studies. Regulatory filings and technology transfer would, however, benefit from some foreknowledge of this framework process.

These considerations are important, especially today. The development of antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) exemplifies the need for high productivity ICB. Due to the global coronavirus disease 2019 (COVID-19) pandemic, many companies have reserved space in Contract Manufacturing Organizations (CMO) with more than 10,000 L bioreactor capacity to make clinical and commercial material for possible antibody therapies (Kelley, 2020), leading to capacity limitations for fed-batch processes. These plants cannot quickly be expanded. Stainless-steel plants take 5 years or more to design and build. Smaller single-use fed-batch cell culture plants can be built in as little as 12 months, but these smaller plants simply will not have the capacity or the cost structure to make sufficient material to prevent and treat COVID-19 if they use conventional batch operations. While many countries would like to control their own manufacturing of the COVID-19 treatments, they cannot build and operate large stainless-steel plants economically. Using the common framework for ICB described in this article would allow quickly built single-use facilities to produce as much as 8 tons of material per year for a fraction of the cost of a traditional stainless-steel plant.

We report in a companion article in this issue (Coffman et al., 2020) the design basis for the framework integrated and continuous bioprocess that is sufficiently detailed to allow CMOs and bioprocessing vendors to design plants and equipment that would enable a global ecosystem high productivity manufacturing sufficient to supply mAbs for treating and preventing COVID-19 and other diseases. This framework ICB is being used as the basis for the "first generation" of ICB for The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), a US government/academic/

industrial bioprocessing consortium, also co-published in this issue (Erickson et al., 2021).

2 | THE COMMON FRAMEWORK FOR INTEGRATED AND CONTINUOUS MANUFACTURING

The known ICB being implemented in clinical or commercial manufacturing are shown in Table 1, based on interviews and surveys conducted by the authors (Charlebois et al., 2020). These processes use a mixture of N-stage perfusion, and some form of multicolumn chromatography with linked processes. Some processes are integrated end-to-end, from the bioreactor through to the final concentration step. Others pool the product in-process to define a lot. Some processes run for 14 days, many run for 20–30 days. Some use single-column chromatography; most use some form of multicolumn chromatography. While it may seem that a single set of equipment cannot enable these different permutations on ICB, a common framework has been designed.

The common ICB framework that fits these processes is shown in Figure 1. The common framework is possible because each ICB was derived from a common batch mAb platform processes for monoclonal antibodies (Kelley, 2009).

The bioreactor in the ICB framework is run continuously, harvesting the product through a filtration step. The bioreactor links to an integrated downstream that is based on batch chromatography processes. Instead of simulated moving bed chromatography, the framework uses dual-column chromatography, which can also be used for processes designed for single-column chromatography. In many cases, the chemistry (resins, buffer composition, and membrane composition) is identical to the historical platform. Product pools are replaced with smaller surge tanks. The low pH virus inactivation step is flexible, allowing batch or continuous inactivation. Some processes pool the lot after the virus inactivation stage; others after the first polishing step; still others just before the virus reduction filter. The common framework uses media and solution concentrates, more preferably, or in-line conditioning to enable large batch sizes. In-line titration is also required for many linked unit operations. Process Analytical Technologies (PAT) are used in combination with feed-forward and feed-back control, but in this common framework, they are not much more complex than those used in fully automated batch processes. The common framework also supports fed-batch bioreactor processes with a filtration harvest step.

2.1 | N-stage bioreactor

The framework uses perfusion bioreactors to achieve high specific productivity in either a steady state or dynamic perfusion (Wolf et al., 2020a). The industry has many years of commercial perfusion manufacturing scale experience at large scale up to 4000 L (Pollock et al., 2013b). Over 17 commercially launched biologics have used

TABLE 1 The implementation of integrated and continuous bioprocessing largely follows the same framework

	N-1	N Brx	Number of columns	Virus inact.	Number of columns	Number of columns	Virus filter	UFDF	GMP/NA
Amgen	NA	Perf	Single	Batch	Single	Single	Batch	Batch	Y
AstraZeneca	Perf	Perf	Multi	TBD	Single	Single	Batch	Batch	N
Bayer	NA	Batch	Multi	Cont.	Multi	Multi	NA	NA	NA
Biosana	NA	Perf	Multi	Batch	Multi	Multi	Cont.	Batch	Y
BMS	Perf	Batch	Multi	Batch	Single	Single	Batch	Batch	Y
Janssen	Perf	Batch	Single	Batch	Single	Single	Batch	Batch	Y
Just	Perf	Perf	Multi	Batch	Multi	Multi	Cont.	Cont.	Y
Lilly	Batch	Batch	Single	Batch	Single	Single	Batch	Batch	Y
Merck (KgaA)	NA	Batch	Multi	Cont.	Multi	Multi	Batch	Batch	Y
Merck (MSD)	Perf	Perf	Multi	Batch	Multi	Multi	Cont.	Batch	Y
Novartis	Perf	Perf	Multi	Batch	Single	Single	Cont.	Cont.	Y
Pfizer/BI	Perf	Perf	Multi	Cont.	Single	Single	Batch	Batch	N
Sanofi	TBD	Perf	Multi	Both	Multi	Multi	Cont.	Cont.	Y ^a
WuXi	Perf	Perf	Multi	NA	Multi	Multi	NA	NA	NA

Abbreviation: ICB, integrated and continuous bioprocesses.

^aSanofi's current GMP ICB is a standard batch process after the multicolumn capture. End-to-end ICB is the ultimate goal.

