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INTRODUCTION

Propelled by single-use systems (SUSs), biopharmaceutical companies are approaching the ideal of continuous bioprocessing. In addition to improving process integrity and decreasing production costs, SUSs have enabled exciting ways to configure, operate, and evaluate manufacturing steps. Sensitive process analytical technologies (PATs) and discriminating data analysis platforms are supplementing those developments, helping researchers to study and modify workflows in unprecedented ways. The goal now is to intensify: to apply increasingly nuanced process knowledge and growing technological capability in ways that simplify, condense, abbreviate, and integrate distinct processes.

Intensification is easier said than done. And considering the industry's ambiguous uses of the term, researchers need clear instruction about how to accomplish it. The writers, speakers, and interviewees featured in this *BioProcess International* compilation offer such guidance. These articles detail how experts from Sartorius Stedim Biotech (SSB) characterize "process intensification" and how end users can transition toward the increased safety and economic sustainability of continuous processing.

Miriam Monge (BPI editorial advisor and head of segment marketing MAb, recombinant proteins, and intensified bioprocessing at Sartorius Stedim Biotech) commences this collection by highlighting the need for intensification. Her interview with Synthon Biopharmaceuticals' head of upstream biotechnology Nienke Vriezen reveals that the increasing number of manufacturing possibilities has complicated process management, introducing new concerns with technology transfer and process integrity. But Synthon has flourished because it has partnered with SUS suppliers that can deliver technologies to integrate seamlessly with extant processes and reduce transitions between steps.

Other articles herein survey bioprocesses that sorely need intensification and explore innovations that can address intensification needs. Gerben Zijlstra observes that SSB is concentrating on three key areas: seed train operations, perfusion systems, and high-throughput bioreactors. Featuring SSB's Cellca cell expression platform and the ambr bioreactor line, Zijlstra emphasizes that companies must leverage integrative upstream technologies to optimize those three distinct but potentially continuous aspects of bioprocessing.

Those innovations are possible because predictive analytical tools offer new ways to scrutinize process development. The works of Dan Kopec, Priyanka Gupta, and Anna Perrson illustrate how improved spectroscopy and data analysis tools such as SSB's Umetrics and SIMCA platforms offer deep profiling of bioprocessing materials, systems, and transitions. Equipped with such information, researchers can streamline and invigorate existing workflows — and move their companies closer to continuous processing and the next generation of biopharmaceutical manufacturing.

—Brian Gazaille, associate editor
BioProcess International

A Small-Scale Perfusion Mimic for Intensified Process Development with CHO Cells

Dirk Müller

Drivers for process intensification include lowered manufacturing costs, shortened production times, and improved flexibility of facilities that ultimately should help improve patient access to biological therapies. The first requirement of successful process intensification is to select a cell line that is robust, that produces high titers, and that has good stability to make long growth cycles possible to achieve high cell densities. Selecting the right clone and determine optimum conditions for intensified processing requires equipment that is fit for purpose, that supports high cell densities, and that has protocols for cell retention. Such systems also need to be supported by software with the right process recipes to harmonize devices with advanced interlinking control schemes. Sartorius Stedim Biotech (SSB) ambr 15 technology was developed for use as a perfusion mimic. Here I detail its performance with Chinese hamster ovary (CHO) clone selection, scalability, and early bioprocess development for process intensification.

Small-Scale Perfusion Mimic

Effective scale-up of intensified processes requires the right clones, media, and process conditions. The ambr 15 cell culture (cc) system has been used successfully for clone and media selection of Chinese hamster ovary (CHO) cell lines cultured in fed-batch conditions since its launch in 2010 (**1**). This system uses a culture workstation for parallel control of 24 or 48 single-use stirred microbioreactors (10-mL working volume). They have similar geometries to larger stirred-tank bioreactors, which helps users select optimum clones and conditions for further process development.

SSB scientists have developed a method for intensified processing, which uses the ambr 15 system in perfusion mode with a media-exchange protocol. That procedure involves culturing cells in the microbioreactors, bleeding them using the system's liquid handler, and then centrifuging vessels in specially designed centrifuge inserts that hold up to three microbioreactors each. Using that method, cells form oblique pellets to facilitate media removal. Then the vessels are placed back into the culture workstation, supernatant is removed and replaced with fresh media, and cell culture continues, enabling high–cell-density growth.

To demonstrate the performance of that media-exchange method for process intensification, CHO cells were cultured with a 1 volume/day media-blend exchange. Using this method, a cell density of >30 × 10^6 cells/mL, with a viability >90% and titers of up to 1.5 g/L/d were achieved. In 2019, SSB introduced the second-generation ambr 15 cc system, which has new functionality to bleed large volumes of culture and quickly remove spent media from microbioreactors to speed up media exchange.

Clone Selection

In fed-batch conditions, distinct CHO clones perform differently. To determine whether that is the case in intensified processing, SSB scientists cultured seven Cellca CHO clones (designated CL1–CL7) expressing different products for 14 days in an ambr 15 system using the media-exchange method with a 1 v/v/d media exchange. Using intensified processing yielded over threefold volumetric productivity increases for some clones, whereas others exhibited reduced productivity compared with standard fed-batch conditions (Figure 1). So clones selected for fed-batch culture often work well in intensified processing, but performance gains differ greatly. Thus, screening is advisable to identify the best clones for that application.

To compare the volumetric productivity performance of CHO cells using different intensification approaches, one of the best-performing CHO clones (CL2) was cultured for 14 days in either the ambr 15 system using the media-exchange perfusion method with a 1 v/v/d media exchange or in shake flasks with high-inoculation or regular fed-batch conditions. The results (Figure 2) showed that in standard fed-batch conditions, CL2 produced a titer of 0.35 g/L/d in 11 days; the high-inoculation fed-batch culture achieved 0.94 g/L/d in 10 days; and the intensified process in microbioreactors produced a titer of 1.41g/L/d in 14 days. That represents a cumulative titer increase from 3.8 g/L to 19.7 g/L and indicates that up to a fourfold increase in volumetric productivity could be achieved within two weeks culturing the same clone in an ambr 15 small-scale perfusion mimic (**2**).

Figure 1: Comparing volumetric productivities of Cellca Chinese hamster ovary (CHO) clones cultured in an intensified and a fed-batch process (dotted line)

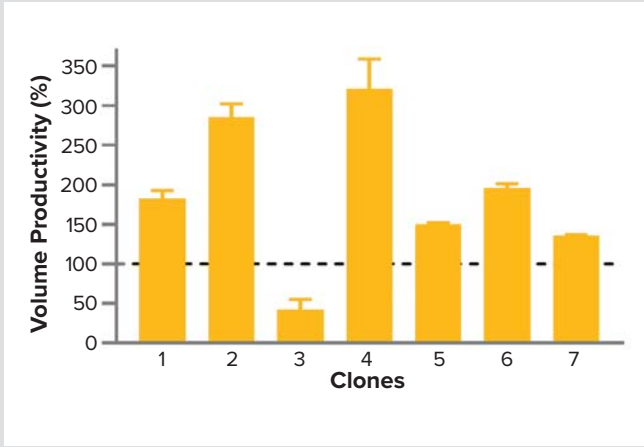
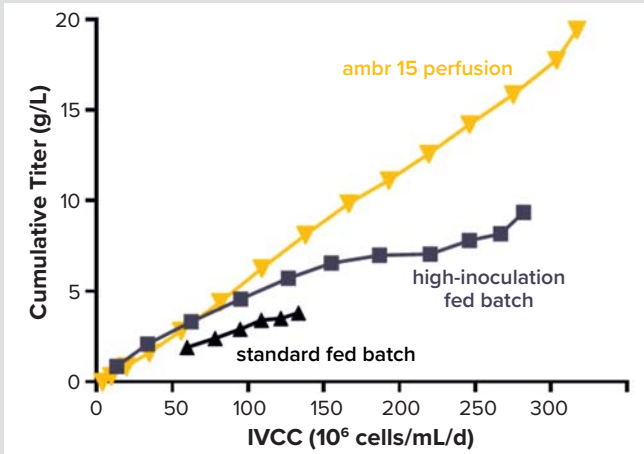


Figure 2: Comparing cumulative titers of Cellca CHO clone CL2 cultured in an intensified, standard, and high-inoculation fed-batch processes



Scale-Up

To determine whether a small-scale perfusion mimic could produce predictive intensified processing conditions, one of the best-performing clones, CL2 was cultured in the ambr 15 system alongside a 1-L rocking motion (RM) perfusion bioreactor and a 5-L fed-batch benchtop bioreactor. The results showed (**2**) that using the fed-batch conditions in the 5-L bioreactor, CL2 produced a titer of 0.5 g/L/d in 14 days, whereas the RM bioreactor produced 0.95 g/L/d in 14 days. The intensified microbioreactor process produced a titer of 1 g/L/d in 14 days, indicating that the performance of the ambr 15 perfusion mimic correlates with 1-L perfusion process in the RM bioreactor. Thus, scientists could use that system in perfusion mode to make meaningful predictions of how their cell lines will perform at larger scales without having to use a benchtop bioreactor model.

Process and Media Optimization

To establish whether the ambr 15 system in perfusion mode could be used to optimize processes and media for biosimilar development, SSB scientists set up screening using BioPAT MODDE design of experiments (DoE) software and the microbioreactor system. The system was used in perfusion mode to run different process conditions such as pH and DO, as well as different types of media mixes and supplements with the media exchange protocol as described above to culture CHO clones over three weeks. Cell growth, productivity, and culture longevity were assessed alongside product-quality indicators such as N-glycans and charge variants to determine the optimal media and process conditions in a multifactorial objective approach that balances productivity and quality tradeoffs.

Results showed that using the small-scale perfusion mimic in a DoE setting improved the process by reducing deviations of charge-variant profiles from an innovator molecule by 44% and deviations from the innovator molecule's N-glycan profile by 17%. There also was an 11% increase in volumetric productivity over the three-week perfusion culture. These results indicate that the ambr 15 system can be used in perfusion mode to select the best process and media conditions to help improve product quality and increase titer in intensified processes.

Conclusion

Intensified processing requires robust cell lines and tailored tools for clone selection and early process development. The results described herein establish that the ambr 15 cc system can be used as a small-scale perfusion mimic to predict which clones will be most suited for use in process intensification as well as to help increase volumetric productivity by up to fourfold and produce cumulative titers of >19 g/L in just two weeks. Such titer increases are comparable to those seen in a 1-L RM perfusion bioreactor, indicating that this small-scale perfusion mimic also can predict how cells will behave at scale.

