

Revamping Recovery & Purification Strategies

Search Continues for Effective Alternatives

K. John Morrow Jr., Ph.D.

Increasing bioreactor titers bring an economy of scale, but no such effect is realized in the subsequent chromatographic separation, where an almost linear increase in cost is seen, reported Uwe

Gottschalk, Ph.D., vp, purification technology at Sartorius (www.sartorius.com) at the recent IBC "Bioprocessing Conference" held in Boston.

Process chromatography for bioseparation is favored by the industry and will remain so for the foreseeable future, driv-

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ing the search for alternatives without the mass-transfer limitations of resin-based chromatography, according to Dr. Gottschalk who was chairman of a session on recovery and purification.

As Dr. Gottschalk sees it, the initial capturing step, although highly selective, is the bottleneck in current manufacturing processes. This carries dramatic consequences for column sizes, buffer preparation, and holding capacity. Even with operational excellence, dynamic binding capacities cannot be pushed beyond their inherent physical limits.

In the case of flow-through polishing steps, the mass-transfer limitations are principally the same, although product molecules are flushed through the column whereas all contaminants bind. Packed-bed chromatography leads to oversizing because of flow rate and pressure restrictions, an exigency that drove the introduction of membrane chromatography and monolithic chromatography matrices.

As a result, contaminants can be removed in a short cycle time with packaged, disposable membranes requiring only a minimal buffer volume. In addition to technical demands, the quality requirements of cGMP regulations are pushing tighter specifications and higher safety margins for targets such as small, nonenveloped viruses.

"The future may bring a generic process built around an initial precipitation or crystallization operation after which only flow-through-based polishing steps are necessary," Dr. Gottschalk predicted.

Escape from Protein A

Antibodies are ordinarily purified using affinity columns or affinity membranes, to which either Protein A or target antigen molecules are embedded in order to bind the desired antibody material. But these are costly approaches, especially when they must be scaled up for large batches. The resin and the reagents alone can cost millions of dollars, so there is a spirited search for alternatives.

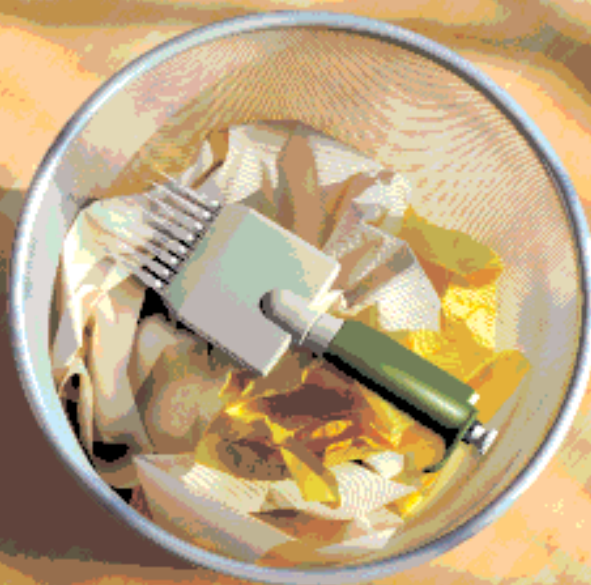
Michelle Wang, Ph.D., a senior manager in purification and process development at Medarex (www.medarex.com), has pursued, with her colleagues, a search for suitable alternatives to affinity purification. She presented their studies on the primary-recovery tangential-flow filtration technology in human monoclonal nonaffinity purification. "In our studies, we focus on scalability and robustness on the one hand and process improvement and economics on the other," she stated.

Tangential-flow filtration (TFF) is a separation technique in which the fluid to be processed is pumped tangentially to the separation medium, rather than through it. As it sweeps by, the retained components do not build up at the surface of the membrane but are pulled along by the fluid force of the tangential flow.

