

Biotech Manufacturing Is Coming of Age

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Process integration, economy of scale, optimized development and manufacturing — aspects present in all other areas of the pharmaceutical industry — are now becoming recognized as highly relevant for the ultimate success of a biotech enterprise. Full pipelines further driven by genomic research and still unmet medical needs, limited GMP manufacturing capacity, growing competition between companies and products, economic problems of health care systems worldwide, and higher quality demands: These are the driving forces toward higher efficiency and productivity. Access to manufacturing capacity is becoming a critical strategic factor

PRODUCT FOCUS: RECOMBINANT ANTIBODIES, VACCINES, AND GENE THERAPIES

PROCESS FOCUS: TOTAL PROCESS

WHO SHOULD READ: PROCESS DEVELOPMENT, MANUFACTURING, FORMULATIONS, QUALITY ASSURANCE AND CONTROL, MANAGEMENT

KEYWORDS: CELL CULTURE, FERMENTATION, PURIFICATION, ANALYTICAL METHODS, FORMULATION, OUTSOURCING, PROCESS OPTIMIZATION AND DESIGN, PROJECT MANAGEMENT

LEVEL: INTERMEDIATE



Mammalian cell culture operation FROM BAYER AG (WWW.BAYER-AG-DE)

and may limit the industry's growth potential. As biotech matures and faces significant consolidation, efficient development and use of technology is an important factor in both upstream and downstream processing. Furthermore, complexity of biotech products is reflected in the rapid progress and growing complexity of analytical development that allows comparability studies between different production versions, scales, and manufacturing sites — and will eventually build the basis for biogeneric drugs.

“Scaling Up of Biopharmaceutical Proteins: Shrink Development Time and Increase Results of Your Manufacturing Process from Bench to Clinic to Market” was the title of a recent IBC Life Sciences conference (23–24 January 2003 in

Basel, Switzerland). It addressed many of the current trends in state-of-the-art upstream and downstream processing as well as the manufacturing of drug products.

Product development and revenue generation is a major driver for the growth of biopharmaceutical companies. A number of speakers at the conference focused on current requirements for the manufacturing of clinical trial and commercial materials. Low cost of goods, increased expression rates, optimized product yields, and robust, scalable manufacturing operations without compromising product quality were identified as key aspects of success. Antibodies represent the fastest-growing segment of biopharmaceuticals, so they not surprisingly received the most attention at the conference.

PRODUCTION ISSUES

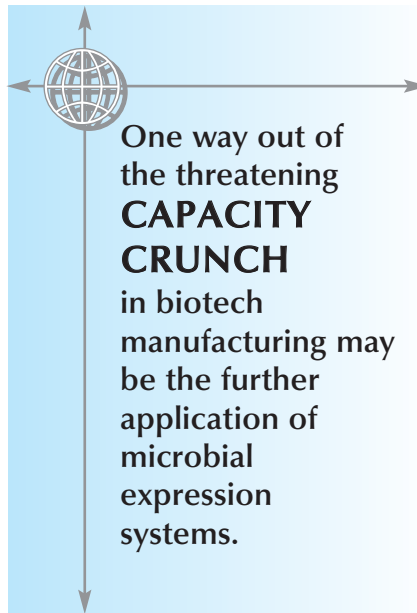
Mammalian cell culture is the reference process for the production of marketed biopharmaceuticals, with Chinese hamster ovary (CHO) cells being the industry standard. Because productivities dramatically increased over the past two decades, the vision now prevails of routine fermentation processes yielding glycosylated proteins in the 1–3 g/L range.

According to Florian Wurm of the Swiss Federal Institute of Technology (www.epfl.ch), premature hopes have been raised outside the biotech community regarding the speed of progress, although the overall expectations are realistic and justified (1). Upstream process development comprises three phases, with considerable room for improvement in all three: cell line generation, cell line development, and fermentation development. Stable chromosomal integration and/or plasmid copy number of the desired product genes is a prerequisite for high expression. New vector targeting and transfection approaches have recently been reported: replacement targeting to high transcription rate loci, homologous recombination, and retargeting.