Additionally, using the small-scale perfusion mimic in a DoE setting can help optimize productivity and product quality attributes (PQAs) of CHO clones cultured in an intensified process. In summary, SSB's ambr 15 technology in perfusion mode is suitable for clone selection and process development to achieve optimized process intensification of CHO cells, which could contribute to delivering more affordable biologics.

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Integrated Tools for Upstream Process Intensification

Gerben Zijlstra

Changes to process methodologies in the biopharmaceutical industry are driven by the need for increased speed, lowered cost of goods (CoG), and improved flexibility (**1**). To meet those challenges, the industry is adopting strategies such as intensified and continuous processing, which regulatory bodies such as the US Food and Drug Administration (FDA) are endorsing. However, for intensified bioprocessing to deliver on its quality and productivity promises intelligently designed process development and manufacturing tools will be required. Sartorius Stedim Biotech (SSB) has developed a toolbox of technologies for that purpose.

Currently the biopharmaceutical industry is concentrating on three areas where upstream bioprocessing can be intensified. The first is in seed trains, which can be intensified by direct inoculation of cells from high–cell-density banks into 50-L rocking-motion (RM) bioreactors — a strategy that eliminates the need to use shake flasks in post-thaw expansion. Also in seed train operations, perfusion approaches that keep cells in their exponential growth phase throughout the entire culture help companies skip steps or generate higher cell densities to inoculate their production bioreactors. For example, *N* – 3 culture in a 50-L RM bioreactor can seed a 500-L bioreactor directly to skip the *N* – 2 (200-L) bioreactor stage. Or *N* – 1 (1,500-L) culture in a stirred or RM bioreactor can seed a production culture with a final volume of 2,000 L.

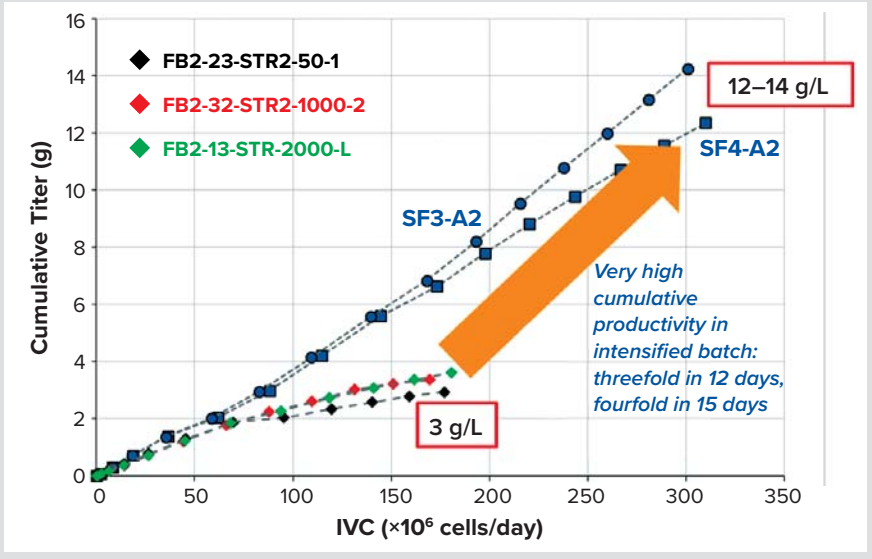
Intensification approaches also can be used in production bioreactors — for example, in a hybrid approach whereby the seed-train runs in perfusion mode, with *N* – 1 (500–L) culture used to seed a main bioreactor (2,000 L) with a high cell density that runs in fed-batch mode to purify product from the bioreactor. This approach is used as a platform by companies such as Bristol-Myers Squibb (BMS). Other companies have installed or are developing platforms running production bioreactors in perfusion mode and either retaining product in the bioreactor for batch harvesting (e.g., Amgen) or purifying their product from the permeate (e.g., Pfizer and WuXi).

Intensification approaches also can be used in PRODUCTION bioreactors — for example, in a hybrid approach whereby the seed train runs in perfusion mode and a main bioreactor runs in fed-batch mode.

Cell Line and Media Platform

For process intensification in a seed train or main bioreactor, cell lines that can be perfusion cultured are required, and SSB offers the Cellca CHO Chinse hamster ovary cell expression platform for this application. To demonstrate their performance under perfusion conditions, Cellca CHO cells expressing a model mAb (IgG1) were cultured in fed-batch and simulated-perfusion conditions with a shaker-

Figure 1: Expression titer produced by CellCa CHO cell line in fed-batch cultureunder intensified batch conditions



intensified batch-simulation protocol using daily centrifugation and medium replacement of standard Sartorius cell culture media and feed mix (1 volume/day perfusion over 15 days). Using the fed-batch conditions, 25 × 10^6 cells were produced; a higher cell density of 30 × 10^6 cells was produced in the simulated perfusion. That cell density was restricted deliberately with a temperature-drop–induced cell growth arrest (**2**).

Although the cell density achieved was not significantly higher, the titers were (Figure 1). With the fed-batch conditions, 3 g/L was produced; CellCa CHO cells cultured using the simulated perfusion conditions produced a threefold greater titer in 12 days and a fourfold titer increase to 12–14 g/L in 15 days (**2**).

Process Development Platform

For intensified processes to be scaled up effectively, process conditions need to be modeled accurately. Moreover, to do this in competitive time frames as regular fed-batch cultures would require high-throughput mode. In 2018, SSB introduced the ambr 250 high-throughput perfusion system for this application.

The system uses up to 24 single-use stirred microbioreactors (250 mL working volume) which have similar geometries to larger stirred-tank bioreactors, which helps users develop process-intensification conditions for seamless transfer to manufacturing scale. To demonstrate the performance of ambr 250 high-throughput (HT) perfusion, a biopharmaceutical partner perfusion-cultured a cell line expressing a coagulation factor over 30 days in two such bioreactors and two 5-L benchtop bioreactors using alternating tangential-flow (ATF) mode. Crossflow and dilution rates were within typical operating ranges for ambr 250 HT perfusion, and automated bioreactor bleeding was based on daily cell counts.

Results showed that cell growth and viability were consistent between ambr 250 HT perfusion replicates, which were comparable to those seen with the 5-L benchtop bioreactors that represented the current down-scaled model (**3**). Additionally, four quality attributes were tested, designated each week from the ambr and 5-L bioreactors for three weeks of culture. Results showed that there was good consistency between both bioreactor types (**3**), indicating that product quality in ambr 250 HT perfusion bioreactor is representative of larger scale perfusion cultures.

Bioreactors for Intensified Processing

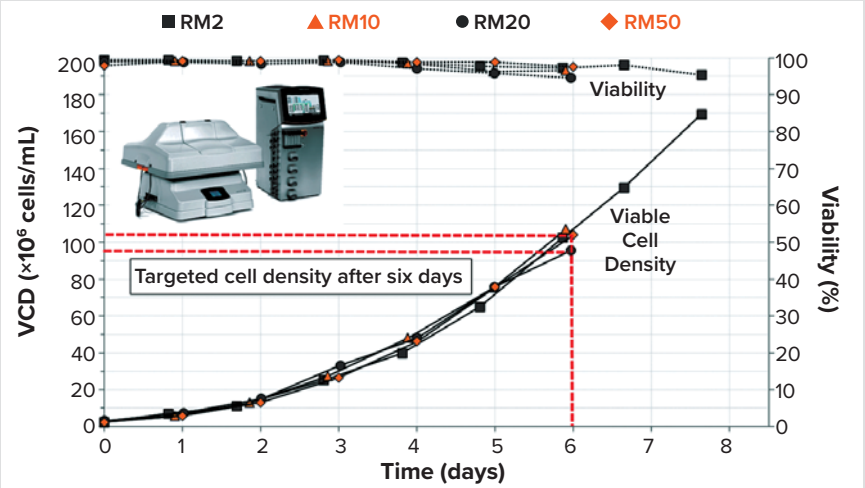
For seed-train process intensification, SSB offers the GMP-compliant BIOSTAT RM wave-mixed bioreactor with single-use bag sizes from 2 L up to 200 L (a maximum 100-L working volume). RM perfusion bags (constructed of Flexsafe film with proven cell growth properties) feature an integrated perfusion membrane that precludes the need for external cell-retention devices, making them simple to operate.

For intensified processes to be SCALED UP effectively, process conditions need to be modeled accurately. To do so in competitive time frames with regular fed-batch cultures requires high-throughput mode.

To demonstrate the performance of those bags, CellCa CHO cell lines were cultured over six days in RM2, RM10, RM20, and RM50 perfusion bioreactor bags. A BIOSTAT RM platform was used to automate them along with online process analytical technology (PAT) sensors, including a BioPAT ViaMass instrument to monitor and control exponentially growing culture and allow for automatic feeding and bleeding. Results (Figure 2) showed that these bioreactors produced high cell densities (≥100 × 10^6 cells/mL), scalable up to a 25-L working volume (**4, 5**). That indicates that using these bioreactors at 20-L or 200-L scales, operators could reduce their seed trains to a minimum number of stages and inoculate a production bioreactor at higher cell density.

For intensified processing at bench, pilot, and manufacturing scales, SSB offers the BIOSTAT STR range of stirred-tank bioreactors from 12.5 L (STR50) to 2,000 L (STR2000) working volumes. For several years, these have been capable of high–cell-density culture and have been shown to produce up to 200 × 10^6 cells/mL in concentrated fed-batch conditions, with CHO cell lines cultured over 12 days in 50-L and 500-L bioreactors using a tangential-flow filtration (TFF) retention system. Both bioreactors

Figure 2 Viable cell density and viability of cells cultured over six days in RM2, RM10, RM20, and RM50 perfusion bioreactors



Leveraging integrated upstream technologies from SSB is helping the biopharmaceutical industry to achieve process intensification, which could help ACCELERATE manufacturing speed and productivity using smaller facilities and fewer resources.

showed comparable viable cell densities and product titers, indicating scalability up to 500 L (**6**).

Conclusion

Intensified processing enables biopharmaceutical companies and contract development and manufacturing organizations (CDMOs) to use smaller single-use bioreactors and manufacturing facilities with increasingly smaller footprints than ever before. However, this requires upstream tools capable of culturing higher cell densities, and SSB offers suitable solutions for every stage of intensified upstream biomanufacturing, beginning with cell-line development.

