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INTENSIFIED BIOPROCESSES

UNITING PRODUCTIVITY AND SUSTAINABILITY

Brian Gazaille, with Miriam Monge

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Intensified Bioprocesses

Uniting Productivity and Sustainability

by Brian Gazaille, with Miriam Monge

Biopharmaceutical companies gradually are exploring options for process intensification (PI) and continuous manufacturing. Such strategies are known to increase process economics and decrease equipment footprints, both of which help to reduce cost of goods and capital expenditure. But as this eBook shows, most companies have yet to consider another critical driver for PI adoption: sustainability. Herein, BPI's managing editor speaks with editorial advisor Miriam Monge about how intensification strategies can reduce water use and minimize equipment footprints for biomanufacturing operations, driving down production costs while alleviating negative environmental impacts.

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Although complete connection of upstream and downstream biomanufacturing processes remains an elusive prospect for much of the biopharmaceutical industry, companies generally acknowledge the advantages of intensified and continuous operations. In May 2023, BPI editorial advisor Miriam Monge (head of single-use technology implementation at Sartorius) described *process intensification* (PI) to me as a holistic framework for maximizing productivity in biomanufacturing processes and facility outputs. Many approaches are possible within that scheme. Biomanufacturers can leverage advanced technologies to enhance particular unit operations. For instance, using high-density cell culture can increase seed-train productivity. Alternatively, connections can be established across two or more unit operations. By implementing such strategies, companies hope to increase process economics and decrease the facility footprint of manufacturing equipment, both of which help to reduce cost of goods sold (CoGS) and capital expenditure (CapEx).

Such benefits are compelling, but as Monge explained to me, the biopharmaceutical industry should consider another critical but seldom-discussed reason to adopt PI: sustainability. Drug makers increasingly are launching ambitious environmental initiatives (**1**). Such plans indeed represent a step in the right direction regarding stewardship for our planet. However, companies generally have yet to associate PI and continuous manufacturing (CM) with sustainability despite the concepts' shared goals of reducing resource consumption and minimizing waste.

Thus, Monge and her colleagues have begun making a case for treating sustainability as a key business driver for bioprocess intensification. The Sartorius team published a white paper on the subject in February 2023 (**2**). The following May, Monge spoke about the environmental advantages of intensified processes at the 2023 BPI Europe Conference and Exhibition (Amsterdam, Netherlands) (**3**). I spoke with her shortly after BPI Europe to identify environmental "pain points" in biomanufacturing operations and to explore how intensification strategies could reduce such impacts. Monge also described the state of PI and CM in the industry, calling attention to trends in uptake, examples of successful implementation, and current gaps in sustainability data. Having more information at the ready, Monge emphasized, will empower more companies to adopt intensification strategies that increase the cost-effectiveness of biomanufacturing while decreasing its environmental costs.

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Editorial advisor Miriam Monge presents at the 2023 BPI Europe event in Amsterdam. To find the presentation and other resources for process intensification, visit https://srtrs.info/keynote-bpi-202306. (PHOTO CREDIT: SARTORIUS)

Reckoning Environmental Impacts

What aspects of traditional, batch-mode biopharmaceutical manufacturing exact the largest environmental costs?

Typically, the largest impacts come from the water and chemicals that are required to clean and steam stainless-steel equipment. About 70–80% of the water consumed in a traditional biomanufacturing facility with multiuse equipment goes toward

