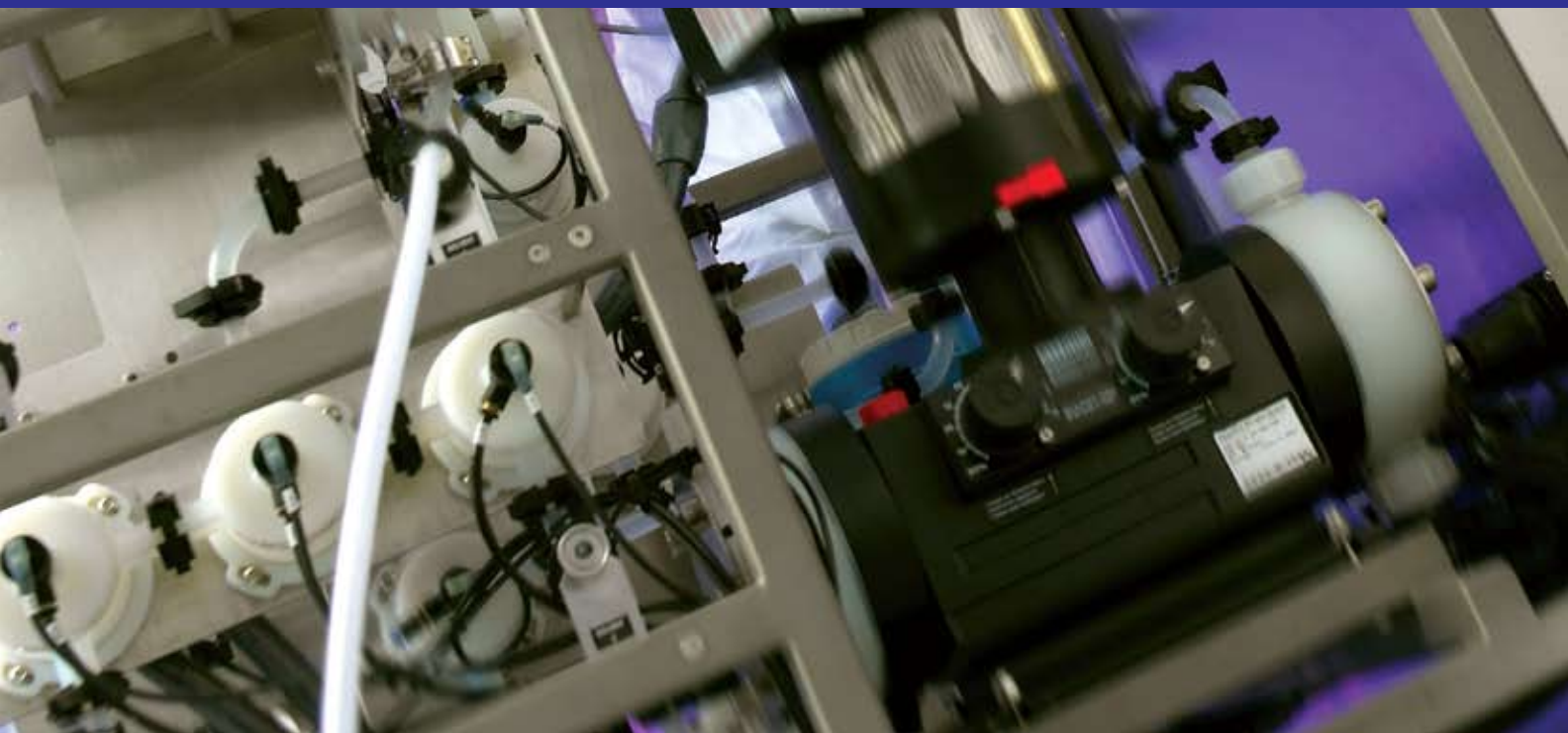




## Proactive Debottlenecking Planning Ahead for the Downstream Bottleneck

by Cheryl Scott, senior technical editor of *BioProcess International*

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# Proactive Debottlenecking

## Planning Ahead for the Downstream Bottleneck

by Cheryl Scott, senior technical editor, *BioProcess International*

It wasn't so long ago that people in the biopharmaceutical industry talked about a "capacity bottleneck" to describe the difficulty faced by bioprocessors as their many products moved forth through development to require production at larger and larger scales (1). Expression technologies at the time were making proteins at levels suggesting that huge amounts of manufacturing capacity would be needed soon. Just after the turn of the century, product titers (in terms of protein present per liter of culture broth/supernatant) of 0.5 g/L were common, and 1 g/L was considered impressive. Downstream processes that took those production titers and ultimately yielded around half of the original protein content in purified form were once considered good enough — but everyone could see that technological limitations were presenting an increasingly daunting obstacle to manufacturers of biological drugs. There were over a thousand products in the development pipeline, many of which would ultimately be needed in large quantities. Immunex's Enbrel troubles became the oft-repeated cautionary tale (2).

Cell and microbiologists stepped up to the challenge. They knew that the best way to lower the square-footage of manufacturing space needed to make the many drugs coming down the development pipeline would be to increase expression titers (3). More powerful producers would yield more protein in smaller bioreactors, requiring less water and media, less power and utility use, and so on. The solution was trifold: cell line engineering (4–6), optimization of growth media (7–9), and bioreactor improvements (10, 11). And the results have been spectacular. In just five years, production titers have improved to the point where 1 g/L, once respectable, is now quaintly old-fashioned — the new baseline. Regularly we've seen announcements of 2 g/L, 3 g/L, 4 g/L, 5 g/L, and even 10 g/L — with recent reports of record 15 g/L and an astounding 27+ g/L by the DSM/Crucell alliance (12).