Cellca CHO cells can be cultured to high densities in SSB cell culture media and are suitable for both seed-train and main-bioreactor process intensification. For process development, SSB provides for ambr 250 HT perfusion, a game-changing system that for the first time allows high-throughput perfusion process development to deliver robust and optimized processes to manufacturing in substantially reduced timelines using less resources than full-size systems require. To complete upstream-intensified process integration for manufacturing scale, RM perfusion bioreactors can be used for seed-train intensification; benchtop and single-use stirred-tank bioreactors for achieving intensified cell cultures up to 2,000-L scale; and proven cell densities of up to 200 × 10^6 cell/mL at 500-L scale.

In summary, leveraging integrated upstream technologies from SSB is helping the biopharmaceutical industry to achieve process intensification, which could help accelerate manufacturing speed and productivity using smaller facilities and fewer resources. Ultimately this should help improve global access to relatively affordable biologics and vaccines.

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Successfully Implementing End-to-End Single-Use Technology in a Biomanufacturing Facility

An Interview with Synthon Biopharmaceuticals' Nienke Vriezen

Miriam Monge

Synthon Biopharmaceuticals is a leading company that develops innovative medicines with a focus on next-generation cancer drugs. It has implemented single-use technology successfully throughout its monoclonal antibody (MAb) manufacturing process. To discover the secrets behind this company's single-use strategy, I took the opportunity to interview Nienke Vriezen, director of upstream process development and manufacturing at Synthon's company headquarters in Nijmegen, The Netherlands.

Nienke Vriezen (Synthon Biopharmaceuticals)

Miriam Monge (Sartorius Stedim Biotech)

Design Concept

Monge: Synthon is an industry reference for end-to-end integration of single-use systems. What drove you to base the process design for your current good manufacturing practice (CGMP) facility totally on single-use systems?

Vriezen: We needed the flexibility that disposables offer. Over 10 years, we've revamped our plans several times before finally deciding on a plan to implement. We use disposables integrated through all the buffers, media, and bioreactors, which offers us speed by reducing a lot of studies that we would need for cleaning and changeovers.

Monge: How did you set up your task force for single use?

Vriezen: We included upstream, downstream, and even analytical people to ensure the right handover of materials and samples. One challenge was to envisage what it would look like physically to have all the buffer supplies in place. There are significant volumes to handle, and you need to design the area to be safe and comfortable for people to move around in.

Choosing a Supplier

Monge: How did you go about selecting a single-use supplier, bearing in mind that the breadth of different companies' portfolios differ greatly?

Vriezen: We tried to simplify our lives by getting a supplier that could deliver a range of technologies across the whole process. We envisaged that we would have to tie together different companies chosen for different parts of our process, and we wanted to limit the need to do that as much as possible. The bioreactor was a prominent choice because we knew we were starting out with experience in one process, but we also knew that we wanted whatever we chose to work with for future processes as well.

Monge: What were the key criteria in your single-use bioreactor selection?

Vriezen: We had some experience at a contract manufacturing organization (CMO) with one of the available brands, and we sought two other brands. We took the opportunity to test both of those at 50-L scale in our research and development (R&D) laboratories, and those studies taught us a lot. We learned about different approaches to control that came with the systems and about the limitations of disposable bioreactors. We got to test-drive the vendor support in our selection process — which is important whichever brand you choose. Ultimately, I could get any of those three brands to work with this specific process, but I wanted confidence in the support throughout the rest of the life cycle. And I wanted to see what it would be like if we found something challenging with our next project. That was a key element in choosing the bioreactor supplier.

> The most important thing is to develop a **GOOD UNDERSTANDING** with your supplier.

Monge: Can you report on your experience with Sartorius in supporting implementation of your bioreactor?

Vriezen: We have people here who were comfortable with the controller type because it's used in smaller bioreactors in our laboratory. But we had to get accustomed to the single-use concept, and we saw the support that Sartorius brings with experts on site. We could share information easily, with people supporting our observations based on technical knowledge in your organization. This gave me confidence that I would get the support I needed if ever I required it.

Implementing Single-Use in Manufacturing

Monge: In addition to 50-L to 2,000-L single-use bioreactors, you also implemented an extensive range of single-use technologies in downstream processing. How did you go about operator training with such a broad range of different technologies throughout your process?

Vriezen: As much as possible, we included the technicians who would be performing the process operations. Even during selection, we had a couple of people try different brands with different setups, and they reported back with some good-to-know, daily-use common issues. The design process of these single-use items was certainly a challenge. We had been warned at the start of the project that it's not just about choosing the hardware, but also thinking clearly about what you really need from the disposable parts as well as how they interact.

Monge: How did you handle the logistics of transfer from one unit operation to another?

Vriezen: We tried to work out the physical flow as much as possible. First on paper, we worked out where the path should be connected with the first upstream unit to the downstream unit operation. Then, on what used to be a parking lot but is now is our MAb plant, we had almost three-dimensional (3D) trial runs to work out where those elements should be and what would be the most natural places to make those connections.

Monge: How involved was the Sartorius Integrated Solutions team in supporting that phase?

> We **INVESTED EARLY** in a common understanding of the project — the wider teams here at Synthon and at Sartorius as well.

Vriezen: We had a good rapport with the disposables team, who had their design issues to handle with our team working on the hardware. That's where we discovered the difference between the hardware of skids or controllers and what disposables actually could deliver.

Monge: How will you implement process data capture and transformation into real, usable knowledge?

Vriezen: We're used to taking as much data as we can from our cell cultures and working through that throughout upstream. As we mature, we will have some learning to do. Integrating all those data toward a continuous process verification will be exciting next steps.

Monge: How did you approach the testing requirements needed for this new single-use facility?

Vriezen: We go through a systematic approach to assess the potential for removing leachables and extractables in the rest of the process. Most of what we needed was covered by the vendor package information.

Monge: Many different working groups working on industry guidelines and standards creation: the Bio Process Systems Alliance (BPSA), the BioPhorum Operations Group (BPOG), the International Society for Pharmaceutical Engineering (ISPE), and the Parenteral Drug Association (PDA). How did you go about selecting which guidelines to follow?

Vriezen: We keep aware (as best as we can) of all the different guidelines, and we double-check that we've got a proper, justified position in how we approach things relative to the guidance coming out.

Monge: When you move to single use, you become heavily reliant on your supplier. What key elements should be considered when verifying your choice of supplier?

Vriezen: The most important thing is to develop a good understanding with your supplier. You might make some choices that sound very simple from a process perspective but that put the supplier in hot water by requiring an element that just isn't available. Understanding is key, and I think all suppliers in this area are trying to cope with an accelerated rate of growth.

Benefits of Single Use

Monge: Is your single-use facility more sustainable than a stainless steel one?

Vriezen: Yes. The amount of cleaning water that would be needed is quite large for a stainless-steel version of this facility, and it would probably make it too rigid to fulfill the portfolio we require of the plant. We have calculated that the water requirements balance out the plastics use, so I'm convinced that we've got a plan that is sustainable at the moment.

Monge: Thinking about project management of implementing this new single-use CGMP facility, what were your expectations of your supplier to ensure success?

Vriezen: We invested early in a common understanding of the project

— the wider teams here at Synthon and at Sartorius as well. I feel that was a very useful investment upfront. Then we had subteams working on different areas upstream and downstream, and they had their counterparts at Sartorius. If it started to become more complicated, then nothing substituted for a face-to-face meeting. We had a clear structure in the documentation and project management on both sides, and that helped us stick to pretty tight timelines.

Monge: How much time did you save in implementing single use rather than stainless steel?

Vriezen: We talk about it here as a series of "births," the physical building done in about nine months, the selection and creating of all the equipment was done in another nine months. Then we went through all the validation in about nine months as well.

Monge: That seems incredible! Were a lot of activities done in parallel?

Vriezen: Yes, we had discussions about certain utility layouts, and I was no longer surprised when somebody walked into my office and said, "The guy with a diamond drill is here now. Can you point to where you need the drain?"

Lessons Learned

Monge: How satisfied are you with the solutions you have in place at your single-use facility today?

Vriezen: From the principles of design, we absolutely got what we wanted with the knowledge we had at the time. The challenge now is in getting continuity of supply and keeping up the quality of responses to questions and issues to resolve. That is where we see the industry becoming more active.

Monge: What was the most exciting or impressive moment for you in this project?

Vriezen: For me, it was seeing the big, shiny, 2,000-L bioreactors go in in four parts, with one of my engineers having worked out in detail that those elements could get through the door if the door was removed. The guys doing the moving had planned ahead. They had all the support they needed the day they came in — and to me, the shiny, nice picture we can present now is one of the highlights of this whole project.

Monge: What would you change in your next single-use facility project?

Vriezen: Working out the physical, 3D part of which bags can be where at what point in the process is not easy. You need to think through the whole logistics and timing. I have learned that you shouldn't underestimate the footprint needed for storage of buffers and media. We designed that as a corridor around the core process. It's hard to defend open spaces during a plant design, but that would be my point of learning to take forward.

Monge: If you were to advise someone considering single use in a new facility for the first time, to what key points would you recommend that they pay attention?

Vriezen: Be very clear on the scope of the plant. If you think it needs to be flexible, you have to plan in flexibility from an early point. Try to get a core crew of operations people involved early on because they are the ones who actually will make your process happen. We had a great experience with our team being on board early. Not everybody's wish gets to be fulfilled every time, but the more of those wishes you can fulfill in the design process, the better it all turns out in the end.

Monge: Any last comments?

Vriezen: It's been a fantastic experience to put this plan together. We couldn't have done it without supplier cooperation. We knew that our choice of bioreactor was one of the drivers for that, and I'm happy to see that we now have a process up and running. We've progressed a stage further and nearer to CGMP operations, and we're looking forward to overcoming the next hurdle.

You shouldn't underestimate the FOOTPRINT needed for storage of buffers and media.

*BPI editorial advisor **Miriam Monge** is head of segment marketing mAb, recombinant proteins, and intensified bioprocessing at Sartorius Stedim Biotech. **Nienke Vriezen** is director of upstream process development and manufacturing at Synthon Biopharmaceuticals' company headquarters in Nijmegen, the Netherlands. Highlights from this interview are detailed herein. Find the full video online at https://www.sartorius.com/us-en/knowledge/resources/case-studies#id-33762.*

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Process Intensification Scenarios for Predicting the Most Cost-Effective Strategies

A Summary of a Presentation Given By Priyanka Gupta

Process intensification (PI) — defined as a chemical engineering developmental strategy leading to smaller, cleaner, safer, and more energy-efficient technologies — is widely applied in Sartorius Stedim Biotech's push to expand productivity. Priyanka Gupta (bioprocessing modeling manager at SSB) presented her experiences in moving manufacturing productivity beyond the capabilities of initial facilities during a recent company conference. She directs a team that analyzes production processes to define the most cost-effective framework. Her team uses BioSolve software (an analytical and economic modeling package) to model and select the most significant impact on overall process economics.