In cell culture development, high-level productivity so far is not a predictable phenotype of cells, and high yield will continue to depend on efficient screening techniques. Higher cell densities ($>5 \times 10^6$ cells per mL), extended life spans (>10 days), and higher specific productivity (10–50 pg per cell each day) is recommended. Scale-down strategies to the 10-mL scale and even multiwell plates are useful but not entirely satisfying. A tube-spin model using disposable 50-mL tubes filled with 5–10 mL of cell culture and shaken at 150–200 rpm under a controlled CO₂/air atmosphere allowed the assessment of many variables in less time. Wurm concluded that decisions towards cell culture-based production technologies are still long-term commitments. They require well organized and partially overlapping

development activities before finalizing the decision on the best cell line for each individual phase of manufacturing. Eventually, large-scale transient gene expression with plasmids may provide a powerful alternative for the extremely fast expression of complex proteins.

Mohamed Al-Rubeai from the University of Birmingham (www.bham.ac.uk) presented new trends in the monitoring of mammalian cell culture (2). With no doubt, the physiological and intracellular state of cells beyond the



general parameters “viable” or “dead” allows a well-directed selection of high- and stable-producer cell lines suitable for industrial production. Flow cytometry facilitates a multiparametric selection of stable secreting cells with a variety of antibody-based cellular detection methods. It also can be applied to the development and implementation of control strategies for large-scale bioreactor operations, such as online cell cycle analysis and identification of apoptosis.

A CHO cell culture laboratory model predictive of full-scale production processes was the central aspect of Ron Taticek’s (Genentech, www.gene.com) presentation (3). A valid model is key to successfully implementing process

improvements, establishing limits on process parameters and excursions, and troubleshooting during manufacturing (“satellite” production runs). Scale-up and scale-down are challenging because the inevitable cellular environment changes are leading to different characteristics in cell growth, viability, specific productivity, product quality, and impurity levels. A number of parameters must be adapted, some of which are nonlinear. Taticek identified the osmolality profile as a lead parameter to match during scale changes because it reflects the metabolic state of the cells. As a result, scale-down models for production of monoclonal antibodies (MAbs) were established that include purification and characterization. Charge heterogeneity and galactosylation as lead parameters were virtually identical between small- and large-scale runs. The model supports implementation of robust market-ready processes, with multiple improvements after phase II and accurate prediction of the product quality profile at full scale.

One possible way out of the threatening capacity crunch in biotech manufacturing is the further application of microbial expression systems that are very well established in industrial biotechnology. This point of view was put forth by Bo Kara of Avecia Biotechnology (www.avecia.com/biotech), who referred to established techniques and elucidated points to consider in the scale-up of microbial fermentation — of which a worldwide capacity of 260,000 L should be available by 2005 (4). He introduced a proprietary, stable, inducible *Escherichia coli* vector that shows no growth inhibition, can be tightly regulated, and contributes to maximizing productivity at large scales. For a given product, fermentation costs were reduced by 20 percent relative to a prior pET11a based process. In a second example, a 30-kD glycosylated



Overall process design and integrated manufacturing could be another **STRATEGY** to optimize productivity.

protein with seven disulfide bridges was expressed in *Pichia pastoris* at about 1,000 mg/L in a fed-batch fermentation. Scale-up challenges with this system are related to the high biomass involved (oxygen supply, heat, regulation) and the use of methanol for growth and induction (a safety issue). Production under current Good Manufacturing Practice (cGMP) conditions was, however, possible at the 1,000-L scale with those key issues properly addressed.

Transgenic production of proteins and comparability with human products were also topics covered at the conference. Scott Borneman of EpicYTE (www.epicyte.com) described the characterization of IgA (the native first-line defense against infectious agents) with microgram levels of material obtained from transgenic corn in various structural and functional assays including glycoanalysis (5). This analytical strategy for early phase products provides a rationale for scale-up with the most suitable engineered plant material.

An innovative moss bioreactor system for the production of complex biologicals including humanization of the glycosylation pattern through genetic strain design was reported by Sabrina Wagner from Greenovation Biotech (www.greenovation.com) in Germany (6). Phototropic moss cultivation combines a number of advantages: a safe and robust

eukaryotic organism with high genetic stability growing in a simple and low-cost medium. Moss is easily accessible to genetic engineering and would permit the introduction of whole metabolic pathways. Secreted proteins can be obtained in the order of 30 mg/L per day in a perfusion system with a biomass of 1 g/L in a 10-L fermentor and up to 5 g/L in perfused glass tubes. From those models upscaled, a cost calculation for manufacturing MAbs was derived suggesting a 40 percent reduction potential of the moss bioreactor as compared with CHO-cell cultivation.

