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Designing Out Up- & Downstream Mismatches **Thoughtful Planning Equals Efficiency, While Built-in Flexibility Leaves Wiggle Room**

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Discrepancies between the material output of cell culture and fermentation and the capacity of downstream purification has long been a point of debate in biotechnology. The bad news, if one can call it so, is that protein titers are rising, even for established processes. The good news is that mismatches between upstream output and downstream capacity need not be a problem for well-designed processes.

In biotech's early days most products were expressed in bacteria, with protein products isolated within inclusion bodies and frozen until a purification train freed up. Bacterial fermentation was fast, with doubling times of 20 minutes, and manufacturing runs lasted on the order of a day. Purification was the rate-limiting operation then as it is now, but processes were so inefficient that few companies paid serious attention to downstream productivity.

Today, high-value, high-titer, high-volume process streams from cell culture increasingly tax downstream operations, but manufacturers have several attractive solutions at their disposal.

Harmonizing upstream and downstream capacities involves a combination of three approaches: slowing down protein expression, speeding up purification, or designing expression and purification to work more harmoniously together through integrated, holistic process design.

The first option may be dismissed out of hand since no one is interested in lower titers. Speeding up or improving throughput of downstream operations, while hardly trivial, is generally viewed as an engineering exercise: membranes scaled up linearly, chromatography by increasing column diameter.

There is considerable disagreement over how easily the latter may be accomplished. Some companies achieve incredible chromatography scaleup. But according to Paul Miraglia, director of biotechnology at **Integrated Project Services** (IPS; www.ipsdb.com), widening chromatography columns comes at the expense of ease of handling.

Harmonization

Given limited wiggle room with respect to equipment, it appears the answer to upstream/downstream harmonization lies in more thoughtful, comprehensive design of the entire process, which can be approached from several angles.

Interestingly, companies undertaking comprehensive process optimization and modeling will likely discover bottlenecks in unexpected places, far from center stage. "The limitations are often not with equipment but in support systems," Miraglia says, for example clean-in-place (CIP) equipment and cleaning turn-around. He therefore recommends that companies build redundancy into their CIP capabilities and not count on CIP skid utilization much higher than 70%.

Another often-encountered limitation is in the ability to deliver water-for-injection (WFI) or highly purified USP water. Even after calculating water needs rigorously, examining the process configuration, and interviewing operations personnel to learn how much of what type of water is required when and where, IPS will routinely add an extra 20% to pure water specs in a facility design.

The idea that downstream processing is continually playing a game of catch-up arises, in part, from the fact that upstream productivity is open-ended whereas purification systems are limited by size, materials, equipment, and the quantity of material feeding into them. "In downstream operations, the changes in dynamic yield are much broader than for cell culture," says Ron Snee, Ph.D., a principal at Tunnell Consulting (www.kwtunnell.com).

Downstream operations can play catch-up through conventional capacity-expanding strategies, for example by scaling up downstream equipment, employing multiple controllers for more rapid cleaning between steps, running fewer purification cycles (by scaling up column dimensions), or using media with higher throughput or binding capacity. Each solution has its benefits, as well as costs.

Upstream yield improvements of 10% per year are not unusual for established processes. Before long, plants designed to purify one quantity are expected to process significantly more. One strategy for keeping downstream options flexible is to modularize by adopting recovery and separations skids. "When the load gets high, you simply add a skid," notes Dr. Snee.

Modularization also works for orphan plants, engineered for one protein product but used for an entirely different one. "Designing separations for one protein and using them for another opens up capacity and throughput problems," says Pete Latham, president of **Biopharm Services** (www.biopharmservices.com).

That is not to say that genuine mismatches between upstream and downstream capacities cannot occur under seemingly ideal conditions. But when they do, it is often a result of the evolution of upstream operations toward greater efficiency, not faulty design.

Flexibility

Bioprocessors therefore engineer flexibility into manufacturing processes to allow for upstream and downstream variability, or improvements at either end. Otherwise, situations where one side unexpectedly operates more efficiently than the other can be handled only up to a point. Engineers may increase the number of chromatography cycles per batch to accommodate upstream productivity enhancements, for example, but at some point this strategy strains support systems like buffer and media supply, or even basic needs like purified water supply.

Demetri Petrides, Ph.D., president of process modeling software firm **Intelligen** (www.intelligen.com), says these diversions, or peripheral bottlenecks, are easily overlooked when designing or streamlining a process. "Even seemingly simple process changes can create operational challenges."

Established Processes

Manufacturers bristle at the suggestion that their processes harbor upstream/downstream mismatches. By the time a company registers a process such mismatches are in fact rare, says Erica Shane, Ph.D., director of manufacturing sciences at **MedImmune** (www.medimmune.com). Glitches do occur, for example in the scaleup of chromatography media or in the event of unexpected surges in upstream productivity. But these events are the exception, not the rule, for well-designed processes. The materials science and physics of chromatography media has evolved to the point where even off-the-shelf resins deliver predictable, if not linear, scalability.