In her discussion of analyzing manufacturing productivity, Gupta described strategies for improving productivity as an overall process of guiding the evolution of a processing facility from the first through fourth generations, with "next-gen" facilities scheduled to come on line in the next 10 years. "Why does the industry need process intensification for increased productivity?" she queried, then elaborated on many problems the biopharmaceutical industry is facing.

"In the first place," she said, "it will enable the application of faster-to-market strategies with attendant cost-of-goods reduction through flexible manufacturing." The introduction of next-generation molecules such as bispecifics and fragments, aging current infrastructure, new technologies, and enlisting regulatory support all are demands that will be incorporated into process intensification.

Currently, single-use facilities solve many of these needs, but with severe limitations on their productivity. Disposables typically generate only 500 kg/year by contrast to an average productivity of 1,500 kg in stainless steel facilities. But application of process intensification easily can triple single-use output, driving it up to match that 1,500 kg/year and making it competitive with traditional commercial facilities.

Process analytical technology (PAT) automation strategy is another concept that can help companies realize the full potential of intensified single-use processing. This strategy includes

Figure 1: Activities in existing hybrid facilities toward intensification

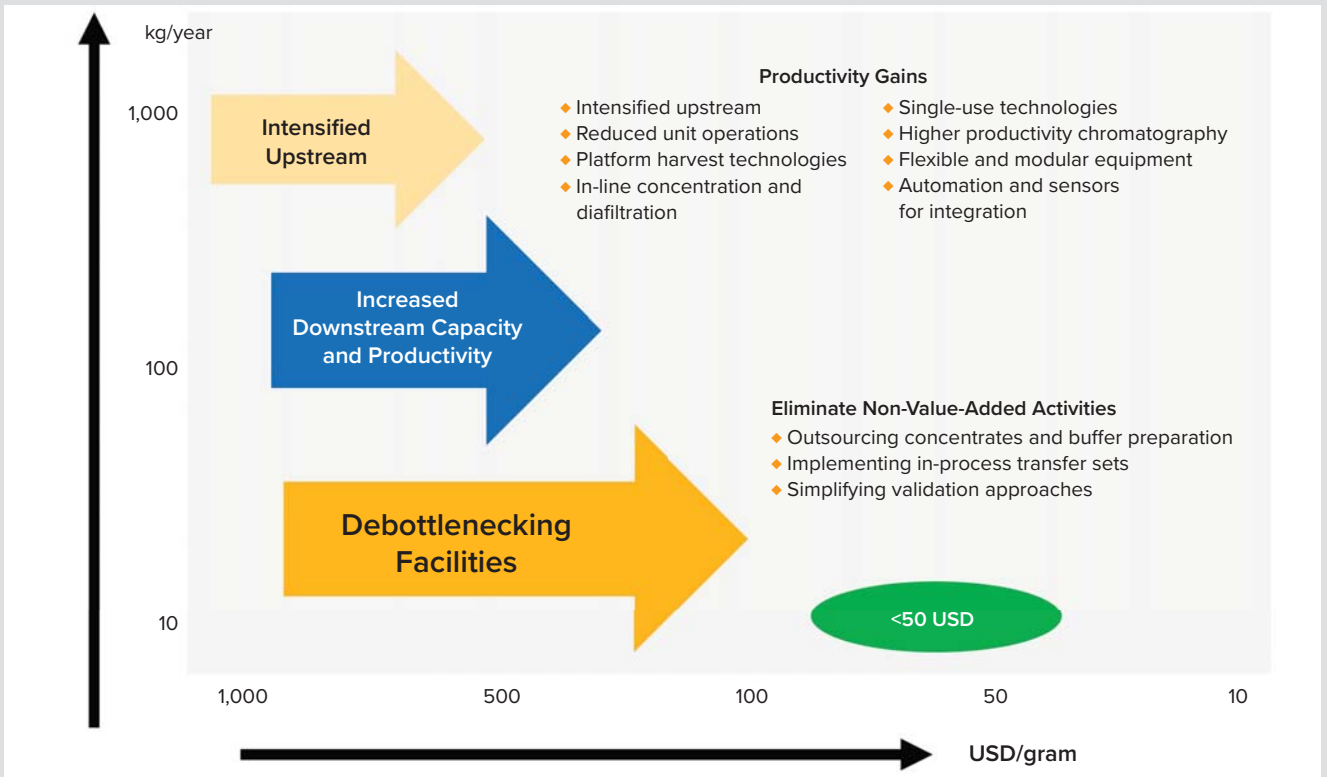
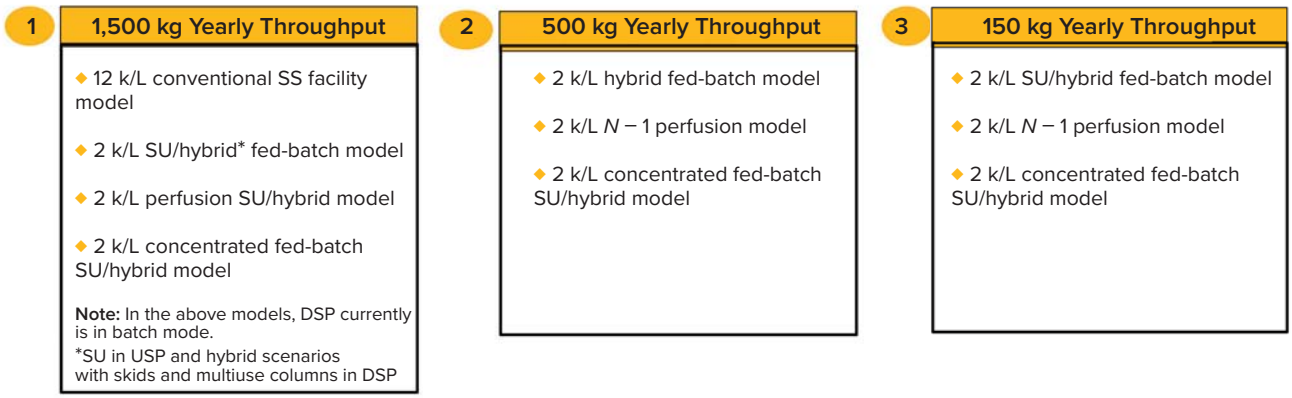


Figure 2: Three industrial scenarios for production of biologics



ViaMassPAT-based automated feed, bleed, and process monitoring to improve process control and output, reducing labor and risks associated with manual sampling and control.

Gupta described the role of process modeling in maximizing the quality of decision-making for process intensification. The goal is a holistic picture that makes predictions and evaluates alternatives, factoring in cost implications through a cost breakdown by unit operations and targeting changes that can be made cost-effectively. The team examined three industrial scenarios for production of biologics: 1,500, 500, and 150 kg/yr (Figure 2). The analysis identified those steps with the greatest impact on overall economics, allowing outcome optimization.

To attain a 1,500 kg/year output, the team considered four permutations: a 12,000-L conventional fed-batch stainless steel facility; a 2,000-L single-use fed-batch hybrid model; a 2,000-L *N* – 1 perfusion, single-use hybrid model; and a 2,000-L concentrated fed-batch single-use hybrid model. "*N* – 1 perfusion" uses a cell-retention device to achieve high cell densities in the seed train step before the production bioreactor (*N*), improving process speeds and robustness while maintaining an existing fed-batch–based production scheme.

Those approaches result in different distribution of costs for the various inputs. The lowest overall cost was for the single-use hybrid concentrated fed-batch perfusion model at 42 €/g. That model yielded much lower media costs than the single-use hybrid concentrated fed-batch approach (55 €/g).

Costs were substantially higher in the 500 kg/yr model. Three possibilities were evaluated: a 2,000-L single-use hybrid model, the cost of which was 68 €/g; the 2,000-L perfusion, single-use hybrid model with a cost of 50 €/g; and the 2,000-L concentrated fed-batch single-use hybrid model (65 €/g). The 150 kg/yr was far and away the most pricey: For batch sizes of 2,000 L, the costs were 96 €/g at a 3 g/L expression titer, 77 €/g at 6 g/L, and 102 €/g at 10 g/L. Results garnered from analyses of the three yield scenarios are the expected outcome based on recognition that the larger the volume generated by a specific scenario, the lower its cost per unit of yield.

Gupta and her team then considered further seed intensification in the perfusion process. They examined three approaches: a classical fed-batch seed train; an *N* – 1 perfusion seed train with a stirred-tank reactor (STR); and an *N* – 1 perfusion seed train with RM perfusion as an *N* – 1 seed efficiency seed train. The goal as always is to seed the production process, provide economical modification, increase capacity with more batches per year, and produce higher expression titers.

According to Gupta, the classical fed-batch seed train has the merits of a proven technology with a cost efficiency that fits the industry standard reliably and robustly. The second protocol (the intensified stirred-tank seed strategy) commands less hardware investment in a lower footprint, with up to a twofold increased facility output, enabling a large inoculum for the main bioreactor. The third design (an *N* – 1 perfusion seed train with RM perfusion) requires minimal hardware investment and low consumable costs in a lower footprint than the classical option and delivers an increased facility output.

In drawing conclusions from her team's investigations, Gupta stressed that the models predict a 50% footprint reduction for intensified upstream processing scenarios in all possible throughput demands. Applying the intensified upstream processing approach within an existing footprint can yield a two- to three-fold productivity increase.

N – 1 intensification appears to be the ideal choice for increasing productivity and flexibility while reducing capital expenditure, operating cost, and facility footprint. An important finding from these analyses is that media volumes and costs are the major drivers of concentrated fed-batch costs. Therefore, media optimization represents a critical route to economic gains. These findings also can be applied to to generate faster turnaround time during the clinical phases of development when multiple products are in the pipeline.

Finally, application of efficient seed-intensification protocols can contribute further to reducing costs per gram, to increasing productivity, and to reducing overall operating costs without increasing initial investments.

Gupta ended by outlining future goals for her team. These include the study of perfusion options in upstream processing and analysis of downstream processing and its combination with the upstream phase.