BETTER PROCESSES, BETTER BUSINESS

Overall process design and integrated manufacturing is another strategy to optimize productivity. Richard Francis, operations support manager at Protherics (www.protherics.com), exemplified how the introduction of a number of improvements in the production of marketed polyvalent FAb-fragments — used as antidotes — resulted in increased product recovery, supply, and hence revenue stream (7). The value of bench-scale development to study processing conditions with downscaled process models was also described. In his case study, the original FAb manufacturing process cost-of-goods were at >40 percent of the sales price, with manufacturing accounting for the largest part thereof. After a number of parameters were refined for serum production in sheep, IgG purification and freeze-drying costs, digestion and formulation, as well as revising the whole downstream process, the optimized procedure met a target of <15 percent COG with a <1 percent failure rate. Overall yield was increased from 24 to 60 percent. A key learning point of the study was that, after product approval, a commercial success would not have been possible without major improvements of the manufacturing process allowing for a better use of the existing facility.

Kevin M. Egan from ICOS (www.icos.com) explained his company's generic MAb production process (8) in CHO cells, with proprietary vector and cell technology that enables the initiation of clinical studies within 48 weeks after transfection (so-called rapid development). For phase III and commercialization, a second-generation process is implemented with animal protein-free medium and a typical antibody titer of 1,000–1,500 mg/L (referred to as commercial development).

“Value by Process Optimization,” the title of a talk by Helmut Hoffmann, Boehringer Ingelheim (www.boehringer-ingelheim.com), reflected the approach of the conference overall (9). He introduced concepts for designing fast-track process development in both the upstream and downstream areas. Examples included optimization of expression systems, screening of high-producer cell lines, and removal of bottlenecks in primary recovery and polishing steps. Lower cost-of-goods through more efficient use of an existing facility was the subject of Hoffmann's case study. In one MAb process, a fourfold throughput with 850 mg/L in fed batch culture and a final yield of 70 percent was reported. Comparability observations attributable to glycosylation changes could be solved after feed optimization for the second-generation process. With further upstream improvements (cell lines, media design, process control) fed batch processes could be realized at up to 2,500 mg/L. Protein recovery and downstream processing, Hoffmann reported, will soon see breakthroughs in improved methodology such as expanded-bed absorption and other forms of continuous chromatography.

Once you can select the best candidates from your research pipeline, you need strategies in place to help your company take maximum advantage of those choices by taking into account the probability of success and

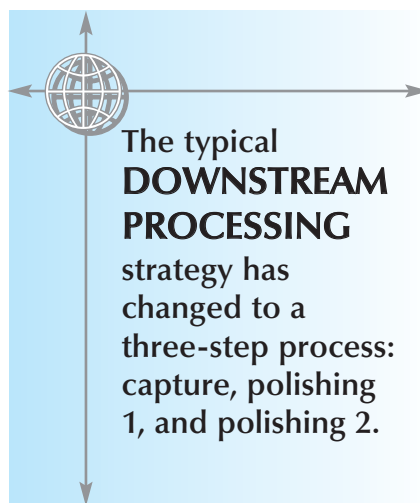
development limitations as well as manufacturing capacity and commercial potential. Thomas Seewoester (Amgen, www.amgen.com) explained how, at his company, educated go/no-go decisions are made at key milestones to manage a strong product pipeline, to allocate resources adequately, and to develop competitive enabling processes (10). Making and executing rational decisions is an integral part of Amgen's development strategy, supported by a large toolbox of generic modules in all areas of cell biology, upstream and downstream processing, and economic models to evaluate relevant scenarios. With that approach, the company goes for commercially viable processes from phase I onward in less than 10 months from transfection to released preclinical material for mammalian cell culture processes and antibody titers exceeding 2 g/L. With microbial processes, product titers up to 18 g/L can be achieved with a few months of development work.

Outsourcing: When it comes to contract manufacturing, how can the client-CMO relationship be effectively managed for mutual benefit? Wieland Wolf of Rentschler Biotechnology (www.7-schwaben-apotheke-laupheim.de/bio) said that interests, risks, and expectations of both parties — client and CMO — must be clearly defined to prevent unrealistic anticipations (11). For the customer, access to cGMP manufacturing capacity together with a higher financial and operational flexibility is key, whereas a minimized risk share and calculable capacity use is important for the contract manufacturer. In any case, Wolf concluded, a long-term partnership potential, effective project management tools, open communication, and a well-designed and comprehensive contract are the ideal bases for a successful cooperation.

