GEN Bioprocessing

Culture Media Underlies Productivity Gains

Optimization Has the Potential to Create Higher-Quality Products and Robust Processes

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To say that biomanufacturers owe the volumetric productivity gains of the last decade solely to improvements in cell culture media would be a stretch, but not by much. Media products, feeds, supplements, and additives are areas of intense interest and innovation precisely because their implementation has been so successful.

Many cell culture media today exist as historical relics, notes James Blackwell, Ph.D., senior consultant at BioProcess Technology Consultants (www.bioprocessconsultants.com). "In the past, we lacked the experimental power to determine if every medium ingredient was actually needed, and if their quantities were optimal."

Legacy media often contain unnecessary components, or useful ones in insufficient quantities. Some probably hold ingredients that, unknown to processors, inhibit cells or protein expression. "It used to be that if you weren't sure, you threw it in," says Dr. Blackwell. High-throughput testing of media components can save money by identifying unnecessary ingredients that may be safely deleted from the mixture.

Culture medium optimization will always involve some degree of trial and error, but high-throughput techniques add systemization and parallelism to these efforts. High-throughput micro- and mini-bioreactor technologies such as Bioprocessors' (www.bioprocessors.com) SimCell™ platform allow investigators to vary any medium component while holding everything else constant. SimCell runs hundreds of cultures simultaneously, which is useful in selecting production clones, defining process limits, identifying optimum medium and feed conditions, and understanding process variables.

Optimized media formulations dramatically and positively affect media costs, process productivity, and capital investments, and can thereby help justify reengineering of even established processes. Media optimization has the potential to create higher-quality products and a more robust process, in addition to the ability to run smaller or infrequent batches. "The return on investment for a company with several pipeline or commercial products growing rapidly can be tremendous," says Dr. Blackwell. "Some product companies have been able to scale back on their future capacity demands in this manner and still meet market needs without the huge capital costs of new facilities."

Production line inspection of cell culture media at JR Scientific

Belt-Tightening

The cell culture market has experienced belt tightening over the past 18 months, says Hans Huttinga, strategic marketing director for pharma products at Kerry Bio-Science (www.kerrygroup.com). The company, which sells media supplements for cell culture and microbial fermentation, claims that five of the top biotech blockbusters, and 25% of currently marketed therapeutic protein products, are produced with its culture supplements.

Growth in annual demand for media products, according to Huttinga, has fallen from 15–20% to 7–10%. Some cost-cutting measures are a consequence of current economic conditions, but a fair amount results from biomanufacturers becoming more sophisticated in how they use and acquire media and supplements. Nevertheless, Huttinga believes that all indicators point toward significant growth and "lots of optimism" moving forward.

Kerry develops media supplements that are vegetable-based and serum- or protein-free, but not chemically defined. Products include HyPep™ 1510, a soy-based hydrolysate currently used in several marketed drugs, and UltraPep™ Soy, which provides hydrolyzed carbohydrates in addition to amino acids and peptides. UltraPep is produced enzymatically from soy, wheat, and cottonseed protein sources. The company claims that UltraPep simplifies production processes and gives a more consistent product, but unlike chemically defined media it does not require extensive optimization.

A Reflection of Biotech Itself

Cell culture media and production capacity are closely interdependent. Some experts, like Professor Florian Wurm at the Swiss Federal Institute of Technology, believe that media have been responsible for most of the recent rise in protein titers and volumetric productivity. Higher titers mean smaller reactor volumes or fewer batches, which explains why growth in demand for cell culture media is slowing somewhat. "But while less is needed, media tend to be richer," says Bruce Lehr, marketing director at SAFC Biosciences (www.safcbiosciences.com).

Instead of using more concentrated media formulations, bioprocessors begin with simpler media and supplement it with highly concentrated feeds during growth and production phases. Because production cells have been engineered to utilize nutrients more efficiently, somewhat fewer ingredients are employed today per production unit than years ago.

The evolution of cell culture media reflects the maturity of bioprocessing and biotech itself. "Fifteen years ago, companies were worried about getting product to market. Today, they're more concerned with streamlining processes, lowering costs, and supply chain management," says Lehr.

Chemically defined media and to a lesser degree ACF media play into the trends of process streamlining, simplification, and standardization with the goals of predictability and consistency. The platform idea that has worked so well with cells and unit operations has become attractive for processes as well.

Media optimization appears to be at odds with one-medium-fits-all standardization, but it actually is not because standard media have become so good. SAFC, for example, offers 14 chemically defined formulations in its CHO Media Library. Processors can try several of these for their specific cells, or begin with the CHO Fusion Media formula-

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News Bioprocessing Highlights

MannKind Inks $2.5M Option Agreement for SemBioSys' Plant-Produced Insulin

MannKind (www.mannkindcorp.com) purchased an option to license SemBioSys Genetics' (www.sembiosys.com) plant-produced recombinant human insulin for $2.5 million. Mannkind will primarily use this plant-produced insulin for Afresa™, its ultrarapid-acting insulin that recently completed Phase III studies.