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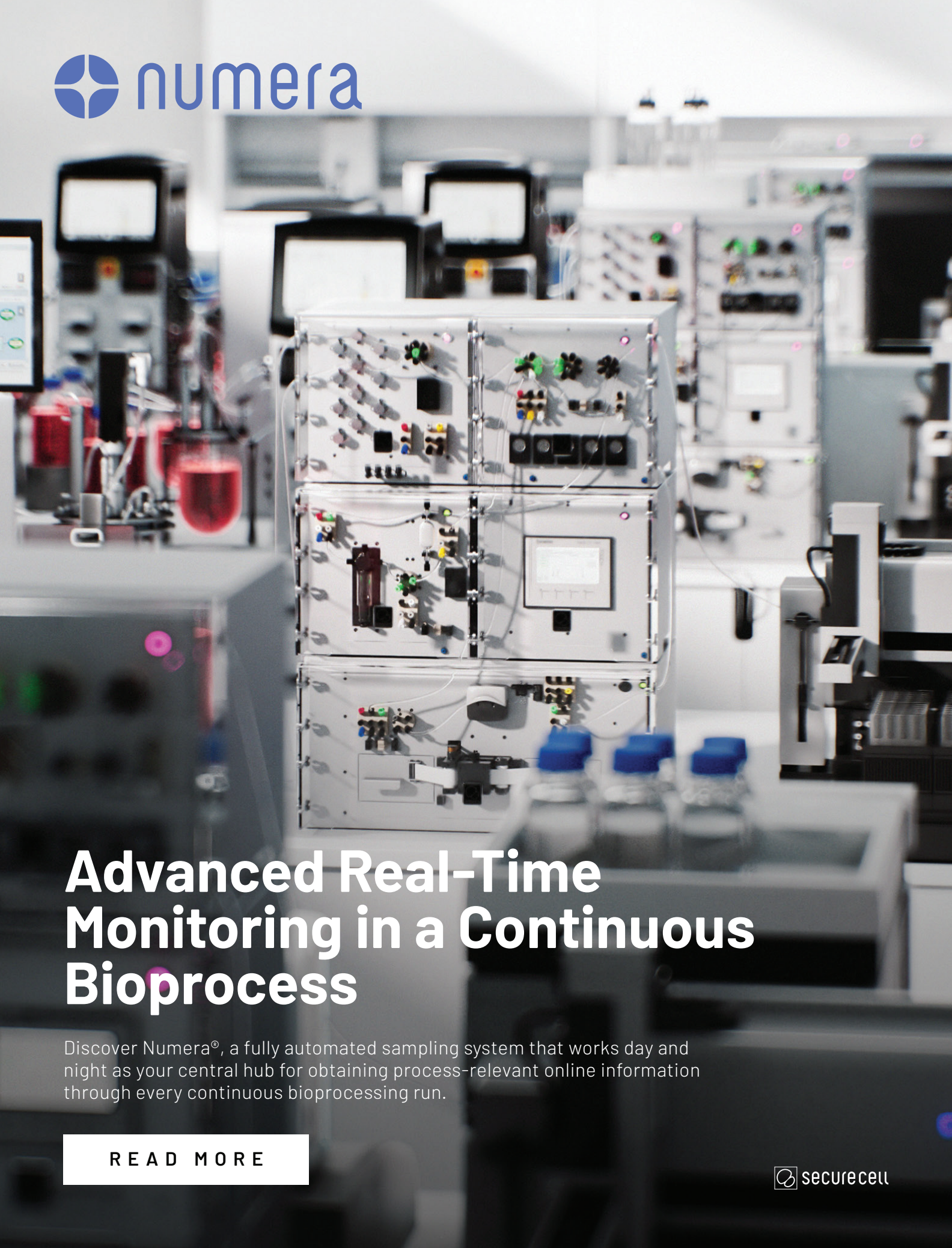
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those purposes, although the exact amount depends on the scale and type of manufacturing performed at a given facility. Of course, electricity is required to generate pure water for cleaning and steaming, so additional impacts come from energy generation and consumption. Then, you need to manage effluents and chemicals from the cleaning procedures.

Facility space represents another major contributor to the environmental impacts of a given manufacturing process. Compared with facilities based on single-use technologies (SUTs), manufacturing plants with stainless-steel equipment require larger cleanrooms, which puts larger demand on heating, ventilation, and air conditioning (HVAC) systems. As Kristi Budzinski et al. highlighted in a 2022 article about life-cycle analyses (LCAs) of single-use bioprocess equipment, HVAC for cleanroom filtration is the single biggest contributor to energy consumption in a biomanufacturing facility (**4**).

Anything you can do to reduce the amount of equipment — and thus the equipment footprint — requiring cleanroom protection helps to decrease a facility's environmental impact. So by intensifying a seed train, for example, you can eliminate a process step, which immediately reduces your cleanroom footprint. Also, your overall facility footprint can be reduced by moving away from stainless steel because you can considerably downsize utility systems.

In terms of manufacturing processes, purification steps create the largest environmental impacts. Chromatography is easily the most water-intensive aspect of biomanufacturing (**5**) (see the "Gauging Impacts" box). Again, anything that can be done to reduce water use is of critical importance. Here, I should note the distinction between water-use requirements for cleaning/steaming of stainless-steel equipment and for chromatographic purification. In our calculations, we consider cleaning equipment to be peripheral to the systems that produce and purify biological material. Given that distinction, purification processes use more water than any other process step.

More generally, traditional batch manufacturing involves sequential processing: Operators need to wait for one process step to finish before starting the next one. That is not an optimal way to work — hence the need for PI. Companies can move from a strictly sequential approach to a staggered set of processes or even a concurrent approach in which several operations are performed in parallel. During my BPI Europe presentation, I gave an example of a downstream process that originally required five days but that could be performed in less than two days and with the same output when using a concurrent approach. It is rather like lean manufacturing in that you think about doing some activities in parallel to decrease waiting time for the next parts of the process.

What metrics do biopharmaceutical companies use to evaluate the sustainability of their processes? My BPI Europe presentation focused on *process mass intensity* (PMI) *analysis*, which calculates the potential environmental impact of a

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—M. Monge

Gauging Impacts from MAb Manufacturing

In 2019, Kristi Budzinski et al. published "Introduction of a Process Mass Intensity Metric for Biologics," an article that has spearheaded ongoing investigations into the environmental impacts of biomanufacturing (**5**). Applying the concept of *process mass intensity* (PMI) devised by the American Chemical Society (ACS) Green Chemistry Institute Pharmaceutical Roundtable to assess production processes for small-molecule drugs, the team analyzed data from six large pharmaceutical companies to determine how many kilograms of water, raw materials, and consumables are needed on average to produce a kilogram of monoclonal antibody (MAb) drug substance.