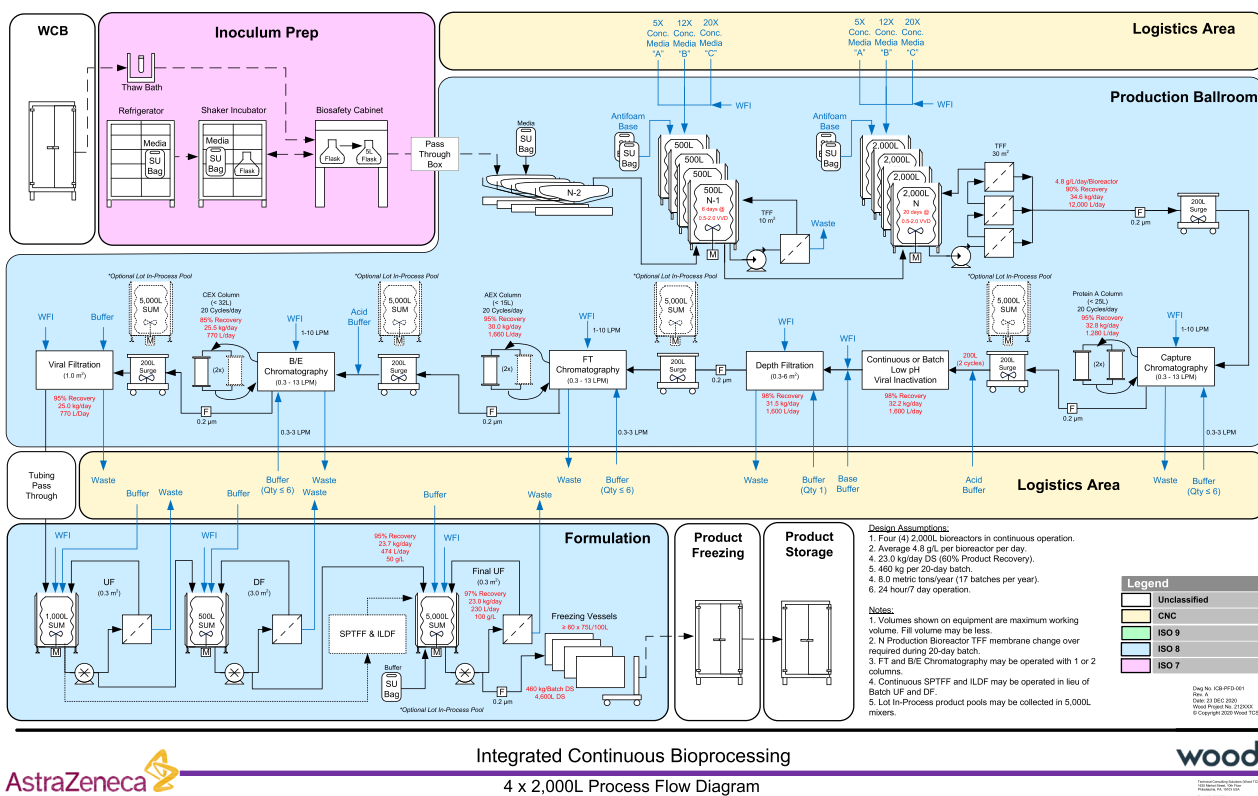


FIGURE 1 The common framework for integrated and continuous bioprocessing, showing the process flow from seed vial through to drug substance

perfusion over the last 25 years using a range of cell retention devices including gravitational settlers, alternating tangential flow filtration (ATF) and tangential flow filtration (TFF; Clincke et al., 2013). The industry has seen cell specific productivities in the range of 20–90 pg/cell/day range (Allread et al., 2014; Li et al., 2010) with cell densities in the 30–70 million cells/ml. Typically constant cell-density perfusions have been achieved at 2–4 g/L day at 70 M cells/ml for up to 60–80 days (Brower et al., 2015; Warikoo et al., 2012). Dynamic perfusion, which allow variable cell densities, has seen cell densities between 100 and 200 M cells/mL (Wolf et al., 2020a). Given this industry experience and that this framework is designed to be compatible with the future productivities, a relatively high bioreactor productivity of 4.8 g/L/day (Tables 2 and 3) was chosen. This assumes the relatively high cell specific productivity, 40 pg/cell/day and a high average cell density of 120 million cells/ml which has been achieved at lab scale. We expect this productivity to be routine during the next five years. Indeed, as discussed below, the only upper limit to the framework process with respect to bioreactor productivity is solution preparation and storage, and the maximum pump flow rate on the Protein A skid.

We have found that cell lines that have been selected for fed batch adapt easily to perfusion cell culture. The volume of media required for perfusion bioreactors is many-fold higher than that of fed-batch. Perfusion media must be developed that minimizes the volume of media required. Perfusion media can be adapted directly from fed-batch media by mixing a portion of the basal media with the

concentrated feed media, removing components unneeded for perfusion, and separating acid, base, and neutral soluble components (Dennis & DiSorbo, 1995). This media can be from three to five-fold more concentrated than the original fed-batch media. The example below assumes that three-fold media concentrates are used. Expensive media components such as insulin, di-peptides, or hydrolysates should be avoided. The diluted perfusion volume is set to 1.5 volume per volume per day (VVD). The media feed rate is likely linked to cell density, so PAT that includes measuring cell density is required. The most common of these method is dielectric spectroscopy followed by Raman and infrared spectroscopy (Wolf et al., 2020b).

TABLE 2 High-level process description used for the bioreactor operation

N-stage bioreactor size (L)	500–2000
Number of bioreactors	1–4
Avg cell density (million cells/ml)	50–120
Cell specific productivity (Q_p) (pg/cell/day)	20–40
Permeate perfusion rate (bioreactor volumes/day, vvd)	0.5–2
Lot (batch) duration (days)	20
Number of concentrated media feeds	1–5
Media concentration factor	1× to 5×

TABLE 3 The bioreactor productivity and downstream capacity dictates the media and solution utilization

	Productivity/capacity/diafiltration product concentration	Volume used (L/L)	Yield (%)	Number of solutions per Step	PMI (L/kg)	Concentrate factor	PMI for concentrates	ILC concentration factor	PMI for ILC
Bioreactor	4.8 g/L/day	1.5 L/L/day	100	3	529	3	176		
TFF	1000 L/m ²	NA	90	NA	NA	NA	NA		
ProA	60 g/L	21 CV	90	7	534	5	107	10	53
VI	NA	0.1 Protein A CV	100	NA	15	1	15	1	15
AEX	100 g/L	16 CV	95	4	220	5	44	10	22
CEX	40 g/L	16 CV	85	5	521	5	104	10	52
VRF	700 g/m ²	NA	95	NA	15	5	3	5	3
UFDF	50 g/L	6 DV	95	2	126	1	126	10	13
Overall			59	14 ^a	1960		576		158

Note: Shown are the process description for each step that impacts solution use. The PMI or process mass intensity is the mass of material used in production per mass of product produced. Here, the PMI refers only to the solution mass used. Downstream uses 400 kg buffer/kg BDS equally divided among 14 buffers means each kg of BDS requires 29 L of each buffer. Bioreactor yield includes 90% yield of cell retention step.

