Whitepaper

Shifting the Biomanufacturing Paradigm

Intensifying Upstream Processes

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The first commercial monoclonal antibody (mAb) was approved in 1986 and today, the global market is large and growing. Reports estimate the size of the market to be approximately $100 billion with expectations of it growing to more than $130 billion by 2023, a robust compound annual growth rate of more than 5.5%.[1,2]

The demand for mAbs and other antibody-based biologics continues to be fueled by a number of factors including:

- A growing elderly population experiencing age-related conditions including cancer, cardiovascular and respiratory diseases
- The opportunity for significant breakthroughs in cancer, diabetes, liver and kidney diseases which continue to have high mortality rates
- The availability of biosimilars and novel modalities including bispecifics and antibody-drug conjugates

While demand increases, so does pressure on the biopharmaceutical industry to accelerate development, improve flexibility and reduce costs all while sustaining or improving drug product quality. New, innovative approaches are needed to address these challenges.

In 2017, the BioPhorum Operations Group (BPOG), an industry consortium, published the Biomanufacturing Technology Roadmap in an effort to establish a dynamic and collaborative technology management process to accelerate change. The roadmap is intended to focus the effort of the biomanufacturing community and provide direction by identifying technology and/or manufacturing targets and prioritizing potential technological solutions.

Adoption of next generation processing and technologies can enable improvements in productivity by streamlining workflows, reducing the manufacturing plant footprint and increasing flexibility. And while the benefits of next generation processes are recognized, there are many options to consider and multiple pathways to success. We are developing technologies and approaches to intensify upstream and downstream processes, delivering both short term improvements as well as game-changing solutions to enable substantial gains over a longer timescale.

This whitepaper explores several upstream strategies to increase protein titers which can translate into higher throughput, improved flexibility, and compressed timelines. The topics to be covered are:

- Perfusion culture and media
- High cell density cryopreservation
- Modified amino acids
- Cell culture media compaction
- Buffer and media concentrates

Perfusion Culture

Perfusion involves the constant feeding of fresh media and removal of spent media and product while retaining high numbers of viable cells within the bioreactor vessel. This strategy has a long history in the industry.

Starting in the early 1990s, a number of commercial molecules were manufactured using perfusion processes. Most of these molecules are sensitive and their indication range is small, expressed in micrograms per kilogram and the cultures used adherent cell types.

Industry moved away from perfusion, however, as suspension cultures improved as did the yield of batch processes. In the mid 1990s, attainable cell concentrations using fed-batch processes were about 5×10^6 cells/mL, with product concentrations of 1–2 g/L. Today, attainable concentrations are greater than 15×10^6 cells/mL, with product concentrations

of up to 10 g/L. While these numbers are impressive, pressure to drive down manufacturing costs and the need to become more flexible are not necessarily compatible with building new multiple 20,000 liter fed-batch bioreactor facilities.

New perfusion-based, ultra high-density cell culture processes such as concentrated fed-batch and steady-state perfusion are well suited to new manufacturing environments and are now fostered by the increasing adoption of single-use technologies. Perfusion operation allows for high-density cell cultures in both the seed train and production bioreactors (**Figure 1**), and can result in a 3-fold increase of volumetric productivity.[3]

Implementation of perfusion-based approaches for upstream intensification can:

- Compress the seed train
- Maximize bioreactor utilization
- Maximize facility capacity and efficiency
- Reduce the size and number of bioreactors
- Reduce the overall upstream footprint
- Increase volumetric productivity
- Improve protein quality