In addition to calculating an average PMI (7,700 kg/kg), the team found that >90% of mass input stems from water consumption — primarily during chromatography. Below are percentages indicating an activity's share of total water use during biomanufacturing, excepting water required for cleaning/steaming:

- chromatography — 62%
- cell culture — 18%
- ultrafiltration/diafiltration — 9%
- centrifugation — 7%
- viral filtration — 2%
- fill–finish — 1%
- viral inactivation — 0.7%
- filtration — 0.3%

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particular biomanufacturing process based on the amount of resources — raw materials, consumables, water, electricity, and so on — that are used to produce a kilogram of biological product (**5**). My company uses BioSolve Process software from Biopharm Services UK to perform such analyses, and the program can feed our PMI data (e.g., about electricity consumption, HVAC use, and carbon-dioxide emissions) into models for our product LCAs.

I should note that PMI studies measure environmental impacts for a given process within a given facility. LCAs, on the other hand, are designed to examine the creation and postuse fates of materials involved in manufacturing a given product. Many organizations around the industry are performing LCAs (**6–10**). But some difficulties remain with conducting them. For instance, companies cannot accurately account for stainless-steel beginning- and end-of-life impacts because the industry has little if any information about what happens to stainless-steel tanks after they are dismantled.

Connecting Intensification and Sustainability

What PI strategies are possible in today's biopharmaceutical industry? We have many examples of how intensification helps to optimize processes. Several strategies are possible, and many technologies have been introduced. For instance, my company's BioSMB multicolumn chromatography (MCC) system enables users to decrease downstream buffer and resin use by 30% and 70%, respectively, while accelerating processing times. Overall, such technologies increase facility productivity significantly in terms of both time and materials. Because water use represents a major contributor to PMI, reducing buffer requirements helps to increase process sustainability.

Considering the many productivity and sustainability advantages that can come with intensified processing, it surprises me that biopharmaceutical companies have not moved more quickly in that direction. At the end of my presentation, one attendee pointed out that many biotherapeutics are still working their way through the development pipeline. Some of those products will move into their companies' existing facilities for commercial manufacturing, which usually are set up with stainless-steel equipment. Thus, the attendee explained, companies have opportunities to reimagine their workflows and incorporate intensified/fully continuous processes only when they are constructing facilities or undertaking significant renovations to existing plants.

Different levels of intensification are possible for both up- and downstream processes depending on needs for annual product output (in kilograms) (Figure 1). In my presentation, I highlighted some strategies for PI of upstream operations (Figure 2) (**3, 6**). Traditional fed-batch culture represents a benchmark. Beyond that, companies can choose to intensify one unit operation, connect two unit operations, implement semicontinuous processes, or move into fully continuous operations. One specific method is seed-train intensification ($N - 1$). Companies also can perform PI for a main

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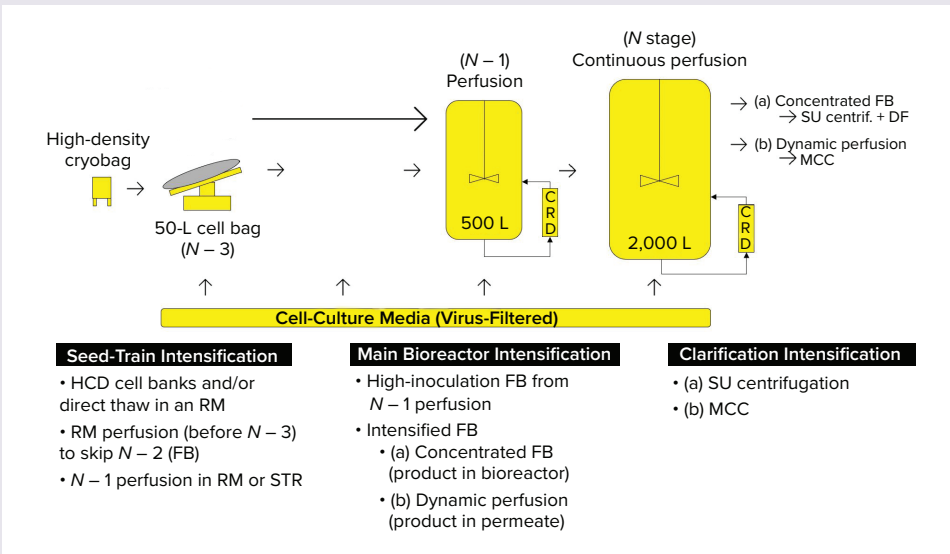
bioreactor. That can take the form of concentrated fed-batch production, during which product proteins remain in the bioreactor and are harvested directly after processing, or dynamic perfusion culture, during which they are harvested daily from the permeate.

