Advances in Biopharmaceutical and Vaccine Manufacturing Plants

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OVERVIEW: The development of innovative pharmaceuticals with potential for meeting unmet medical needs and vaccines that protect against infectious diseases is very important for ensuring people's health and welfare. However, biopharmaceuticals and vaccines that make these developments possible are more easily affected by the production process than are chemically synthesized low-molecular-weight pharmaceuticals, and they demand a higher level of technology to manufacture reliably and efficiently. Hitachi is contributing to advances in biopharmaceutical and vaccine production technologies by working to improve the productivity and quality of pharmaceutical manufacturing.

INTRODUCTION

BIOPHARMACEUTICALS (pharmaceuticals produced using biotechnology) such as monoclonal antibody drugs have attracted considerable interest in recent years to satisfy demand for treatments for currently untreatable diseases, and a series of large production plants have been built in different parts of the world. Meanwhile, although vaccines have already played an important role in protecting against infectious diseases, growing threats such as pandemic influenza or bioterrorism have prompted a major renewal of interest. In addition, a number of companies are working on therapeutic vaccines that take advantage of the immune system.

The following lists the challenges facing the production of these distinctive biopharmaceuticals and vaccines.

(1) Compared to chemically synthesized pharmaceuticals, many of these drugs have a high molecular weight and variations in structure, and thus are limited in the extent to which their uniformity can be verified.

(2) Problems such as allergies will arise unless impurities derived from cultured cells are adequately removed.

(3) Small changes in the production process have the potential to cause changes such as to the quantity of impurities or the quality of the resulting product.

(4) The construction of facilities needed for high-quality production is very expensive.

Considerable effort is being devoted to productivity and quality improvement in order to overcome these challenges.

This article gives an overview of biopharmaceutical and vaccine production technology and developments in the field, and describes the work Hitachi is doing to improve the productivity and quality of pharmaceutical production technology.

OVERVIEW OF BIOPHARMACEUTICALS PRODUCTION TECHNOLOGY AND ASSOCIATED DEVELOPMENTS

The production of biopharmaceuticals consists of a culture process, in which the desired product is produced by a biological reaction, and a recovery and purification process, in which contaminants are removed to purify the product.

Compared to chemically synthesized low-molecular-weight pharmaceuticals, biopharmaceuticals are inherently more expensive to produce for a number of reasons, including their long reaction times, low concentration of product, stringent sterility and cleanability requirements, and the use of expensive chromatography resin. This creates a strong need to improve productivity.

Improving the productivity of the culture and purification processes requires more than just increasing production quantities; what is also needed is to identify which components make up the largest part of production costs, such as the cost of materials, utilities, labor, or equipment, and then to make process improvements that reduce those costs. While the conventional approach to productivity improvement is to increase the concentration of product in the plant, in cases where raw materials such as the culture medium make up a large part of the cost, it is necessary to

focus on the amount of production per unit of material consumed.

Culture Process

Most biopharmaceuticals are produced from mammalian cells. As mammalian cells are susceptive to the culture environment, the productivity and quality of the product will potentially be affected if an appropriate culture environment is not maintained. For this reason, when scaling up mammalian cell cultures from the laboratory to commercial production, it is necessary that the design of the process and equipment take full account of how this will change the culture environment and what effect it will have on productivity and quality. Fig. 1 shows a large-scale cell culture plant.

Typical examples of culture environment that influence productivity and quality include hydrodynamic forces generated by stirring, dissolved oxygen, dissolved carbon dioxide (CO_2), uniformity of mixing, and foaming.

(1) Hydrodynamic forces

The effects of excess shearing stress or other hydrodynamic forces generated by stirring on mammalian cells include increasing their rate of oxygen consumption, reducing their rate of protein production, reducing their rate of cell growth, and causing cells to be destroyed.

(2) Dissolved oxygen

A low concentration of dissolved oxygen in the culture causes low cell growth rate and can also influence the quality, such as altering the composition of the product.

(3) Dissolved CO_2

The consequences of a high level of dissolved CO_2 include reducing the rate of protein production and cell growth.

(4) Uniformity of mixing

It is essential to achieve uniform mixing inside the bioreactor to maintain a uniform distribution of nutrients in the culture fluid and to prevent localized increases in concentration when pH control chemicals are added.

(5) Foaming

While direct sparging (gas flushing) of the bioreactor is the most efficient way to supply oxygen and remove CO_2, the presence of proteins, fats, and other materials in the culture medium can cause a layer of foam on the liquid surface that can overflow into the discharge line. Accordingly, a sparging method that can avoid this is essential. Measures include reducing the amount of gas used for sparging, addition of an antifoaming reagent, fitting of antifoaming devices, optimization of bubble diameter, and optimization of stirring.

Fig. 1—Large-scale Cell Culture Plant.
This large-scale cell culture plant used to produce monoclonal antibody drugs was supplied by Hitachi.