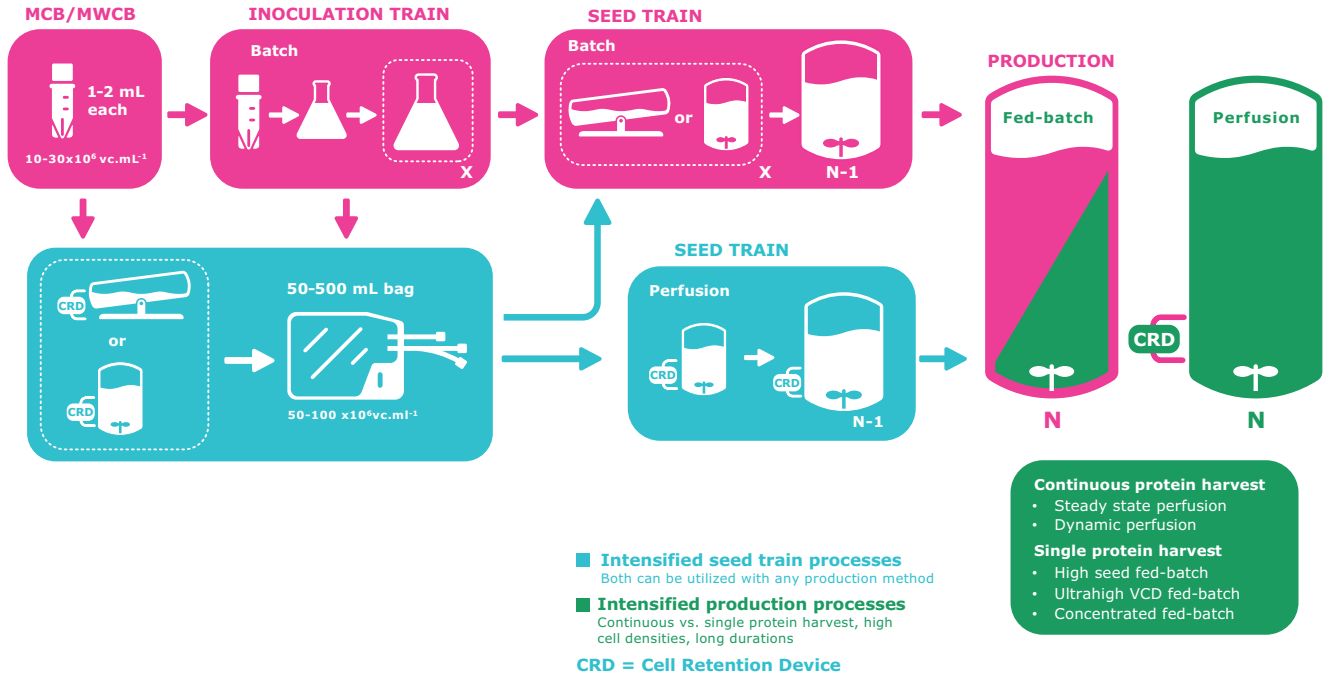

Figure 1. Perfusion can be used to increase the viable cell density or in the seed train to inoculate the production bioreactor with a higher cell density and in the production bioreactor.

Perfused bioreactors allow a better control of process conditions during seed train and production due to continuous exchange of culture medium and reduced overall residence time of proteins. These factors combine to improve the quality of proteins. Use of perfusion for intensified production can also provide cleaner harvests and facilitate the downstream process by reducing charge variants, glycosylation heterogeneity and protein aggregation.

Increased cell density in the N-1 bioreactor enables spiking of the production bioreactor with a higher quantity of cells and reduces the time needed to reach the desired level of protein production (**Figure 2**). Additional productivity gains and elimination of a traditional bottleneck can be realized as multiple production bioreactors can be inoculated from a single N-1 bioreactor operated in perfusion mode.

In production processes, capacity can be increased with a reduced footprint. For example, the same amount of protein can be produced in a 2,000 liter perfused bioreactor compared to a 15,000 liter fed-batch bioreactor. Additional benefits of perfusion in single-use bioreactors include elimination of cleaning validation, increased plant flexibility, and capacity utilization.

An additional benefit of perfusion is the ability to generate high cell density process intermediates which can compress timelines. Single-use 250 or 500 mL bags with high cell densities can be generated, frozen, stored, and used to inoculate upstream processes whenever required, increasing flexibility in manufacturing. This is a more streamlined approach compared to the time-consuming, cumbersome and risky process of transitioning from small vials and several steps in shake flasks, to the first bioreactor.

