Overview

Production Unit

Production Overview

The pharmaceutical industry has been manufacturing traditional drugs or pharmaceuticals since the late 19th century; and the biopharmaceutical industry, a specialized segment of pharmaceuticals, has expanded rapidly over the past 30 years. The objective of the biomanufacturing industry is to develop cost-effective, robust, and reproducible biomanufacturing processes to manufacture safe and efficacious product for various clinical applications. Biopharmaceuticals are large and much more complex molecules than traditional pharmaceuticals (which are produced using chemicals synthesis and oftentimes referred to as "small molecules," as their molecular weight is in the order of a few hundreds). The typical size of biopharmaceuticals ranges from 10,000 Dalton (10 kiloDalton (kD)) to 160,000 Dalton (160 kD) and they are produced in living cells, which can be mammalian, yeast, bacterial, or insect cells. These living cells will express slightly different molecules depending mostly on the expression system but also on the integration site and cell culture conditions.

Since biopharmaceuticals are administered to patients, the development and production process has many regulatory components—the raw materials must be safe and the removal of contaminants must be demonstrated during processing. For example, mammalian cell cultures use serum or serum components of animal origin and these components can contain harmful prions (*see* ***Microbiological Control*** *chapter*) or adventitious viruses. Using such serum is acceptable for research and diagnostic purposes but poses problems for biopharmaceuticals. If serum must be used (e.g. first step of cell line development), then it should be of New Zealand origin, as no prion-related diseases (such as mad cow disease) have been detected in the country. This is an example of the importance of safe raw materials. Through the entire production process similar thinking must be applied.

Process development starts when research scientists first express a potential drug in a cell and prove the correct identity. It continues through evaluation and optimization of various steps, followed by scale-up to full scale production, which involves upstream and downstream production. The process development phase can take many years, and during this phase the objective is different than that for full scale production. The objective during the early research phase is to produce material for preliminary *in vitro* studies. The focus is not on cost but on producing material to be evaluated for biopharmaceutical production. As the product goes through the pre-clinical evaluation and human clinical trials, the focus turns to developing a satisfactory manufacturing process.

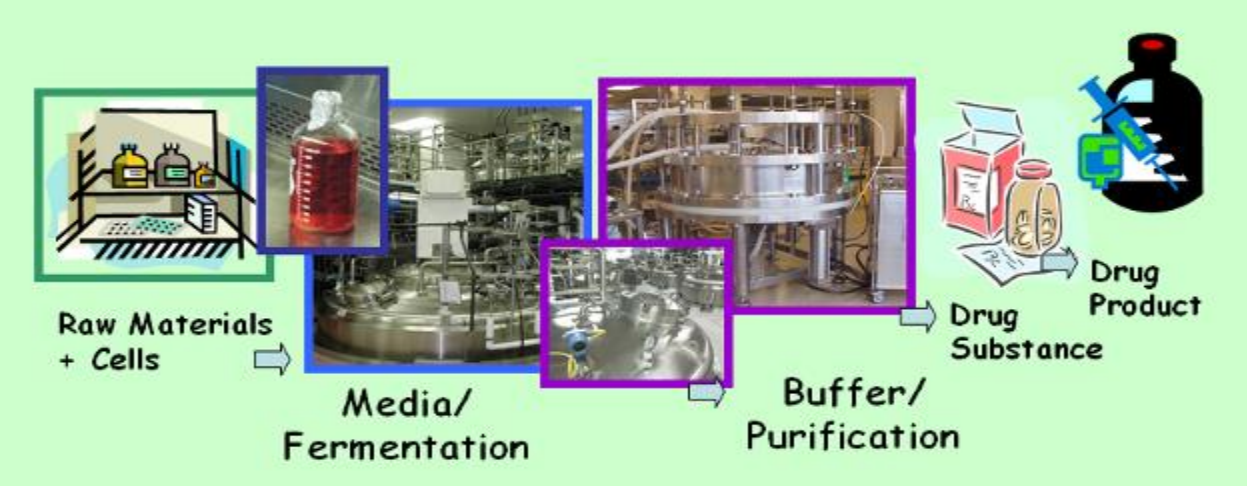
For a product to be safe, efficacious, and economical it takes several years and many groups, including the process development team, to develop all the analytical methods for raw material testing, API, and impurity testing. Furthermore, a facility and applicable equipment must be designed (unless the manufacturing is sent to a contract manufacturing organization), the Quality Assurance department's guidance documentation has to be written and approved, and SOPs, batch records, and calibration must be in place, including an EH&S program addressing issues such as safety and hazardous waste.

