Biopharmaceutical Manufacturing Facility

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Biopharmaceutical shows promise as major part of pharmaceutical products since it is applicable to a wide range of diseases such as cancer, rheumatism, and influenza vaccination with high pharmaceutical efficacy but little side effect. Bioplant engineering is one of main business unit of IPEC. Therefore, IPEC has been conducting many development projects in the field. This report gives the outline of biopharmaceutical manufacturing process, features of facilities, key points of the scale up, and design procedures of bioreactor and major purification equipment. In addition, IPEC's remarkable ability in optimization of bioprocess using pilot scale facilities is introduced

1. Introduction

Major processes for manufacturing pharmaceuticals include chemical synthetic procedures, which use chemical reactions to mainly produce low-molecular compounds, and biochemical synthetic procedures, which use the vital functions of living organisms, such as microbes, mammalian cells, and plant cells, to mainly produce high-molecular compounds.

Some biopharmaceuticals produced by a biochemical synthetic procedure are low-molecular compounds including antibiotic drugs, but most of them are proteins and other high-molecular components that cannot be produced by chemical synthesis.

Because of their specificity to certain diseases, high activity, and low side effects, these high-molecular components show promise as major parts of pharmaceutical products in the future. Typical biopharmaceuticals include antibody drugs and vaccines.

Bioplant engineering based on biotechnology is the main business unit of IHI Plant Engineering Corporation (hereinafter called "IPEC"). Therefore, IPEC has been conducting many development projects in the field.

This report gives an outline of the biopharmaceutical manufacturing process, features of used facilities, and key points of the scaling up and design procedures of bioreactors and major purification equipment. In addition, IPEC's remarkable ability in the optimization of bioprocesses using pilot scale facilities and recent development results are introduced.

2. Biopharmaceutical manufacturing process

An outline of a biopharmaceutical manufacturing process is shown in **Fig. 1**. The process is roughly divided into a cultivation process, a purification process, and a formulation process.

In the cultivation process, microbes or mammalian cells are cultivated to produce a desired substance. In the purification process, the culture solution, which contains numerous foreign substances along with the desired substance, is purified to increase the purity and concentration of the desired substance. The products obtained through these processes are called active

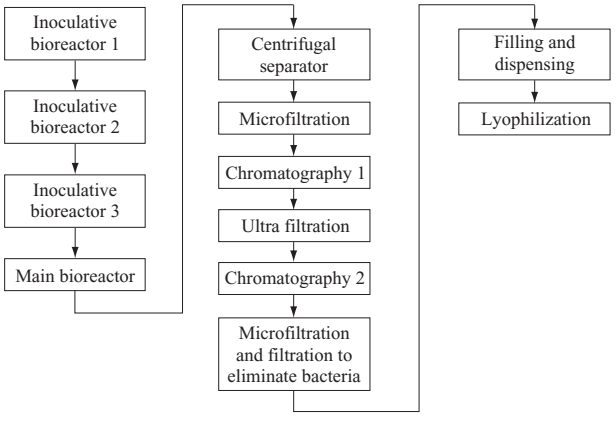


Fig. 1 Biopharmaceutical manufacturing process

pharmaceutical ingredients, and they are processed into formulations, such as injections and internal medicines, in the subsequent formulation process.

In the cultivation process, genetic engineering has been often used to cultivate microbes such as bacteria, molds, and yeasts, but complex structures required for antibodies, as seen in a protein having a three-dimensional structure with sugar chains, cannot be produced by using genetic engineering with microbes.

Cultivation techniques using mammalian cells are solutions to this problem. The desired product productivity when mammalian cells are used is lower than that when microbes are used, but these techniques are essential to producing pharmaceuticals with a complex structure and high pharmaceutical efficacy.

