



Whole in One



How can biomanufacturers ensure fast-track results in their fixed-cost environment? Dr Manfred Papaspyrou at Rentschler recommends a holistic approach offered by partnership with a single CMO

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Biomanufacturing is a risky business driven by fixed costs. A highly regulated environment, long lead times, high capital investments, and demanding processes define this sector. Today, the most dynamic driver behind the use of CMOs in the biopharmaceutical industry is becoming the unique, innovative and state-of-the-art process and production technology they offer. Collaboration with one single CMO partner from development to production has several advantages. It avoids frictions and delays at interfaces with various partners for subsequent project parts. It prevents duplicated and therefore redundant activities such as auditing, tech transfers and supervision, and thus streamlines project activities and shortens time-to-market. At each stage of the development process, activities can be evaluated in view of regulatory and manufacturing considerations, thus avoiding costly redevelopment endeavours. A holistic, integrated development concept, involving recombinant protein expression, product development and overlapping activities, may therefore permit rapid but economically and technically-feasible product development.

FAST ESTABLISHMENT OF RECOMBINANT CELL LINES

One of the first important steps during the development process for a recombinant protein is the choice of producer organism and the subsequent establishment of a recombinant cell line producing the protein of interest. Today's state-of-the-art production processes are performed in well-defined serum- or protein-free media. It is therefore important to have a cell line development process in place that can also be performed under

serum-free conditions. The development of recombinant production cell lines is usually performed under serum-containing conditions and the cells are later adapted to serum-free medium, which does, however, bear the risk of losing productivity or product quality and significantly extends development time.

The development of recombinant cell lines is much faster when using optimised cell lines such as Chinese Hamster Ovary cells, dehydrofolate reductase negative (CHO dhfr cells). They allow the serum-free performance of all steps essential to the development of the recombinant cell line, including transfection, selection, amplification and single cell cloning. Single cell cloning is not only an important regulatory step, but also has substantial impact on cell-specific productivity of the cell line, which in turn has a dramatic influence on the cost and the realisation of the development process as a whole.

Single cell cloning traditionally requires 2-20 per cent foetal bovine serum (FBS) or other sera. Through substantial screening of media, it has been possible to identify media formulations that are suitable to support single cell cloning by limiting dilution without the addition of serum at any step. Besides regulatory compliance, this procedure significantly shortens the time for cell line development compared to a serum-containing development process followed by adaptation to serum-free media.

Subsequent to single cell cloning, the clones have to be assessed with respect to growth characteristics and specific productivity in order to identify those clones most suited to the development of an economically feasible fermentation process. Once the ideal clones have been selected, they can be used to develop fermentation processes and downstream processes. Therefore, having upstream and downstream development in the hands of one single CMO partner, it may pay off in terms of time and cost savings.

REPEATED BATCH FERMENTATION

Fermentation in a stirred tank fermenter is the most widespread technique for the production of recombinant proteins such as biopharmaceuticals using suspension cell cultures. Fed-batch processes are predominantly used for the production of antibodies, which are relatively stable and generally support long residence times in culture. In the case of sensitive proteins

Figure 1 : GMP production suite in upstream processing (USP)





Figure 2: GMP production suite in downstream processing (DSP)

such as growth factors, which are unstable or toxic to the cells, or whose accumulation in the media inhibits cell, other production processes, such as perfusion, are required.

One option for perfusion is the repeated batch process. This process can be performed with or without reuse of cells, which impacts on the simplicity, control and yield of the process. The repeated batch process combines the advantages of fed-batch and perfusion fermentations. By fermentation in the repeated batch mode, the production period can be prolonged compared to standard fed-batch processes, which results in a significant increase in the final product yield. On the other hand, medium consumption is lower than in perfusion processes.

For repeated batch fermentation, cell recovery is performed under sterile conditions, using a centrifuge. For harvesting, cells are separated from the supernatant by centrifugation, re-suspended in fresh media and re-transferred into the fermenter. The cell density for re-inoculation can be adjusted, if required. Depending on the cell culture needs and product stability, the harvesting is carried out in pre-set intervals (usually one to four days). Cellular growth, viability, productivity and product quality in terms of glycosylation, remain constant during the whole fermentation processes. Repeated batch fermentation shortens the time for process development considerably, and has been applied to the GMP-production of several recombinant proteins using CHO cell lines.

DOWNSTREAM PROCESSING

Meanwhile, the current purification technology is not able to follow the upstream productivity revolution. Therefore, innovative downstream processing technologies that can catch

up with the improvements described above are desperately needed. Conventional column scale-up is reaching its physical limits and the cost of column fillings is going through the roof. Therefore, revisiting simple robust, and controllable technology such as precipitation, crystallisation, extraction, centrifugation, filtration and UV-inactivation may be a viable way to overcome these challenges. High-throughput capture and platform manufacturing technologies with generic, modular unit operations is the key to modern downstream processing. The adaptation of single and generic modules rather than the *de novo* design accelerates process train assembly.

