Listicle

A Closer Look at Biomanufacturing

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Biomanufacturing is a process that involves the production of commercially important biopharmaceuticals using various biological systems such as animal cells, microorganisms, plant cells, tissues, genetically modified cells and organisms and enzymes.

Unlike small-molecule drugs, biologics are much larger and possess greater structural complexities. Their function is defined by their structure and metabolic pathways. In this listicle, we will outline some of the key biomanufacturing process steps.

Origins and subsequent development of biomanufacturing

Researchers have classified the history of biomanufacturing into three revolutions:

- **Biomanufacturing 1.0** – Primarily focuses on the synthesis of primary metabolites by using mono-culture microorganism-based fermentation. In 1990, Chaim Weizmann produced primary metabolites, namely, acetone, butanol and ethanol (ABE) via anaerobic liquid fermentation.

- **Biomanufacturing 2.0** – Originated from penicillin fermentation during World War II. It primarily focuses on the production of secondary metabolites via two methods: utilizing specific mutants and aerobic submerged liquid fermentation. Some of the products linked with this category of biomanufacturing are penicillin and streptomycin.

- **Biomanufacturing 3.0** – Started after 1980 with the production of large size biomolecules, including proteins and enzymes. During this time, recombinant DNA technology and advanced cell culture helped develop many important biopharmaceuticals such as insulin, erythropoietin, amylase, DNA polymerase and growth hormone.

Key steps in biomanufacturing

Upstream bioprocessing is the first step of biomanufacturing that involves media preparation, cell culture and harvesting when the cells reach their desired density. These steps occur in a bioreactor. Upstream bioprocessing is followed by downstream processing, which entails the recovery of bioproducts from microbial, plant or animal cells (intracellular metabolites) or fermentation broth (extracellular metabolites) collected from the bioreactor. The downstream process also encapsulates steps involving the purification of a particular product and proper disposal of biological waste.

In the biotech industry, process development requires hundreds of million dollars of investment. Hence, any changes made to improve speed and reduce manufacturing timelines mustn't compromise the quality of the bioproduct.

Advancements in upstream bioprocessing

Recent advancements in upstream processing have led to high yield and rapid and cost-effective production of biopharmaceuticals. Some of the innovative technologies associated with upstream bioprocessing include high-throughput (HTP) technologies, optimized microbial culture media and growth parameters and continuous upstream processing. Many companies have improved established protocols or manufactured products (biosimilar) using various methods, after the expiry of the patent. Such processes reduce the time required for designing an optimized protocol, significantly.

Improving cell lines, culture and media

Innovations in the formulation of media and improvements to host cell lines have revolutionized upstream bioprocessing. These cells can be genetically engineered to optimize the production of the biopharmaceutical product, ensuring a higher concentration and/or quality. Genetic engineering requires an understanding of the "omics profile" of the cell line to enable an accurate prediction of the phenotype from an engineered genotype. Techniques used for omics analysis, such as next-generation sequencing (NGS), mass spectrometry (MS) and spectroscopy, are increasing in speed and sensitivity, making it easier to characterize biological systems and genetically manipulate them.

Bioprocessing optimization is essential for perfusion cell culture, which involves continuous amendment of fresh media into the bioreactor with simultaneous removal of spent media and product. Continuous assessment of cell viability is an important aspect of this method. Optimization of cell expression systems is also crucial to attain large quantities of a high-quality product.

Novel bioreactors

Bioreactors that intensify the process of biomanufacturing are rocking motion, single-use stirred tank and alternating tangential flow filtration bioreactors. These bioreactors are regarded as the latest generation bioreactors that are known to be more efficient and gentler on cells, which enhances cell viability and provides lesser impurities for downstream processing. With respect to the size of a bioreactor, typically, smaller bioreactors (micro and milli scale volumes) are used in *in vitro* research that offers miniaturized, high-throughput solution to bioprocessing. Larger scale bioreactors (approximately 20,000 liters volume) are used for clinical use, for example, production of adipose-derived stromal cells for tissue engineering, megakaryocytic progenitor cell line for regenerative medicine, etc.