Since the multiplication of microbes or mammalian cells requires a nutrition source and oxygen, a medium that provides a nutrition source, such as a carbon source, a nitrogen source, or phosphorous, is put in a bioreactor. Microbes or mammalian cells are multiplied in the bioreactor kept at a certain temperature through agitation and aeration. The structure of a general bioreactor is shown in **Fig. 2** [(1)].

Cultivation is started with a flask with a small capacity, and scaled up gradually using tanks with various capacities. In the cultivation of mammalian cells, the cultivation scale is generally expanded about five-fold at a time, ie: 1 *l*, 5 *l*, 25 *l*, and so on. On the other hand, in the cultivation of microbes, the cultivation scale is expanded 10- or 100-fold at a time. This is because the difference in the multiplication speed and fungus density between microbes and mammalian cells causes their optimum expansion scale factors to be different. The size of a bioreactor for final production, which is designed depending on the production amount of the desired substance, is about 500 m^3 for a large-scale plant for microbes and about 20 m^3 for cell cultivation.

The purification process is divided into a separation process and a purification process.

In the separation process, the desired substance is collected by separating microbes or cells in the culture solution from the supernatant fluid. Devices commonly used for this purpose include centrifugal separators, which use centrifugal force to separate components with different specific gravities from each other, and membrane separation devices, which prevent the passage of cells to separate them from the supernatant fluid.

If the desired substance is contained in cells, the collected cells may be broken by cell breakage equipment.

In the purification process, column chromatography, ultra filtration, microfiltration, and other processes are combined to increase the purity and concentration of the desired substance.

Column chromatography is a method of separating a desired substance using the difference in molecular size, ionic strength, hydrophobicity, and other properties between the substance and impurities. In antibody production processes, highly selective chromatography using biochemical affinity (affinity chromatography) is often used.

Filtration is a method of separating substances by utilizing the difference in their molecular sizes. Depending on the pore size of filtration membranes, filtration may be called "ultra filtration" (fractionation on the molecular weight level) or "microfiltration" (0.1-10 μm). These types of filtration are used appropriately for different purposes. The scaling up of purification and key points in designing

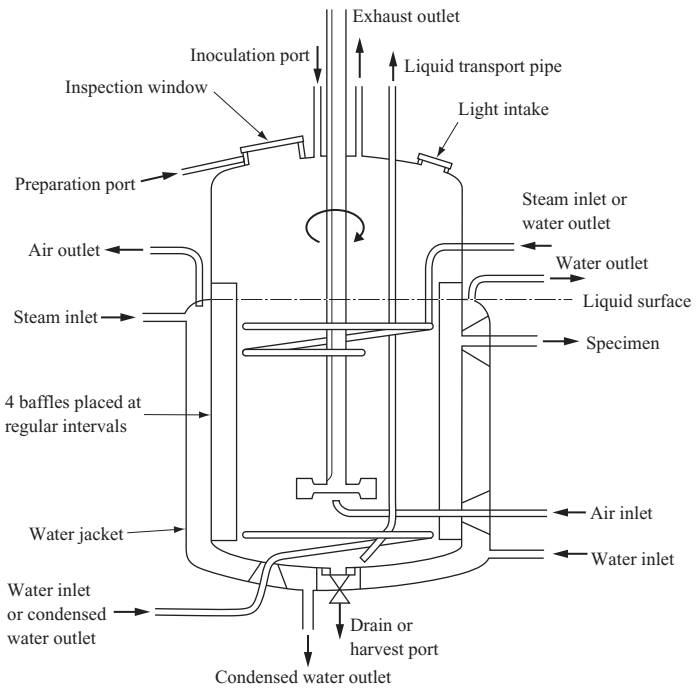


Fig. 2 Bioreactor [(1)]

purification equipment are described in **Chapter 4**.

In the formulation process, active pharmaceutical ingredients obtained through the cultivation and purification processes are processed into pharmaceuticals through the addition of auxiliary ingredients, sterilization, dispensing, lyophilization (if used as a powder), or other processes.