Abbreviations: AEX, anion-exchange chromatography; CEX, cation-exchange chromatography; CV, column volume; DV, diafiltration volume; ILC, in-line conditioning; PMI, process mass intensity; ProA, protein A; TFF, tangential flow filtration; UFDF, ultrafiltration/diafiltration; VI, virus inactivation; VRF, virus reduction filtration.

^aThe overall number of buffers does not include the VI/VRF buffers, as those will not go into large buffer tanks. The sanitization solution is assumed to be common among all steps. The PMI does include the buffers from these steps.

Some ICB will not have three-fold media concentrates. The framework process would require either more frequent media preparation during the batch or the use of fewer bioreactors.

The regulatory perspective on the bioreactor is well-established (Fisher et al., 2019). In addition to the normal critical process parameters (CPPs) seen in batch bioreactors, feed rate and the ratio of feed rates of the concentrates may be critical.

2.2 | Cell retention

Cells are retained in the bioreactor with tangential flow filtration, operated in one of two modes: ATF system, or TFF. This approach has enabled high cell concentrations up to 100 million cells/ml and established up to 2000 L scale. The high cell density increased the rate of membrane fouling (Pinto et al., 2020; Wang et al., 2019). These can lead to shortening of manufacturing campaigns (van Reis & Zydney, 2007) and to the routine cumbersome switching of filtration systems during manufacturing operations. The fundamental mechanistic understanding of membrane fouling with undesired product retention and membrane plugging remains poorly understood. Mechanistic studies propose biological material deposits onto the microfiltration membrane forming a cake that blocks the pores (Kelly et al., 2014) causes product sieving (Bolton & Apostolidis, 2017; Wang et al., 2019). Performance comparison of ATF to TFF show a lower sieving decay for ATF, potentially due to the bidirectional flow, minimizing the biomaterial deposit on the hollow fiber membrane (Radoniqi et al., 2018; Wang et al., 2017). However the ATF has been associated with operational instability at higher cell densities (Pinto & Brower, 2020; Pinto et al., 2020). Backflushing of the ATF and TFF filters with perfusate or fresh media has shown potential to extend filtration life during a manufacturing campaign (Wang et al., 2017; Wang et al., 2019). Researchers have evaluated different membrane chemistries and pore size structures as opportunities to reduce product sieving that typically can reach as low as 50%–60% transmission after only 20 days of operation. Recently the product sieving & fouling has been successfully mitigated to allow more than 85% transmission for 40 days by the use of wide pore filters (Pinto et al., 2020; Wang et al., 2019). Despite these improvements, it is clear that opportunities remain to improve the understanding of fouling and the potential for pursuit of non-membrane approaches (Bettinardi et al., 2020; Kwon et al., 2017).

ATF systems are often used for 500 L bioreactors and below; two or more ATF systems may be used to support a 1000 L perfusion bioreactor, but TFF is the preferred choice. Some authors have developed processes that use as many as four ATFs to support a 2000 L perfusion bioreactor. They take up considerably more floor space than the TFF systems and must be immediately adjacent to the bioreactor. ATFs require less development time than TFF systems, and thus may be used in early phase clinical trials or commercial products that do not have high material needs. Many, but not all, of the authors of this paper agree that TFF systems are preferred.

The common framework would use the same permeate pumps for both the ATF and TFF set up. The retentate pumps and associated controllers would be unique to either the ATF or TFF set up.

From a regulatory perspective, the degree of product retention may become a critical performance parameter, since this will impact product residence time in the bioreactor, which may in turn impact product quality attributes such as acidic species level.

2.3 | Dual-column chromatography

The chromatography steps are the most variable from company to company. Many companies use three or more columns (Zydney, 2016). Some use only one. Some use different numbers depending upon the step. The simplest common framework for these various chromatography processes is two columns. Although many processes use more than two columns, the multicolumn nature is not used in any step but the load step; the systems use two columns during the load step to overload one column while capturing the product in the flow through onto the second column (Angarita et al., 2015; Baur et al., 2016a, 2016b). All other steps are effectively single-column operations for the Protein A capture step and the two polishing chromatography steps. Thus, the simplest embodiment of the multicolumn chromatography skid is a two-column system for all chromatographic steps. A gradient elution is not considered. A two-column system has about 1/4 fewer valves and ½ the number of pumps as a three-column system.

Rather than use a dual-column system, it may be possible to link two standard single-column systems side-by-side. Many single-column systems are less than half the cost of a multicolumn system. Each single column system could, in addition, be used separately for more conventional processes, increasing the flexibility of the plant. Automation allowing the tandem operation of two single-column skids is not a trivial task. Loading the flow-through material from one skid onto a column on the second skid would require commonly used orchestration software or distributed control software that could communicate both skids.

The dual-column system can be used as a replacement for one or more columns without impacting the regulatory process description. Since the operation of each chromatography column is the same independent of the number of columns, the virus removal validation should not have to be redone. It is important to note here, however, that the regulatory filing should not refer to three or more columns, but rather two or more columns.

More columns do, however, increase the apparent continuity of the product flow into and out of the step. Also, above a certain load concentration, the two-column process cannot be continuous because the load step is completed faster than the subsequent wash, elution, regeneration steps (Angarita et al., 2015; Baur et al., 2016a, 2016b). A dual-column process therefore requires a surge tank for the load when the load product concentration exceeds a threshold. A surge tank is almost required before the polishing stages. It may not

be required between the two polishing steps if these can be operated in sync.

The polishing steps benefit only marginally from multicolumn chromatography by allowing continuous flow into and out of the step. Multicolumn chromatography does not benefit the overall performance by increasing the capacity of the columns. The first polishing step often uses flowthrough anion-exchange chromatography, which has a fixed capacity independent of residence time. Since the flowthrough chromatography is preferred for one of the polishing steps because it is easy to integrate with other steps, requires fewer buffers and uses less solution volume (Table 3) than bind/elute chromatography, and allows for a more continuous product flow through the process (Brown et al., 2010; Ichihara et al., 2018, 2019; Liu et al., 2011; Reck et al., 2017; Vogg et al., 2020). Typical flowthrough polishing steps include AEX or mixed mode resins with loadings of more than 100 g/L resin that capture impurities and aggregates as the product flows through. Another option is frontal CEX chromatography, in which the product binds to the resin but is displaced by aggregates and impurities that bind more tightly (Reck et al., 2017; Vogg et al., 2020). This format has achieved loadings exceeding 1000 g/L resin. Load conditioning occurs inline via pH titration, salt solution addition or water for injection dilution, depending on the requirements of the step. Since the multicolumn chromatography does not benefit the polishing steps as much, many companies use single column chromatography.