How does the full-scale production/manufacturing process differ from the process research and development efforts? As biopharmaceuticals are not synthesized but produced by living cells,

there will be some variability in the production that can only be monitored and not specifically controlled. Slight changes in cell culture conditions could lead to subtle changes to the API, which could trigger unwanted reaction in patients. The cause and effect of these changes, however, cannot be predicted. The main way to address this is to consistently use the same process, chemicals, and conditions, keeping everything in the acceptable range as defined in the batch records.

Bacteria or mycoplasma content must be routinely monitored. Cells can contain adventitious viruses, and individuals working with the cells can transmit those viruses in the culture. The process must be able to inactivate (kill) any viruses that are introduced. Furthermore, potentially harmful chemicals can be introduced through the process stream. Whether virus or chemical, however, the development scientist has to consider ways in which to remove these potentially harmful intrusions.

The following three chapters will discuss the most important aspects of biomanufacturing. The process of protein manufacturing has two main stages: *upstream* and *downstream.* Upstream manufacturing (***Chapter 10, Upstream Processing***) is the process that initiates, monitors, and supports cell culture and subsequent protein production. Once the cell culture reaches certain parameters, the protein must be purified during downstream processing (***Chapter 11, Downstream Processing***). The last chapter (***Chapter 12, Process Development***) actually examines the first step in the entire process of creating a biopharmaceutical. It explains the research, development, and additional tasks required to express a potential drug with well-defined biological activity. The reason for this first-is-last approach is that most of the topics covered in the rest of this textbook will explain many of the aspects of process development. This makes it easier to explain and understand why certain systems are chosen and applied—this includes expression systems, selection of bioreactors, and choice of chromatography techniques, buffer exchange, and formulation. It also helps to understand Operational Excellence, EH&S, metrology, validation, Quality Assurance, Quality Control, and other related topics.



Overview of the Production Process

The Production Unit covers a wide variety of topics focused on bulk product processing (drug substance); it does not cover final fill and finish (drug product) processing. The chapters in the unit mainly focus on the most successful and largest volume production efforts, such as those that produce monoclonal antibodies in mammalian cells but the guiding rules are the same, whether production is done in mammalian cells or bacterial cells.

Dispensing chemicals, preparing media and buffers, and filtration processes are performed in the same manner. Cleaning and sanitization guides are the same as well. The two main steps that differ are: 1) the harvest step, when the bacterial cells are broken up to facilitate the isolation of the API, and 2) for bacterial processing, protein refolding is performed in case the API is overexpressed as inclusion bodies.

The upstream processing chapter describes the large scale (20,000 L production bioreactors) processing of mammalian cells. The advantage to describing the largest size production scale is that not only are cell culture conditions covered, but other aspects of large-scale processing are examined as well. This includes scale-up, engineering, facilities, and the widely-used harvest techniques of centrifugation and filtration.

The downstream processing chapter uses a similar approach. Monoclonal antibody manufacturing is the model described. Not only is mAb production a nearly $100 billion business, but it also results in the largest volumes to be handled. Recent improvements in cell line development have resulted in a huge cell productivity increase. Ten years ago the typical expression levels were in the 0.1–1 g/L; new cell lines often produce 3–5 g/L protein. The effect of high expression systems will be that bioreactors could get smaller as the size of purification suites remain constant.

The downstream processing chapter also covers the various types of filtration and scale-up considerations, both for purification and filtration, whether it is tangential flow filtration applied for buffer exchange and product concentration, or for aseptic filtration.

The process development chapter provides a basic understanding of the factors that must be considered when a product and process are developed in the regulated biomanufacturing industry.

Method Overview and Introduction

This section briefly describes some of the methods explained in more detail in the following chapters.