Instead of disappearing, however, the so-called capacity bottleneck has simply moved downstream — because while those cell biologists and production engineers were busy improving the upstream output, separation and purification technology has pretty much remained the same. And now, it's being challenged with new impurities, higher concentrations of protein, and ever-increasing cost pressures as biopharmaceutical companies seek to push their products through development and to market as fast as

possible without compromising quality, safety, or efficacy.

Back in 2006, Eric Langer of BioPlan Associates identified the trend with his coauthor Joel Ranck:

Upstream production efficiencies and yield improvements have, in effect, created more capacity. Methods of filtering and purifying product streams are now the focus of attention for many manufacturers and suppliers. Changes on the downstream side in material recovery and purification are growing more urgent. However, such changes may not be as fast-moving or revolutionary as the industry would like.

"You don't have the same opportunity for dramatic changes downstream," said Scott M. Wheelwright, PhD, president of Strategic Manufacturing Worldwide. "We are likely to only see incremental changes. On the downstream side the challenge is how to handle that amount of protein. To date, there have not been a lot of advances. People still rely on chromatography, but there hasn't been much capacity increase. We're still limited in our ability to process protein.

Constraints may be particularly acute in monoclonal antibody (MAb) manufacturing, where product titers continue to increase. "The main bottleneck is how the protein A MAb purification process can be adapted to cope with the high yields of product without resorting to unfeasibly large columns with large inventories of protein A," says John Liddell, PhD, head of separation science for Avecia Biotechnology. Liddell explains that for non-MAb protein purification, challenges arise in the lack of any general platform purification approach and the necessity to approach development of purification schemes case by case. (13)

### TECHNICAL ASPECTS

Langer's annual survey of biopharmaceutical manufacturing capacity and production has tracked this trend. In elucidating the 2005 survey's results, he showed us that most manufacturers were greatly expanding their production capacity (and use), and many were concerned about their downstream processes' ability to handle those increases. Some respondents were advocating a "systems" approach to solving the problem strategically, saying that

incremental “piecemeal” improvements were inefficient and would be too expensive.