Priyanka Gupta *is process modeling manager at Sartorius Stedim Biotech; priyanka.gupta@sartorius-stedim.com.*

Making Downstream Processing Continuous and Robust

A Virtual Roundtable

S. Anne Montgomery, Cheryl Scott, and Peter Satzer, with Margit Holzer, Miriam Monge, Ralph Daumke, and Alexander Faude

Current biomanufacturing is driven to pursue continuous processing for cost reduction and increased productivity, especially for monoclonal antibody (MAb) production and manufacturing. Although many technologies are now available and have been implemented in biodevelopment, implementation for large-scale production is still in its infancy.

In a lively roundtable discussion at the BPI West conference in Santa Clara, CA (11 March 2019), participants touched on a number of important issues still to be resolved and technologies that are still in need of implementation at large scale. Below, moderator Peter Satzer (senior scientist with the Austrian Center of Industrial Biotechnology, Vienna) summarizes key points raised in that session. Based on his highlights, BPI asked a number of industry representatives to comment on those points further, and their responses follow.

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Requirements for Continuous Downstream Applications

by Peter Satzer

Minimizing Buffer and Hold Tanks: One critical parameter is integration of unit operations on manufacturing shop floors using minimum numbers of buffer tanks and hold tanks. The benefit of continuous manufacturing can be realized fully only if auxiliary equipment for buffer preparation and hold tanks are minimized between downstream operations. Although continuous buffer preparation and reduction of buffer hold tanks are being investigated (and also were the subjects of BPI West presentations), direct integration without the need for hold tanks between unit operations requires further exploration.

Chromatography Operations: Some unit operations such as flow-through chromatography and single-pass, tangential-flow filtration (TFF) can be integrated easily into a continuous downstream process scheme at any point because they offer constant inflow and outflow of material. Such operations can be called truly or fully *continuous*.

Other unit operations — especially bind–elute chromatography — are much harder to implement because they don't provide truly continuous operation, but rather produce individual elution peaks with fluctuating product and impurity concentrations. Usually the unit operation following such a step cannot handle such changes in flow and concentration directly, requiring inclusion of surge vessels and hold tanks that interrupt the process. This makes continuous periodic countercurrent chromatography (PCC) a unit operation that is harder to implement than other operations such as flow-through chromatography and new, alternative purification methods, including continuous precipitation.

Viral Inactivation: Current implementation of continuous processing in the MAb world could make use of viral inactivation steps as surge vessels. Biomanufacturers could fill parallel vessels periodically and empty them to provide constant flow and product concentration for subsequent unit operations. This might not be possible for all antibody and nonantibody products, however. Alternative purification techniques such as continuous precipitation offer a constant inflow and outflow of material and prevent integration issues.

Residence-Time: Another important point to consider when thinking about continuous integrated unit operations is the residence-time distribution through all unit operations. Understanding of this concept is limited, but it is critical to ensure batch definition and ensuring process robustness. Although a narrow residence-time distribution is preferred for batch definition and to minimize product loss in case of contamination, a broad residence-time distribution smooths process irregularities and leads to a more robust process. The trade-off between those two approaches will need to be discussed in the future.

PCC Chromatography: PCC can be detrimental to our understanding of residence-time distribution because it can split it during an operation. A PCC operation will produce one or two residence-time distribution peaks depending on the state of the operation, and no easy model can be applied. If bind–elute polishing also is implemented using PCC, the resulting residence-time distribution can be split into up to four individual distributions traveling through the system, which complicates decisions about when to discard material. Currently, this issue is too poorly understood and modeled. It either is not addressed, is addressed with wide safety margins for discarding material, or is addressed by implementing a hybrid process that uses batch manufacturing after a PCC unit operation.

Alternative technologies to bind–elute chromatography such as flow-through chromatography, precipitation, crystallization, and flocculation can offer solutions to those issues in the future to prevent integration problems. Developers of future technologies should aim for a constant mass flow through all unit operations, enabling integration and a seamless downstream process train.

The Roundtable Discussion

Based on the discussion highlights above, BPI invited further insights from several advisors, end users, and supplier companies. Included below are additional comments from Satzer along with responses from participants listed in the box at right. BPI invites further comments and welcomes submitted manuscripts detailing how your organization approaches these issues.

Capacity Mismatch

We've heard recent discussion about a "capacity mismatch" between upstream and downstream aspects of biopharmaceutical drug-substance manufacturing. A couple decades of production improvements have created challenging process streams for separation and purification. Can continuous downstream processes offer a partial solution to this problem?

Satzer: Continuous downstream processes can offer solutions in two separate ways. The first way is to increase downstream equipment use by using equipment 24–7 and therefore increasing downstream productivity. The second way is through a change in mindset, in which up- and downstream operations are integrated directly rather than remaining distinct and separate — which forces them into direct communication. That opens the possibility of solving problems earlier (upstream), where solutions might be easier to achieve.

Holzer: Great improvements have been made in cell-specific productivity, media development, and process design during recent decades, resulting in some cases up to 100× increased product concentration in fermentation broth. The product output of the same upstream installation could be increased proportionally.

However, improvements in chromatography media capacity and membrane performance have not given the same range of productivity increase, instead doubling or tripling it.

Roundtable Participants

Peter Satzer, senior scientist with the Austrian Center of Industrial Biotechnology, Vienna

Margit Holzer, principal at Ulysse Consult

Miriam Monge, BPI editorial advisor and director of marketing for integrated solutions at Sartorius Stedim Biotech (whose comments include insights from colleagues **Ganesh Kumar, Tom Erdenberger,** and **Hemanth Kaligotla,** members of the Sartorius MAb and intensified market segment team)

Ralph Daumke, biotechnology market manager life science, FILTROX Group

Alexander Faude, PhD, director DSP development at Rentschler Biopharma (whose comments include those of colleagues **Thilo Grob,** director DSP process design/validation, and **Anja Trapp,** senior scientist)

Therefore, work is needed on process design to overcome the downstream bottleneck. Related design studies easily show the great potential of continuous processing.

Monge: Recent advances in upstream processing have led to processes in which expression titers have increased beyond 10 g/L or even 20 g/L. Availability of concentrated fed-batch options on a pilot and commercial scale has resulted in DSP handling product amounts ranging from 5 kg to 40 kg per batch. On one hand, process intensification efforts have reduced overall footprint and time in the upstream train by eliminating intermediary seeding steps. But this has led to an increased footprint in downstream processing (DSP) that has required some biopharmaceutical companies to switch from single-use to hybrid/stainless steel technologies.

One way to address the challenge is to implement multicolumn chromatography at the capture step to ease the burden or to have continuous DSP with perfusion-based upstream processing (USP) — enabling biopharmaceutical companies to debottleneck this "capacity mismatch" because the amount of product to be processed is distributed daily. The approach also provides high flexibility in product output, reduces the overall

For **CONTINUOUS PROCESSING,** choosing ready-made buffers connected at the point-of-use, along with in-line dilution and stream conditioning for steps with low buffer demand, could be an option.

DSP footprint, and enables closed processing and cleanroom declassification. With the same scale for clinical and commercial production, the technology transfer process is seamless.

Daumke: Different options can help solve the mismatch. One option would be a single-use centrifuge, and another would be filtration. In the latter case, alluvial filtration can be developed and built as a continuous skid working similarly to a chromatography system. When capacity is reached in the first filter, the system switches automatically to the next filter, and so on.

Faude: Continuous processing contributes to facing DSP challenges in two ways. First, it usually goes in hand with increased automation compared with conventional batch processing. For example, using more sophisticated chromatographic devices allows manufacturing to run efficiently over 24 hours. Second, the continuous processing mode enables higher resin use, resulting in lower buffer amounts needed for processing. Both can have significant impact on costs. Moreover, continuous processing can trigger optimization of simple process steps such as flow-through applications and should push forward the development of resins, membranes, and other technologies that can deliver fast mass transfer.

Buffer Preparation

Whereas continuous upstream processing uses more buffers and media, continuous downstream processing should reduce the number of buffers required for separation and purification operations. Does the end result balance out? And does it make more sense to buy and store premade, ready-to-use buffers for continuous processing — or to make them on demand based on powders and/or concentrates?

Satzer: The final goal has to be preparation of buffers and media based on powders or concentrates. For concentrated buffers (and reduction of different buffers used in the complete downstream stream), efforts have been made already and the first prototypes created (some presented at BPI West). Media tanks especially occupy a significant portion of a shop floor and either take up significant space or have to be refilled regularly (requiring personnel, analytics, maybe cleaning, and so on). Buffer preparation is an essential part of DSP, so development of continuous buffer preparation from powders is fundamental to a fully continuous process with minimal shop-space requirements.

Holzer: Perfusion or chemostat cell culture processing typically need more media while significantly improving plant productivity. Depending on the process design of unit operations in DSP, the amount of necessary buffers and solutions may be reducible.

For example, continuous downstream processing (cDSP) could be achieved by connecting staggered batch operations, but that would not reduce buffer consumption. Yet in the case of countercurrent multicolumn chromatography processes or single-pass TFF, typically more product can be processed with less buffer. It is important to analyze an individual product, plant, and strategy to come up with an adapted, balanced, and cost-effective processing platform.

Cost for buffer preparation, analysis, and storage are significant for recovery and purification steps and can become an operational bottleneck for some plants. That might be not seen during clinical phases, but it becomes evident during commercial production. Cost studies and risk assessments should support decision making. Results often bring about interest in working with concentrated solutions using in-line dilution systems. Currently, technologies that allow buffer or media preparations starting from powders are under development.

Monge: Performing chromatography in multicycle, sequential batches with smaller columns/adsorbers that require a high degree of saturation has led to a considerable decrease in downstream buffer demand. Choosing the right buffer-management strategy would require a company not only to evaluate the buffer demand on a unit operation and process level, but also to evaluate the impact of each proposed preparation and distribution concepts on mobility, adjacency, and room classification.

As far as continuous processing is concerned, choosing ready-made buffers connected at the point-of-use, along with in-line dilution and stream conditioning for steps with low buffer-demand, could be an option. So could choosing a buffer-on-demand system with powder/concentrate that eventually will be formulated into buffers and released in real time for steps with moderate to high buffer demand.

Adapting that approach would mean that buffer needs/demands in DSP could be addressed continuously by a modular, intelligent buffer skid that is adjacent (e.g., in close vicinity) to the DSP skid but in a different room. That would eliminate the need for high-volume single-use mixers for buffer preparation and significantly reduce the area required for buffer preparation and distribution.

Faude: Buffer reduction in continuous downstream processing seems unable to adjust to increased media amounts used in USP. Using buffer concentrates is a very interesting possibility for reducing downstream buffer volume substantially, especially when DSP occurs in facilities that are not designed for continuous processing.