The dual column operation benefits the Protein A step significantly more than the polishing steps. The continuous operation of the dual-column process reduces the Protein A column volume from about 1500 L for a 10 ton/year plant (Kelley, 2007) to two 25 L columns. The difference in cost is about \$23 M versus \$0.8 M, assuming \$15,000/L as a reference point. The 25 L columns reach their lifetime in 200 cycles, or at the end of every 500 kg batch; the 1500 L reaches its 200-cycle lifetime in about 1.5 years if the plant makes only one product. Many plants make 3–8 products, meaning that the 1500 L may reach its expiry (typically 5 years) before reaching its lifetime. Furthermore, the 1500 L should purify 15 tons of material before reaching its lifetime. There is significant risk that a product that is initially forecast to require 15 tons will never actually need 15 tons. These multi-use stainless steel plants may have more money invested in their partially used Protein A inventory than the cost to build an entire ICB facility.

From a regulatory perspective, a major difference between integrated downstream and a batch downstream is the bioburden control strategy. Since the Protein A step is attached to the bioreactor, and the bioreactor has more stringent bioburden control criteria, the downstream bioburden control strategy should be more comprehensive than typical batch downstream processes. This control strategy should consider the initial state of the state of the fluid path (for instance gamma irradiation) including the Protein A column (for instance, the use of peracetic acid or sodium hydroxide), the maintenance of the closed system, ongoing sanitization procedures, the location of sterile filters, and the frequency and location of bioburden sampling. The Protein A step does not require sterility; it

requires the demonstration of a sanitary state appropriate for linking to a bioreactor.

2.4 | In-process pooling strategy

In-process pool stability is a key consideration for integrated processes. The process should be integrated and continuous at least 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 can be defined as the lot. Some companies pool the entire lot after the virus inactivation step, which would require four to five 5000 L single use bags, indicated in Figure 1. Pooling after the flow through step increases the pool volume but not beyond the five 5000 L SU bags. Pooling after the CEX step reduces the lot volume so that it can be contained in four 5000 L SU bags.

The hold time surrounding this pool may be the largest regulatory consideration for the framework process. Unless the process was designed with such a hold in mind, in-process hold studies will be required. These studies are not difficult, unless an instability is found that affects product quality. In this case, the lot pool should be made in a more stable part of the process.

The hold in the surge tank is also a consideration. The surge tank may have a volume as low as 5 min of process flow to as much as 24 h. A hold time of 4 h would require hold studies out to three or four time constants, or perhaps 12–16 h. Less stable products would require smaller surge tanks commensurate with the validated hold time.

These in-process holds, though continuous, provide a sampling point for quality control. The samples represent an average of the product stream over a relatively short duration (typically hours). After validation, the sampling of a small amount of process material is acceptable. During process validation, slip-stream samples may need to be taken to validate process performance and bioburden control.

2.5 | Solution management strategy

The solution and water utilization of the downstream is a major consideration, and in many cases the main bottle neck in the ICB downstream. The standard productivity measure used in the literature for this step, mass of product per volume resin per unit time (Budzinski et al., 2019; Cataldo et al., 2020), is not the most relevant to manufacturing because the resin is almost never the limiting factor. Instead, one should consider a process mass intensity (PMI; also known as E-factor) for the solutions, which is the solution mass per mass of product produced. The PMI for step i is:

$$PMI_i = \frac{\hat{V}_i}{C_i} \frac{1}{\prod_{j=i}^n Yield_j}$$

with product concentration C_i (for instance, the product concentration on a chromatography column or during diafiltration), with a

specific wash volume \hat{V}_i (for instance, column wash volume, or number of diavolumes), and yield for remaining steps.

The use of solution concentrates is essential to maximizing the productivity of ICB. Floor space for production would be mostly solution preparation and solution storage without solution concentrates. Even with five-fold concentrates, many authors have seen that the solution preparation and storage area is larger than the main production area. Also useful would be the use of the same solutions from step to step. For instance, the equilibration solution for the anion-exchange chromatography step can be used to equilibrate the Protein A, and as the last wash. It can also be used to equilibrate the VRF. The high-salt strip solution can be used on the anion and cation exchange, as well as the high salt wash on the Protein A. The same sodium hydroxide solution can also be used throughout the process. These changes would decrease the number of solutions from 14 to 10 or so. It does not decrease the total volume of solutions used. The volume and number of solutions drives the size, cost, complexity, and throughput of high-capacity ICB facilities.

In-line conditioning (ILC) or In-line formulation (ILF) may provide a better solution management option, providing over 20 \times concentration factor for many solutions (Carredano et al., 2018). ILC does not simply dilute a solution with water, but mixes acid, base, concentrated untitrated buffer, and salt with water to achieve the correct solution composition. ILC has the capacity to impact manufacturing more than multicolumn chromatography in terms of solution management reduction. ILC systems could be attached directly to each chromatography step, which might be expensive. A full cost analysis for the common framework process has not been done. Alternatively, one ILC system could supply concentrated downstream solutions that are then diluted by the skids, as above. Since the process uses over 14 different solutions, this method of solution management uses nearly the same amount of floor space as the in-line dilution alone.

ILC works best, and allows the best pH control, where the pH is near the pKa of the buffer. The use of ILC should be considered during development so that the solution compositions and buffer choices are simple and consistent across steps.

These considerations also benefit classic batch processing, though not to the same degree.

2.6 | Low pH virus inactivation

The low pH inactivation step typically follows the Protein A step. The step can be operated in either periodic batch mode or with a continuous plug-flow reactor (PFR). The framework process can satisfy both modes with in-line titration of the product stream. For typical product concentrations, the acid solution flow rate is proportional to the product concentration. In-line titration should allow the adoption of the common framework without requiring new virus inactivation studies for batch processes.

