



Integrating and Streamlining Biopharm Purification Processes

Advances in fermentation technology have pushed the bottleneck in biopharmaceutical production further downstream and the emphasis is now on developing effective purification processes to cope with higher yields.

By Andrew Clutterbuck at Eden Biodesign Ltd



Andrew Clutterbuck is responsible for the Downstream Process Development Team at Eden Biodesign Ltd, a company that develops and optimises purification strategies for recombinant proteins, antibodies, viruses and virus-like particles. He also plays a key role in the subsequent scale up and tech transfer of processes into the cGMP processing facilities at the National Biomanufacturing Centre (Liverpool, UK) and in providing technical support during manufacturing activities. Previously Andrew was a Senior Research Scientist for Avecia Biotechnology in Billingham, working within both the development laboratories and cGMP production facilities developing and running purification processes over a wide range of scales.

Over the past decade, biopharmaceuticals have consistently been the fastest growing segment of the pharmaceutical market, showing 19 per cent compound annual growth (CAG) since 1998. This – coupled with pricing pressures and the advent of competition from biosimilars – has resulted in growing pressure to increase manufacturing productivity and maximise the potential of existing facilities. In 2008, the global biopharmaceutical market was estimated at \$75 billion (1), with an expected growth rate of 12 per cent per annum for the next decade (2).

Historically, the major bottleneck in biopharmaceutical production has been fermentation capacity. The industry has responded by producing ever-higher yields as a result of using both new and novel expression systems, developing and optimising new and existing growth media, and increasing cell densities. All of these improvements have helped to increase the productivity of upstream fermentation processes, with the best examples of optimised monoclonal antibody fermentation achieving yields of over 27g/L (3). This has meant that, over the past few years, an increasing emphasis has been placed on developing effective downstream purification processes to cope with these higher yields. At the same time, in-process impurities have increased, mainly due to more complex growth media and higher cell densities with lower cell viabilities. This introduces elevated levels of host cell proteins, lipids and nucleic acids into the process and offers a greater challenge to the purification process (4).

With these advances in fermentation technology, the bottleneck has been pushed further downstream where filter and column sizes, and larger processing volumes and times, are becoming an issue. There are two main solutions to this problem. The first is to invest in greater infrastructure, which can be a costly and risky venture and, ultimately, is limited by column sizes. The second is to use current capacity more efficiently – this being the preferred option to meet current and future challenges. Seeking advice early in the development programme, and gaining a sound knowledge of manufacturing capabilities and regulatory requirements, can pay dividends and reduce the requirement for additional process development further down the line. Coupled with the general trend within the industry towards disposable technologies as opposed to fixed equipment, this makes the integration and combining of unit operations, with a view to reducing the number of overall processing steps, an increasingly attractive prospect. Increasing the efficiency of any process has a number of other cost benefits too, including a reduction in raw material requirements, buffer consumption and waste disposal considerations, and downstream processing facility requirements.

DOWNSTREAM PROCESS DEVELOPMENT

Typically, during the development of any purification strategy, processes can be split into a number of defined steps or unit operations, each mainly dependent on the activity to be performed at each stage. An understanding of how each operational step affects the performance of the subsequent downstream step is fundamental to



commercial success. The strategy should aim to achieve the highest yield and level of contaminant removal with the fewest number of unit operations.

Taking the model of a therapeutic protein secreted from cells into the growth medium, a simplistic view of the downstream process can be seen as:

- ◆ Primary separations – removal of cells and cellular debris from the process stream
- ◆ Primary capture – initial product capture and concentration
- ◆ Intermediate purification – removal of bulk in-process impurities
- ◆ Polishing chromatography – removal of specific, low level in-process impurities
- ◆ Final formulation – final dosage concentration and buffer formulation also includes bio-burden reduction filtration

PRIMARY SEPARATIONS

The primary separation of cells from the fermentation medium is a key area of consideration during process development and scale up. Advances in cell technology – which have led to increased yields – in some cases have also led to increased cell densities from both mammalian and microbial systems, both of which place an increased demand on the initial separation step. The primary separation can be the most significant bottleneck in any process because centrifugation – the classical cell and cell debris removal technique – is effective at small scales but becomes more problematic when dealing with increased volumes.

