Assure Bioreactor Sterility

Sterile operation is critical for bioreactors. Here are guidelines on how to address this during design.

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Bioreactors invariably comprise the core component of a biopharmaceutical production operation. The biocatalysts — animal cells or microorganisms that produce the desired product — are grown and maintained in bioreactors. In a majority of industrial processes, the biocatalyst is suspended in a nutrient medium in one of four types of bioreactors, as illustrated in Figure 1: external-loop airlift (Figure 1a), internal-loop airlift (1b), stirred tank (1c), or bubble column (1d). The success of the production process depends on the reactors being kept free of all unwanted organisms — that is, maintaining monoseptic or sterile operation. Indeed, loss of sterility during operation is the most common cause of process failures.

Here, we examine some features of bioreactor design for sterile operation, including sterilization of bioreactors, sterile sampling, and aseptic transfers between bioreactors. These features, proven by routine use in large-scale animal cell culture, obviate such practices as addition of antibiotics to the culture medium.

Design for sterilization

Industrial bioreactors are usually large: 300–3,000 L vessels are common in animal cell culture processes; still larger bioreactors (for instance, 100–250 m^3) are encountered in microbial fermentations. These bioreactors must be sterilized in place.

A typical bioreactor with the valves needed for sterilization of the vessel, the air inlet, and the exhaust gas outlet is shown in Figure 2. Although the figure depicts a stirred tank bioreactor, similar arrangements apply to airlift and bubble column varieties. Because almost all biopharmaceutical production processes involve aeration (air or oxygen for microbial fermentations; mixtures of air, oxygen, nitrogen, and carbon dioxide in animal cell culture), the system in Figure 2 shows aeration and exhaust groups that also must be sterilized.

The air inlet and exhaust lines have *in situ* sterilizable gas filters. Either absolute (such as 0.1–0.4 μm hydrophobic filters) or depth filters may be employed *(1)*. Often two filter cartridges in series are used at both locations; the first, or the prefilter, serves to protect the final filter. Additional valves (not shown in Figure 2) may be added for integrity testing of the filters after sterilization.

Apart from the harvest valve, all other valves shown in Figure 2 should either be diaphragm or pinch valves. The harvest valve is usually a piston valve with a metal-bellows-sealed stem. The valve closes flush with the internal surface of the bioreactor, and there is an unobstructed flow path through the valve body.

The valves may be operated manually, but operation via automatic control is more efficient and reproducible. Saturated clean steam (1.1–1.4 bar gauge) is used for sterilization. The air inlet and exhaust groups are sterilized first and, then, in a second step, the bioreactor. The two steam crosses (valve groups 2, 3, 10 and 15, and 6, 7, 13 and 17) allow for the replacement of filters during a fermentation so long as a temporary interruption (of up to ≈ 40 min) in air supply is acceptable. Arrangements with parallel standby gas inlet/outlet filters are also feasible, but rarely necessary.

Sterilization of the air inlet (exhaust) group starts with valves 1, 2, 3, 9, 10, 14, and 15 closed (6, 7, 8, 13, 17, 18 and 19 closed). Valves 10 and 15 (13 and 17) are opened (for ≈ 30 s) to preheat the steam cross through the valves. Valve 10 is shut, while 9, 2, and 14 (7, 18 and 19) are