“You have to consider how the whole system will react,” explained Adam Goldstein, engineering manager for downstream process development at Amgen. He said the problem would not be solved by larger columns or companies using more purification runs, but rather that chromatography resin vendors would have to develop products that could offer increased capacity. “The way things are done now is to run multiple elutions over a column and then gang them up,” he explained. “But it’s not the prudent thing to do. A better solution is to get a higher number of protein loads on the columns themselves.”

Manufacturers using a systems approach would project and anticipate future upstream technological improvements, integrating those developments into their projections. As an example, Liddell mentioned single-use technology, which he said “allowed tankage to be largely replaced at early phase by disposable systems. Developments in this area are likely to continue . . . to have a significant effect on the throughput that can be achieved in an early phase facility.”

About a year later, Langer revisited the topic by reporting for us the results of his 2006 survey, and not a lot had changed (14). The downstream bottleneck had not yet fully manifested — but it was still anticipated — and neither had any real improvements in the technology come about. Almost half of respondents were seeing at least some capacity bottlenecks attributable to downstream processes, but few had any solutions to the problem. Interestingly, contract manufacturers were experiencing more trouble, with 64% of them reporting that downstream processing severely affected their overall capacity (compared with 39% of biopharmaceutical companies making the same claim).

The concern then was that research and development into downstream technologies on the part of vendor companies and such would not translate into solutions any time soon. Yuling Li, director of purification sciences in process development at Human Genome Sciences, warned that, “Innovation successes in downstream may not be just around the corner. For innovation to reach the commercial stage, we may still be years out. Yes, we need to optimize areas such as chromatography media, but the real breakthroughs will come from innovation. These downstream innovations may include membrane technologies, which are very promising in terms of improvements to throughput and capacity. They may also come from reoptimization of technologies such as aqueous two-phase systems, crystallization, and others. There are a number of technologies being developed today in response to the need for lower costs and higher throughput.”

Geoff Hodge, vice president of process development and technology at Xcellerex, Inc., explained that “with typical downstream yields for a monoclonal antibody process at ~60–70%, a twofold improvement in purification efficiency would be overly optimistic.” He felt that significant improvements in purification would not be made by incremental, continuous improvements to current technology — but would come from new technologies instead.

## WHAT THE EXPERTS ARE SAYING: VALIDATED BIOSYSTEMS INC.

### Pete Gagnon, consultant, Validated Biosystems Inc.:

“In one sense, the bottleneck is artificial. Cell culture production takes about two weeks (not counting preparation of seed stock) and purification takes about a week. In another sense, the bottleneck is real, and a genuine concern. Process time for the protein A capture step from 20,000 L of cell culture supernatant (CCS) commonly requires 72–96 hours. This represents multiple cycles. The long hold time for IgG produced in the early cycles increases the risk of degradation by proteolysis, deamidation, etc. It also increases the risk of contamination. The bottleneck is also real from an economic perspective. Long process times tie up expensive manufacturing space and limit overall facility capacity. They also inflate labor costs per unit of finished product. Anion-exchange membranes have accelerated flow-through polishing purification of IgG and established the industrial value of convective mass transport, but they have not proven competitive for bind-elute applications.” (26)

Improvements in separation/purification are not like those that were made upstream. Production engineers at the biotherapeutics companies, themselves, were able to tackle those problems. But the industry is much more reliant on its suppliers for answers to downstream capacity questions.

Respondents to Langer’s fourth annual survey expected that improvements in membrane technology and simulated moving-bed chromatography were the most likely technologies to improve their downstream processes over the coming five years. Langer warned, however, that the industry should be prepared for a long learning curve and slow regulatory acceptance of new technologies. The more unfamiliar the technology, the more questions both users and regulators will have about it.