High-throughput devices

Some of the high-throughput devices (HTPDs), designed for an upstream process, are multi-well-plates, microtiter plates, miniature shaken vessels and mini bioreactors. HTPDs enable optimization of experiments before scaling up the production. Miniaturization and parallelization permit laboratories to screen multiple experimental conditions and help design optimal, fast and accurate processes in a fraction of the time and cost.

Process analytical technologies

Scientists have also introduced tools for several online process analytical technologies (PAT), e.g., cell density monitors based on capacitance sensors. This technological advancement, i.e., sensor-based bioreactors, has enabled bioreactor automation with a supervisory control system. Importantly, these sensors can monitor the cell density of viable cells without collecting samples and, therefore, avoid the risk of contaminating the culture. PAT tools based on spectroscopic techniques (near-infrared, fluorescence, infrared and Raman) are also used to analyze samples. For instance, Raman spectroscopy is used to assess structural and chemical changes in proteins.

Single-use technologies

Advancements in technical equipment, such as single-use technologies [SUT] and inline testing technologies, are regarded as crucial upstream innovations. There are different types of single-use disposable cultivation systems, such as stirred tanks and orbital shaken and pneumatically mixed bioreactors. The SUT-based biomanufacturing system is associated with low contamination risk as cleaning and sterilization are not required. These advancements have enabled biomanufacturing companies to develop more complex biomolecules in shorter durations with reduced manufacturing costs.

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Advancements in downstream bioprocessing

Generally, conventional downstream bioprocessing methods, to obtain purified and biologically active proteins, include various steps such as cell lysis, centrifugation, diafiltration, chromatographic purification etc. Downstream processing has been immensely benefitted by revolutions in technology and equipment, suchas HTPD, SUT and PAT, similar to upstream bioprocessing.

Recent advancements, such as in-line cell washing and acoustophoresis (ultrasonic) cell separation, have enabled significant enhancement in biomanufacturing. For example, in the case of harvesting ***E. coli***, HT-compatible bead mills have been designed for cell disruption. Also, researchers have developed a 24-well-HT sonication device for bacteria, fungi and yeasts cell disruption/lysis. HT-chromatography systems with different capacities have also helped researchers immensely.

Scientists have developed a novel system that assists acoustophoresis in a high-performance microfluidic device, which enables label free cell separation. This system is used to isolate mononuclear cells from a mixture of cells. This device can separate clinical blood samples without requiring centrifugation, which has successfully accelerated the downstream process. Further, some of the SUTs that have fast-tracked downstream bioprocessing are single-use continuous centrifuges, single-use filtration techniques and single-use chromatography systems, etc.

PAT tools

In downstream processing, PAT tools, using spectroscopy, high performance liquid chromatography and circular dichroism, help determine the concentration and purity of proteins, endotoxin and process-related impurities. Recently, scientists used Fourier transform infrared spectroscopy as a PAT tool for real-time in-line estimation of PEGylation in chromatography.

Transition to continuous bioprocessing

The transition from conventional batch processing to continuous bioprocessing has enabled a reduction in the cost of production. Continuous centrifugation is one of the crucial methods used for cell harvesting or cell removal. For instance, during the large scale production of antibodies (IgG1 mAb), disk-stack continuous centrifuge with periodic and continuous elution has been used. Scientists have engineered a continuous-flow electroporation module and in-line buffer exchanger, which allows the introduction of RNA, mRNA and DNA into a cell without requiring viral vectors or even activation steps. To obtain highly pure bioproducts, continuous chromatography processes (e.g., continuous annular chromatography, countercurrent chromatography, multicolumn countercurrent solvent gradient purification chromatography and countercurrent tangential chromatography) have played a crucial role.

Future of biomanufacturing

Biopharmaceutical companies are subjected to complex regulatory requirements and high development costs. Advancements in biomanufacturing processes and tools help these companies to comply with all the regulations and develop novel products in a cost-effective manner. Owing to advancements in technology and equipment, the current biomanufacturing industry is moving towards biomanufacturing 4.0. This type of biomanufacturing would strongly depend on the advancement in digitalization and automation and is expected to bring about a substantial increase in productivity and value of biopharmaceuticals.