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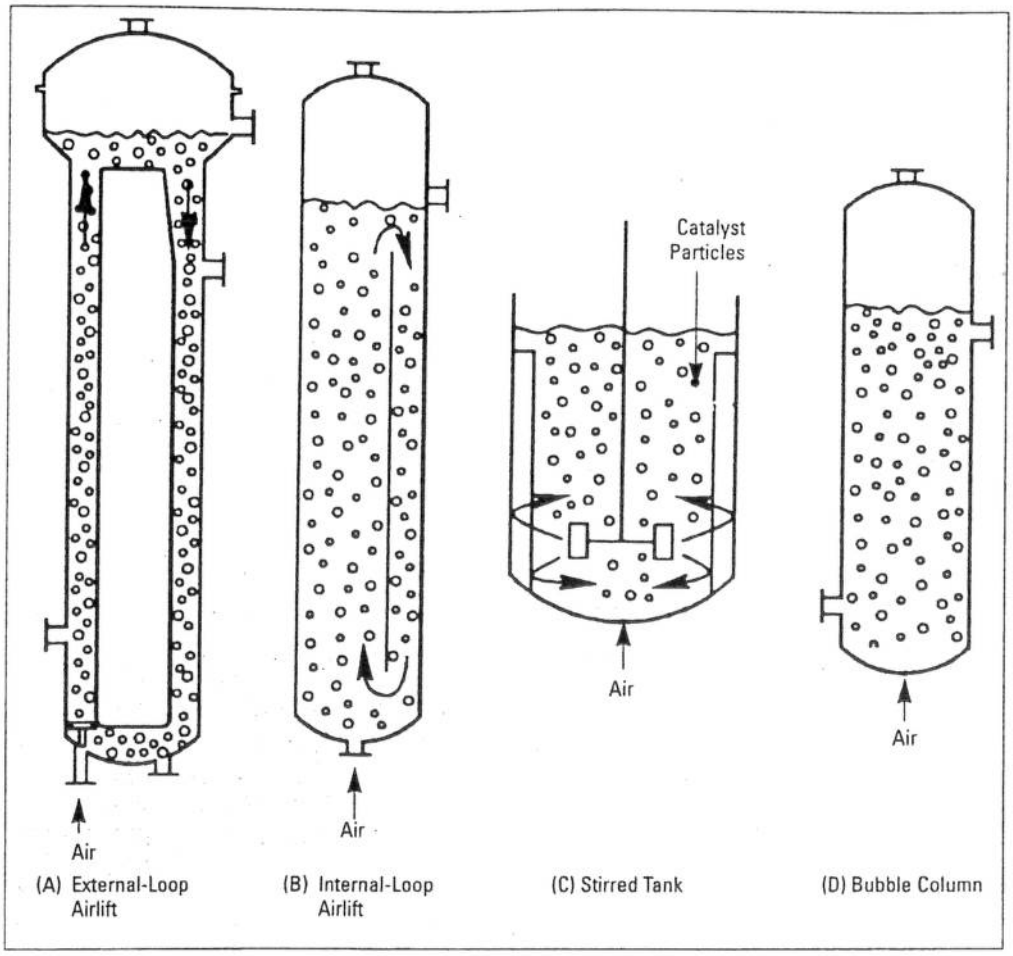
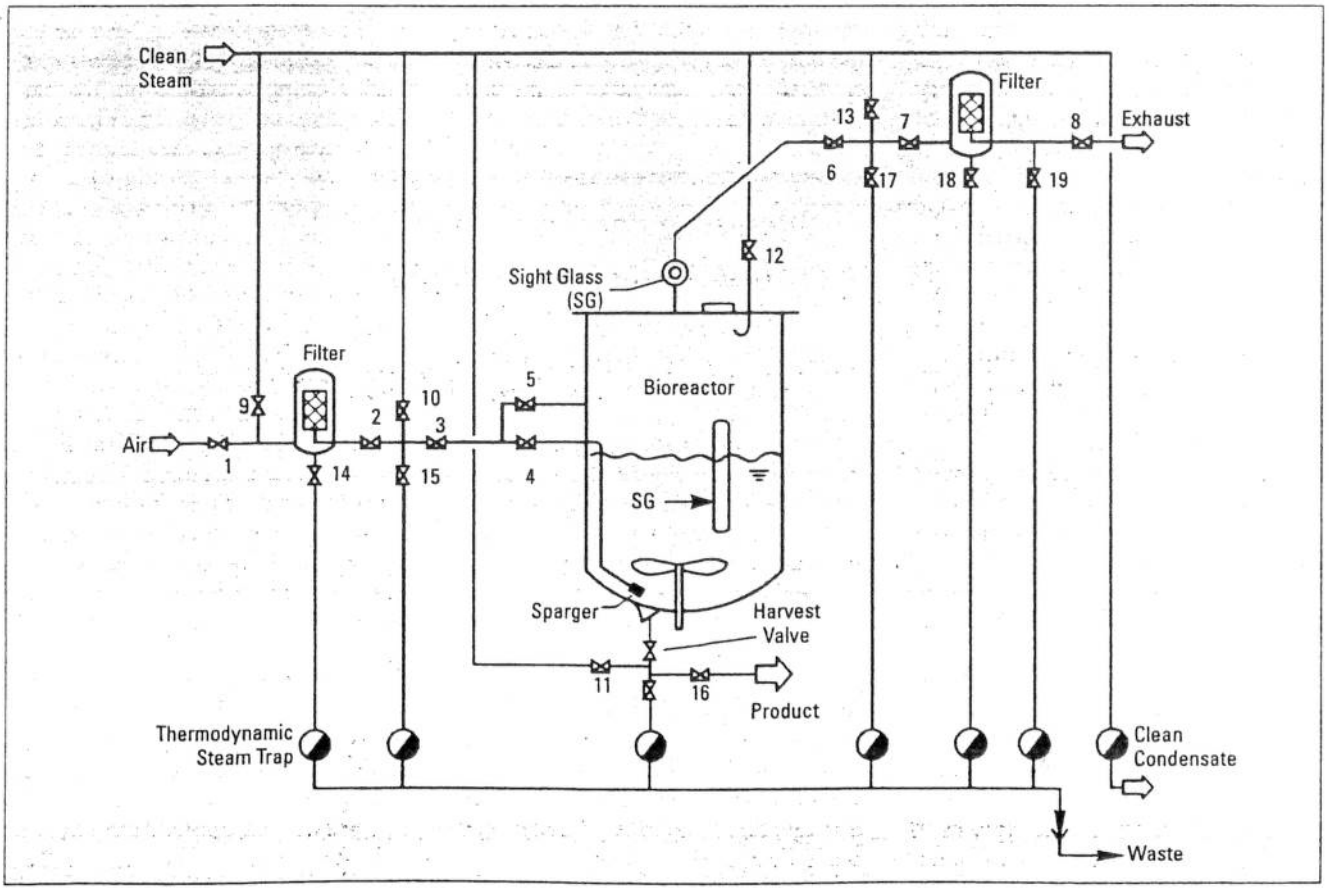