3. Key points in designing cultivation equipment

As described in the previous chapter, a cultivation process is intended to multiply microbes or mammalian cells and produce a desired substance by using its metabolic capability.

In designing cultivation equipment, the following points must be kept in mind:

(1) Ensure that microbes other than the microbes or mammalian cells to be applied do not get mixed (prevention of contamination by bacteria).

(2) Ensure that optimum cultivation conditions are maintained (a bioreactor with an optimum structure is controlled to keep it under optimum conditions.)

First, measures to prevent contamination by bacteria are described. Microbes or mammalian cells are cultivated in a medium that helps them multiply. This medium can also provide a nutrition source for other kinds of microbes.

If several kinds of microbes exist in the medium, all the microbes are multiplied, and the multiplication of the microbes or mammalian cells to be multiplied is hindered. Generally, bacteria, including molds existing in the air, are multiplied easily. To prevent contamination by bacteria, the bioreactor and the medium must be sterilized by heating with high-pressure steam or subjected to microfiltration to eliminate or kill bacteria in the bioreactor and the medium before the microbes or mammalian cells to be applied are inoculated. It is also important to prevent bacteria from getting mixed in with the bioreactor during cultivation.

The tank, piping, and other parts of the bioreactor must be sterilized with high-pressure steam before the medium is put in it. By doing this, the inside of the bioreactor can be made free from bacteria or sterile.

A medium that is less subject to denaturation by heat can be sterilized by heating. However, a medium that is likely to denature by heat, such as vitamins, must be subjected to microfiltration to eliminate microorganisms. Now, preparation for cultivation has been completed, and a culture solution obtained by scaling up cultivation can be inoculated in the bioreactor to start the cultivation process.

Next, the optimization of the design of a bioreactor is described.

In cultivation, optimum cultivation conditions or conditions that make the concentration of the desired substance as high as possible must be provided. Environmental factors in a bioreactor that have an influence on cultivation include the following:

(1) Dissolved oxygen (oxygen dissolved in the culture solution) / distribution

(2) Dissolved carbon dioxide (carbon dioxide dissolved in the culture solution) / distribution

(3) Temperature / distribution

(4) pH / distribution

(5) Substrate concentration (uniform dispersion) / distribution

(6) Product concentration / distribution

(7) Shear stress

Since these factors influence each other, comprehensive optimization is required.

The optimum values of these factors depend on the type of microorganism. In the cultivation of mammalian cells, the influences of factors (2) and (7) are larger than in the cultivation of microbes, and the range of optimization is narrowed.

To keep these factors in optimum conditions, the primary parameters in **Fig. 3** should be controlled. The optimum design is achieved when there is a design that makes this possible, setting the optimum operating conditions.

Figure 4 shows the influence of temperature on the multiplication of mammalian cells and **Fig. 5** shows the influence of dissolved oxygen (DO) on the viability of mammalian cells.

As shown in these figures, by setting conditions in the bioreactor at optimum values, the multiplication of cells can be encouraged to lead to improved productivity.

Scale-up factors that decide the conditions in a bioreactor include the following bioreactor structural elements:

(1) Shape of the bioreactor (L/D: Ratio of the height to the diameter)

(2) Aeration system, aeration flow volume

(3) Shape of the impeller, agitating rotation speed, agitating power

According to **Fig. 5**, the DO value must be kept at 1.5 mg/*l* or above to cultivate cells without reducing their viability. To achieve this, k_La should be used to design the specifications of the bioreactor.

k_La is a parameter that represents an oxygen transfer rate, and is expressed by the following formula (1) [(1)]:

$$\frac{dC}{dt} = k_La\,(C^* - C) - Q \quad \cdots\cdots (1)$$

where the parameters represent the following:

C : Dissolved oxygen level in the bioreactor

C^* : Saturated dissolved oxygen level at cultivation temperature

Q : Respiration rate

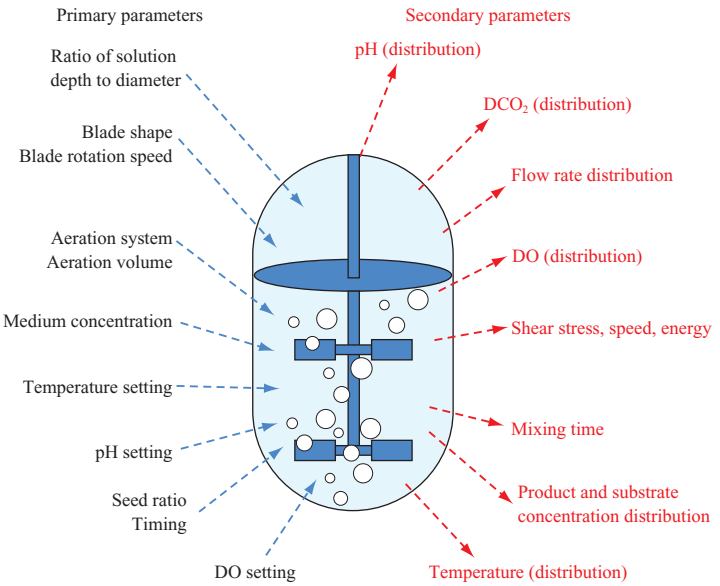
k_La : Oxygen transfer capacity coefficient

t : Time

C, C^*, and Q in formula (1) are decided in development experiments, and the necessary k_La is then decided. Thus, the bioreactor can be designed (scaled up) by using this k_La as a parameter.

Generally, the following formula (2) [(1)] is used for a design using k_La as a parameter:

$$k_La = b\left(\frac{P_g}{V}\right)^{\alpha} V_s^{\beta} \quad \cdots\cdots (2)$$



(Note) DO : Dissolved oxygen concentration
DCO_2 : Dissolved carbon dioxide concentration
Primary parameters : Parameters that can be set by designers and operators
Secondary parameters : Parameters decided by the primary parameters

Fig. 3 Factors that influences cultivation

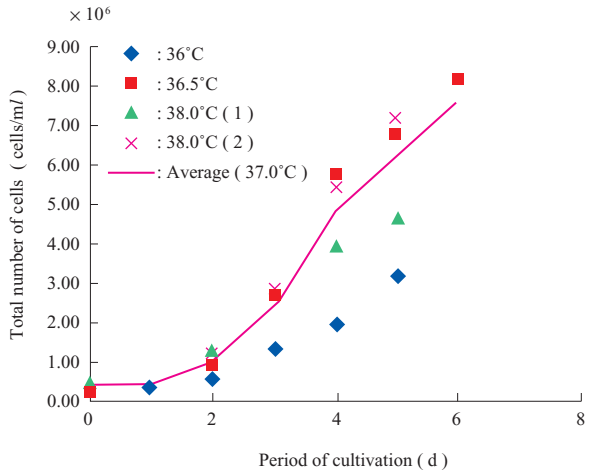


Fig. 4 Influence of temperature on multiplication of mammalian cell

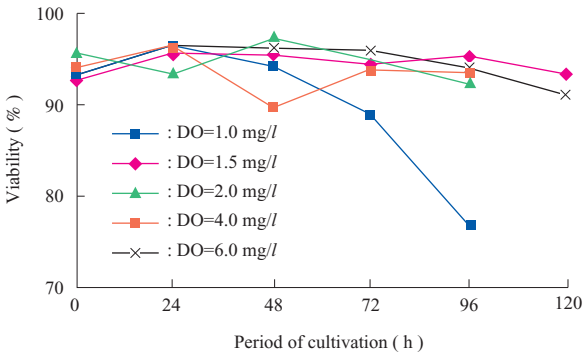


Fig. 5 Influence of dissolved oxygen (DO) on viability of mammalian cell

b, α, β : Coefficients
P_g : Agitating power at aeration
V : Liquid volume
V_s : Linear velocity of aeration (value obtained by dividing the aeration volume by the cross-sectional area of the bioreactor)

b, α, and β are decided mainly by the shape, the size, and the number of stages of the impeller. Thus, selecting an impeller with an optimum shape, size, and number of stages from those available is optimum design. In this case, it is important to select an impeller that realizes the target k_La with minimum shear stress (agitating power) to avoid the cell disruption that would be caused by a large shear stress. **Figure 6** shows the influence of shear stress on the viability of mammalian cells.