Recovery and Purification Processes

In the production of proteins using mammalian cells, a supernatant containing the desired protein is obtained by separating and removing the cells. As proteins and deoxyribonucleic acid (DNA) from inside the host cell will leak out if this process results in cell disruption, it is essential that cell separation be done in a way that does not damage the cells.

As the proteins used as biopharmaceuticals are prone to denaturation, and as proteins with a similar structure are also produced, column chromatography plays a central role in the purification process, because it allows this separation to be performed with precision. Monoclonal antibody drugs are typical examples of biopharmaceuticals. In their production, the culture fluid after the cell separation process is supplied to a column filled with a gel embedded with Protein-A. The bulk of the antibodies bind to this Protein-A and are then eluted using an acidic buffer solution (pH: 3 to 4). Next, apparatuses such as a cation exchange column or anion exchange column are used to purify the product.

Because of the risk that the host mammalian cells used in protein production may be infected with a virus, it is essential that the purification process reliably perform virus inactivation and viral clearance. Methods for inactivating viruses include lowering the pH (acidification), heat treatment, and treatment with surfactants, and methods for viral clearance include viral clearance filters and column chromatography.

After undergoing these purification processes, the resulting product can achieve high purities of 99.9% or better, with cell-derived admixed protein levels of 5 ng/mg or less, cell-derived DNA levels of 10 pg/mg or less, and pyrogen levels of 0.005 endotoxin units (EU)/mg or less. However, this also means that the bulk of the target protein produced by the culture process is lost in processing.

OVERVIEW OF VACCINE PRODUCTION TECHNOLOGY AND ASSOCIATED DEVELOPMENTS

Use of mammalian cell cultures for the production of vaccines has been on the rise since its use for rabies vaccine in the 1970s. In recent years, it has been used for the production of vaccines for diseases such as hepatitis A, Japanese encephalitis, polio, and influenza. Vaccine production can also be spilt into a culture process and recovery/purification process.

Culture Process

To produce a vaccine in large quantities as soon as possible after a new form of influenza appears, it is necessary to use cell-culture-based manufacturing systems rather than the egg-based manufacturing systems used in the past. After multiplying cells in the culture process, the cells are infected with the virus, which is allowed to multiply, and then the antigen that forms the basis of the vaccine is harvested. Methods that use genetically engineered cells to produce the antigenic protein directly, in the same way as a biopharmaceutical, have also been developed.

Recovery and Purification Processes

In the vaccine recovery and purification processes, the cells and contaminants are first removed from the culture fluid to obtain the supernatant containing the virus. This is then subject to virus inactivation, destruction, concentration, and filtering.

(1) Cell separation

Removal of cells and cell debris from the culture fluid is typically performed using a continuous-flow centrifuge operating at about 12,000 G.

(2) Virus separation

Removal of viruses from the recovered fluid requires use of an ultracentrifuge that generates an even higher centrifugal force (about 110,000 G) than that used for cell separation. Furthermore, centrifugal separation based on sucrose density gradient is performed to achieve an even higher degree of precise fractionation. First a sucrose density gradient is established in the centrifuge rotor, then separation is performed based on the sedimentation coefficient and suspension density of the various compounds, including viral particles. Next the viruses are inactivated and separation using an ultracentrifuge performed again. Fig. 2 shows an ultracentrifuge used for virus separation.

(3) Chromatography

In processes that use genetic engineering to produce antigenic proteins directly, the proteins are separated out using chromatography in the same way as monoclonal antibody drugs and other biopharmaceuticals.

MEASURES FOR IMPROVING BIOPHARMACEUTICAL PRODUCTIVITY AND QUALITY

This section describes what Hitachi is doing to improve productivity and quality for the biopharmaceuticals described above.

Culture Process Optimization

It is essential that the operation of the culture process keeps hydrodynamic forces, gas exchange, mixing, and foaming in the bioreactor within appropriate ranges. The expression of the limits to

Photograph courtesy of Hitachi Koki Co., Ltd.

Fig. 2—Ultracentrifuge Used for Virus Separation.
This ultracentrifuge for virus separation made by Hitachi Koki Co., Ltd. operates at approximately 110,000 G to separate viruses from the recovered fluid.

these ranges in terms of the stirring speed and sparging rate is called the "scale-up window"[1]. As the scale of a culture becomes larger, the scale-up window becomes progressively narrower until it disappears entirely above the upper limit on scale-up[2].

For large scale production, it is important that the process design make this scale-up window as broad as possible, and achieving this requires that optimization be performed through a large number of shapes and operating conditions. Use of computational fluid dynamics (CFD) to analyze bioreactor performance is an effective way of doing this. Having already applied this technique at many commercial plants, Hitachi has established biological, chemical, and physical models, input conditions, and calculation methods, and has also conducted testing. Meanwhile, Hitachi has a pilot plant for evaluating the growth characteristics of cell cultures for biopharmaceuticals and vaccine production that can also be used for tasks such as verifying CFD results, determining growth characteristics when using the customer's cells, and optimizing culture conditions.