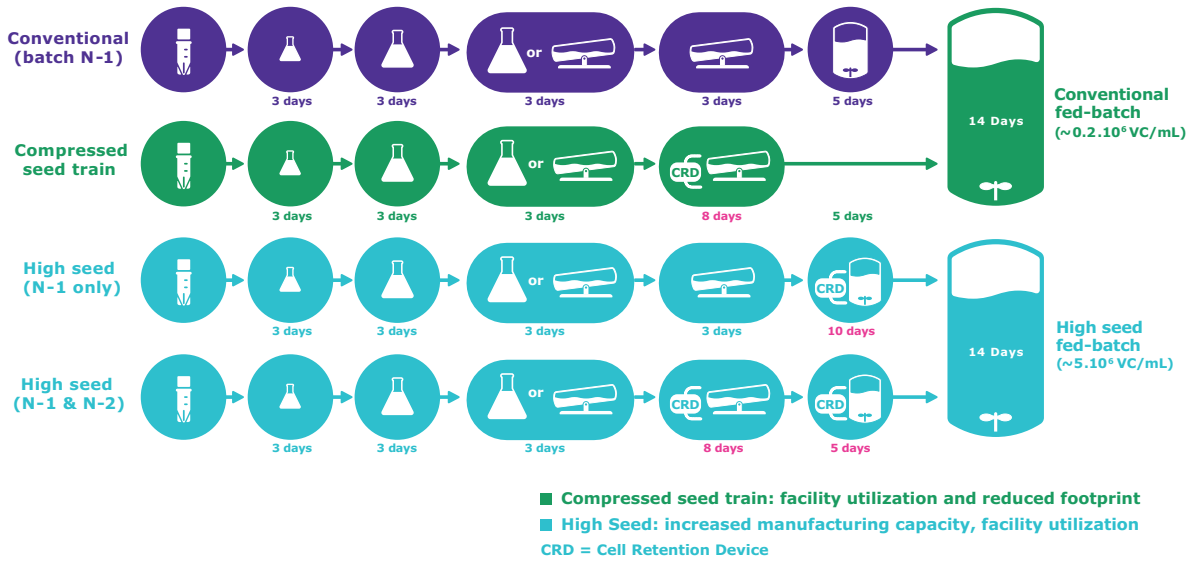

Figure 2. Perfusion can be used to dramatically increase the cell density of the N-1 bioreactor and accelerate the production process to achieve gains in efficiency. Introduction of perfusion culture within the seed train to increase cell density can reduce the number of bioreactors needed or the time required to reach the desired titer at harvest.

Perfusion Media

Media specifically designed for intensified perfusion reduce costs and may increase volumetric productivity.[4] To support an intensified process, the medium must be able to sustain high cell densities (≥ 50 million vc/mL) and maximize specific productivity while using the lowest possible perfusion rates.

When compared to fed-batch media optimized for perfusion, perfusion-specific media support a reduced time to steady state and an increased volumetric productivity while achieving a 40% reduction in medium usage (**Figure 3**).

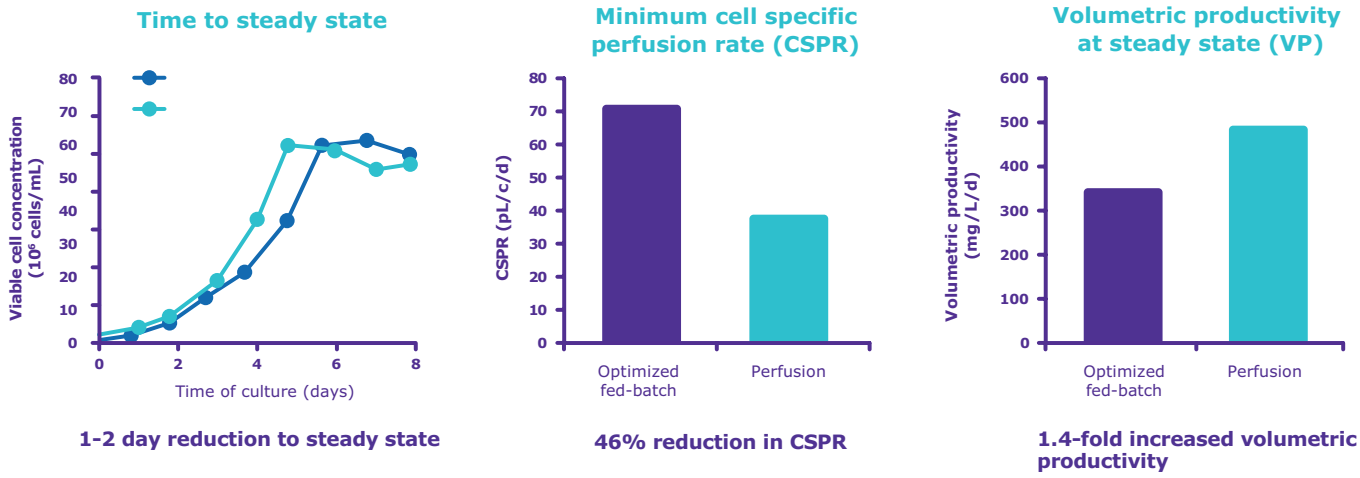

Figure 3. Evaluation of optimized fed-batch versus perfusion media. Perfusion media supports reduced time to steady state and 1.4-increased volumetric productivity with a >40% reduction in medium usage.

Why Intensification?