The most obvious area for improvement, about two-thirds of survey respondents agreed, was fouling of filter membranes — and Langer said the vendors were responding with improvements already (14, 15). “The major problem experienced by biomanufacturers involving microfiltration steps is primarily lost productivity,” said Jerold Martin, senior vice president of scientific affairs at Pall Life Sciences. “If an operation has to be halted to change a filter, that time can equate to significant money.” Other areas of concern in filtration were throughput, production scheduling, and filter cost (14).

User concerns about all the above have kicked technology providers into gear. Vendors know a good business opportunity when they see one — and just as with therapeutics, the first to supply a workable solution to a problem will get the lion’s share of the market. We at *BioProcess International* had noted a lot of activity in the upstream area between 2000 and 2005, evident even in just the number of submitted articles on related topics. Since then, the volume of submissions has shifted slowly downstream topically. Now lots of solutions are being suggested, but it remains to be seen which will survive the gauntlet of biopharmaceutical development and regulatory scrutiny.

According to Uwe Gottschalk, vice president of purification technologies at Sartorius Stedim Biotech, some

## WHAT THE EXPERTS ARE SAYING: DIOSYNTH RTP

**George Koch, chief scientific officer at Diosynth RTP, Inc. :**

“It is likely that smaller bioreactor volumes of more precise antibodies will become the rule and thus that current purification technologies will prove to be quite adequate for the task of meeting both the health needs of patients and the business needs of the industry.” (42)

methods of opening up the downstream bottleneck include further optimization of chromatographic methods and media, process intensification (a concept borrowed from other process industries, in which “out-of-the-box” engineering has allowed for reductions in plant size), nonaffinity chromatography strategies (e.g., mixed-mode separations and monoliths), technology transfer from other industries (e.g., expanded-bed and simulated moving bed), new/alternative ligands (mimetics, etc.) and matrices (membrane adsorbers, monoliths), and the “anything but chromatography” approach (15, 16). Moving away from chromatographic separations, some answers may be found through new ways of looking at old technologies: centrifugation, extraction/precipitation, filtration, crystallization, and ultraviolet inactivation of viruses. And the concept of process intensification encompasses several approaches that have been considered separately in the past: moving from batch to continuous processing, a multidisciplinary systems approach, and process technology platforms that provide flexibility in multiproduct environments.

**Protein Capture and Refolding:** For many microbial processes, the first and foremost rate-limiting step in downstream processing is protein refolding. This is because microbes tend not to secrete recombinant proteins into their culture medium (like most eukaryotic cells do) but rather keep the proteins within their cell walls, where they form aggregated clumps called inclusion bodies. The cells must be disrupted mechanically to free the product, but the aggregated proteins are seldom correctly folded — and correct folding is necessary for their *in vivo* function. Traditional methods of refolding have included dilution and filtration with special buffers, and liquid chromatography has recently begun to be applied.

Langer found in his third annual survey that protein refolding presented a major opportunity for improvement with microbial-based production (13). Wheelwright said, “If you have higher expressions, it doesn’t help in the refold buffer. That remains a challenge unless we come up with better ways to refold.” Langer suggested in-line refolding as a potential alternative, with protein injected into a flowing stream and mixed in a pipe. That technology remains to be optimized, but a group of authors in China suggested an approach based on high-performance hydrophobic-interaction liquid chromatography (HPHIC) that could both separate and refold aggregated proteins in a single step (17). They reduced an eight-step manufacturing process of recombinant human granulocyte colony-stimulating factor produced by *Escherichia coli* to five steps: fermentation, extraction, HPHIC, buffer exchange, and fill/finish. Doing so at large scale could save time and money for companies using microbial production.

Even those processes that don’t require a refolding step face difficulty right from the start. Combining simple harvest with separations, as described above, could help companies speed up their downstream operations. Methods of doing so are typically based on membrane filtration or liquid chromatography (18, 19). Centrifugation separates components of a mixture by size using the simulated gravity of centrifugal force, and it is best applied to clarifying an initial harvest. The mechanisms of filtration and chromatography are more complex — and thus more capable.