In terms of scale and total output, perfusion-based production requires much less facility space than does a traditional batch process performed in stainless steel. I find that feature to be particularly interesting. For instance, I know that a Sanofi site replaced its large-scale stainless-steel bioreactors with perfusion-capable single-use bioreactors that were smaller by several orders of magnitude (**11**). The company made that change for commercial manufacture of its Fabrazyme (agalsidase beta) enzyme-replacement therapy for patients with Fabry disease. Again, downsizing a manufacturing process can make a significant impact on a facility's footprint and, thus, on its energy and resource consumption. I also know that Enzene Biosciences Ltd., a biologics contract development and manufacturing organization (CDMO) from India, has a 1,800-ft^2 good manufacturing practice (GMP) manufacturing suite that can produce 3–5 kg of monoclonal antibodies (MAbs) per month at 200-L scale using its proprietary, commercially validated EnzeneX technology for fully continuous manufacturing (**12**).

Figure 1: A preengineered, off-the-shelf intensified upstream process of 200-L scale (HTTPS://WWW.SARTORIUS.COM)

Figure 2: Options for upstream process intensification, with *N* representing the production bioreactor (**3, 6**); CRD = cell-retention device, DF = diafiltration, FB = fed batch, HCD = high culture density, MCC = multicolumn chromatography, RM = rocking-motion bioreactor, STR = stirred-tank bioreactor, SU = single use



What factors should biopharmaceutical companies consider when evaluating PI methods? Developing a PI strategy requires careful analysis, with multiple scenarios to consider linked to drug demand, molecule type, and existing process infrastructure (Figure 3). Seed-train intensification is the most common PI strategy in the industry because it can be implemented easily into an existing stainless-steel facility. But if your company is establishing a new site, then you have an opportunity to install capabilities for dynamic perfusion culture and/or continuous processes, which would need completely different manufacturing

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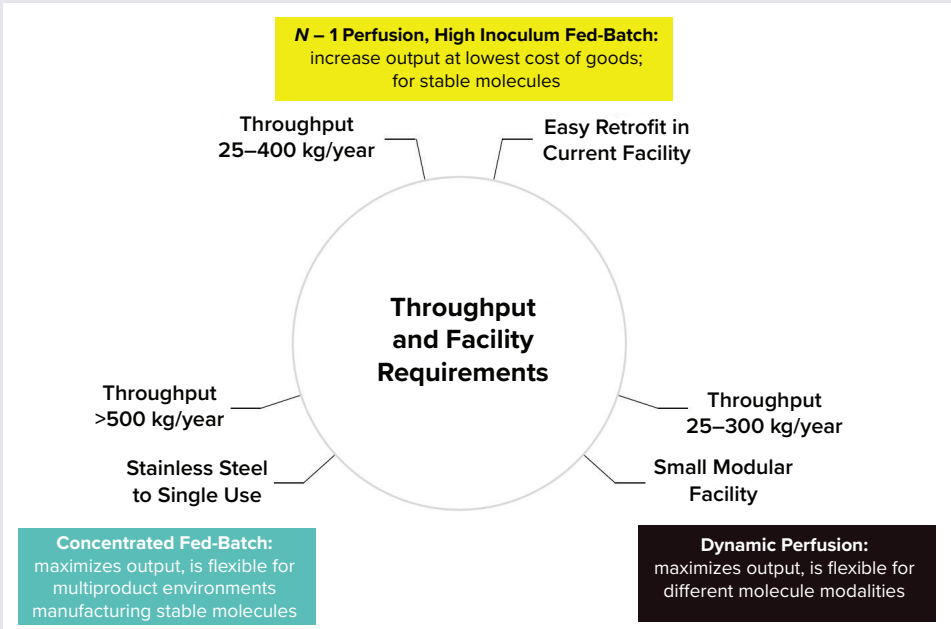
setups than what are used for traditional batch processing.

Which level of PI to pursue also depends on the characteristics of the biotherapeutic being made — e.g., its stability during manufacturing. Concentrated fed-batch culture can be a great alternative to traditional batch culture in massive stainless-steel tanks. In 2014, Amgen leveraged licensing agreements with DSM Pharmaceutical Products to establish capabilities for high-density cell culture at a new production facility in Singapore. The company reduced its facility footprint significantly while maintaining the same level of output as one of its stainless-steel facilities. Some years later, Amgen replicated that strategy and its success at a new site in West Greenwich, RI (**13**).

Figure 3: Facility and throughput requirements are critical criteria when deciding which intensification strategy to pursue.



Dynamic perfusion can be a good alternative, particularly for unstable proteins. That factor was part of Sanofi's rationale for perfusion-based production of its Fabrazyme drug. The product was relatively unstable, and perfusion turned out to be suitable for its cultivation. Overall, the company has been so impressed with the productivity of its perfusion process that it is cloning the operation and investigating its utility for other biologics.