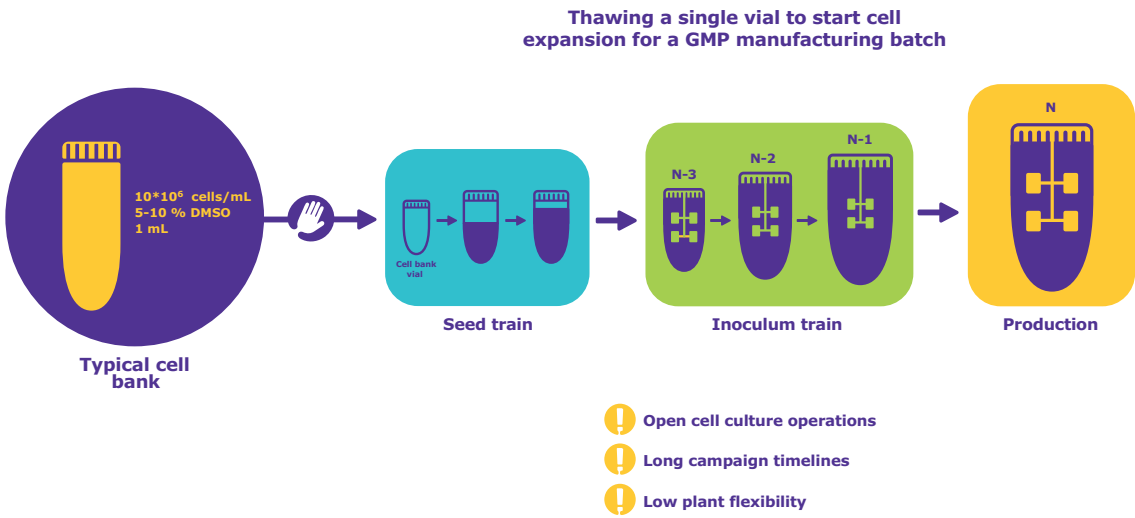

Figure 4. Thawing a single vial of cells to start expansion is a time-consuming process.

The traditional process of thawing a single vial of cells to initiate cell expansion for a GMP manufacturing batch is time-consuming and requires open cell culture operations (**Figure 4**).

Use of a high cell density cryopreserved cell bank which feeds into the first seed train bioreactor can streamline the overall process.[5] Traditional processes typically require 20-30 days in the seed expansion stage before inoculation of the production bioreactor. In the example shown in **Table 1**, a 150 mL bag of 50x10⁶ cells/mL reduced the standard process by ten days as compared to starting with a 1 mL vial of 10x10⁶ cells/mL. This time-saving is achieved by starting the expansion in the N-3 bioreactor.

	10⁶ cells/mL
Inoculation VCD	0.5
VCD - End of batch	6

	Standard vial	Bag
VCD [10⁶ cells/mL]	10	50
Volume [mL]	1	150
Cell count [10⁶ cells/mL]	10	7500

		Volume [L]	Cell count for inoculation 10^6 VC	Run time (without lag-phase) [d]	
Bioreactors	n (production bioreactor)	15000	7500000	3.5	
	n-1	1250	625000	6.9	
	n-2	105	52083	10.4	
	n-3	8.7	4340	13.8	10.4 days
	n-4	0.72	361	17.3	
	n-5	0.06	30	20.7	
	n-6	0.005	2.5	24.2	

Table 1. Inoculation with vial versus bag. Use of high cell density cryopreserved cells significantly reduced the process timeline.

Specially-designed cryo bags eliminate open cell culture operation steps, lead to better reproducibility in seed train expansion, and decouple cell expansion and batch production, allowing for global distribution of cells to production facilities from a central expansion facility.

A specially-designed medium (currently in development at MilliporeSigma) is also necessary for high cell density cryopreservation. Key requirements include:

- No adaptation from cryopreservation to perfusion media needed
- No cell damage during freezing and thawing
- Fast growth with minimum or zero lag phase after thawing
- A constant growth rate and specific productivity throughout expansion and production

Modified Amino Acids

Fed-batch culture approaches typically rely on concentrated feeds to prevent nutrient depletion, extending culture duration and improving cell growth, viability, and protein titer.

A neutral pH feed is desirable because culture pH should remain stable after feedings. The extremely low solubility of L-tyrosine and the low stability of L-cysteine at neutral pH have necessitated processes that leverage a slight acidic main feed and a separate alkaline feed containing these essential amino acids. Use of separate feeds at different pH levels creates the need for complex control strategies to minimize pH spikes during feed additions. In bioreactors, the alkaline feed can be added slowly while pH is monitored continuously and proportional-integral-derivative (PID) settings are adjusted to reduce CO_2 sparging response to spikes in pH. In large-scale manufacturing, even with slow addition of feeds, precipitation is likely to occur after contact with neutral pH media, especially when residual foam is present.