MOLECULAR MODIFICATIONS

An alternative approach to directing a therapeutic effect with MABs

without raising effector functions is the use of fragments such as the monovalent antibody fragment (FAB) unit as the basis for biopharmaceuticals. With site-specific PEGylation, disadvantages such as reduced valency and shorter half-life can be compensated for. FAbs are efficiently produced in *E.*



coli, an advantage for rapid cell line development, accessible manufacturing scales, low cost of goods, and short process times.

David Glover of Celltech (www.celltechgroup.com) in the United Kingdom presented a process development strategy that results in development timelines of about 10 months from selection of a therapeutic antibody fragment to release of clinical lots (13). It is essential that robust, transferable strategies are followed to prevent compromising scalability and multisite producibility by speed. Process and clinical development need to be aligned, and substantial changes can be introduced only if they offer a significant benefit — and if reliable change control and comparability programs are in place. Introduction of expanded bed absorption in the recovery step was such an example. FAbs have been produced at 1.3 g/L in the 1,000-L scale (fed batch).

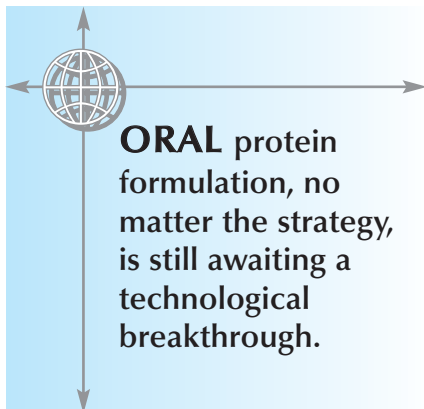
The opposite effect — Fc receptor dependent effects on immune cells — can be increased by modifying the glycosylation pattern

of antibodies through a constitutive overexpression of glycosyltransferase in hybridoma cell lines, as described by Pablo Umana of GlycArt AG (www.glycart.com) in Switzerland (14). The technique is interesting for therapeutic antibodies with antitumor activity through either mediated cellular toxicity or complement activation, and Umana said it can be applied to all standard antibody-producing cells without affecting productivity or cell growth.

DOWNSTEAM PROCESSING

In downstream processing (DSP), innovation is needed and processes must be adjusted to accommodate improvements in fermentation titers, media composition, and cell viability and maximize the productivity of existing capacity. Eric Grund of Sweden's Amersham Biosciences (www.amershambiosciences.com) reflected recent advances in areas like chromatography and filtration that are considered mature unit operations, especially focusing on generic methods for MAB production including whole facility design (15). Modern processes require chromatographic media that are not only selective and consistent, but that offer both high dynamic capacities and low cycle times to be compatible with the large volume and high expression levels of state-of-the-art fed-batch fermentation processes.

Because of a new generation of powerful supports, most bulk impurities are being removed in the recovery step already. The typical DSP strategy has changed to a three-step process: Capture, Polishing 1, and Polishing 2. Requirements are redefined for each individual step, including scale-up approaches, a point that was accentuated by Charles Christy representing Millipore (www.millipore.com) in France (16). With new demands for rigidity of chromatographic media and high flow rates at reasonable back pressures, the dogma to scale up by column diameter is being challenged. Scale-up by column



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length and/or throughput provides a larger window of operation in production columns, reduces investment, and increases flexibility. Every commercial process requires a compromise between economical production, high quality, and time constraints — an interrelation Christy referred to as the “Bermuda Triangle” for biopharmaceuticals, where the different objectives are balanced early on in development.

Once again, a generic approach for MAb production was introduced, with adsorptive depth filtration as the cell removal and tissue culture fluid (TCF) clarification step. A depth filter with a positively charged internal surface is combined with a membrane filter layer in a single element, providing a high capacity for all kinds of particles in the TCF and allowing process compression into a single clarification step. Other trends include removal of small viruses with normal flow filters, membrane adsorbers for the separation of trace impurities, and integration of unit operations.

