The fermentation processes that are used by biopharmaceutical manufacturers have shown to lead to increasing quantities of therapeutic proteins. However, this increase in turn leads to capacity bottlenecks in the subsequent purification process (known as downstream processing) and is associated with high costs. Downstream processing comprises up to 80 per cent of the entire production costs. Producers are increasingly recognising the present need for improvement and have shifted their focus from improving the production process (upstream) to improving the downstream process.

Thanks to the progress attained in molecular biology, which has led to better cell culture methods, pharmaceutical producers can now increase the yield of monoclonal antibodies (the fastest growing marketing segment) from the milligram scale to the gram scale.

Purification processes are very expensive
Experts state that a tenfold (from 0.1 to 1.0 g/l) increase in the fermentation titre causes the ratio of upstream to downstream costs in the process to drop from 55:45 to 30:70. This shows that the upstream costs are inversely proportional to the titre, but the same is not true for the downstream processing costs.

In order to satisfy larger market demands, the protein load on the chromatography steps needs to be increased. This can either be achieved by increasing the number of cycles or by investing in larger chromatography columns (up to a diameter of two metres). This, however, also leads to larger volume loads on the subsequent filtration steps, leading to longer filtration times or the need for larger areas.

Economic limits and difficulties in increasing facility space and equipment size

Experts are not expecting any major problems in the early isolation process, including protein ‘harvest’, the separation of cell material and clarification. However, real problems are expected to show up with the isolation of proteins, since an increase in protein yields will require manufacturers to invest in larger equipment and more materials in order to handle the larger quantities of protein produced. Although large chromatographic columns are technically feasible,
they require larger utilities and floor space to match the larger size of the columns, which is something that is not always possible for the existing facilities. It is, therefore, quite understandable that the biopharmaceutical industry is actively seeking less expensive alternatives, and not only in view of the growing cost pressure in the healthcare sector. One idea to overcome the problem at hand is to replace chromatography resins, for example Protein A (which can bind mammalian proteins), which is very costly, or to reduce the number of column chromatography steps from three to two, and eventually to one. Membrane absorbers might also be a potential alternative for counteracting the downstream bottleneck.

New methods for German production site

For Germany, which is the second-largest biopharmaceuticals producer worldwide, new purification methods are needed in order to remain competitive. The German Federal Ministry of
Education and Research has reacted to this situation with a funding initiative ([link](https://www.gesundheitsindustrie-bw.de/www.bmbf.de/foerderungen/11545.php)) and hopes to address the current situation by increasing cooperation between industry and science as well as by motivating the stagnant German research and education landscape.

The purification problem faced by the biopharmaceutical producers can be better understood by looking at the historical development of red biotechnology. In their incubation phase (1982-1994), biotech start-ups were primarily focused on developing recombinant proteins to be used for substitution therapy and obtaining marketing authorisation for their products. In many cases, the laboratory processes were directly implemented into the production scale. The therapeutic doses of recombinant proteins were usually in the μg range. In bioreactors, E. coli bacteria and animal cell cultures (primarily hamster ovary cells) normally produce un-glycosilated therapeutic proteins. This method led to 10 to 100 mg of protein per litre. At that time, the biopharmaceutical industry was mostly interested in improving production and put purification processes on the backburner.

Process innovation follows product innovation

In the second phase, which is the companies' success phase, the R&D portfolios of all the biotech companies changed drastically. Custom-made fusion proteins, humanised antibodies and FABs (antigen binding fragment of an antibody) were increasingly used for the treatment of tumours and immunological diseases. The throughput had to be increased to the mg scale. As a result, manufacturers had to think of new ways to change their manufacturing processes in order to match the purification capacity with upstream productivity. The manufacturing portfolio shifted from prokaryotic to eukaryotic systems due to the growing importance of glycoproteins.
The growing understanding of molecular and cellular processes led to an increase in the expression rates of existing cell lines and to the development of new expression systems, such as NSO (mouse myeloma cells). Standardised fermentation methods led to protein yields of up to 2 g per litre that were produced with animal cell culture systems, and up to twice as much in the development process. Productivity increased by 50- to 100-fold.

Innovation in the downstream processing area was much more inactive. As upstream productivity increased, the binding capacity of chromatographic materials that are used for the subsequent purification of proteins, increased by no more than a factor of three. "The linear upscaling of the downstream processes was usually the most frequent action keeping pace with upstream innovation" (Allgaier).

Multi-million expansions

Since the first-generation production facilities focused mainly on upstream processing, the existing facilities usually did not have the equipment or floor space to meet the increase in fermentation titres. Expansions became necessary, which in turn led to costs reaching to the two-figure million range.

Two types of large-scale production facilities were primarily used at the end of the 1990s. Both types were used as multi-product facilities employing the fed-batch technology. The reactors had a capacity of 6 x 15,000 litres with turn-over periods of three weeks and a total annual capacity of 500 to 600 kg protein.

Although the productivity increased drastically thanks to improved cell culture methods and fermentation conditions, the technical fermentation process remained, in principle, unchanged. The up- and downstream processes drifted more and more apart. In the mid-1980s, product yields amounted to 50 to 100 mg/l, and just 25 years later, they centupled.