As I mentioned in my presentation, to move into intensified and even fully continuous upstream processes, companies need robust, stable cell lines with quality profiles that are suitable for perfusion-based production. Culture media must be adapted to support long culture durations and high cell densities. Media pricing can be a limiting factor for some companies because dynamic perfusion and fully continuous operations require much more media than do fed-batch processes. The increased need for media also raises questions for handling and storage — e.g., whether to purchase media concentrates and systems for reconstitution. Intensified processes require companies to use highly scalable process equipment that support high culture densities. Consideration also should be given to process analytical technologies (PATs). Sensors for advanced process monitoring and control must be transferable across scales.

How often are biopharmaceutical companies pursuing such PI options? An exact figure is difficult to give, but I estimate that >20% of companies are considering seed-train intensification. That option remains highly popular among companies that are pursuing PI. Right now, movement to dynamic perfusion and fully

continuous operations usually requires an opportunity for installation of a completely new process. My colleagues and I anticipate that, by 2026, about 30% of processes in the industry will have undergone some level of PI. What level of intensification happens will depend on many factors such as the type and characteristics of the therapy being produced, the novelty of its manufacturing process, the requirements and limitations of the production facility, and the requisite format for installation (e.g., incorporation into an existing production facility or implementation at a new site).

In today's industry, the question of how much PI to undertake usually concerns how many unit operations can be connected, not necessarily how a fully continuous process would be implemented. There is much potential for innovation in this respect. Sartorius and other industry suppliers want to develop more single-use components that facilitate intensification. In some cases, we still are developing multiuse equipment but are designing such systems to have smaller facility footprints. For instance, the Resolute BioSC chromatography system (Sartorius) uses a modular skid, whereas traditional purification systems would require two to three skids to perform downstream processing steps. And significant opportunities remain in that area for reducing footprints, increasing productivity, and decreasing processing time.

As an industry, we have much to think about. Ideally, biomanufacturing processes would be completely connected and closed (and thus supported by SUTs). If a company achieves and validates such a process, then it can start thinking about reclassifying its cleanrooms and downgrading its HVAC systems. By and large, the industry continues to work on demonstrating fully closed and continuous operations. From a sustainability perspective, many advantages would come from using intensified and/or continuous operations to diminish equipment footprints, cleanroom requirements, and associated HVAC needs.

But the question of which PI strategies will be adopted most depends on the factors that I mentioned earlier: volume demand, product characteristics, and the like. Intensified seed trains and concentrated fed-batch processes might be more suitable than dynamic perfusion for large-scale production of "typical" recombinant proteins, for instance. Companies also need to consider whether they can transition from stainless-steel to single-use equipment. Most clinical-scale processes today are performed with SUTs, but much of the overall global biomanufacturing capacity for commercial-scale processes remains in stainless steel. Concentrated fed-batch in single-use bioreactors could provide companies with an alternative to stainless steel even if they have large kilogram demand, and adopting SUTs could help to establish more sustainable facilities, certainly in terms of water and energy use.

Some questions remain about plastic consumption, although the industry is studying how much plastic is used in a biomanufacturing facility for each batch of product (**8–10**). Thus far, the industry has

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found that its plastic use generates far less environmental impact than does water and energy consumption.

Gathering Information To Create Awareness

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What does the industry still need in order to evaluate PI and sustainability? Are the needs primarily technological?

Technology developers and biomanufacturers still are adapting downstream equipment to accommodate upstream PI scenarios, although recent advances in MCC have been quite compelling. And whereas the industry already has commercially available systems for dynamic perfusion, suitable solutions for concentrated fed-batch have been more difficult to develop for large scales.

More generally, I believe that the PMI data from my BPI Europe presentation (and my other presentations on the same topic) have generated interest in PI as a strategy for sustainability. I remember presenting some of those data to biopharmaceutical companies in 2022 and having attendees say that they hadn't considered PI from a sustainability perspective until then. Certainly, those people knew about PI's benefits for process economics and business, but they had not given much thought to positive environmental impacts from intensifying and/or connecting unit operations.

The industry is conducting considerable studies into sustainability right now, particularly LCAs. Organizations such as the US National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) and the BioPhorum Operations Group (BPOG) are performing some in-depth studies along those lines. I am concerned, however, that current studies might not be comparing "like with like." One problem involves accessing relevant data, which companies often are unwilling to share for reasons of intellectual property (IP). The industry needs all companies to collaborate, even with their competitors, to perform comprehensive LCAs.

Let's say that we want to compare the overall carbon emissions of a stainless-steel facility with those created by a single-use facility with the same manufacturing output. In my BPI Europe presentation, I gave examples of my company's modeling and simulations, but it would be extremely beneficial to compare such data with those from a real-world facility. The current question is whether we can compare like with like; right now, it is not obvious that we can.

Like Sanofi, WuXi Biologics has done considerable work to intensify processes (**14**). Within its network, the company can perform nearly every commercially available biomanufacturing methodology, including perfusion-based and concentrated fed-batch processes. Those processes have shown strong results in terms of process productivity, antibody titers, and other key metrics. Transcenta has had similar success with its own intensified processes (**15**). To my knowledge, though, those companies have yet to consider such processes specifically from a sustainability perspective. The industry needs to speak more with such companies to measure PI's potential impacts — e.g., how traditional and concentrated fed-batch processes compare in terms of PMI.