Typically, a nonaffinity scheme for antibody purification contains a primary-recovery step, a capture column, a membrane-chromatography step, and a polishing column. Dr. Wang noted that in designing a nonprotein A purification platform, a

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totally different approach is required. “When protein A is used to capture the antibody, the clarified cell supernatant can be placed directly on the affinity column,” Dr. Wang explained, “but when using a nonaffinity capture, the diafiltration at the primary-recovery stage gives us the leverage to have a consistently cleaner load for the nonprotein A capture.”

Diafiltration is a membrane-based method wherein pore size dictates the retention and elution of material from a sample. Samples can be recycled, gradually removing unwanted low molecular weight materials and making the sample acceptable for further purification. A major benefit from the diafiltration step is that DNA contamination is significantly reduced, which makes subsequent steps easier. The Medarex team found that processing time can be reduced by using a buffer with lower pH and conductivity than those of the diafiltration endpoint.

By including this TFF primary recovery step and other purification conditions, Dr. Wang and her team were able to increase the column lifetime and performance as well as obtain a better hold on the stability of the product. “The buffer exchange conditions play a crucial role by reducing batch volume and processing time and also providing partial purification by lowering host-cell contaminants,” said Dr. Wang.

The critical factor guiding the adoption of nonaffinity-based purification methods is the cost of doing business. With increasing bioreactor productivity and the optimization of the primary recovery through tangential-flow filtration, nonaffinity-based technology becomes more economically appealing. Given that many producers are realizing much higher concentrations at the upstream end, Medarex is excited about the significant promise of this new strategy.

Optimizing Cell Line Performance

How does quality by design apply to cell line development? Pranhitha Reddy, Ph.D., scientific director, process and analytical sciences, at Amgen (www.amgen.com), spoke about designing and developing formulations and manufacturing processes to ensure predefined product quality. Previously, this concept was applied during commercialization but now it has been adopted at Amgen to screen and select the overall properties of a mAb product in the early stages of development.

The product quality attributes of an antibody therapeutic are influenced by its amino acid sequence, cell line, and process design. These parameters can be selected for by screening a panel of clones that harbor multiple antibody candidates for acceptable product quality traits. Those that fit the downstream process development platform and deliver acceptable product quality attributes are corralled by the screening process.

Dr. Reddy presented an example of the manner in which the cell line and molecule sequence influence product quality and

downstream process development. Her data detailed the elution pattern of high molecular weight peaks as they eluted a mAb off a size-exclusion column.

Clearly, the downstream process had to be optimized to limit this species to an acceptable range, which prolonged the development timeline. Her analysis revealed that the amino acid sequence in the CDR3 region was the root cause of

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According to Sartorius, the initial capturing step, although highly selective, is the bottleneck in current manufacturing processes.



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Medarex (right) advocates the use of tangential-flow filtration in which the fluid to be processed is pumped tangentially to the separation medium rather than through it.



Tarpon Biosystems (below) seeks to lower downstream purification costs by combining the technologies of chromatography media and columns in a novel format.



this undesirable heterogeneity profile. Other features of the molecule that can influence the purification efficiency include the VH3 domains, the presence of extra cysteines, and the isoelectric point of the molecule.

By setting parameters and limits for the desired end product at the start of the study, the investigator can ensure that the platform delivers the best process and desired product results. The bioactivity and product expression levels are vital, as is the purification fit, which includes the molecular parameters of the particular protein including its propensity to form aggregates and maintain stability. Conditions that influence capacity to withstand harsh purification protocols are used to screen candidate molecules. Further along, molecule solubility, critical to purification and formulation, is also screened.

Using case studies, Dr. Reddy expanded her discussion to illustrate how clonal and molecular screening methods can reveal the path to the elimination of undesirable levels of product aggregation in purification or solubility in platform formulation during the final candidate selection process.

With a surfeit of clones to select from, Dr. Reddy counseled close collaboration with the purification team to balance the properties of the clones and to eliminate early those that could lead to less robust performance. "Exercise restraint and self discipline," she advised, "Supply representative cell culture supernatant from clones and upstream process for purification development."