To address this challenge, we have developed a simplified approach to fed-batch processes in large-scale manufacturing using a highly soluble phosphotyrosine disodium salt (PTyr) and a new derivative, S-sulfocysteine sodium salt (SSC), in neutral pH, highly concentrated feeds (**Figure 5**).

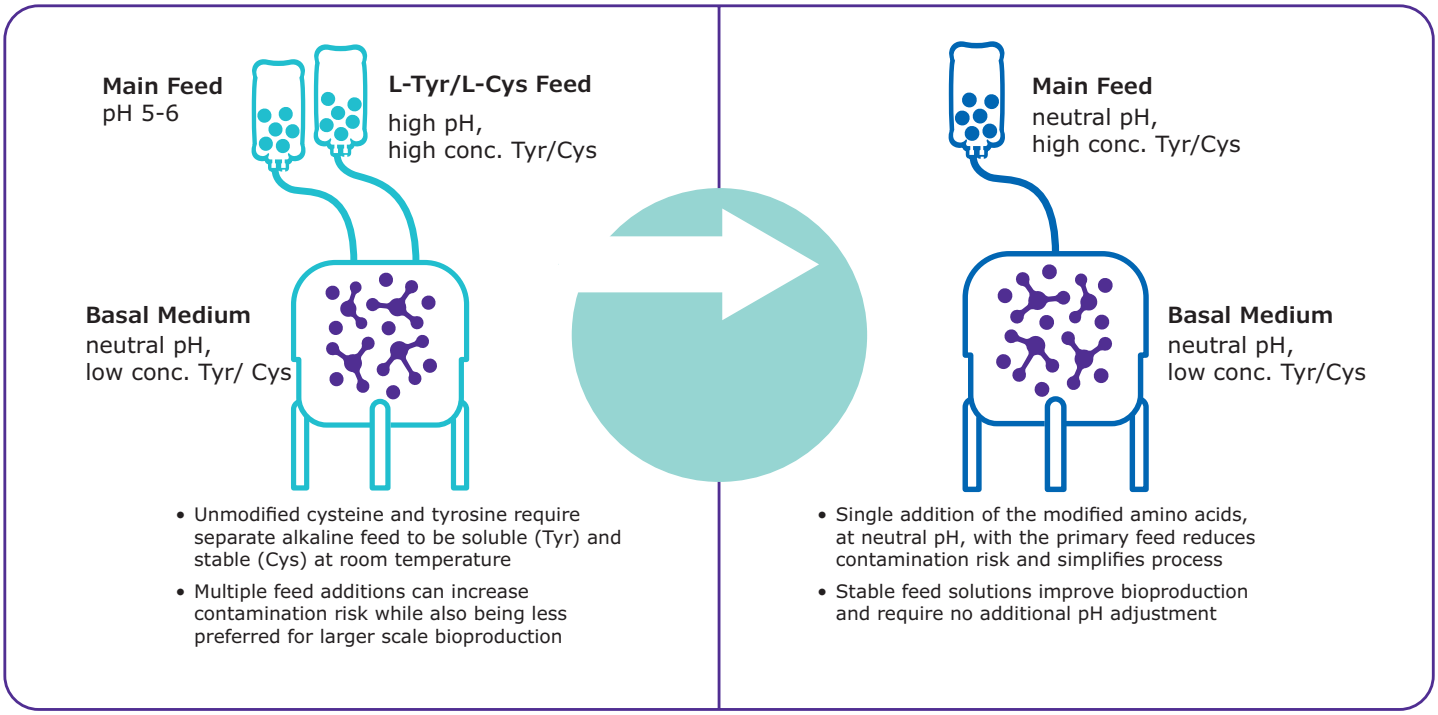

Figure 5. Process simplification with incorporation of modified amino acids into one neutral pH main feed.

Incorporation of these modified amino acids as components of chemically defined, neutral pH, highly concentrated feeds provides an alternative to the commonly used separate cys/tyr alkaline feed.

The advantage of this approach is that it can be incorporated into existing strategies to simplify fed-batch processes in large-scale manufacturing and improve overall process performance (**Figure 6**).