MedImmune says it is eager to tweak processes at any stage, even during clinical trials and post-commercialization. So if an upstream/downstream mismatch were to arise due to some unforeseen event, Dr. Shane and colleagues are ready. "As long as we can show product comparability, we feel it is never too late to improve upstream and downstream processing."

The use of specialized software for process modeling is growing among bioprocessors. MedImmune, like many biotechs, still uses spreadsheets to model and optimize processes, but the company is looking into dedicated applications for this purpose. (Software vendors often comment that their main competition comes not from dedicated software packages but from generic spreadsheet applications.)

Bayer Technology Services (www.bayertechnology.com) uses several software packages to simulate and model critical processes to avoid mismatches. Sebastian Schmidt, Ph.D., manager for bioprocess technology, mentioned aspenONE® from **Aspen Technology** (www.aspentech.com), which includes modules for process development and production management. Aspen claims that firms adopting the aspenONE process development module can save \$1M within one year of deployment.

Another of Bayer's preferred simulation packages is Intelligen's SuperPro Designer®. Among SuperPro's 140 unique modules are modeling packages for reactors, energy balances, sizing, costing, scheduling, resource/utility management, and economics. SuperPro's main limitations are its restriction to single-product facilities and narrow scheduling options. Both shortcomings are addressed through Intelligen's SchedulePro product, which may be used as a standalone modeling package for multiproduct facilities with variable production scheduling.

SuperPro and aspenONE help Bayer to simulate equipment failures and their impact, and especially to calculate costs associated with various scenarios.

One general strategy for harmonizing upstream and downstream operations is to design the process appropriately, to scale, as chemical and polymer processors have done for decades. However, the upstream yields from chemical processes are much more predictable than from cell culture or fermentation. Nevertheless, by drawing on its chemical processing experience, Bayer has achieved process streamlining and eliminated upstream/downstream mismatches.

Dr. Schmit recommends that viewing and designing processes as a whole, rather than as two consecutive but distinct sets of unit operations, provides insights not only into downstream efficiencies, but upstream strategies that may enhance purification as well as "sidestream" operations like waste treatment, utilities, and logistics.

Because of the specialized nature of biopharmaceutical development timelines, harmonizing upstream and downstream operations for many companies means exploiting a narrow time window during development—after proof of principal but before clinical trials.

"The gap where you have enough knowledge to optimize a product, but are not so deeply into clinical and regulatory activities is quite small," notes Dr. Schmidt, who believes the upstream/downstream mismatch is over-rated as a business or manufacturing issue. The interface between any two steps, he explains, is a possible bottleneck. Upstream and downstream disconnects may be somewhat more pronounced when the respective processes are designed separately, by different teams. But even manufacturers who find themselves "stuck" with a mismatched process can implement work-arounds, for example addition of a hold step.

The real issues, says Dr. Schmidt, are not fermenter size or chromatography column diameters. "Process optimization is about space, people working in it, utilities, waste treatment, and a host of things going on around the actual production. Vessels and columns are easy to dimension. Scaling utilities and facilities are where the real bottlenecks occur."

The opening of **Wyeth Biopharma's** (www.wyeth.com) Grange Castle, Ireland, and Andover, MA facilities in 2005 represented a \$2.5 billion infrastructure investment, and a turning point for Wyeth.

Model of Efficiency

The company hoped that these facilities would become the model of efficiency, eventually handling up to a dozen development projects simultaneously. This goal has since been achieved; Wyeth developed platform manufacturing technologies and eliminated inefficiencies in the upstream-downstream handoff. In addition to not having to re-invent process technology for each product, Wyeth discovered that modularization improved technology transfer of successful molecules to new facilities.

In many ways Wyeth was prepared for high protein titers long before they happened. The company had instigated downstream improvement efforts years before, when it was believed that transgenic technology would take off and stress downstream operations as never before. Now, with cell culture so productive, Wyeth's foresight has paid big dividends in the form of established downstream efficiencies.

"Our objectives were to make biomanufacturing and the processes surrounding it as efficient as small molecule manufacturing and bring the cost of those drugs in line with that of small molecule," says Jeff Deetz, Ph.D., senior director for drug substance development at Wyeth's Andover facility. Like Bayer, Wyeth had the advantage of also operating a small-molecule pharmaceutical business, which provided a frame of reference.

A decade ago, biomanufacturers routinely produced 100 grams of protein per batch. Today 10 kilos is not extraordinary. "This has completely changed how we approach the business, how we think about capital investment, technology, and innovation," says Dr. Deetz. Wyeth uses the term "campaigning" to describe how molecules and facilities are interchangeable, provided the underlying manufacturing technology is sufficiently standardized. As a result of platform technologies that rely on standardized equipment, Wyeth does not

experience upstream/downstream mismatches. Last fall, a Wyeth researcher presented a paper at an American Chemical Society meeting in which he described a ten-ton antibody process that used conventional column chromatography for purification.

“The folklore among bioprocessors is that conventional chromatography isn’t amenable to those scales, but in fact it is, and perhaps the main reason why our upstream expression and downstream purification capacities and throughput have always matched nicely,” Dr. Deetz explains.