SemBioSys' plant-produced insulin is human insulin produced from genetically enhanced safflower. According to SemBioSys, it is physically, structurally, and functionally indistinguishable from pharmaceutical-grade human insulin as demonstrated in its analytical tests and preclinical subchronic toxicology studies in rodents and primates.

SemBioSys is currently conducting a Phase I/II trial of its plant-produced insulin. The three-arm study, of up to 30 healthy volunteers, is designed to demonstrate the bioequivalence of safflower-produced insulin to two commercial insulin standards. Full results are expected to be available during the first half of 2009.

Afresa is a pH-sensitive therapy that dissolves upon contact with the lung surface, releasing insulin monomers that enter the bloodstream. It achieves peak insulin levels within 12–14 minutes of administration, thus mimicking the release of mealtime insulin observed in healthy individuals, MannKind points out. The company expects to finalize an NDA for Afresa to submit to the FDA in early 2009.

Talecris Biotherapeutics Signs Second PER.C6 Deal

Talecris Biotherapeutics (www.talecris.com) signed a second exclusive, commercial license agreement with Crucell (www.crucell.com) for access to the PER.C6® cell line for the production of an undisclosed and specific protein.

Crucell will receive an upfront payment of $1.5 million following the execution of the agreement and will be eligible for milestone payments of approximately $20 million.

The first deal was signed in September, also for an undisclosed and specific protein and the exclusive rights to produce that protein using the PER.C6 cell line. For that deal Crucell received an upfront payment of $2.5 million and is eligible for milestone payments of approximately $30 million across multiple indications. ■

Cell Culture Media

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tion, which SAFC claims provides the highest growth and productivity across the widest range of clones.

"If you had to try one medium, this is the one formula we'd recommend," Lehr says. "It does not replace media optimization, but it does offer an alternative to full-blown optimization for customers who don't have time, money, or expertise to optimize their own system." Lehr points out that most large biotech companies optimize in-house.

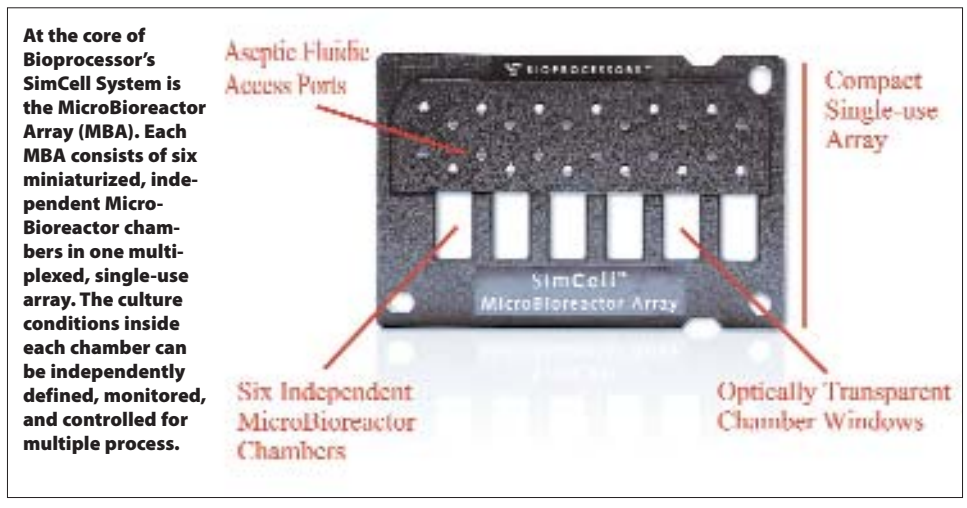
SAFC plans to introduce a chemically defined CHO feed supplement to replace hydrolysates early in 2009.

Animal-Free Media

FDA has for years urged biomanufacturers to avoid ingredients of animal origin despite the fact that the industry relies heavily on mammalian cell culture production methods. Of 31 therapeutic proteins approved in the U.S. since 2003, 17 are produced in mammalian cells. Suppliers of media, feed, and ingredients have responded with raw media ingredients of plant origin or manufactured through recombinant expression. Animal component-free is also big in stem cell development and regenerative medicine, since tissues are designed for implantation directly into humans.

While regulatory and market pressures are nudging biomanufacturers toward serum-free media for new and older processes alike, diagnostics and academic researchers still rely on classical media based on fetal bovine serum, calf serum, and other serum-based platforms for basic research. And many industrial groups still use animal component-based media for early-stage production of therapeutic proteins and proof-of-principle experiments.

InVitria (www.invitria.com) sells animal component-free recombinant albumin, lactoferrin, and lysozyme for cell culture and microbial fermentation media. Customers include biomanufacturers, media companies, and diagnostics firms. Lactoferrin is a growth factor that CEO Scott Deeter says out-performs insulin and insulin-like growth factors used to boost cell growth and productivity. "It's a new product, not yet widely used, but we hope to change that."