Filtration

Continuous filtration can involve problems related to low flux. Does this — or do other challenges — limit its potential in downstream processing? What kinds of technological solutions are needed?

Faude: Prepared backup filters placed in parallel might solve the problem. Switching to a parallel filter could be automatic depending on the flux course. Doing so might make filtration a simpler and better-controllable process step compared with continuous column chromatography.

Satzer: Filtration can be made continuous in two different ways: by exchanging filters or by using membranes in TFF mode (either single pass or not) with regular cleaning and regeneration or. In the end, all filters tend to foul over time in a continuous process and will cease to function at some point. The exchange of filters and continuous filtration can be used (and must be used in the case of dead-end filtration) and has been demonstrated by our group and others for viral filtration. In my opinion, the technology exists and can be used, but large-scale implementation is still missing.

Holzer: Many different filtration principles (e.g., micro-, ultra-, and nano-depth filtration and membrane adsorption) are applied during downstream processing. Most filters are used in a frontal-filtration mode batch process, in which concentrations change over time and reach the maximal limit of filtration capacity. Bioburden reduction, depth, or sterile filters are examples.

Redesign of those filters and unit operations into a tangential continuous process mode often is not of any process and economical interest. Therefore, the most efficient way is to work with staggered-batch or parallel-batch configurations. The flux is proportional to the membrane surface area or the number of filters used.

TFF processes allowing for continuously run unit operations are applied typically to cell separation, buffer exchange, and product concentration. Because the flow of a continuously run operation usually is lower than in a batch operation, technology is available or can be designed properly (for example, in the case of single-pass TFF).

Reducing the number of unit operations during downstream processing — such as by buffer exchanges or concentration between steps — is a great help for reducing cost and improving productivity. This is facilitated by the availability of chromatography media that can accommodate higher salt concentrations and flow rates with more targeted buffer choices.

The integration of viral filtration steps into continuously run DSP lines continues to be challenging. Process models and software could help us further characterize this unit operation, capitalize on historical data, and assist in process design and control. Use of process analytical technologies (PAT) to reveal rapid information about filter integrity or the amount of filtered product are of utmost importance as well.

Monge: Continuous processing created an additional driver to understand further the technical and operational aspects of establishing filtration steps toward manufacturing implementation. The worst-case criteria for batch-mode filtration do not translate to newer process scenarios. Besides a lower volumetric flow and prolonged exposure, significant challenges that need addressing include filter installation, integrity testing, and the impact of process variations and physicochemical feedstock variations.

The challenges mostly involve virus filtration because the number of other filtration steps is decreased in an integrated processing approach. Small, smart surge tanks are one way to reduce many concerns. They can serve to bridge downtime and compensate for process perturbations from other unit operations.

Daumke: In my opinion, the whole liquid flow needs to be optimized to reach the flow needed. Clarification the use of (for example) depth or alluvial filtration requires a certain flow to become effective. Therefore, other options need to be investigated (e.g., continuous centrifuges or TFF).

Chromatography

Continuous bind–elute chromatography involves concerns over residence time and loading capacities. Do you see this as a chance for more flow-through operations to succeed? What about alternative purification technologies such as precipitation/flocculation?

Satzer: Bind–elute chromatography cannot be run in fully continuous mode. Flow-through operations would be preferred, but in many cases they cannot offer the same purification efficiency as bind–elute chromatography. We demonstrated that precipitation/flocculation can yield similar purities to affinity chromatography in bind–elute mode while offering a constant mass flow. So I believe that those technologies (along with flow-through chromatography for polishing) will solve the issues of bind–elute chromatography.

Continuous processing created an additional driver to **UNDERSTAND** further the technical and operational aspects of establishing filtration steps toward manufacturing implementation.

That said, I think that bind–elute chromatography is here to stay. There might be separation and purification tasks for which no suitable precipitation technology exists, and the biopharmaceutical world is conservative when integrating new methods. It will tend to implement more familar methods (such as PCC), at least in the near future.

Holzer: Control of residence time and loading capacity is important for batch and continuous (countercurrent multicolumn chromatography) bind–elute as well as for flow-through chromatography (where impurities are bound and can break through if residence times and process conditions are not controlled). Such applications require tighter control and more process understanding for continuous bind–elute chromatography because robustness is reduced.

Flocculation and precipitation steps for impurities and target products are applied in several industrial production processes (e.g., impurity precipitation/flocculation mainly for products expressed in microorganisms and product precipitation in Cohn plasma fractionation). Based on the performance of protein A capture steps for antibodies, it might be difficult in this case to compete with bind–elute chromatography. Such alternative technologies need to be evaluated case by case, taking into account precipitation behavior, solubilization

conditions, processing times, product quality, yields, and so on.

Monge: Bind–elute chromatographic operations are by nature discontinuous processes and can become a bottleneck. Other techniques such as multicolumn and simulated moving-bed chromatography can bring resin-based systems closer to a continuous mode. However, the approach requires sophisticated hardware systems with tedious automation and validation needs.

On the other hand, membrane-based flowthrough polishing provides a cost-effective and highly productive alternative to resin-based bind–elute chromatography. Many experts anticipate that eventually all polishing flowthrough steps could be membrane-adsorber–based. Current concerns about larger volumes could be addressed with new process schemes. Given that chromatography steps comprise most of the process bottlenecks and complexity in DSP, alternative approaches such as precipitation or flocculation that increase product purity without compromising product quality/activity before chromatographic steps will help streamline the process.

Faude: Independent and continuous processing flowthrough operations are used wherever possible to shorten process times and achieve more economic separations. Even for chromatographies that traditionally were applied in bind–elute mode for MAb purification (e.g., cation exchange), suppliers are beginning to provide new resins designed especially for flowthrough applications.

Alternative technologies are under consideration, but platform processes are still in development. Technologies that can be used more generically will attract greater interest. An example is the recent trend toward considering flocculation technologies to optimize harvest-filter capacity and precipitation for optimizing impurity reduction.

Virus Safety

Low-pH and detergent treatments are difficult to automate, which is necessary for continuous processing. Viral safety is not a single unit operation, but rather is expressed as the cumulative effects of many operations. Does continuous processing bring new challenges to achieving such results? And which viral safety operations will be most difficult to adapt to a continuous approach?

Faude: Low-pH treatment especially needs equipment and technology for appropriate process control. Detergent treatment can be implemented easily from a technical point of view, but detergent removal, potential side reactions with the detergents, and the potential presence of trace impurities increase development and analytical costs. Additional challenges in virus clearance studies for continuous processes are to acquire representative starting materials and addressing ramp-up and shut-down phases as well as transient operation ranges of steady-state unit operations.

Satzer: The first parameter to assess when moving to continuous operation is a new definition for exposure time. Typically in batch processes this is defined as a certain time period, but for continuous operations there is a residence-time distribution (meaning that some molecules stay longer, some not as long in the low-pH environment). It has to be ensured that even molecules with shorter residence times will have adequate viral inactivation. This issue can be prevented by implementing larger safety margins or by using hybrid processes in which viral inactivation remains a batch operation.

Another approach is to redefine the viral inactivation step itself. Historically, this is set (for antibodies) to be around one hour. New research shows that a few minutes are sufficient for total viral inactivation. So in comparison with the current approach, there already is a very large safety margin. I think the greater challenges in viral clearance has to do with filtration, because filters have to be exchanged, and exchanges have to be validated. In general, technologies for implementation of continuous viral safety have been shown at laboratory scale, so I think implementation is rather straightforward for this step.

Holzer: New technologies such the BioSC pilot system (Novasep) allow for perfect control of unit operations such as low pH- or detergent-based viral inactivation, definition of different unit operations (such as staggered-batch, parallel-batch, or continuous multicolumn chromatography), integration of several different unit operations to achieve cDSP, and the possibility for different process scheduling of simultaneously run unit operations. Control and integration of viral filtration into cDSP needs more development.

Monge: Virus inactivation (VI) is considered one of the two orthogonal steps required for virus safety in a biotherapeutic production process. Making the process automated and continuous poses several challenges. Various industry groups have adopted different approaches — including a tubular plug-flow reactor, a continuous stirred-tank reactor, and column-based reactor — and successfully demonstrated the implementation.

The challenge currently is a lack of standardized and validated viral inactivation strategy at scale or in a proven scale-down model. With continuous processing, the significant problem is dividing an integrated operation into discrete unit operations for virus clearance testing. Virus filtration is a robust orthogonal method in downstream applications that is amenable for use in a continuous process. Issues that need addressing here are process variation/perturbations and physicochemical feedstock changes. However, in both cases, validation-scale challenges are real and need to be understood before implementing a full-scale continuous process.

Residence Time

Can you describe the distribution of residence time across a downstream process and how it affects products and the bottom line? Is this a problem for continuous operations, or is there a way to address it?

Satzer: Residence-time distribution through complete processes is poorly understood. Research has been limited because batch processing does not have or need a description of residence-time distributions. This question is unique to continuous processing and therefore relatively new in the biopharmaceutical world. Models are available for some

unit operations and for some molecules, but to fully understand all residence-time distributions of all unit operations and impurities and products, we still need a lot of research. To assess the question for product quality, we have to define what components to track, because not all have the same residence-time distribution. For instance, aggregates tend to elute at the front and back of a protein-A elution peak, creating different residence-time distributions for the product and for the impurities. Both have to be known before you can make any decision on product quality at the end of your process.

Only the generation of true "digital twins" with process models for all involved molecular species (such as product, impurities) can solve this completely. But discussion has only just started on what parameters have to be tracked and what might be omitted from those models.

Monge: Processing time ranges need to be characterized for unit operations and integrated downstream processes to ensure product stability and control of impurities. Based on the criticality of product residence time at a specific unit operation, additional studies might be necessary to define control strategies (e.g., tight control of pH and time for low-pH virus inactivation). The characterization of residence-time distribution becomes even more important in cDSP because it serves also as information for material traceability and helps during impact analyses in case of investigations.

Timing/Transition

It's a general belief that the transition from batch to continuous processing should be made as early as possible in product development. If you consider the challenges that come with transitioning too early and too late, where does the "sweet spot" typically come between them?

Satzer: At the moment we phase some issues in process development. In my opinion, the switch starts with the cell line. Current cell lines are adapted for fed-batch high production, so they might not be at peak performance for use in perfusion cultures. We experience quite drastic changes in impurity patterns and therefore downstream development when switching from batch-produced material to perfusion material.

SPEED to clinic and market are significant drivers for biopharmaceutical companies.