IPEC has found the optimum conditions with respect to

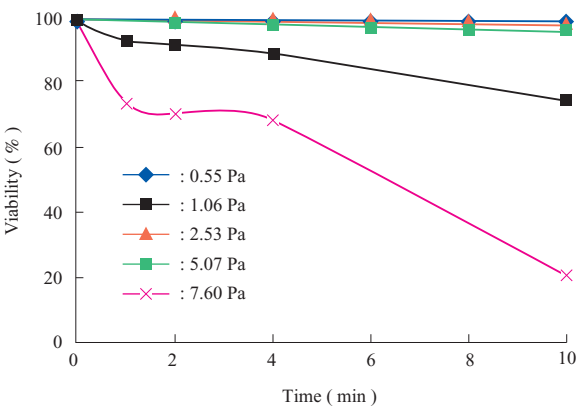


Fig. 6 Influence of shear stress on viability of mammalian cell

this in development experiments.

Thus, the optimum design of a bioreactor requires the optimization of various parameters, but finding the optimum values for all the parameters in experiments takes considerable time and cost, and is inadvisable. Recently, it has become common to use computational fluid dynamics (CFD) in combination with experiments for optimization. The author also uses CFD for designing optimum bioreactors.

Figure 7 shows an example of the application of CFD. This figure shows that as the scale is expanded, the shear stress is increased. Thus, attention must be paid to shear stress when scaling up.

4. Key points in designing purification equipment

The scaling up and design procedures of major equipment used in the purification process are described here.

4.1 Centrifugal separator

A centrifugal separator is equipment that separates a culture solution into microbes (or mammalian cells) and a supernatant fluid.

There are many types of separators, and disc-stack centrifugal separators and cylindrical centrifugal separators are mainly used in a bioprocess.

The scale-up of a disc-stack centrifugal separator is decided by a factor called the centrifugal spin down area, which is decided by the flow rate and the settling rate of cells and such like in centrifugal separation.

However, depending on the properties of the culture solution, centrifugation efficiency may be lowered because of the influence of foam formed following rotation. For this reason, a test should be conducted with a pilot separator to check for such lowered efficiency.

4.2 Column chromatography

Column chromatography is very important in the purification process of a bioprocess.

The supernatant fluid separated by a centrifugal separator contains the desired substance as well as various proteins and low-molecular compounds produced as the result of the metabolism of the living organism. Column chromatography is a very effective method for purifying the fluid to obtain only the desired substance. **Table 1** [(2)] shows types of chromatography and their respective features.

In an actual purification process, the following four types of chromatography are mainly used, and two or more of these are often used in one process:

(1) Gel filtration chromatography (separate by difference in molecular sizes and shapes)
(2) Ion-exchange chromatography(separate by difference in ionic strength)
(3) Hydrophobic chromatography(separate by difference in hydrophobicity)
(4) Affinity chromatography (separate by biological affinity)

In scaling up chromatography, the column size is decided depending on the amount of the substance to be purified. Chromatography is scaled up so that the liner velocity (value obtained by dividing the inflow velocity of the solution to be processed by the cross-sectional area of the column) is kept constant. Thus, the packed column height cannot be increased, and the diameter becomes very large for a large-size column. Since the carriers used to fill a column are expensive, the number of processes should be increased to minimize column sizes instead of using a large column for processing at a time.

4.3 Ultra filtration

As described in **Chapter 2**, ultra filtration uses membranes with a molecular weight level of accuracy.