**Product- and process-immanent impediments**

Several steps are required to purify the therapeutic proteins produced in bioreactors. These contaminations are the result of the processes, or the products themselves, and can pose a danger to those patients treated with impure substances. Therefore, care must be taken to reduce the amount of undesired substances down to a harmless amount. The contaminations, moreover, might also have detrimental effects on the product itself (denaturation).

The process-related contaminations include the remains of the host cells (proteins, nucleic acids) or of the cell culture (components of the culture media used) or can be the result of the processing (salts or detached chromatographic ligands). The contaminations might also be related to the product itself, i.e. undesired molecular variants or shortened forms, precursors, hydrolytic degradation products or modified forms (e.g. through erroneous glycosylation). Polymers and protein aggregates are also undesired product-specific variants.

It is also necessary to remove all the other substances of microbiological, biochemical or chemical origin, which are directly part of the production process, for example viruses that might occur in cell cultures.
Large-scale purification under economic aspects

It is certainly possible to improve biopharmaceutical purification. However, the large-scale production of proteins is associated with exorbitant costs. Production and purification must not put the profitability of the biopharmaceutical product at risk. The time required to establish a new purification process also plays a major role in process economics. In addition, the development of the process must be coordinated with the preclinical and clinical development of the drug.

And last but not least, there is no "one and only" purification process. Every substance requires a specific purification process, which is adjusted to the substance's specific biochemical and biophysical profile and its specific fermentation conditions.

Downstream processing operations, i.e. the processes used to turn a product from its natural state into a pure protein, can be divided into four steps. The first step involves the isolation, concentration and stabilisation of the target protein (capturing), followed by the removal of viruses. The third step involves the removal of contaminations, such as nucleic acids, unwanted proteins and endotoxins (intermediate purification). The final processing step involves the removal of trace contaminants that might compromise the safety of the product (polishing). The typical operations are filtration and precipitation, as well as (column) chromatrophic methods, wherein a broad range of methods and materials are available.

Process development according to the trial and error principle

The purification of the target protein or molecule depends on its form, size, solubility, surface charge or its biospecific affinity to binding partners. This also increases the number of processes that can be used to purify the proteins. Considering the enormous cost and time pressure, as well as the production-plant-specific constraints, manufacturers are having a tough time developing optimal purification steps for the target molecule or protein. Usually, these processes are developed according to the trial and error principle and are established as soon as the process "functions".

It is difficult to omit steps in the purification process and not compromise thereby the safety of the protein (such as the removal of viruses or DNA). However, representatives of the pharmaceutical industry believe that it is possible to optimise the procedure and potentially also combine individual or partial steps of the process. Column chromatography is usually regarded as the major cost driver. Besides for the fixed costs, the column membranes that are used for the purification of the proteins swallow a large proportion of the costs.

Those looking for reasons as to why downstream processing has been neglected for such a long time are well advised to take the following into account: while the establishment of master cell banks and the optimisation of the cell culture techniques were achieved without the help of others, the purification of proteins, however, has always involved third parties. This has made innovation quite difficult.

From a manufacturer's perspective, downstream processes can be effectively improved by using less complex culture media (so-called minimal media, which only contain the minimum nutrients required). High priority is also given to efforts trying to reduce the purification steps. This would save a lot of time and money and simultaneously reduce the substance losses occurring with any further purification step. Innovative gels and double binding capacity is also expected to increase the effectiveness of the chromatographic processes.
Paradigm change in the industry

Some of the authors discussing the problems experienced in upstream and downstream processing seem to deny the fact that the biopharmaceutical industry is experiencing a downstream bottleneck, claiming in turn that the most severe bottlenecks occur in facilities that are 10 to 20 years old, which is where those operators were unable to anticipate the increase in upstream productivity. Other people believe that the purification issues are over-hyped since only very few products are manufactured at a very large scale.

What seems to speak against the downstream bottleneck idea, is the notion that modern biopharmaceuticals are becoming more potent, which reduces dosing and in many cases also the batch sizes.

One thing seems clear: the biopharmaceutical sector is undergoing a paradigm change. Some people are increasingly making suggestions to reverse the current way of thinking. This is also the case with many other mature technologies. Why not split the batches or purify the proteins in two partial steps in order to not run into capacity limitations? Why is it always necessary to purify all the fermented proteins? Is it really necessary to spend a two-figure million sum on reorganising a downstream process that only leads to a productivity increase of 10 to 20 per cent?

The trend towards new substance classes also affects production plant concepts and technology. "In the future, I'm sure that single-use disposables will come into greater use in order to reduce equipment and other types of costs," anticipates Dr. Hermann Allgaier of Merckle Biotec. He also believes that fast 'frigates' will outpace the heavy and slow 'ocean liners' as a result of the productivity progress made: Nowadays, 2k fermenters are used for the production of 2.5 t for which 12k fermenters were previously required.

Walter Pytlik, BioRegionUlma BIOPRO Baden-Württemberg GmbH

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