Formulation: An important success factor for biopharmaceuticals is the identification of suitable formulations and dosage forms, taking into account the high sensitivity and limited permeability of macromolecules through biological membranes. Fast-release parenteral liquid administration is still the predominant application form for biotech drugs, with so far only a few exceptions. A session involving three experts from the industry was held at the end of the conference to discuss current trends

in alternative formulations and dosage forms.

Karoline Bechthold-Peters from Boehringer Ingelheim (Germany) highlighted the advantages of alternative approaches such as nasal, topical, oral, and pulmonary application of proteins (17). In three case studies, administration problems were addressed by innovative dosage forms as new approaches and life cycle management options for existing drugs:

- subcutaneous application of a growth factor as if in situ, forming sustained-release depot gels or biodegradable microcapsule preparations
- pulmonary delivery of a highly glycosylated protein with a nebulizer or as a spray-dried powder for inhalation
- chronic administration of a cytokine with needle-free injections or osmotic driven, implantable pumps.

Biodegradable implants, microcapsules, and microcrystals are other trends in delivering large amounts of proteins and peptides as well as viral and nonviral gene therapies. Oral protein formulation, no matter the strategy, is still awaiting a technological breakthrough.

According to Hanns-Christian Mahler of Merck (www.merck.com) in Germany, critical steps during production can be directly correlated to chemical and physical instabilities of proteins (e.g. deamidation, oxidation, denaturation, and aggregation) that may ultimately result in reduced bioactivity and even immunogenicity (12). Robust formulations meet product requirements and withstand temporary stress conditions. Formulation design involves the determination of degradation routes, selection of excipients and/or conditions, and models for the assessment of long-term stability.

For a human MAb formulation, Steven Chamow of Abgenix (www.abgenix.com) described a

lyophilization method suitable for high-dose subcutaneous injection as compared with the first-generation product, which was based on a liquid formulation at 10 mg/mL (18). The new procedure was statistically developed with a design-of-experiments (DOE) approach to identify critical excipients and optimize the formulation matrix for stability, tonicity, and viscosity while optimizing the freeze-drying cycle. The improved formulation contained, among other components, histidine as a cryo- and lyoprotectant and arginine to reduce viscosity after reconstitution. Lyophilization is performed in a 48-hour cycle with a reconstitution time of <15 minutes and a final MAb concentration of 200 mg/mL.

Analytical Methods: Regarding analytical development and safety testing, new and improved methods are developing rapidly: quantitative polymerase chain reaction (QPCR), for example. Discussing QPCR methods for virus and DNA clearance studies and novel assays for prions, Martin Wisher from BioReliance (www.bioreliance.com) in the United Kingdom picked two hot topics out as a central theme of his presentation (19). QPCR is now widely accepted as a sensitive and robust method to quantify the retrovirus load after mammalian cell culture and to assess the virus clearance potential of a process. Increased precision allows better mass balance studies than those performed using electron microscopy and infectivity assays. The method is also suitable for validating host cell DNA removal, with sensitivity down to a threshold of about 0.5 fg/mL.

In validation of transmissible spongiform encephalopathy clearance, where infectivity studies are long and expensive, Western blot assays for the presence of Pr^{Pres} show a good correlation and are therefore useful for first evaluation of potential removal steps in a purification process. So far, up to a 5.0 log₁₀ clearance has been demonstrated with this semiquantitative method.



Microbial fermentation FROM BAYER AG
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A MATURING INDUSTRY

Although the biotech industry is set to justify some of the front money it has received over the years with full pipelines and a number of new, innovative drugs, it faces unknown challenges. Our business sector lacks the infrastructure for manufacturing the biopharmaceuticals to come. Despite major investments, the high demands for results and unavoidably long lead times prohibit a short-term solution. Growing competition within the industry and margin reduction overall has made development toward higher efficiency vitally important for biotech companies. Fermentation development is setting the pace. Perfecting cell lines, bioreactors, and media leads us to believe that routine production of proteins at 3 g/L seems feasible. Technical developments will facilitate the production of proteins like human serum albumin in the ton range. Higher titers and throughput from fermentation — and eventually transgenic organisms — will require breakthroughs in recovery and purification. Upstream and downstream processing are growing together, filtration and chromatography complement each other, and new formulation strategies should enable novel drug

delivery routes. Overall process integration will help to close gaps in the supply chains for manufacturing future biotech drugs.

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