The main steps of protein purification:

- Capture is an initial protein purification and concentration step in which a large volume of the main impurity, water, is removed. For capture, high capacity and fast techniques are needed, as the feed typically contains proteases that could quickly break down proteins.
- Intermediate is a step that requires good resolution and removes most of the impurities. Ample effort goes into the development of the intermediate step.

- Polishing is the final step of purification intended for the removal of impurities—often used for cellular DNA and bacterial endotoxin removal.

The most widely used protein separation/purification methods are the various chromatography techniques applied in analysis (QC), development, and manufacturing. There are several types of chromatography modes

- Size Exclusion Chromatography (SEC): separation is based on the size of the molecules; it is widely used as an analytical tool in QC but used less often in preparative scale processes; it is not a large scale processing technique; selectivity is extremely low and the volume of sample is very limited.
- Affinity chromatography: based on specific recognition, which makes the purification method highly selective; affinity chromatography provides good capture and is sometimes used for intermediate purification.

  A ligand of choice is immobilized to the chromatography matrix that will only recognize a certain 3D structure on the target molecule. The ligand used in most of the antibody purification is Protein A. Protein A was isolated from *Staphylococcus aureus,* where the biological role of Protein A was to bind immunglobulins in an orientation which disrupts phagocytosis. As native Protein A also binds albumin, a modified r-Protein A is used. The biorecognition of Protein A and immunglobulins has been used in numerous applications. As biorecognition occurs easily, the dissociation of the ligand-target may not always be obvious. It does mean that the target molecule might be denatured under the conditions of the dissociation, which somewhat limits the use of high-binding affinity ligands for preparative purposes.
- Hydrophobic Interaction Chromatography (HIC): a versatile method for the separation of biomolecules based on differences in their surface hydrophobicity

  HIC is the ideal candidate for intermediate separation. It couples nicely with Ion Exchange Chromatography where elution occurs at high salt concentration (which is the starting condition of HIC). HIC couples well with a polishing IEX step, as the HIC elution is a low salt concentration (the starting condition of IEX). Proteins usually have hydrophobic amino acids in domains away from the surface of the molecule. Therefore, the protein needs to be slightly denatured prior to separation to expose the hydrophobic patches. High salt concentration is used to expose the hydrophobic areas. This feature promotes the high selectivity of HIC and elution conditions at low salt concentration help to preserve biological activity.
- Ion Exchange Chromatography (IEC or IEX): relies on the reversible charge-charge interactions between proteins in the sample and the charges immobilized on the resin.

  IEC is excellent for capture because of its high capacity. It is also good for the intermediate and polishing step due to high selectivity. Ion Exchange Chromatography can be subdivided into 1) Cation Exchange Chromatography (CEX), in which positively charged ions bind to a negatively charged ligand and 2) anion exchange chromatography (AEX), in which the binding ions are negative and the immobilized functional group is

positive. Salt or pH gradient can be used to separate the components. This is called the "bind and elute" method. A different approach is the "flow-through mode," when the IEX is performed under conditions in which the protein of interest is not binding. This is a particularly good mode to use in the case of anion exchange, where it is used to bind endotoxins, cellular DNA, or other highly negatively charged substances—at the same time product is not retained by the matrix.

The chromatography matrix is important. The ideal matrix will only react through the special ligand. In other words, the carrier will not interact with biomolecules. The ideal matrix will allow rapid processing and take high flow rate while being cleaned and sanitized hundreds or thousands of cycles without loss of performance. Unfortunately such an ideal matrix does not currently exist. Agarose-based matrices are closest to perfection at this point. They are inert and hydrophilic but the applied flow rate can be limited. A high flow rate can cause a high pressure drop on the column that can crush the chromatography beads. Polymer-based resins are resistant to high pressure and to many chemicals. However, they can exhibit hydrophobic properties. Using a hydrophilic coating is one way to overcome this issue. There are many options to choose from, as the various chromatography matrices all exhibit different properties. Organizations have been trying different ways to overcome these issues with various degrees of success.

In the third and last ***Production Unit*** of this ***Introduction to Biomanufacturing*** textbook, ***Chapter 10*** covers Upstream Processing, ***Chapter 11*** covers Downstream Processing and ***Chapter 12*** covers Process Development of biopharmaceuticals.