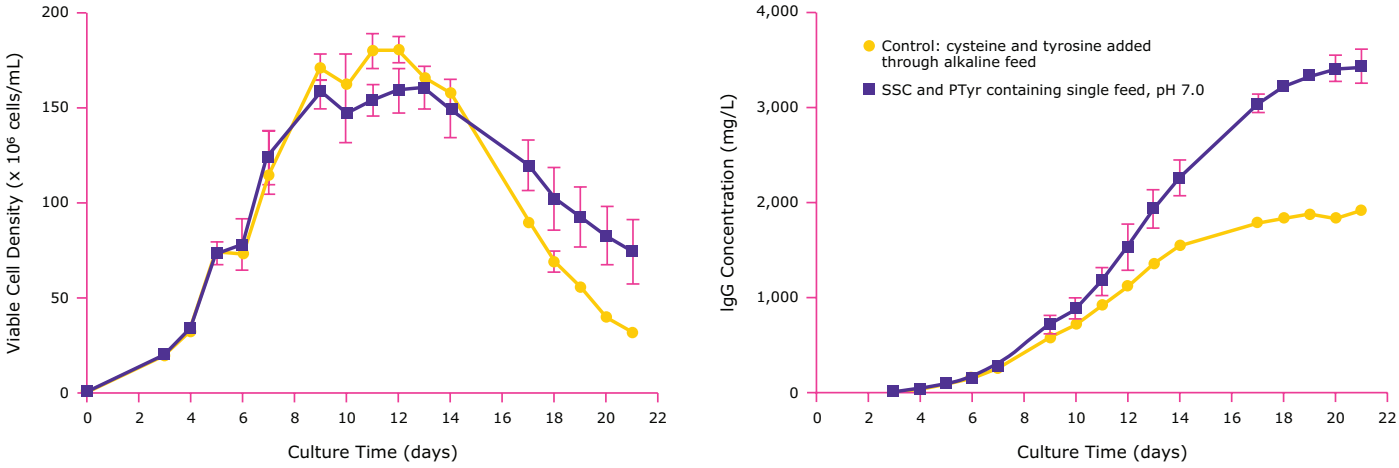

Figure 6. Comparison of culture duration and titer using a single-feed strategy with PTyr and SSC versus a separate cysteine/tyrosine alkaline feed (pH 11); data are represented as mean ±SEM with n = 4 replicates for each condition.

Cell Culture Media Compaction

The use of dry powder media presents a number of challenges including:

- Poor solubility / long solubilization times
- Dust formation and exposure of staff
- Caking which leads to inaccurate weighing and difficult preparation
- Low bulk density requiring extensive storage space and difficult handling

Compaction of dry powder media strongly improves dissolution kinetics and enables safer, convenient handling (**Figure 7**).

Our compaction technology is completely water- and additive-free and applies compression force to homogenous dry powder media, fixing homogeneity in place and creating granules several millimeters in size. The process does not alter the media's amino acid or vitamin composition, and leaves intact the physicochemical parameters of dissolved media and feeds, preserving the media's ability to support cell growth and productivity.

Vertical lines indicate time point of visual dissolution of all particles

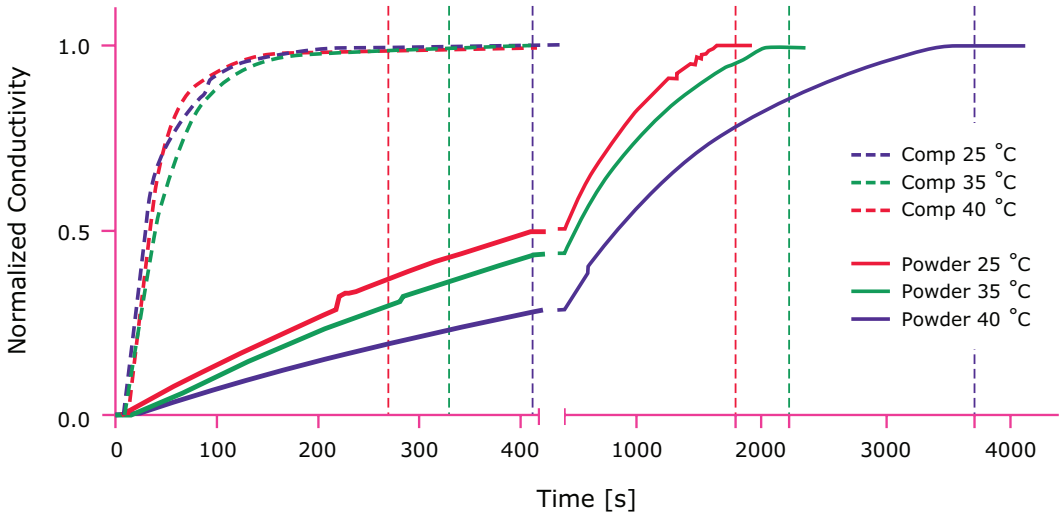

Figure 7. Dissolution kinetics of Cellvento® 4Feed compacted media versus powder. Dissolution of powder at 40 °C required 4.4-fold longer compared to compacted feed at 25 °C.