**Filtration:** In their simplest form, filter membranes act like sieves to retain larger particles while allowing smaller ones to flow through. Anyone who makes coffee knows that. However, some filters defy common sense by trapping particles smaller than their pore sizes. They use electrical charges and other properties to trap particles within the pores (depth filtration) or on the filter surface (as in tangential-flow filtration). Depending on its properties, a desired product can be trapped or left in solution.

The problem with high-titer product streams comes from their high concentration of molecules in solution. Membrane filters can clog (“foul”) pretty quickly when faced with highly viscous solutions or simply those carrying a high load of proteins and impurities. As far back as February 2005, we published an article that proposed an approach to the fouling problem through understanding its actual causes and selecting filters that counteract those effects (20). Later, the same author joined with others to discuss the topic in light of process scale-up (21). Referring to the caked solids that accumulate on a fouled membrane, they wrote

The thickness of that solute cake depends on a number of factors, including the rate at which permeating liquid brings solute to the membrane surface, the rate at which solute back-diffuses into the feed stream, and the hydrodynamic shear of the tangentially flowing stream. Successful exploitation of membranes in crossflow filtration therefore largely depends on effective fluid-management techniques. (21)

Scaling up is clearly the main issue when it comes to the downstream bottleneck. And the physics of filtration scale-up are far from simple or straightforward. NCSRT, Inc. is one company focusing on improving fluid dynamics and

## WHAT THE EXPERTS ARE SAYING: LAUREATE PHARMA

**Yamuna Dasarathy, senior marketing manager of Laureate Pharma, Inc.:**

“Yes, a downstream bottleneck does exist in purification process development. It is less of a bottleneck for antibodies than for other proteins. Non-protein-A affinity-based purification processes can involve several unit operations, with packing of different columns, validation, and qualification slowing down the process. For small companies, equipment availability, turnaround time, and stability studies can add to the bottleneck. Simplified purification processes as well as very careful planning and coordination between upstream and downstream processing will minimize it somewhat.” (43)

combining harvest with separations with disposable, scalable devices (22).

Filter vendors were among the first to offer single-use options to biopharmaceutical customers: e.g., Sartorius Sartobind, Pall Mustang, and Millipore Intercept membranes. In the process of developing such technologies, these companies (and others) have managed to create an entirely new class of products: membrane chromatography, whereby filter membranes perform chromatographic-like separations (23). They can provide equivalent separations to large anion-exchange columns, for example, in a relatively smaller space, using relatively less buffer. And their disposability eliminates the cleaning (and related validation) and equilibration of column chromatography. Membrane chromatography is currently touted as one of the most promising ways to open up the downstream bottleneck.

**Chromatography:** Even though their products are traditionally paragons of reusability (e.g., glass and stainless steel columns, resins that are washed and reused many times), vendors of chromatographic supplies have also found ways to offer single-use technology. These include prepacked columns and monoliths (24–26). Disposable prepacked columns were first used in analytical applications, but companies such as Pendotech, DSM Biologics, and Upfront Chromatography are working to develop disposable chromatography systems based on the concept for process purification. Disposables save money in other areas of bioprocessing; they should do so here — so long as their economies aren't outweighed by the proven savings of reusing column packings.

Monoliths are solid discs of porous methacrylate material that come in different heights and depths roughly correspondent to a packed resin bed inside a traditional chromatography column (24). They are disposable and offer several benefits that can help companies address the downstream bottleneck. Because they're solid, monoliths are unaffected by a broad range of flow rates, even those high enough to disrupt packed beds of loose beads. Monolithic media have high capacities for binding large biomolecules (e.g., 10 mg plasmid DNA/mL, 20 mg genomic DNA/mL, and 25 mg virus/mL). Scale-up is facilitated by switching out otherwise identical supports of different sizes. And monoliths can provide the same separations as deeper column bed heights in a more compact depth, minimizing conformation changes and inactivation of biomolecules with less nonspecific binding (24). We first saw this concept in *BioProcess International's* October 2004 issue, and since then it has received increasing attention as the downstream bottleneck becomes more of an issue.