Fujifilm Diosynth Biotechnologies (FDB) has provided in-depth proof of concept for an integrated, fully continuous biomanufacturing facility based on its MaruX platform process (**16**). The proof-of-concept run was performed in a non–good manufacturing practice (GMP) environment at 500-L scale, which is considered suitable for much of clinical-stage manufacturing and potentially for commercial manufacturing. FDB expects to install the MaruX platform into GMP facilities (also at the 500-L scale) over the next couple of years. Facility output can be increased by multiplexing 500-L bioreactors to feed a single purification train or by scaling up to a larger bioreactor. When such facilities are online, it will be good to speak with FDB to compare PMI data from stainless-steel and MaruX-equipped facilities producing the same annual drug output. I am also interested to find out how the company implements its highly intensified platform process because it continues to leverage stainless-steel systems for commercial-scale biologic production. Clearly, one size does not fit all. Going forward, the primary question for contract manufacturers might be how to best meet the needs of a given client and therapeutic protein. Perhaps having traditional and intensified operations makes sense in that regard.

Especially in the wake of the COVID-19 pandemic, the biopharmaceutical industry is experiencing significant pressure to decrease prices for drug products and contract services. I believe that interest will surge in the fields of PI and continuous biomanufacturing, both from the cost and environmental perspectives. The more that companies can adopt such strategies — and, more generally, a lean approach to biomanufacturing — the more cost-effective and sustainable our operations will be.

Companies cannot change their business models overnight. They still have legacy stainless-steel systems — and now facilities and capabilities that they established during the pandemic. Often, substantial changes to biomanufacturing workflows must happen early in drug development; if you developed a product in a traditional fed-batch process, then it will be extremely difficult to adopt a different production method at later stages. Thus, companies typically begin with intensified seed trains and minor alterations during downstream purification.

Nevertheless, my colleagues and I believe that the biotechnology industry has major opportunities in the areas of PI and continuous biomanufacturing. That is especially true for new treatment modalities, which struggle from a cost perspective. The industry knows that cell and gene therapies (CGTs) can cost 10× more than products based on MAbs. PI could help to drive down costs for advanced therapies while realizing benefits for sustainability and economics. Water and electricity are operating expenses, after all.

What measures would help to increase uptake of intensified and continuous biomanufacturing processes? More industry awareness will help. That will come as Sanofi, WuXi Biologics, FDB, Transcenta, Enzene Biosciences, and other such

MORE DATA are needed to demonstrate the advantages of process intensification and continuous manufacturing — and to educate the industry about their implementation.

—M. Monge

companies continue to bring more intensified processes through to commercial manufacturing and begin to provide concrete data on both cost and environmental impact. For instance, Sartorius is collaborating with Enzene Biosciences to drive an end-to-end intensification process. The collaboration has proven most successful. We hope to publish data soon about that high-productivity process.

More data are needed to demonstrate the advantages of PI and CM — and to educate the industry about their implementation. Once the industry has that information, companies will begin rethinking their processes in terms of costs and overall productivity. I hope, though, that companies also will start thinking about the sustainability benefits of PI. Even current data are so compelling that the industry cannot ignore sustainability as a driver of PI activity. As I mentioned, legacy processes and facilities tend to be the major obstacles for our clients. For such customers, our discussions often focus on how to retrofit existing facilities for more productive capabilities. In some cases, companies decide to remove their stainless-steel systems and install new processes and equipment, but doing so requires a strong business case. More data can help companies to make the case for highly intensified processes and facilities.

Historically, the biopharmaceutical industry has been slow to adopt new technologies and processes. The pandemic has shown that the industry can move much more quickly than we realized. The same can be said for recent increases in the number of applications approved for "breakthrough therapy" designation. Such developments might be just the kind of change that industry needed to start implementing novel manufacturing methodologies more rapidly than it has, not just for MAbs, but also for emerging modalities. As I said, advanced therapies have major obstacles to overcome. Who will pay the extremely high prices assigned to such products? Until that issue gets sorted out, and until costs come down considerably, CGTs will encounter big challenges to uptake.

What else would you like to tell our readers about the interplay of PI and sustainability? I have tried in this interview and in my BPI Europe presentation to highlight a change in the industry. Sustainability, intensification, and other topics that had been considered "nice to have" are coming to the fore. I am interested to find out how our current overcapacity situation will influence the industry's trajectory, especially in terms of launching intensification and sustainability initiatives. I also want to monitor how drug prices will influence manufacturing strategies. I assume that the influence will be significant. But even though companies seem to have less money to spend on facility upgrades and changes (at least compared with what funds were available before and during the COVID pandemic), the industry needs to investigate PI and sustainability much more rapidly than ever before, especially considering the complexity of our current geopolitical situation.