In addition to accelerated dissolution, benefits of compacted cell culture media include:

- Minimized dust formation for improved handling and operator safety
- Less caking for better flowability
- Easier handling during dosage and mixing
- Reduced bulk volume requiring less storage space

Results of a customer evaluation of our compacted media are shown in **Figure 8**. Typically, a 1x media preparation required 11 hours which included a 5-7 hour dry powder media dissolution time plus sterilizing grade filtration and/or virus filtration. A total of 150 kg of compacted media was hydrated in 2500 liters of water in less than 50 minutes, representing an 85-90% time saving.

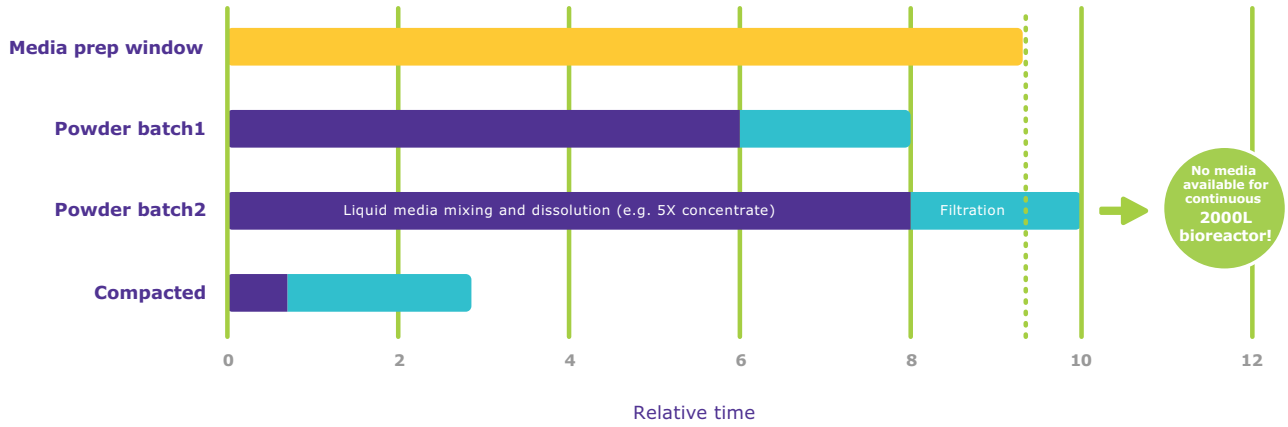

Figure 8. Significant time savings were achieved with use of compacted media.

Buffer and Media Concentrates

A 2,000 liter bioreactor operating at 5 g/L has an approximate downstream buffer demand of more than 10,000 liters. The time and space required to produce, store, and then distribute this quantity of buffer can be significant, and so the use of buffer concentrates provides the opportunity to streamline the process, significantly reduces the facility footprint, and enables greater flexibility when switching over a production line. Current approaches to dilute these concentrates include in-line conditioning and in-line dilution.

With in-line conditioning, a set of stock solutions consisting of at least one acid, one base, and one salt, all in concentrated form, is used to prepare most of the necessary downstream buffers (**Figure 9**). Blending different amounts and combinations of these stock solutions with WFI and adjusting pH and conductivity, allows most, if not all, downstream buffers to be formulated.