I think the earlier that transition is made, the better, and one limiting factor (availability of small-scale equipment for running perfusion) has been lifted recently with combining the ambr system (from Sartorius Stedim Biotech) with automated screening of chromatography performance using Robocolumns technology. Additionally, this might depend a great deal on the product produced.

For antibodies, we know that protein A will perform, regardless of what material you use, and minimal adjustments are necessary when the feedstock changes. But for products that do not have a high-performance affinity capture option, providing material that adequately resembles that produced in pilot/large-scale DSP as early as possible is crucial for process development.

Holzer: Typically, process design of countercurrent chromatography or membrane steps, plug–flow reactions, in-line dilution (ILD), and so on need additional developments and dimensioning studies. Therefore, this development stage would be perfect for implementation. The results of these studies allow also for specifying processing equipment requirements. However, changes that mainly concern process scheduling and not development of the unit operation to achieve cDSP could be implemented later in product/process development. In some cases, facility-fit challenges might be addressed with implementation of in-line buffer dilution or continuous multicolumn chromatography for one single unit operation even for commercialized products.

Faude: The development of continuous processing seems to be more time consuming unless you have a robust platform for some types of molecules. But that could conflict with the importance of time to [reach] toxicology testing and time-to-market considerations in early phases. The transition to continuous processing might be during late-stage development with a stronger focus on robustness and economical aspects.

Monge: Speed to clinic and market are significant drivers for biopharmaceutical companies. In this context, it would be better first to develop a batch-based platform process and manufacture product for clinical, process validation, preapproval/registration, and commercial scale. That would enable companies to enter the market and cater to its needs quickly without having to worry about uncertainties that continuous processing could bring from quality, manufacturing, automation, and regulatory perspectives. A parallel work stream needs to be in place where the same product is developed using a continuous process along with evaluation of the transition from batch to continuous manufacturing and identifying a bridging strategy. Once that is completed, then a postapproval change to a continuous process can be made by filing a prior approval supplement (PAS) as outlined by the US Food and Drug Administration (FDA) in its latest draft guidance covering quality considerations for continuous manufacturing.

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A Look into the Future of Bioprocessing

Maximizing Process Productivity, Efficiency, and Flexibility

A Summary of a Presentation Given By Dan Kopec

At the Conference on Bioprocessing, recently held by Sartorius Stedim Biotech (SSB) in San Francisco, one critical issue examined was the need to move new bioprocessing technologies ahead with much greater rapidity than has been common in the past. Dan Kopec, specialist for process analytical technology (PAT) at Sartorius Stedim Biotech, examined some key challenges currently facing the biopharmaceutical industry: maximizing process productivity, efficiency, and flexibility.

Kopec stressed that drug approvals today move forward too slowly and that the rate of approval is quite low. According to a report by the Sloan School of Business Management, only 14% of drugs in clinical trials eventually win FDA approval. There is ample evidence that a more rapid and expeditious system is badly needed in the bioprocessing industry today.

In his presentation, he outlined the application of process analytical technology (PAT), an approach to controlling and monitoring product quality during manufacturing steps. Its application is designed to move from a rigid approach to cell-specific productivity to a more flexible feed strategy based on a combination of inputs, including biomass and oxygen uptake and nutrient concentration. "This enables consistent high product quality," Kopec stated.

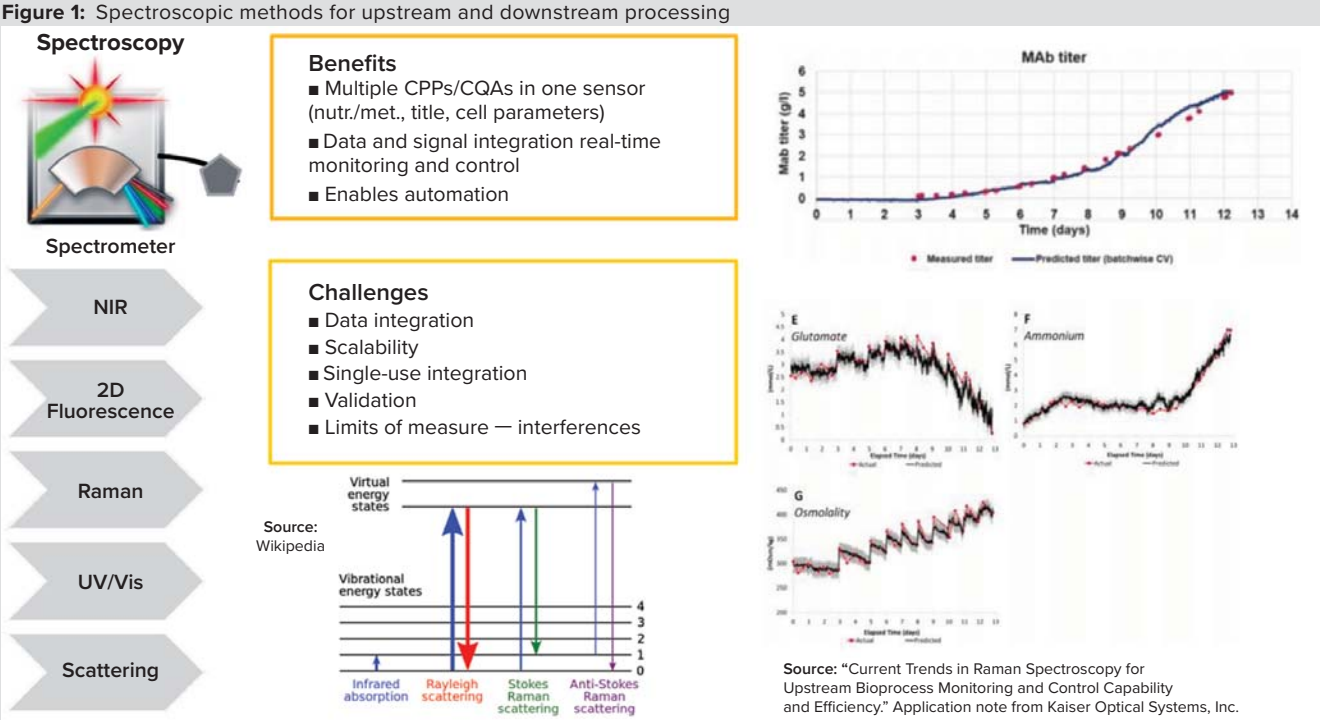
Moreover, the dynamic control provided by PAT enables a fast and predictive response to changing conditions, thus freeing up operator time. Conditions are monitored by a combination of spectroscopy — e.g., near-infrared spectroscopy (NIR), 2D fluorescence, Raman, UV/vis — and light scattering.

Spectroscopic methodologies are becoming more widespread in both upstream and downstream processing. Because they are label-free, they can be initiated online to monitor different quality attributes and thus are superior to offline techniques. The benefits of such an approach include data and signal integration and real-time control.

"Using real-time sensors allows a much clearer picture moving from research to development to manufacturing," said Kopec. However, he stresses that it will be necessary to advance other technologies, such as biocapacitance and dedicated nutrient and metabolic sensors to monitor processes that cannot be reached through spectroscopic technologies.

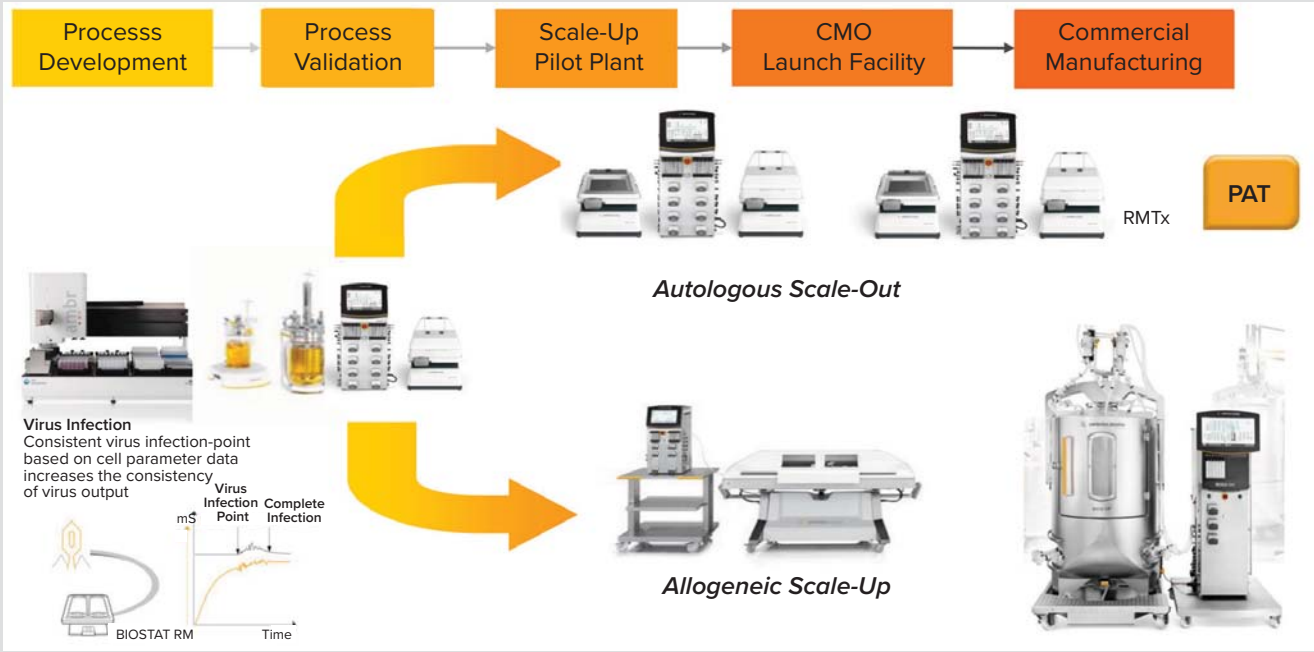
The expert argued that combining PAT with advanced data analytics offers important advantages to deliver a stronger, more functional technology. That provides for consistent, high-quality products; reduced cost of both up- and down-scaling; and an ability to account for variations in cells extracted from different patients (again delivering improved consistency). More reliance on automation lessens operator errors while freeing up staff to concentrate on other tasks.

Figure 1: Spectroscopic methods for upstream and downstream processing



Source: "Current Trends in Raman Spectroscopy for Upstream Bioprocess Monitoring and Control Capability and Efficiency." Application note from Kaiser Optical Systems, Inc.