Ultra filtration equipment is used to concentrate a desired substance or for buffer exchange. Ultra filtration equipment consists of a tank to store a process solution,

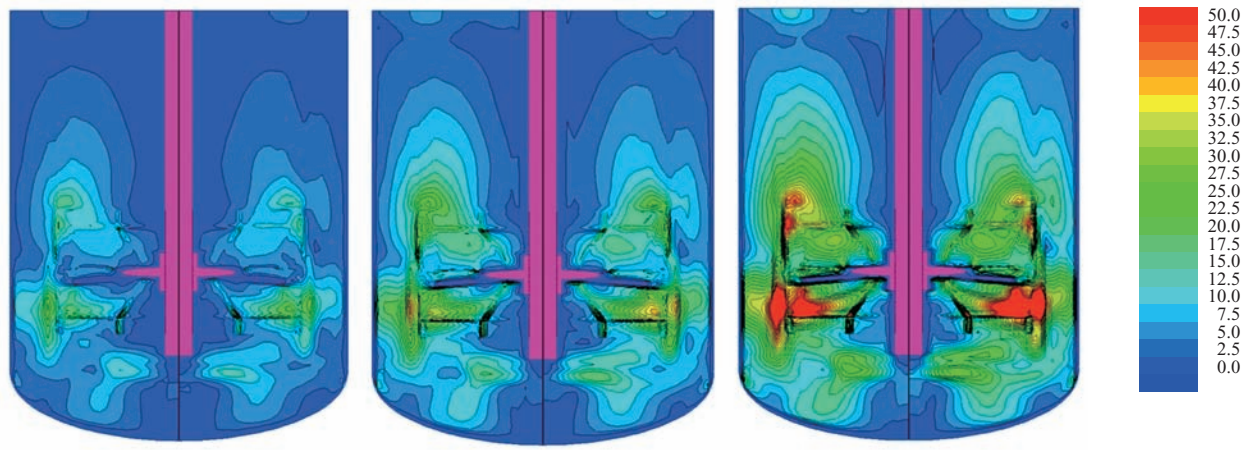


Fig. 7 Shear stress distribution in bioreactor

Table 1 Types and features of chromatography [2]

Liquid chromatography	Mechanism of separation	Elution method	Features	Target substances
Gel filtration chromatography	Molecular size and shape	Elute with the same fluid	- Because there is a partition coefficient of 0 to 1, a column of a certain length is needed for separation. - Processing conditions are eased. - The recovery rate is high.	Desalination, buffer exchange, and molecular weight fractionation of proteins, etc.
Ion-exchange chromatography	Static electricity	Stepwise elution Gradient elution	- Can be used with a wide range of substances. - Elution conditions can be changed to adjust the degree of separation. A large amount can be processed. - Concentration of desired fractions.	A wide range of substances including low- and high-molecular electrolytes
Hydrophobic chromatography	Hydrophobicity	Stepwise elution Gradient elution	- The adsorbability of adsorbents can be adjusted widely. - Adsorb by high ionic strength. - Various elution methods can be used for fractionation.	Proteins, cells, and proteins with a conformation
Hydrogen bond chromatography	Hydrogen bond	Gradient elution	- A column of cellulose with no ion-exchange groups can be used at any pH value. - Since most proteins are adsorbed by cellulose in the presence of 3M ammonium sulfate, and eluted in the presence of 1M ammonium sulfate, this chromatography is suitable for purifying enzymes stabilized by a high concentration of salt.	Proteins
Salting-out chromatography	Difference in solubility depending on the salt concentration	Gradient elution	- This chromatography can be conducted by two different methods or by forming a concentration gradient of a salting agent in a column in advance or by salting out a target specimen first and then adding to a column.	Proteins
Chromatofocusing	Difference in isoelectric point	Gradient elution	- High separation capacity (difference in pI: 0.05) - High level of concentration of desired fractions	Isozyme and other proteins that are difficult to separate
Affinity chromatography	Biological affinity	Stepwise elution (Gradient elution)	- High selectivity - High level of concentration of desired fractions - A large amount can be processed. - Ligand and elution conditions must be decided carefully.	Low concentration of biologically active substances