By following these guidelines, data can be mined for better predictive tools, and a better understanding of the clinical relevance will expedite the drug development process.

mAb Capture by Multicolumn Chromatography

Tarpon Biosystems (www.tarponbiosystems.com) seeks to lower downstream purification costs by combining the technologies of chromatography media and columns in a novel format. With industrialization of biotechnology moving at a rapid pace, purification has become the dominant expenditure of biomanufacturing, now up to 65% of total downstream processing costs.

Tarpon, through a deal with BioFlash Partners (www.bioflashpartners.com), has exclusive access to a product line of prepacked, disposable, presanitized cartridges. BioFlash DFC™ are disposable-format chromatography cartridges for multicolumn chromatography applications. According to bioprocessing director Marc Bisschops, Ph.D., these cartridges can provide high chromatographic performance with virtually any medium and eliminate the labor-intensive task of cleaning and revalidating columns. "Moreover, the units are scalable from bench to large-scale manufacturing," Dr. Bisschops stated.

The company has adapted traditional simulated moving bed technology (SMB) to address the requirement for sanitary processing in biotherapeutic manufacturing through the use of biocompatible, cost-effective disposable-format components such as valve cassettes. These items are proprietary to Tarpon, which has assigned them the product name of BioSMB™.

SMB was invented within the petroleum industry a half century ago and has since found application in pharma, the food industry, and in biotechnology. The rationale for adoption of SMB is to minimize solvent use and separation media in order to reduce the cost of operation as compared to traditional batch chromatography.

The modular column units can be assembled in series, so one feeds into the

next, and products can be shunted off when their concentration is optimized. This operation mode provides a continuous chromatography system. BioSMB as a purification strategy is a volume-driven process, rather than mass driven.

This means that as cell culture titers continue to increase upstream, a BioSMB downstream system can handle varying titers efficiently without changing system hardware, simply by adding more disposable valve cassettes and cartridges. In a cost of goods analysis, taking into account production, labor, consumables, materials, and equipment, the BioSMB system was far superior to conventional separation, Dr. Bisschops concluded.

Another firm seeking to optimize its downstream processing technology is Boehringer-Ingelheim (www.boehringer-ingelheim.com). Dorothee Ambrosius, Ph.D., director of downstream development, and her coworkers are probing the limits of these manipulations. The company has a significant commitment to contract manufacturing of biologics, using both mammalian cells and microbial systems.

Dr. Ambrosius' approach is to think commercially from the beginning of a biologics production project, with the aim of reducing the time to the clinical phase and shortening the overall time to market.

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is one of monoculture that tries to grow one type of cell in isolation, to the next level where we must deal with populations. Controlling cell differentiation and maintaining equilibrium cell populations will be another huge challenge in bioreactor design.

- **Development of organ and tissue culture bioreactors:** These devices will need internal structures that in some way mimic vasculature to provide nutrients and remove metabolic products. Nanotechnology and self-assembling structures may prove to be the way to finally grow organs.

- **High-density production of cells:** The human body is remarkably efficient, producing 7×10^9 erythrocytes/day at a concentration of 2×10^9 cells/mL. This is about 100 times the capacity of the best bioreactor currently available.

- **Bioreactors with better attachment surfaces for cell growth:** The key feature for this application will be new techniques to attach and detach cells under complete control and with minimal damage.

- **Bioreactors for the culture of difficult cells:** New bioreactors need to be developed that can be used for the expression of cells such as astracytes and neurons.

These reactors could be constructed so that they incorporate layers of feeder and stromal cells to provide the necessary growth factors. Perhaps even microbes can be incorporated into these reactors to present antigens.