Figure 2: A flow chart for application of process analytical technologies (PAT) in next-generation bioprocessing for cell and gene therapies



In addition to spectroscopy and multivariate data analytics, he described the third technological innovation: the use of flexible automated skids, which are essential for advancing downstream processing. Such units can handle different types of operations to make use of a "ballroom" operations concept.

Kopec stressed that biomanufacturing faces numerous challenges — including regulatory, logistics, and safety issues — as it moves into the next generation of technological developments. Existing pipelines of thoroughly established processes may be so well optimized that automation could cause a net loss of productivity. Moreover, product qualifications need to be taken into account, such as the differing behavior of dissimilar proteins. Kopec also noted that process parameters and quality attributes do not have workable, robust solutions yet.

Regulatory challenges to bioprocess automation also must be taken into account. Because automation technologies are such recent developments, they may not be covered by appropriate guidelines yet. For instance, there is no clear batch definition. Regulations once established for a two-week bioprocess must be adjusted for processes that potentially can run for months.

Kopec discussed the future outlook for bioprocessing, emphasizing the role of PAT and advanced data analytics. He anticipates that in two years such approaches will have expanded beyond what is now available to encompass wider adoption by the bioprocessing industry of multivariate data analysis and design of experiments. Standardization will be widespread to accommodate multiproduct setups. Hybrid modeling that combines statistical and deterministic principles will advance within the pharmaceutical industry.

Going out on a limb to five years, the PAT expert predicted widespread application of intensified and continuous processing driven by advanced automation. Plant-wide visualization will combine with electronic batch record retention and sophisticated analytical tools, including HPLC and mass spectrometry. Robotics will take over tasks that cannot be automated otherwise.

Kopec envisions a future (just a decade away) dominated by the industry "4.0 approach": a system of cyberphysical control that monitors activity through computer-based algorithms. Its adoption is predicted to generate a massive leap in productivity, enhancing the human workforce in ways that are difficult to conceive at this time. Fully automated, continuous bioprocessing pipelines will be controlled remotely and require no hands-on intervention. Data will be gathered, processed, and stored "in the cloud." Data will be applied to manufacturing processes for which physical location is irrelevant.

AUTOMATED continuous bioprocessing pipelines will be controlled remotely and require no hands-on intervention. Data will be gathered, processed, and stored "in the cloud."

The Sartorius team continues to design and test new concepts to work in the ever-changing regulatory landscape, including batch fingerprinting based on multivariate models and multianalyte sensors (Raman spectroscopy) based on computational models. "We anticipate machine learning, fully automated processes, and advanced simulation and prediction 10 years from now," Kopec predicted.

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A Data-Driven Approach to Design Next-Generation Manufacturing Processes

Summary of a Presentation Given By Anna Persson

In a discussion of continuous bioprocessing, Anna Persson, data scientist at Sartorius Stedim Biotech (SSB), emphasized that "continuous and intensified bioprocessing are attracting much attention from biopharmaceutical companies seeking to maximize the efficiency of their manufacturing assets." Benefits include reduced facility size and scale-up risk and increased throughputs and product-quality consistency.

To drive forward the data analytical process, Persson takes advantage of the Umetrics suite of data analytics solutions, a common, large-scale, automated platform designed for handling large quantities of data. Its predictive power comes through process modeling and data mining, enabling large improvements in real-life situations.

A challenge put forward by Lonza was a record of lower-than-expected average yield in its facility, creating difficulties for project planners and in delivering product to end users. After variability analysis to optimize yield and time-based multivariate data analysis of real-time process parameters, yield was increased and cycle time was decreased, resulting in a significant increase of plant output.

Similar strategies were put to use in a project that improved viable cell density 23% for Amgen. Another effort with Novartis analyzed "out of control incidents," leading to improvement of process consistency.

The Umetrics suite is supported by the US Federal STAR METRICS initiative: "Science and Technology for America's Reinvestment; Measuring the EffecTs of Research on Innovation, Competitiveness, and Science." This program drives similar projects and large-scale advances in the methodology to combine, mine, and analyze large research data sets. Figure 1 outlines its overall organization.

ADVANCED ANALYTICS are engaged in the modeling process and real-time implementation, enabling early fault detection, forecasting, and control.

Persson also highlighted SIMCA, "a powerful data exploratory tool that can chart trends and patterns not identifiable through univariate analysis." It can analyze historical data, increase process understanding, and perform troubleshooting and deviation analysis. The SIMCA-online program is designed to provide multivariate, real-time monitoring and predictions, increasing user confidence in process performance. The SIMCA-online control advisor package can forecast critical quality attributes and batch finish and carry out real-time predictive and prescriptive control.

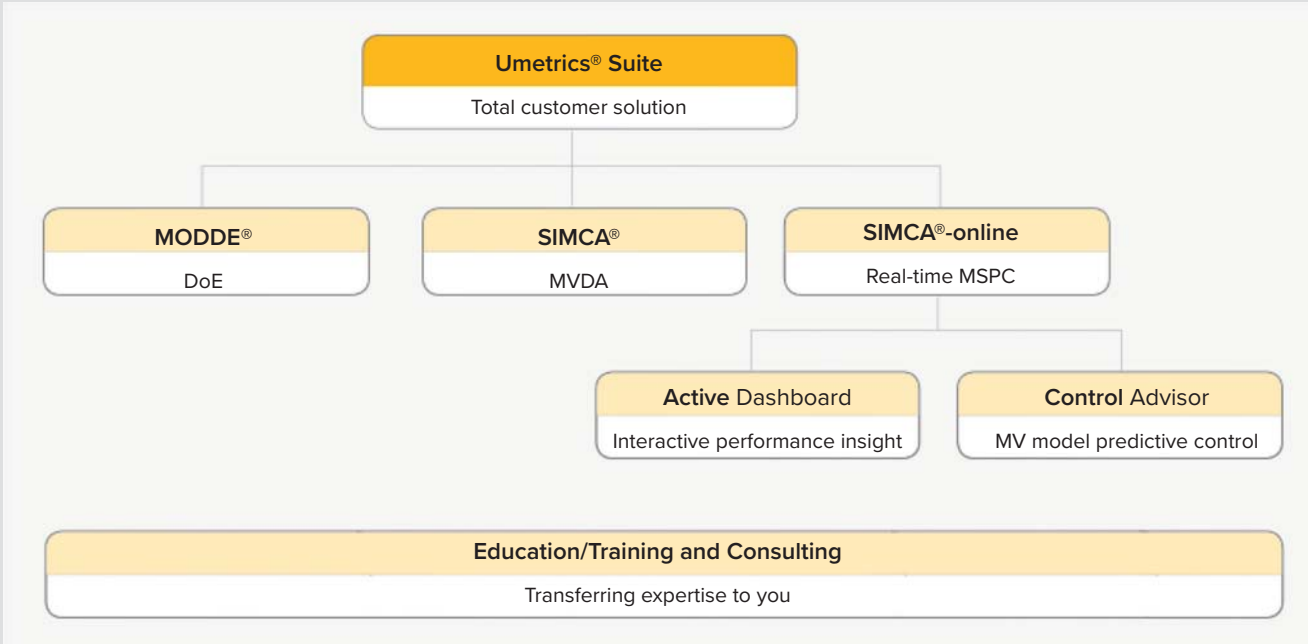
Another tool is MODDE software for analyzing decisions through total risk assessment. It guides users to more robust settings with a balanced multilevel design that provides optimal solutions to complicated design problems.

The data analysis expert discussed the power of Umetrics software to not only aid in description, but also to provide predictive analytics of what will happen and prescriptive analytics — that is, "how we can make a desired outcome happen," or alternatively, "how we can prevent an unwanted outcome from occurring."

Umetrics software has been of great value in driving the move from classic fed-batch to continuous or intensified bioprocessing. Advances in measurement technologies — including process sensors, spectrophotometers and other devices — have improved the overall quality of data available. Advanced analytical capability will help to increase process understanding and control. These advances generate significant value. The ability to optimize expression titers — even with a 1% increase — can provide large savings in cost of goods (CoG) over time. Contamination can be recognized more rapidly than ever before, and getting a handle on titer predictions can help identify technical solutions, such as the number of protein A cycles used for a particular procedure.

The comprehensive quality by design (QbD) approach is widely used in experimental design today. Advanced analytics are engaged in the modeling process and real-time implementation, enabling early fault detection, forecasting, and control. According to Persson, a paradigm shift is occurring from quality testing "postprocess" (after the fact) to

Figure 1: The Umetrics Suite comprises three packages: SIMCA, SIMCA-online, and MODDE. The SIMCA (soft independent modeling of class analogy) graphics package converts data into visual displays to facilitate interpretation of data sets.



advanced analytics supporting QbD. Consistency in product quality is achieved through continuous monitoring and control of critical process parameters.

Overall, the Umetrics suite contributes to a complete solution in intensified manufacturing, with applications in fed-batch, continuous, and perfusion processes as well as in downstream processing (see the "Scale Comparison" box). It supports understanding, process design, and scaling to help ensure consistent quality. It also aids in building a digital footprint. Most important, regulatory agencies recognize the program's value and utility.

Given the many features and applications of Umetrics software, widespread demand for continuing education is not surprising. Ongoing training is available at numerous locations worldwide and through on-site consulting services

The Umetrics analytical tool is applied widely to research in many contexts, as a literature survey indicates. For instance, Zanuttin et al. were able to classify wines from different Italian producers using Raman spectroscopy data analyzed with the SIMCA method (**1**). In a much different application, Jordan et al. modeled drug stability using Umetrics MODDE 10.1 software (**2**). In yet another completely unrelated field, Zolas et al. used the Umetrics package to investigate whether expertise gained by researchers in conducting their projects propagates into the broader economy (**3**). Other examples abound, but these few suffice to illustrate the ability of this software to help resolve a broad range of otherwise intractable research challenges.

Scale Comparison

SIMCA for ambr 15 to STR 2,000-L: Data from three classifications of scales from different campaigns: ambr 15, ambr 250, and a stirred-tank reactor (STR).

Three data types, collected once a day

- Initial conditions
- Daily (IPC) data
- Batch quality data

Data analysis in SIMCA

- Compare initial conditions between scales
- Overview of data to highlight issues for daily data
- Predict the final titer for ambr 250
- Predict titer for ambr 15 and STR scales using only the ambr 250 data

According to Persson, "Multivariate analysis is essential in value generation, and regulatory agencies are now encouraging the use of tools such as the Umetrics suite. Looking to the future, we foresee the use of mechanistic modeling and deep learning and an expansion into hybrid modeling."

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