At the core of Bioprocessor's SimCell System is the MicroBioreactor Array (MBA). Each MBA consists of six miniaturized, independent Micro-Bioreactor chambers in one multiplexed, single-use array. The culture conditions inside each chamber can be independently defined, monitored, and controlled for multiple process.

At the American Society of Cell Biology annual meeting held last month in San Francisco, InVitria showcased several animal-free and cell-culture medium components for production cells, stem cells, and regenerative medicine, including Cellastim (recombinant albumin) and Lacromin (recombinant lactoferrin).

Deeter describes the surging interest in media as "a positive step toward making media ingredients more consistent and well-defined." Part of that consistency, he says, involves the use of recombinant ingredients which, unlike batches pooled from serum, are as close in composition as possible. An unanticipated benefit of recombinant components, which have a reputation for high cost, is economy derived from higher productivity and safety, and lower operating and capital costs.

Animal-free media is not a new idea. Its potential benefits were noted, but not realized, from the earliest days of biotechnology. "There were several times during the 1970s and 1980s when experts predicted that animal-free media would revolutionize the industry," says Jan Baker, CEO of JR Scientific (www.jrscientific.com).

It is only in the last decade, however, that the idea really began to catch on for large-scale processes. Many processes, of course, still use animal serum-based media. Serum-free products are less reliable, nutritionally speaking, than those based on animal sera. Biomanufacturers have at times been disappointed with yields and productivity after adapting a serum-based process to serum-free conditions, Baker notes.

"The biotech industry still buys hundreds of thousands of liters of animal sera per year," he adds. "Classical media are not going away any time soon."

JR produces media-related consumables, ingredients, and formulated classical and synthetic media, mainly for research labs at universities and biotechnology companies. The company is also a primary OEM supplier to several large companies and provides them with private-labeled animal sera, media, and cell-culture reagent. "Biomanufacturing is already well-served

by the major media suppliers," Baker notes, "which leaves a large portion of the research marketplace for smaller and well-qualified vendors."

To maintain manufacturing flexibility, JR produces all its media and components in disposable containers to reduce cleaning and cleaning validation at its facility. Disposables are also a selling point, since it reduces the potential for cross-contamination between batches or products.

Specialty Cells

The use of stem cells and other nontraditional cells for human therapy is becoming an area of increasing importance for media formulation. Nutrient and other requirements for expanding populations of therapeutic cells to commercial scale vary widely from those for production cells.

Many therapeutic cells are anchorage-dependent and have been generated in serum-supplemented or serum-free culture containing components of animal origin. Eventually, since these cells and tissues are introduced into human patients, the cells must be brought into compliance with standards for production cells.

"In many cases, this means redeveloping culture media to replace nonhuman animal origin components with human-derived or animal-origin-free components," notes Stephen F. Gorfien, Ph.D., director, bioproduction products and PD-Direct® Media Services at Life Technologies (www.lifetechnologies.com). "These modifications are difficult owing to the dependence of the cells on various growth factors, cytokines, and attachment factors, recombinant or synthetic forms of which may not be commercially available at reasonable cost."

Invitrogen offers a service, PD-Direct® Bioprocess, which among other things helps companies used to working with suspension cultures of production cells migrate therapeutic cell lines from anchorage-dependent to suspension-dependent status.

Vibrant Business

Despite what may or may not be an industry-wide slowdown, cell culture media remains a vibrant business, particularly for specialty cells. After its acquisition of Serologicals in 2006 Millipore (www.millipore.com)—known for its filtration products—found itself in the business of supplying media and reagents for stem cells and primary cell cultures. In October the company introduced a medium for generating 3-D epidermal keratinocytes.

Louise Rollins, product manager, cell biology products, reports that Millipore's cell culture portfolio "offers unique synergies with classic Millipore products," and provides a "complete integrated solution for cell culture." The company has an ongoing program to commercialize a portfolio of specialty cell culture systems, including endothelial, epithelial, and stem cells. GEN

BD Biosciences Expands Capabilities in Cell Culture Media

While BD Biosciences works toward completing construction of its recently announced animal-free/antibiotic-free (AF^2) cell culture media production facility in Miami by the end of the year, the company's new pilot lab is nearing completion.

The pilot plant will focus on the production of cell culture media samples representative of the new commercial-scale AF2 facility, according to company officials. Custom blends of basal cell culture media and supplements are available currently via the BD Autonutrient™ media design service and its custom product development program, they add.

Once the Miami facility begins operations, "BD Biosciences will fully serve the culture media needs of bioprocessors working with mammalian, microbial, or stem cell lines," notes Tom Isett, vp, BD Biosciences–Advanced Bioprocessing.

Originally designed and built for the manufacture of pharmaceuticals, the 90,000-square foot Miami plant is currently being outfitted with modern cell culture media milling, blending, and sterile liquid filling equipment.