Sustainability, intensification, and other topics that had been considered "nice to have" are coming **TO THE FORE.**
—M. Monge

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How an Integrated, Continuous, Intensified Approach for Manufacturing Biologics Provides Productivity and Quality Benefits

Lisa Connell-Crowley and Magnus Schroeder

The biopharmaceutical industry continues to need to facilitate faster, lower cost approaches for manufacturing biologics such as monoclonal antibodies (mAbs) for treating chronic and life-threatening conditions, as well as infectious diseases. Unfortunately, many biologics-based treatments are not accessible to patients worldwide due to issues including affordability. Alongside affordability there are also challenges which are hampering the speed of delivery of biologics-based drugs including long lead times for designing and building conventional manufacturing facilities, which can impact manufacturing costs. These drivers are fueling technology innovations to improve facility utilization for multi-product use and provide easy-to-install facilities for domestic manufacturing which could improve efficiency and increase yields. Increasing productivity from each production batch means fewer process runs often at smaller scale, all of which can reduce the cost of goods (CoGs) for producing biologics.

Traditionally, to increase drug substance/product mass biopharmaceutical companies and CDMOs have produced larger amounts of unpurified product by scaling their fed-batch cell culture processes to 10,000 L, 15,000 L, 20,000 L or 30,000 L stainless steel bioreactors using process transfer into increasingly larger vessels.

Scaling-up a process, for example, from a 2,000L to a 20,000L bioreactor along with the associated downstream processing step involves considerable risks, resources, and costs because not all unit operations scale-up linearly. This often means that multiple engineering runs are performed prior to Performance Qualification (PPQ) to de-risk the PPQ campaign. These engineering runs can be extremely expensive and time consuming. Additionally, working at larger scale requires the entire facility to be scaled with larger and often inflexible downstream unit operations to purify a large amount of product mass. This requires different skills sets and can even mean transferring the process to a new, larger facility, as well as training new staff.

In CDMOs or biopharmaceutical firms an alternative approach is to multiplex, or scale-out, the upstream process, with for example a "six pack" strategy using 6 x 2000 L SU bioreactors. Although scale-out has less scale-up risks, managing parallel production bioreactors creates complexity and downstream challenges remain due to the large amount of product mass that needs to be purified. Additionally, even though the capital expenditure might be lower, the high number of SU bioreactors required increases operating costs.

An alternative strategy which is increasingly gaining traction, is to use continuous manufacturing. We have developed an integrated, continuous bioprocessing platform, based on intensified perfusion cell culture, to provide a highly flexible and economic alternative to the previously described scale-up or scale-out approaches for increasing mass output of drug substance. With continuous processing the duration of the bioreactor perfusion, rather than bioreactor size (scale-up) or number (scale-out), is the principal variable which can be adjusted to increase mass output. Using our continuous processing platform means the mass output can be 'tuned' to precise requirements with only modest increases in bioreactor volume. The other major benefit is that the product is purified continuously so that equipment remains compact and flexible and covers a much wider dynamic range than traditional facilities, eliminating the need to scale up the process as the drug advances through clinical development to commercial launch. This approach avoids the traditionally accepted risks, as well as the additional cost and resources associated with, for example, having to scale up and transfer an early phase clinical scale process to a larger scale bioreactor in a different facility to support late phase clinical and commercial needs. This can help reduce the critical path timeline to clinic, as well as de-risk the path to market for biologics.

Integrated Continuous Manufacturing

We offer hybrid (Figure 1) or fully end-to-end (E2E) continuous platforms (Figure 1) to produce biologics including mAbs, Fc fusion proteins and bispecific antibodies. Both processes utilize intensified upstream cell culture with highly productive cell lines, continuous perfusion of cell culture media to support high cell density, and continuous product passage out of the bioreactor. Concentrated media is used to reduce production and storage costs.

For purification, an intensified downstream process is used where non-continuous steps including virus filtration for virus removal and diafiltration (DF) for product concentration and formulation are converted to continuous ones with high loading to purify the product as it is being expressed.

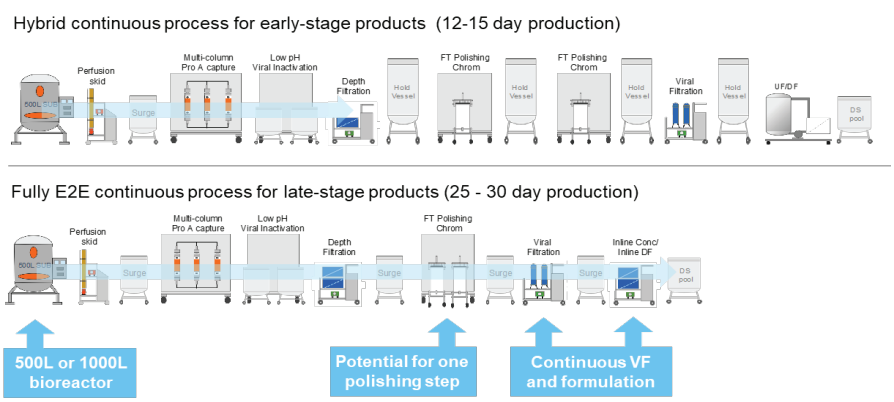

Figure 1: Hybrid-continuous platform for early-stage products (Phase I/II Clinical trials) (above) and fully end-to-end continuous platform for late-stage products (below).