Centrifugation has very high operating costs in terms of energy consumption and processing times, which contributes to decreasing productivity. At volumes above 100L batch, centrifugation becomes impractical and continuous centrifugation becomes the preferred option. This requires further, complicated process development, which may change the impurity profile of the process



material. The high shear forces and stresses involved may damage the cells and release contaminants – such as host cell protein and DNA – into the process material which may, in turn, adversely affect the subsequent downstream process. This also makes microfiltration an increasingly attractive prospect at larger scales, but it also has large energy requirements, as well as a need for expensive, specialist equipment.

Filter technology has improved significantly over recent years, with a number of companies offering a range of process options to allow direct clarification of

Figure 1: 30L Fermenter in PD laboratory



Figure 2: 6mm bioprocess skid in GMP unit

the whole cell medium, and negating the requirement for centrifugation or microfiltration. The technique involves minimal equipment and is directly scalable to multiple thousand-litre volumes. Charged Lenticular-type filters, available from a number of suppliers, may also reduce other in-process impurities such as DNA and endotoxin. The downside of this technique is that the fermentation process must be robust, well defined and reproducible, as any minor changes upstream may adversely affect filter performance or lead to premature filter fouling. However, small variations in the feed material can be compensated for if, during the development phase, a certain

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amount of redundant filter capacity is engineered into the process.

CLASSICAL CHROMATOGRAPHY

At present, monoclonal antibodies or Fc fusion proteins dominate the biopharmaceutical industry pipeline. As such, the use of affinity chromatography resin – now a mainstay of antibody purification – offers a simple and effective way of reducing the number of processing steps by incorporating both a capture and concentration step with a high degree of purification. The major drawback of this approach is that the resin is expensive which, in the long term, may be offset against the cost of plant time as a result of the overall reduction in processing time. A secondary drawback is that the ligand may potentially leach from the column and need to be cleared from the final product. And, finally, for certain mAb-based products, affinity chromatography simply isn't an option and more classical methods must be employed. High-capacity, high-throughput 'classical' chromatography resins are being offered on the market from a number of companies and are becoming increasingly popular and incorporated more frequently into processes.

MEMBRANE CHROMATOGRAPHY

Within the industry, increasing attention has been given to the developing area of membrane-based chromatography. Membrane adsorbers, as they are often known, work on exactly the same principle as resin-based chromatography systems, often with the same basic ligand chemistry available. When clearing specific in-process impurities – such as endotoxins, host cell proteins and DNA – membrane adsorbers used in 'flowthrough' are being used in a growing number of manufacturing processes (5). These easy-to-use, single-use, disposable capsules have significant advantages in terms of throughput due to increased operating flow rates and minimal hardware requirements.

CONCENTRATION AND BUFFER EXCHANGE

Concentration and buffer exchange, both in process and as a final formulation step, are mainly performed by an ultrafiltration and dia-filtration (UF/DF) process. The UF/DF process works on the principle that the process feed stream flows parallel to the surface of the membrane face, creating a sweeping motion, thereby reducing fouling of the pores and the build-up of a gel layer on the surface of the membrane. This is in contrast to normal flow filtration, where the fluid flow path is dead-ended onto the filter surface. This technique is ideally suited for the



Figure 3: Sartoflow® beta crossflow filtration unit in GMP unit

concentration and buffer exchange of larger volumes, as the membrane area can be directly scaled. Advances in filter design and construction materials have been made over the past few years, and each of the main suppliers has further developed and expanded their existing product lines – but no specific breakthroughs have been made, with the exception of fully disposable systems. Selecting the correct membrane and fully optimising the step may take a large portion of the development time, but will ultimately contribute significantly to overall product yields.

INCREASED THROUGHPUT UTILISING DISPOSABLE OPTIONS

Increasing overall facility throughput in a multi-product facility can also be achieved by using disposable systems. This eliminates the requirement for costly and time-consuming cleaning validation and CIP cycles between batches, and is especially attractive for contract manufacturers where cross-contamination may be a problem. Reducing the need for fixed equipment also helps to build flexibility into and minimise the space requirements for each unit operation. The trend within biotechnology over the last few years towards disposable systems will continue to grow with the introduction of disposable fermenters, columns and ultrafiltration systems – making it eventually possible to develop a completely disposable process.

CONCLUSION

The growth of high titre expression technology can be expected to slow, since current cell lines can only be pushed so far and, as downstream processing technology continues to advance, there will be a gradual uncocking of the downstream bottleneck. It is inevitable that once one problem is solved, emphasis will be placed on another aspect of processing. But, with the current and projected growth in the marketplace, the industry will be ready to rise to the challenge.

*The author can be contacted at
andrew.clutterbuck@edenbiodesign.com*

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