an ultra filtration membrane, and a pump to circulate the solution around the membrane. Ultra filtration employs a system called tangential flow filtration, which allows the solution to circulate in the primary side of the membrane and discharges the filtrate into the secondary side of the membrane (passes it through the membrane). **Figure 8** [2] shows a schematic diagram of tangential flow filtration.

The process of concentration, just as the term implies, is to allow only the medium solution of a process solution to pass through a membrane to concentrate the desired substance.

Buffer exchange is a method of changing the composition of the medium solution of a process solution by allowing

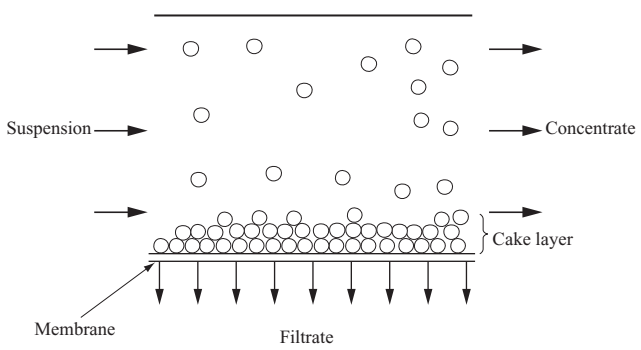


Fig. 8 Tangential flow filtration [2]

the medium solution to pass through a membrane and adding a buffer solution of another composition. This process can eliminate low-molecular impurities that cannot be separated by chromatography.

In the scaling up of ultra filtration equipment, the required number of membranes and the pump capacity should be decided based on the amount to be processed and the process rate.

In the concentration process, the shape of the tank, the length of the piping, and other parts should be designed to be as compact as possible depending on the amount of the final concentrate.

5. Optimization of bioprocesses using pilot scale facilities

In the preceding chapters, an outline of a biopharmaceutical plant was described. In this chapter, the efforts of IPEC are described.

As described before, scaling up in a bioprocess should be conducted in view of various factors.

In order mainly to realize accurate, reliable scaling up, IPEC established a biological laboratory at the IHI Yokohama works. The facilities in the laboratory allow IPEC to conduct joint research or entrusted investigations, helping collect data to be applied to scaling up customers' production equipment.

Major facilities are listed below:

(1) Shaking incubator, jar fermentor with a capacity of 5 *l*, 10 *l*, or 100 *l*
(2) Aerated stirred tank with a capacity of 6 000 *l* (with a bioreactor structure)
(3) Centrifugal separators (pilot scale, cylindrical, and disc-stack type ones)
(4) Column chromatography (10 cm column)
(5) Ultra filtration equipment
(6) Microfiltration equipment
(7) Cell counter
(8) Various kinds of analysis equipment (turbidimeter, high-performance liquid chromatography (HPLC), etc.)
(9) CFD system

IPEC has used these facilities to conduct joint research with customers or entrusted investigations and collected various data on designs described before or conducted CFD analyses. These efforts have been highly evaluated.

So far, IPEC has conducted research on microbes, mammalian cells, insect cells, plant cells, and enzyme reactions, and is currently working on a vaccine production experiment with a customer.

6. Conclusion

An overview of the processes in a biopharmaceutical manufacturing plant, the scaling up and design procedures of equipment used in the cultivation process and the purification process, and the efforts of IPEC in its plant were described.

More and more antibody drugs are expected to be produced in the future. IPEC hopes that its own technique for the optimization of bioprocesses using pilot scale facilities will be applied to as many cases of construction of plants producing these drugs as possible, and IPEC will continue to further pursue optimization to help efficient construction of production plants

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