Culture Process Quality Improvement

Quality by design (QbD) is a technique adopted to modernize quality management systems for pharmaceuticals. It is a systematic development methodology based on verified science and quality risk management that specifies targets in advance and emphasizes process management and understanding of the product and process[3]. QbD is already used for chemically synthesized low-molecular-weight pharmaceuticals, and it is also being applied to biopharmaceuticals with complex molecular structures and production processes. Risk assessment in QbD uses sophisticated statistical methods to identify relationships between the process and quality attributes. This information is then used for establishing production procedures. These methods treat the culture and purification processes as black boxes. However, if it is possible to determine how the culture environment affects the productivity and quality of the product in terms of the cell metabolic mechanisms, tasks such as predicting effects and identifying the causes of deviations can be performed with greater precision and efficiency than when the process is treated as a black box. While metabolic analysis methods have been widely used for microorganisms with simple metabolic reactions in the past, Hitachi has now started applying metabolic models to mammalian cells with complex metabolic reactions[4].

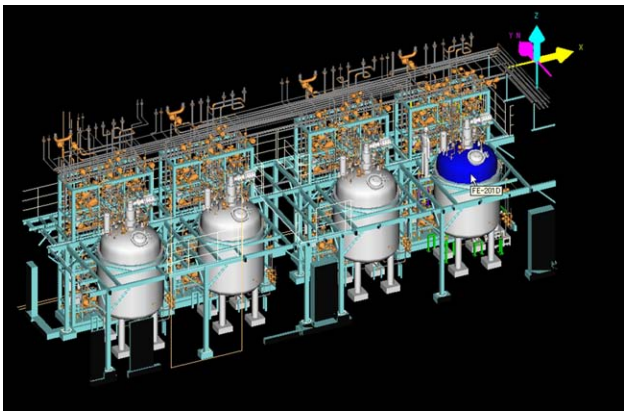

Fig. 3—Three-dimensional Computer-aided Design Image of Tank and Pipe Module.
Construction time is shortened by prefabricating modules in the factory and delivering them to the site for installation.

Modularization

Plants with an important public safety role, such as those used to produce pandemic vaccines, need to improve the quality of their products and they must be able to get supply systems in place quickly using rapid construction practices. To achieve this, Hitachi makes extensive use of modularization design and construction techniques, in which tanks and piping are prefabricated at the factory, and has shortened on-site construction times (see Fig. 3).

Automation

To maintain product quality, the production processes for biopharmaceuticals and vaccines require sophisticated sterilization and cleaning. Large biopharmaceutical plants need to use distributed control systems (DCSs) to ensure that they can control their 1,000 or more valves correctly and avoid misoperation. For the development of control software for these systems, there is a need to standardize process flow and control software to reduce design workloads, and to establish and verify operating practices based on sterilization and cleaning mechanisms that suit the plant's systems. Fig. 4 shows example DCS screens (graphic display and block diagram of process flow) from a large biopharmaceutical plant.

CONCLUSIONS

Biopharmaceuticals and vaccines are well recognized for their potential in treating incurable diseases for which conventional low-molecular-weight pharmaceuticals produced by chemical synthesis are ineffective, and for preventing infectious diseases. However, they also face challenges such as high

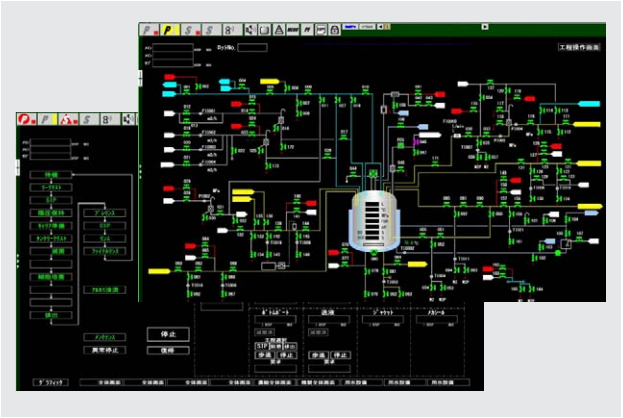

Fig. 4—Example Distributed Control System Screens for Large Biopharmaceutical Plant.
These screens show process graphics and a block diagram of the flow for an operating process.

production costs. This article has given an overview of biopharmaceutical and vaccine production processes and the associated challenges, and described the work that Hitachi is doing in this field.

Hitachi uses CFD simulations underpinned by vast experimental testing and experience to optimize bioreactors, operating conditions, and other parameters. It also conducts analyses using cell metabolic models to understand the influence that culture environment has on productivity and quality, and then uses this knowledge in production procedures. Equipment designed by this work can be used for complex production processes, with automated control and use of modularization to shorten on-site construction time.

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