Scale-up in chromatography has traditionally required larger and larger column diameters while the depth of the packed chromatography resin bed remained the same. A laboratory-scale process might use a 50-cm column with a 100-cm bed height, the same process for making clinical trial materials might use a 1-m column with the same 100-cm bed height, and full-scale production might imply the use of a 3-m diameter column. Oops, that's not really an option, is it? Therein lies the problem.

## WHAT THE EXPERTS ARE SAYING: BAC BV

**Laurens Sierkstra, chief executive officer of BAC BV:** "We are in a very interesting time for downstream process development, and I believe that the advancement of novel technologies will continue to be a challenging activity for the coming years. A number of new technologies will undoubtedly be important in the future. However, it will take a long time before these technologies are used in validated production processes.

"At present, there is no technology in development that promises to satisfactorily replace currently validated techniques such as chromatography for the first capture step. Chromatography in one form or another will always be part of downstream processes. Previously acknowledged limitations with technologies such as affinity chromatography have been addressed over recent years.

"For example, the CaptureSelect affinity ligands we have developed at BAC meet the need for a general platform to purify non-MAb proteins at high yield and in far fewer process steps than with traditional ligands, thereby enabling increased productivity. Our affinity ligands are custom designed for individual projects, and they have been validated by several large pharmaceutical companies."

Meanwhile, the volume of buffer solution roughly doubles with each 5 g/L titer. And of course, wider columns need more chromatography resin to fill them even to a relatively short bed height. Ion-exchange and synthetic media cost about \$2/gram and can be reused about 200 times. Protein A and other protein ligands, however, can cost up to \$16/gram. They provide by far the best-resolution separations — but the price of such affinity media precludes their adaptation to a single-use environment.

Many chromatographic solutions are being offered to the problem of scale presented by the downstream bottleneck, from improving affinity media (27–29) to combining steps and mixing modes of separations (30, 31) to adapting techniques from other industries to work in bioprocessing (32, 33). But a surprising number of people are starting to wonder whether biotechnology has outgrown chromatography entirely (34–36).

**Easy as ABC?** In a recent presentation at a monoliths symposium in Slovenia, purification expert Pete Gagnon told meeting attendees, "The inability of traditional chromatography media to keep up with cell culture production has led many companies to pursue an 'ABC' approach to antibody purification: anything but chromatography." This concept is central to new efforts in downstream bioprocessing because the cost of chromatography columns is a large part of manufacturing costs as a whole. Academics such as David Wood, assistant professor of chemical engineering at Princeton University, are looking into highly scalable alternatives while realizing that many companies will be resistant to ABC because of unknown regulatory implications.

"ABC suggestions to date have concentrated on variations of precipitation methods that were previously used for plasma fractionation," Gagnon explained. "Amgen has developed and presented results from an undisclosed

## WHAT THE EXPERTS ARE SAYING: UPFRONT CHROMATOGRAPHY

**Rob Noel, business development manager of Upfront Chromatography:** “The Rhobust technological platform developed by Upfront has unique advantages for both single-product industrial-scale manufacturing and the multiproduct manufacturing requirements of CMOs.

“Industrial-scale Rhobust technology enables the capture of monoclonal antibodies and other therapeutics directly from a bioreactor, eliminating the need for all clarification unit operations. For an industrial-scale facility producing one dedicated MAb product, it has the major advantage of saving cost and increasing yields by reducing the number of processing operations.

“At the same time, Rhobust disposable chromatography addresses the needs of manufacturing facilities that must process many different MAbs, where the provision of disposable separation solutions is more attractive. In this instance, the ability to load bioreactor contents directly onto a capture adsorbent reduces process development time as well as consumable costs — on top of the known advantages of operating single-campaign, disposable units.”

method capable of achieving 99% antibody purity with a precipitating agent that can be recycled” (26).