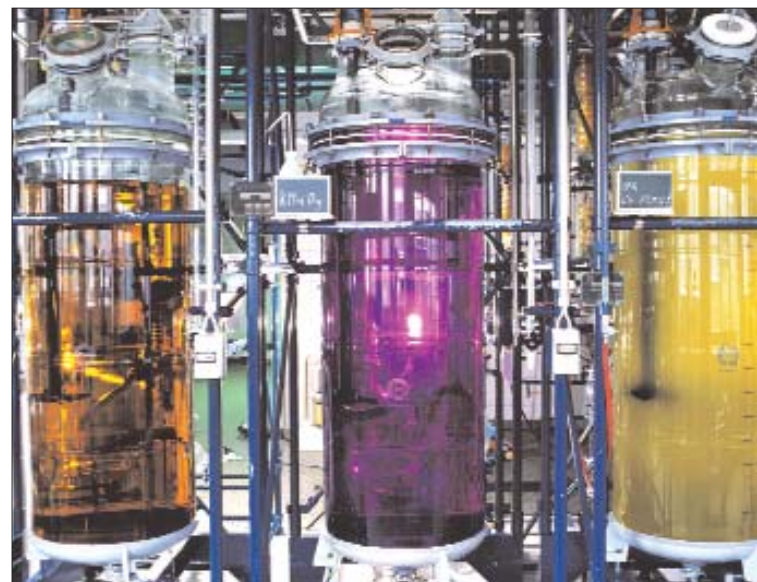
Microbes have been on this earth longer than humans. In fact, the ecosystem of this planet was made habitable for humans by the action of microbes hundreds of millions of years ago. Humans cannot exist without microbes. Consider that the human body is not even a single being but really a host-microbe community. The human body is host to about 10^{12} to 10^{14} microbes. Consider this relative to the 10^{13} or so human cells in the body. By this count we are about half human!

In a sense, the human body is just a bioreactor, which lives on another bioreactor—the earth. So, the future of the bioreactor is really our future.

With this said, the embodiment of the bioreactor in the future will be what we need it to be: a device to grow cells, make replacement organs, produce medicines, grow food, treat wastes, and to generate energy. The bioreactor of the future will

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have many manifestations depending on our needs and our imagination. Like any other tool, it serves a purpose and must change with that purpose.

As Abraham Maslow said, "When the only tool you have is a hammer, every problem looks like a nail." The bioreactor of the future may look like the bioreactor of today or may not. That is not the point. The issue

is what the bioreactor of the future will do for us and how it will change our lives. For that we must understand what we need to build to solve our problems and not be limited by the familiar and traditional.

For inspiration perhaps a final quotation from *Star Trek*, "To boldly go where no one has gone before." This should be our quest. **GEN**

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Regarding the clinical phases, however, opposite goals have to be fulfilled. In early project phases, the primary focus is on fast-track development by means of platform technologies, whereas in later clinical phases, process robustness, scalability, and productivity are the key performance indicators.

A typical platform for mAb purification calls on Protein A affinity chromatography for capture, the decision being based on speed and yield. The details of the platform may require considerable customizing because of variations in the physical properties of individual antibodies as well as differences in impurity levels and other ancil-

lary features of a particular batch. Indeed, Dr. Ambrosius showed that the optimum pH for elution off the protein A column can vary substantially from antibody to antibody. These preliminary investigations may reveal that a certain mAb candidate is not suitable for commercial exploitation.

In later clinical phases and for commer-

cial manufacturing, the typical platform processes are not well-suited and do not meet the demands for robustness and process economy. Further optimization of a platform process often leads to significant improvements in yield, up to fourfold.

Hence, Boehringer Ingelheim has adopted a rapid, automated protein purification technology referred to as RAPPTor®, a screening system for any type of chromatographic resin in a 96-well format. This approach allows rapid testing of the loading, washing, and elution steps of different resins in parallel.

Subsequently, read-out tools have been transferred into the 96-well plates and automatically evaluated for the amount of protein by absorption at 280 nm and purity by different methods. Dr. Ambrosius endorsed the speed of this approach, citing an example in which only three days were required to obtain the best resin and conditions for high-yield antibody purification.

Protein A: Mixed Blessing?

While Protein A is the dominant affinity medium for antibody separation, its robust capture capability comes at a high cost. Alternative approaches that would match the upstream production levels are thus greatly coveted. We can expect that due to their stability, lower cost, and toxicity, engineered ligands, tailored to specific needs will be vigorously pursued in the coming years. While there is at present no simple solution to the vilified downstream bottleneck, it is evident that an intimate knowledge of the physical biochemical properties of the target can open doors to new purification strategies. **GEN**

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