We use intensified continuous process technology within our manufacturing facility, called J.POD®, which is part of our integrated J.Design service. J.POD utilizes modular clean room pods, and SU systems where we connect as many steps as possible to shrink the footprint of the unit operations, reduce buffer volumes, as well as limit or eliminate holding tanks. Reducing the process footprint saves the time and costs associated with larger equipment and consumables thus reducing capital expenses and transferring operating costs from fixed to variable. As J.POD provides relatively fast build and fit-out times it could easily be constructed in different regions to support lower manufacturing costs and faster development timelines of quality biologics from DNA sequence to drug substance/product.

Productivity Benefits

In our hybrid-continuous platform, by using intensified perfusion culture we have achieved up to 10-fold higher productivity with a continuous harvest bioreactor format compared to traditional fed-batch cell culture. This is because the added nutrients and continuous removal of waste products support significantly higher viable cell densities (80-110 X 106 cells /mL) compared with a fed-batch process which supports lower cell densities that are sustained for a shorter time until the culture viability declines. The higher peak cell density for prolonged periods at high viabilities results in productivity of 3–4 g/L/day which translates into 8 kg batches of mAb drug substance in a 500 L bioreactor in 15-days of production.

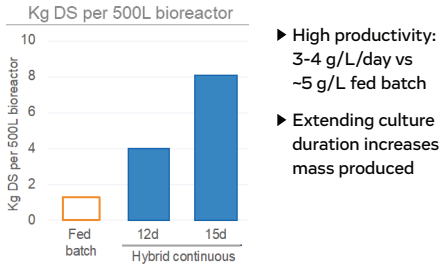

Figure 2: Titer from a hybrid continuous process of mAb drug product for First-in-human (FIH) trials.

Using our intensified, continuous platform, we can achieve 'Industry-Leading' titers and volumetric productivities. The process we develop for FIH drug substance supply at 500 L can remain at that modest scale (500–1000 L) for commercialization to supply multi-kilo amounts of material (up to 50 kg of drug substance mAb in a 1000 L bioreactor over a 25-day perfusion run see Figure 3) or annually metric ton scale production for Phase III and commercial supply by simply extending the culture run times. These high productivities, which are possible due to a combination of process intensification and continuous processing, are also achievable for new mAb modalities with more complex structures, such as bispecific antibodies and Fc containing fusion proteins.

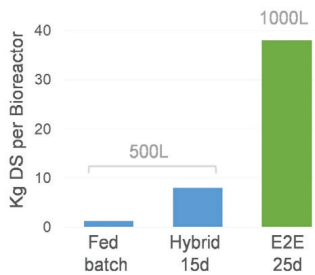

Figure 3: Comparison of Drug Substance Outputs from Fed-Batch versus Hybrid and Fully Continuous (E2E) Processes

Product Quality Benefits

As well as increasing productivity, our intensified perfusion cell culture process also has product quality benefits. This is because cells maintain high viability in continuous perfusion culture as fresh media is continuously being added, and waste products are constantly being removed. Additionally, the mAb product is being harvested for purification from the bioreactor as it is expressed, which means it is in contact for less time with cells, cell culture components and impurities that could cause product degradation. For example, a mAb drug substance we produced in a hybrid-continuous process showed better quality profiles with lower levels of degradation including, oxidation, deamidation and glycation compared to the mAb produced in fed-batch cell culture (Figure 4), indicating that product quality is improved using this approach.

The Future

It will become increasingly challenging for biopharmaceutical companies addressing niche indications or local markets to deliver cost-effective therapeutic biologics using conventional, batch-based bioprocessing methods. This is why new approaches such as continuous intensified manufacturing processes already established at Just – Evotec Biologics are needed. Furthermore, continuous intensified processes run in smaller facilities that are faster to build and less expensive to operate as demonstrated by our Just – Evotec Biologics' J.POD facility. Using our J.POD facility can increase speed, and reduce cost, as well as improve product quality and has the potential in the future to expand global access to more affordable, high-quality biologics.

Location		Residue	Modification	Fed Batch	Hybrid-Continuous
Heavy Chain	Framework Region	Q	PyroGlu	86.4	87.9
		M	Oxidation + PyroGlu	2.9	0.9
		M	Oxidation	1.3	0.5
	Constant Region	K	Glycation	1.0	0.1
		K	Glycation	0.7	0.1
		N	Glycan Occupancy	97.8	99.3
		N	Deamidation	1.3	1.3
		N	Deamidation	2.1	0.5
		N N	Deamidation	1.1	1.2
		M	Oxidation	2.4	0.8
		P	C-term Proline-amidation	5.3	14.2
		G	+ C-term Lysine	9.3	2.5

Figure 4: Comparison of Product Quality of a Drug Substance mAb from a Fed-Batch versus a Hybrid-Continuous Cell Culture Process

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