Gagnon himself sees more potential in chromatographic monoliths, but Wood advocates self-cleaving fusion tags (36): “The discovery of intein-mediated protein splicing in 1990 has led to the development of a variety of self-cleaving fusion tags for protein purification. When combined with existing systems, modified inteins allow conventional affinity tags to become self-cleaving, greatly simplifying this method for protein purification.” This method provides a pure protein without chromatography separations. A target-protein gene is fused to a self-cleaving aggregation tag, which causes it to precipitate out of solution under specific conditions.

According to Matthias Berkemeyer, head of downstream process development at Boehringer-Ingelheim, crystallization may be another option (35). Such processes would be highly specific to each protein, however, and companies with an interest in platform technologies (basic “boilerplate” processes that are adapted to products that are basically similar, such as monoclonal antibodies) may not want to try it. Even if it’s not used in downstream processing, however, crystallization may well replace lyophilization in stabilizing final protein products for transport and storage.

### BUSINESS ASPECTS

The downstream bottleneck isn’t all about science and technology — and it’s not caused solely by the increased protein titers coming from improved cell lines and optimized production upstream. As the biotechnology industry has matured, its investors (from “angels” to venture capitalists, corporate partners to stockholders) have been expecting more returns on their investments — or at the very least, signs that companies are operating efficiently. That means saving money wherever and whenever possible. Downstream processes make up a majority of the

manufacturing cost, which admittedly represents only about a quarter of the total cost of goods for a biopharmaceutical company. Even so, separation and purification are being singled out as an area for improvement.

Higher-titer production shifts even more of the cost burden of manufacturing downstream. Uwe Gottschalk told the audience at the BPI Conference in Europe this year that, “As titers increase from 1 g/L to 10 g/L, batch costs triple, and the proportion of that represented by purification increases from 53% to 81%.” As illustrated above, chromatography is certainly a primary reason for that. Moving from batch to continuous separations — a major tenet of process intensification — would certainly make a difference (37). That’s the premise behind simulated moving-bed chromatography. Because continuous processes can outperform batch processes in yield, purity, and productivity, a continuous ion-exchange process could provide the same separation as a batch protein A affinity process — at a lower cost. Continuous chromatographic methods are so impressive, even makers of small-molecule drugs are beginning to consider them.

One way to improve purification economies would be to decrease waste. Half of a valuable protein of interest has been known to get lost in a single downstream process step — and that kind of wastage is unacceptable, even with high-titer harvests (38). Monoclonal antibodies are usually high-dosage products and expensive to make, for example, so companies pursuing this product category need not only high-titer expression systems, but high-yield downstream processes as well. According to Brian Kelley, director of purification process development at Wyeth Biopharma, “the industry is planning for protein manufacture at the ton scale” (38). The economics of downstream processing come down to scale, cost, and yield. Technological improvements in recent years have increased final yields from around half the original protein of a product stream to something more like two-thirds to three quarters (39). Here, incremental work has paid off — as the yield for each step improves, so too does the overall yield of the process as a whole.

The approach many companies are taking to overall process improvements is referred to as *operational excellence*, a general term that encompasses many business approaches including lean manufacturing, six sigma, and process intensification (40). Not surprisingly, many companies are tackling the downstream problem with these same tools (41). Automation, disposables, and combining steps are all vital to these efforts. The US FDA’s process analytical technology (PAT) initiative encourages incorporation of such concepts as a means by which companies can improve not only the economics but the robustness and reliability of their processes as well.

In the end, it is believed that the answer to this problem, as to the production problem, will probably be threefold: improving yields, reducing batches or instituting continuous separations, and lowering costs by using new and improved resins (or membranes) and buffers. The better, faster, and cheaper production becomes, the more so the same kinds of results will be expected from downstream processing.

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