For the systems that use continuous inactivation, a PFR is required. Many PFR designs have been published for low pH virus

inactivation (Gillespie et al., 2019; Klutz et al., 2016b; Martins et al., 2020; Parker et al., 2018). Since the amount of acid and base changes with product concentration, the stream might require dilution with water to maintain constant ionic strength. The subsequent chromatography step must be developed accounting for variable load flow rates.

From a regulatory perspective, better pH probes would be beneficial for this step. pH probes that do not require daily calibration, or can be calibrated automatically in closed systems, would be beneficial. pH probes that can withstand gamma irradiation would also be beneficial. Use of feed-forward control, as discussed above, would allow more optimal control of the pH. Feed-back control based on the pH probe may also be required. Without these considerations, pH control could be achieved with higher levels of titrant addition and subsequent dilution with water.

2.7 | Virus reduction filter

Earlier investigations about mechanistic understanding of virus filtration have reported the impact of factors such as variability in product concentration, pH, ionic strength of the feed and fluctuations in operating pressure (Bakhshayeshi & Zydney, 2008; Bohonak & Zydney, 2005; Bolton et al., 2005; Ireland et al., 2005; Rathore et al., 2014; Strauss et al., 2017; Syedain et al., 2006; Tateishi et al., 2001; Wickramasinghe et al., 2010). A complex interplay between these factors can impact the overall performance of the VRF process by eventual flux decay and accidental virus breakthrough in the filtrate. Some processes are able to avoid this flux decay and operate essentially continuously for long periods, enabling end-to-end processing. Other processes require operation for less than a day, essentially in batch-wise operation. These processes use a new filter rather than cleaning the used filter.

The filter capacity is often based on the volume passing through the filter. Therefore, the filter area used is the same independent of where the lot is pooled. The flow rates for each mode are quite different. The continuous end-to-end process has about 1/20th to 1/60th the flow rate of the entire lot of material at once on the VRF. The framework process pools material before the VRF for small lot sizes (such as those needed in early phase clinical trials), or changes the virus filter daily for large material requirements. A single use skid with a high-flow and low-flow tubing set and pumps would allow the processing of both modes of VRF. Alternatively, a subplot could be pooled daily before the VRF, thus allowing the use of constant pressure operation.

From a regulatory perspective, the CPPs for this step are similar to the batch operation. Where the filter is changed every day, there are no differences. Where the filter is sized for use over many days, the flux might be so low that monitoring the pressure drop may not be a good measure of filter fouling. In this case, characterization under low flux conditions should establish a wide acceptable range for volumetric capacity.

2.8 | Ultrafiltration and diafiltration steps

The framework process can be configured to allow batch-wise ultrafiltration and diafiltration (UFDF) or single-pass, continuous UFDF. The step uses the same pumps and similar filter areas, while the flow path is redefined to allow both modes of operation.

Many ICB use the same UFDF set up as for batch. Processes that diafilter the entire lot of material at once will suffer from the need for very large pumps and filters. This strategy is not recommended.

The batch-wise process is shown in Figure 2a. First, a small UF system concentrates the VRF stream to a desired level. Periodically, the concentrated material is moved to a small diafiltration system. After diafiltration, the subplot is pooled with other sub-lots. The larger pool is concentrated with a small UF system over the course of the run, allowing fine control of the final pool concentration.

The single-pass, continuous UFDF process uses SPTFF (Jungbauer, 2013) to continuously concentrate the product. The product then passes through an in-line diafiltration (ILDF) step made up of several stages of dilution and SPTFF modules. These modules are most efficient when operated in a counter-current mode (Arnold et al., 2019; Huter & Strube, 2019; Jabra et al., 2019; Nambiar et al., 2018; Rucker-Pezzini et al., 2018). The final drug substance concentration is achieved through a final SPTFF step. A schematic of such a process is shown in Figure 2b. Using countercurrent DF can reduce the diafiltration solution volume by roughly a factor of two,

mostly by allowing a higher concentration of product during the diafiltration operation.

The membrane sizes for the batch-wise and continuous modes of operation are similar, on the order of 1 m². Smaller lots are accommodated by doing fewer sub-batches. The pumps used, labeled A-F, are the same for the batch-wise UFDF and for the single pass set up, with the exception of pump D, which requires a higher flow rate for the batch-wise process.

Material is pooled during the entire lot. As with fed-batch processes, the product quality from the bioreactor may vary with time. The control of the product quality requires a sophisticated GMP and regulatory strategy.

From a regulatory perspective, the CPPs may include cleanability, since the UFDF membranes are likely to see more product per unit area than batch processes. The permeate flow rates will require better monitoring for the SPTFF and ILDF since the total diafiltration volume is less important than the ratio of product flow to DF solution flow at any point in time.

3 | IMPLEMENTATION OF CLINICAL AND A COMMERCIAL 8-TON PER YEAR ICB

A large-scale staged implementation of the framework is shown in Figure 3. The plant is designed with flexibility. It is constrained primarily by two factors: solution and media preparation volume and