The risk of viral contamination is common to all biotechnology products derived from cell lines. An essential component of process validation studies for products derived from eukaryotic cells is therefore to assess the capacity of the purification process to remove or inactivate potential viral contaminants. To date, biotechnology products derived from cell lines have not been implicated in the transmission of viruses. But some manufacturing processes are, by their nature, susceptible to virus contamination from extrinsic sources. Therefore, to assure product safety, validated virus clearance steps must be a part of the manufacturing process.

In a virus validation study, the manufacturer chooses a panel of multiple and appropriate viruses for testing. In spiking studies, those steps within a manufacturing process with the greatest potential for effective removal and/or inactivation of viruses, are challenged with a high titre of virus by adding the model viruses directly to appropriate steps of the validated downscale process. These studies allow the manufacturer to evaluate the overall

Figure 3: Aseptic filling of pre-filled syringes



virus reduction capability of the manufacturing process and determine whether the process is capable of providing an appropriate level of clearance to assure final drug product safety.

FILL AND FINISH

Modern parenteral pharmaceuticals have well-defined and well-established manufacturing requirements to meet regulatory and market needs. First and foremost, injectable drugs must be free of all microorganisms. To achieve that aim, they are filled aseptically in a Class 100 cleanroom with strict limits on viable and particulate contamination.

Biopharmaceutical products are subject to stability problems distinct from traditional sterile pharmaceutical processing and thus require more care in handling and preservation than do classical ‘small-molecule’ drugs. Most biopharmaceutical formulations are aqueous, and protein products have limited stability in their liquid state. Aqueous therapeutics, therefore, are often frozen and then thawed, freeze-dried to be reconstituted when needed, or encapsulated as nanoparticles in liquid form so that there is no contact with the atmosphere.

Delivering temperature-controlled packaging is problematic since validated shipping processes have to be established. Although lyophilisation is rather expensive and necessitates cooling, it is the most appropriate choice for global distribution to a wider market. In freeze-drying, the goal is to design the fastest and most robust cycle possible, consuming the least amount of energy without affecting product quality. The process comprises three steps: freezing, primary drying and secondary drying. As water freezes in the first step, the dissolved components in the formulation remain in the residual liquid – a phase termed the freeze-concentrate. At the point of maximal ice formation, the freeze-concentrate solidifies between the ice crystals that make up the lattice. Under appropriate lyophilisation conditions, the ice is removed by sublimation during primary drying, leaving the remaining freeze-concentrate in the same physical and chemical structure as when the ice was present. Residual water in the freeze-concentrate is removed in the secondary

drying step. Lyophilisation cycle development typically focuses on optimising the primary drying step.

The freezing process can have unexpected consequences on product quality. For example, some forms of sodium phosphate can crystallise upon slow freezing or annealing, resulting in a pH decrease in the freeze-concentrate. A decrease in pH has been shown to affect the stability of a drug when frozen and after lyophilisation. Other excipients that crystallise during freezing (for example, mannitol) can lose their ability to stabilise proteins in the dried state. Some proteins undergo cold denaturation during slow freezing or annealing, which can have deleterious effects on product quality upon reconstitution.

EFFECTIVE QUALITY ASSURANCE AND REGULATORY AFFAIRS

Regulatory compliance must be the number one priority whether your company is a big pharma, a start-up, or somewhere in-between. Quality and compliance are inextricably linked in this industry. Quality control is involved in nearly every step of the production process. From cell line characterisation to final drug product release, the quality control team is in close contact with all departments and guarantees that the product corresponds to all requirements. A typical example can be seen in terms of drug substance properties like glycosylation pattern and peptide map, as well as in regulatory compliance.

Quality assurance allows an enterprise to operate (compliance) and maintain high customer satisfaction (quality) – while optimising costs. The QA/RA team is required by regulation and internal expectation to have expertise and knowledge that is broader in scope and depth than any other technical department in the company. It needs expertise in providing guidance, review, and approval and in maintaining ethical practices in all technical areas of the business. Expertise is required in research, development, scale-up, manufacturing and technology transfer, and QA/RA units need to be aware of all current regulatory compliance requirements. QA/RA units must work effectively with those involved in the production process both inside and outside the organisation, helping them maintain compliance. Process efficiency and cost-effective production are not regulatory concerns. But a well-designed QA/RA unit has regulatory compliance as part of its design, integrating quality engineering to optimise the potential financial return inherent in all existing processes.

The trend is moving towards a holistic, integrated development concept, involving recombinant protein expression, product development and manufacturing, fill and finish and overlapping activities. The pressures within the industry are high: not only to deliver products and results on time, but also within a specified budget. ♦

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