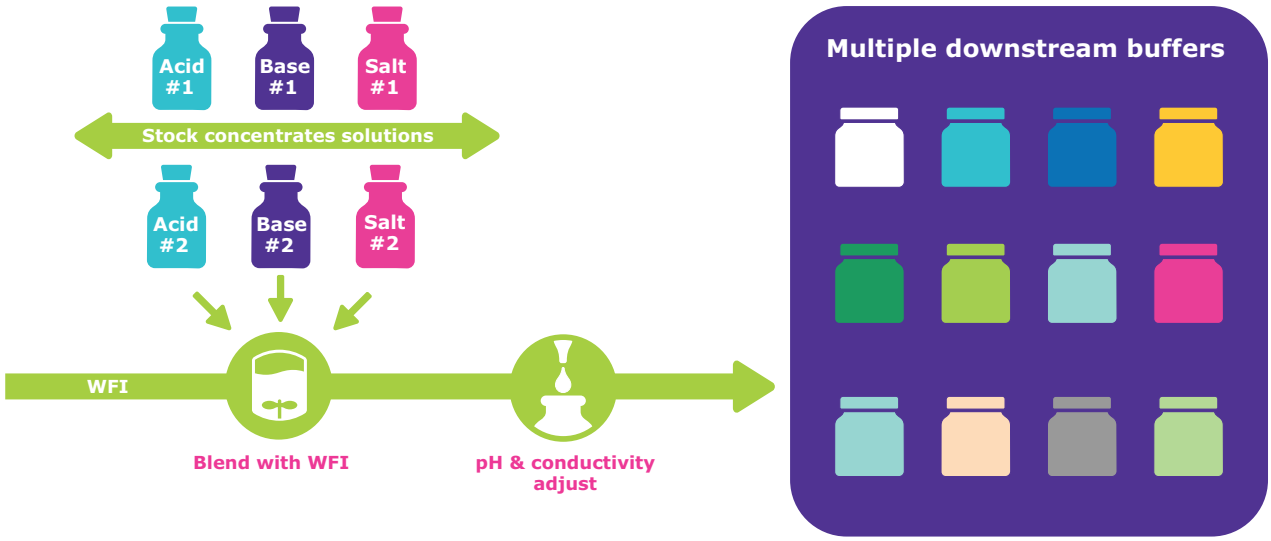

Figure 9. In-line conditioning for multi-component buffers.

With in-line dilution, a concentrated version of each final buffer is created (**Figure 10**). As needed, each concentrate is diluted and minor pH and conductivity adjustments made, if required. This approach might represent a relatively easier and less complex entry into use of concentrates. It does however, require a larger storage footprint compared to in-line conditioning as at least twelve starting concentrate solutions would likely be required to support the entire downstream buffer requirement, as compared to six or seven concentrated stock solutions.

Concentrates of cell culture media can also be used to reduce operational footprint. With media, the concentrate level is more typically 3-4x versus potentially 30x for buffer due to the complexity and stability of some media components. Even at 4x concentration, however, significant space and resource savings can be realized, especially when bioreactors are run in perfusion mode. A 2,000 liter bioreactor running at two bioreactor volumes per day for sixty days will consume nearly a quarter million liters of media; a 4x concentrated media would represent 70,000 liters, a volume that would require significantly less footprint and resource to manage.

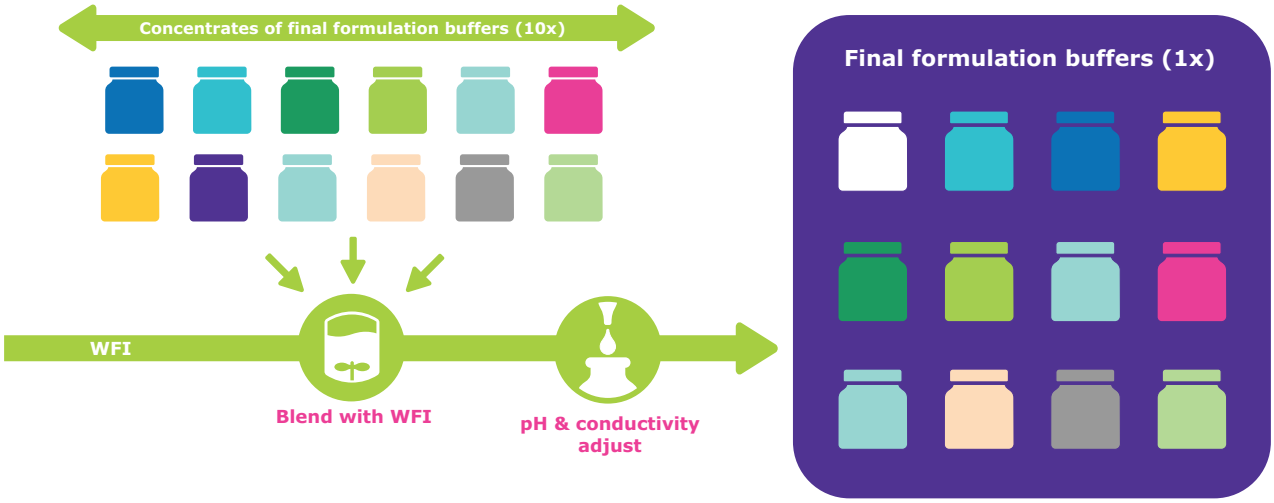

Figure 10. In-line dilution of multi-component buffers.

Conclusion

The biopharmaceutical industry is leveraging next generation approaches to deliver greater productivity, increase agility and reduce costs, all of which are essential to continued growth and innovation. Among these approaches are intensified upstream processes which can increase mAb productivity, shrink the manufacturing footprint, improve facility utilization, and accelerate timelines. And while use of perfusion culture can deliver higher quality protein and help facilitate downstream processes, upstream intensification and the resulting higher titers can lead to downstream bottlenecks if similar advances are not strategically applied across the workflow.

Downstream intensification technologies such as the use of buffer concentrates, single-pass tangential flow filtration (SPTFF) and flow-through polishing are being used to address this challenge.

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