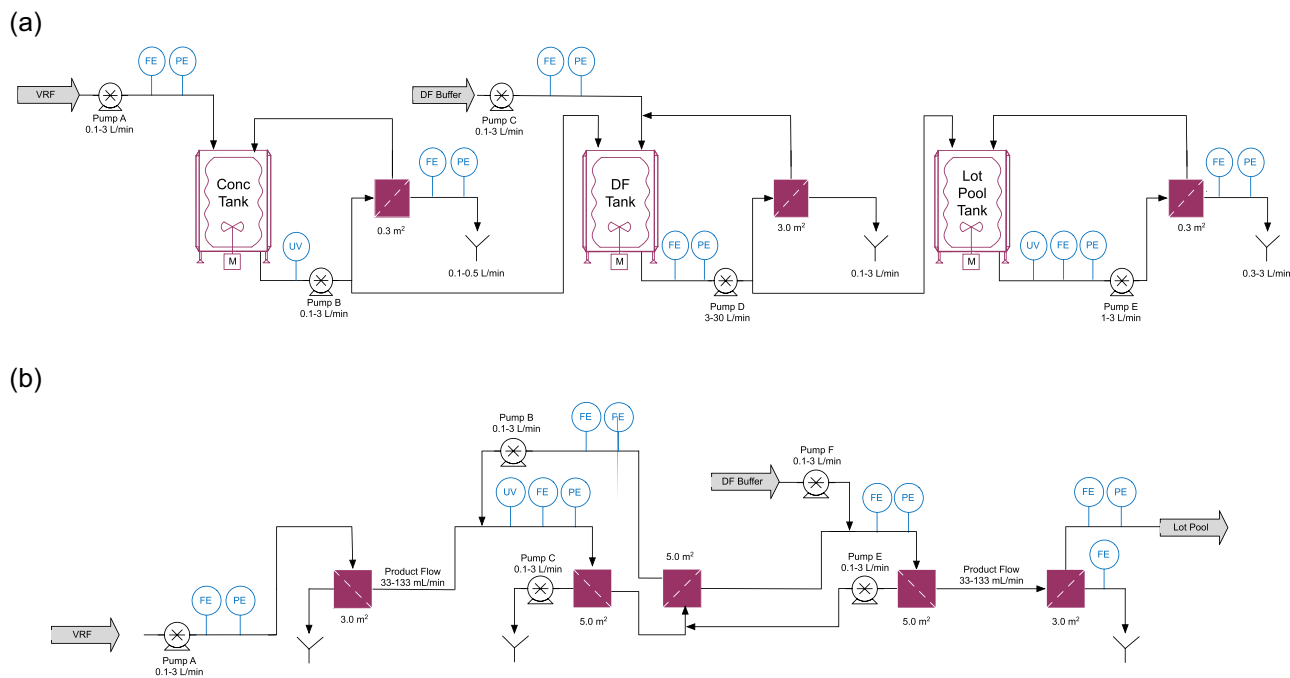
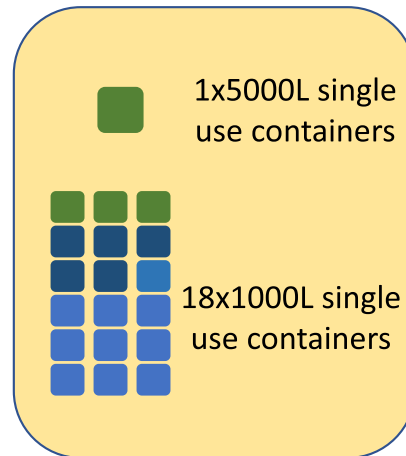
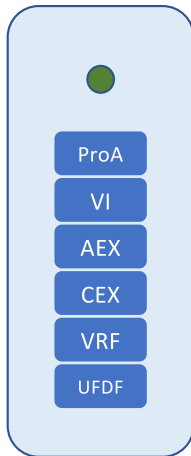


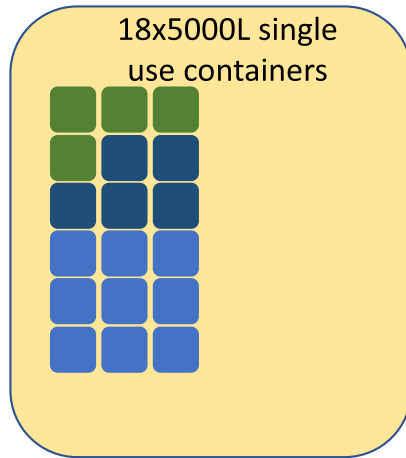
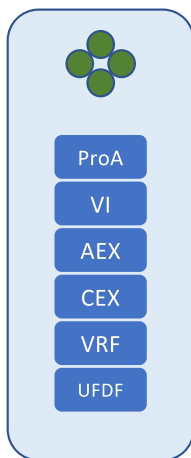
FIGURE 2 The ultrafiltration and diafiltration system (UFDF) that can perform either batch-wise UFDF or a single-pass formulation, depending upon the single-use assembly installed. The pumps A-F are the same in both configurations. Pump D is only used in the batch-wise approach. Single use assemblies are designed to support the flow path of one or the other mode. (a) The batch-wise UFDF, which uses a series of stages to achieve the UFDF. (b) The integrated and continuous single-pass tangential flow filtration and in-line diafiltration process, which uses a series of tangential flow filtration modules in single-pass mode to formulate the drug substance

(a)



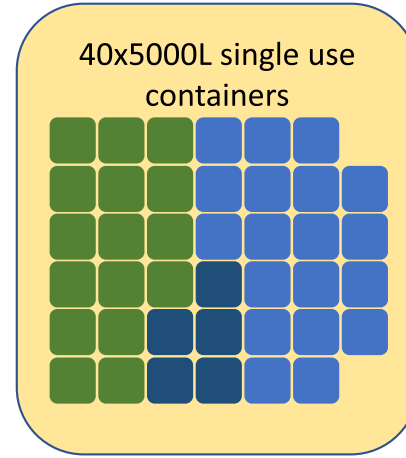
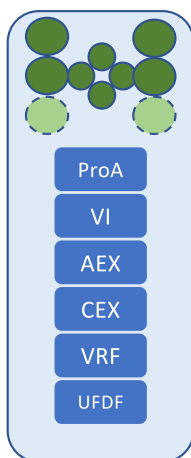
Bioreactors	N: 1x500L
Batch Size	1-30kg
Yearly Plant Capacity	0.5 tons
Media Volume per batch	5000L
Buffer Volume Per batch	12,000L
Water Required Per day	3,000L
Chromatography Skid Maximum Flowrate	0.6 L/min

(b)



Bioreactors	N: 4x500L
Batch Size	1-130kg
Yearly Plant Capacity	2 tons
Media Volume per batch	20,000L
Buffer Volume Per batch	50,000L
Water Required Per day	11,000
Chromatography Skid Maximum Flowrate	3.3 L/min

(c)



Bioreactors	N-1: 4x500 N: 4x2000L
Batch Size	1-500kg
Yearly Plant Capacity	8 tons
Media Volume per batch	80,000L
Buffer Volume Per batch	200,000L
Water Required Per day	50,000L
Chromatography Skid Maximum Flowrate	13L/min

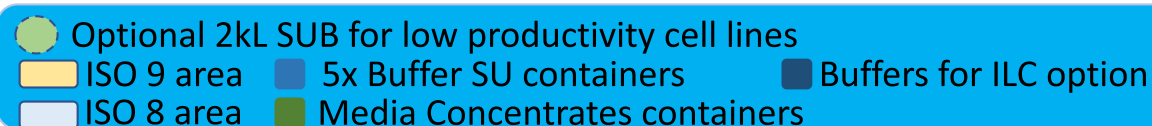


FIGURE 3 A stage-wise approach to implementing integrated and continuous bioprocessing (ICB). Using the same downstream equipment, an ICB plant can start with a small upstream such as the 500 L bioreactor shown here, and expand to as much as six 2000 L bioreactors. As the plant expands, the volume of media and solutions expands as well

the flow rate of the pumps on the capture step. The total amount of media and/or solution that can be prepared was arbitrarily set to 250,000 L, and the maximum flow rate of the chromatography skid was set to 13 L/min. The details around these assumptions are published in the accompanying article.

Stage one of the implementation uses a 500 L SUB. It is capable of producing as small as a 0.5 kg batch, and may be considered representative of a clinical trial manufacturing facility. For many products, 500 L could be the commercial scale ICB. The plant is capable of producing up to 30 kg batches or about 0.5 tons per year.

Some products, however, need higher levels of production. The framework plant can expand by adding three more 500 L bioreactors in Stage Two, as well as more media and solution preparation and storage containers shown in Figure 3b, and increased labor. One remarkable feature of the common ICB framework is that the downstream remains unchanged, as discussed in the accompanying article (Coffman et al., 2020). The “numbering up” of bioreactors does not change the regulatory filing. This Stage Two plant can produce up to 130 kg batches and 2 tons per year (Figure 3b).

The plant can expand further in Stage Three by adding more 500 L bioreactors, or using the existing 500 L as N-1 perfusion seed bioreactors to inoculate between one and six 2000 L SUBs, shown in Figure 3c.

More media and solution preparation and storage are also added, as is more labor. Moving a product from 500 L to 2000 L bioreactors has a moderate regulatory impact and requires agency notification and comparability studies but should require no new clinical studies. This plant can produce 500 kg batches in 20 days and up to 8 tons per year. The capacity is limited by the solution and media preparation and storage, and, to a lesser extent, the maximum flow rate of the pumps for the capture step (Coffman et al., 2020). The plant can operate either the 500 L or the 2000 L SUB as the N-stage production bioreactor to allow legacy production. The plant also supports legacy fed-batch production as well, with some restrictions on the harvest step.

As shown in Figure 3c, the 500 kg batch with four 2000 L bioreactors requires only 80,000 L of media concentrates, which can easily fit into 16 × 5000 L bags. This bag size is suggested because it is the largest single-use container available today. The 500 kg lot would require about 24 × 5000 L bags for the downstream, which is a manageable number. The downstream solution bags require refilling once during the run. Media and solution preparation and storage is therefore not a major consideration for the framework process in new facilities. It is a major consideration for retrofitting legacy facilities.

A bioreactor productivity of 2.5 g/L/day, which is more routinely achievable today, could use six bioreactors running in parallel and produce a 300 kg batch, or 5 tons per year. The total media and solution preparation remains the same, about 250,000 L, in a 20-day period. As opposed to the 500 kg batch, where four bioreactors were used, this operation requires more media preparation and less downstream solution preparation, thus balancing out the total volume prepared during the batch. The perfusion volume from the combined effluent from the six bioreactors is 12.5 L/min, well within the limits cited above.

3.1 | Effect of buffer preparation methods

Since solution preparation is limiting, technologies such as in-line conditioning (ILC) can help. If ILC is used on the chromatography and DF steps, the floor plan is much simpler. The solution PMI for downstream goes from 1400 to 400 by using buffer concentrates. ILC can reduce this further to 160 (Table 3). The 8-ton plant with ILC could look like Figure 3d. The media is contained in 16 × 5000 L SU bags; the concentrates for ILC are in 5 × 5000 L SU bags, which require a refill about twice during the run. The solution management is much simpler with ILC than with ILD. ILC can increase the capacity of the plant beyond 500 kg/batch and 8 tons per year.

Water production is also a consideration. The total amount of water used to make up and dilute the media and solution concentrates over the course of 20 days is 1 ML, or about 50,000 L per day. This volume is not large for large scale facilities. The largest rate of water production would be required for the initial media and solution preparation, which can easily be spread out over the duration of the run. Thus, the water consumption rate for media preparation is not a major factor in this 4 × 2000 L design for new facilities. It may be for retrofitting facilities.

3.2 | Economics

This review does not seek to evaluate the cost of the framework ICB. Some key concerns can be easily addressed.

The capital required for the framework ICB plant is relatively small. Jagschies correlation [Jagschies, 2020], based on installed bioreactor volume, estimates the cost of the plant to be about \$110–150 M. Using a 20-year depreciation schedule, the capital depreciation would contribute about \$0.50–0.8/g to the COGM.

Media costs are often a concern for perfusion-based processes. While they are most likely to vary from company to company, as an example, a media that costs \$20/L (Xu et al., 2017) would add \$3/g to the overall COGM. This cost seems manageable for most products.

As mentioned above, the 10-ton stainless-steel plant described by Kelley (2009) that produces 3–8 products per year on this scale would require an inventory of 4500 L to 12,000 L of Protein A, or about \$68–\$180 M. For the cost of the Protein A inventory, companies could have bought an entire ICB plant. ICB allows staged investment in capital, consumables, and labor to fit the needs of any product lifecycle.

3.3 | Insensitivity to bioreactor productivity

The plant productivity is relatively insensitive to bioreactor productivity when the bioreactor productivity is above 2.3 g/L/day, as shown in Figure 4. At this point, the plant becomes limited by solution preparation capacity, as well as the maximum flow rate of the capture step. With productivities greater than 2.3 g/L/day, the total

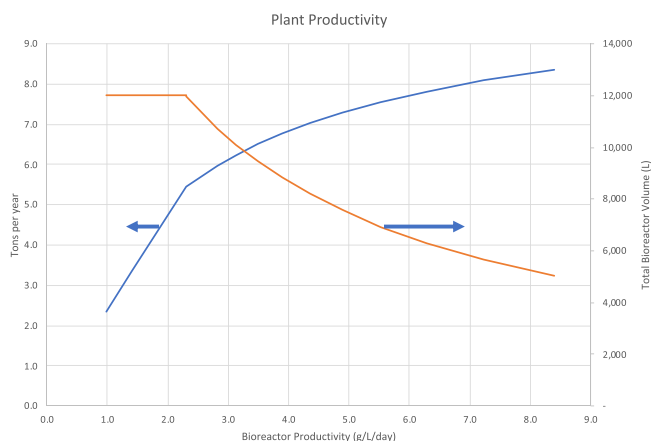


FIGURE 4 The productivity of a plant built on the common framework for integrated and continuous bioprocessing is relatively insensitive to the productivity of the bioreactor since the plant is limited by media and solution preparation, not bioreactor volume. As the productivity of the bioreactor decreases, more bioreactors are used to maintain the plant productivity

solution preparation volume cannot make enough media and solutions to supply the downstream, so less bioreactor volume is used. The space for the media preparation allows more solution preparation. The 4.8 g/L/day productivity used in the example allows for only four of the six bioreactors to be run. While leaving bioreactors empty might be counter intuitive, the cost of not running the bioreactor is much less than the cost of not fully using the downstream and solution preparation capacity of the plant. The flexibility of running more or fewer bioreactors allows the plant to be much less sensitive to the vagaries of cell culture development. As shown in Figure 4, the plant will produce between 5.5 tons per year and 8.4 tons per year over a cell productivity range of 2.3 g/L/day to 8.3 g/L/day, nearly a factor of four! In this range of productivities, the plant is near 100% utilization of all the equipment, except the bioreactors, which cost less than \$1 M each. The idling of \$1–2 M of capital is a small price to pay to enable the full utilization of the \$99 M of capital.

Most companies can achieve a bioreactor productivity of 2.3 g/L/day. This “entry-level” productivity allows huge increases in plant capacity for any network.

3.4 | Sustainability framework

The integrated continuous process can take advantage of intensification, single use and multicolumn chromatography to show the lowest process mass intensity for mAb production. The water intensity of typical batch processes for mAb production in stainless steel has PMI (or E-factor) values ranging between 3900 and 7250 depending on titer (Cataldo et al., 2020; Pollock et al., 2017). The continuous framework reduces PMI to values around 2000 (Table 3); this is corroborated by values reported by Pollock et al. (2017) that lie between approximately 2000 and 5000 depending on perfusion

productivities for similar continuous processes. This reduction is largely due to the increased capacity of the capture chromatography steps, the removal of the primary recovery operations and the use of SUBs.

Consumables PMI values have been reported to reduce from 6–73 to 8–25 as processes switch from the typical stainless steel batch process to integrated continuous bioprocesses employing SUBs for clinical and commercial manufacture (Pollock et al., 2017). This reduction was driven by the significant reduction in resin volume required that outweighed increases due to employing SUBs; ultimately the consumables PMI will depend on the degree of single-use adoption. The electrical energy consumption from HVAC is a dominating factor in the life cycle assessment of bioprocesses, consuming about 70 kWh/kg of product (Cataldo et al., 2020; Pietrzykowski et al., 2013). The energy consumption is reduced by the continuous framework by shrinking the footprint by 50%. The use of fully closed single use integrated processing can support the ball room classification grade reduction from C to D and provide an electrical energy reduction by 15%–20%. Further improvements to the PMI are expected with the reduction in plastics and packaging waste by improved reuse and recycling that will support a circular economy in the coming years (Barbaroux et al., 2020). A full environmental analysis of the common ICB framework is required.

4 | CONCLUSIONS

An 8-ton plant built on ICB and with single-use bioreactors is unprecedented. It rivals the largest fed-batch suites. While all the equipment necessary can be built from existing components, many improvements can be developed. The plant is backward compatible with fed-batch operations, although a process with 10 g/L titer would only produce about 1 ton per year.

The volume of bioreactor(s) that the common framework can support is limited by media and solution storage, and the maximum pump flow rate of the capture step. In these examples, the maximum flow rate was set to 13 L/min arbitrarily to yield a 500 kg batch. Downstream skids with maximum flow rates of 50 L/min are common in large stainless-steel plants. If such equipment were used, the downstream could support a total bioreactor volume of 48,000 L at 1.5 VVD, and yield lots of over 2.5 tons in twenty days. While no product today has such requirements, therapies for wide-spread diseases like Alzheimer's and COVID-19 may require massive amounts of material.

The impact of the manufacture of SARS-CoV-2 antibodies is an example of the need for of high productivity ICB. SARS-CoV-2 antibody manufacturing has caused a sudden shortage of large-scale capacity. In many cases, the existing capacity is not where it is needed. Government funding often requires local manufacturing. We may have to greatly and quickly expand the manufacturing capacity in many different countries to supply the world with lifesaving antibodies to SARS-CoV-2 (Kelley, 2020). We do not know which antibodies are effective. We do not know within four orders of

magnitude how many kilograms of material will be required. We could require ten tons, or we could require none. The uncertainty for material supply for treating COVID-19 exemplifies the uncertainty for many biologics in development.

The common framework for ICB allows a staged investment in new facilities and can grow with market demands, thus mitigating the risk against an uncertain future. The framework ICB allows expansion of the global biomanufacturing essentially at will, for relatively very little capital, in nearly any place in the world.

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CONFLICT OF INTERESTS

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AUTHOR CONTRIBUTIONS

Jonathan Coffman: Proposed the common framework, drafting and critical revision of manuscript. Jonathan Coffman, Mark Brower, and Joseph Shultz: Reviewed literature and designed the common framework. Lisa Connell-Crowley, Sevda Deldari, and Ujwal Patil: Reviewed literature, drafting and critical revision of downstream sections. David Pollard, and Steven Rose: Reviewed literature, drafting and critical revision of upstream sections. Brian Horowski: Drafting and critical revision of common framework process flow diagram. Suzanne S. Farid, Lisa Connell-Crowley, and David Pollard: Reviewed literature, drafting and critical revision of economic and sustainability sections. Maen Qadan and Eugene Schaefer: Reviewed literature, drafting, critical revision of the common framework. Jonathan Coffman, Mark Brower, Joseph Shultz, Lisa Connell-Crowley, Sevda Deldari, Ujwal Patil, David Pollard, Steven Rose, Brian Horowski, Suzanne S. Farid, Maen Qadan, and Eugene Schaefer: Final approval of the version to be published.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that does not issue DOIs.

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