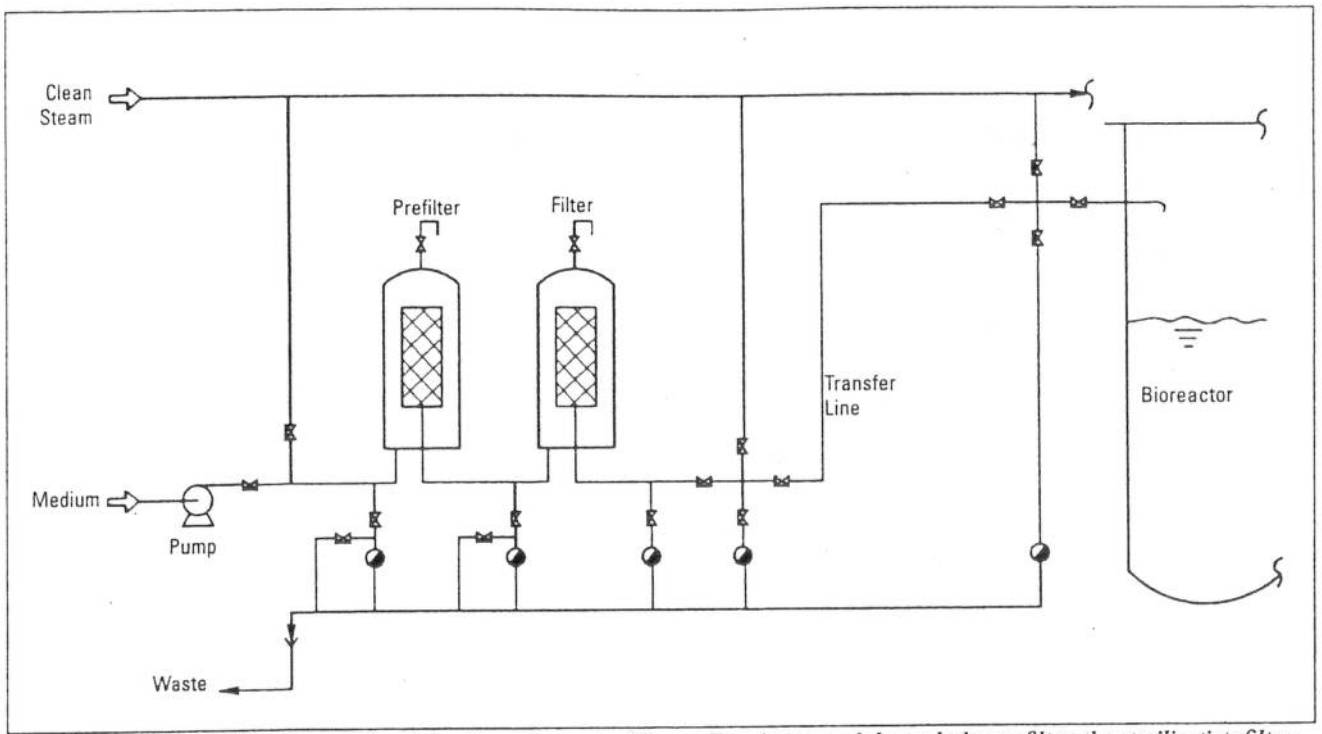


■ *Figure 1. (left) The four common types of industrial bioreactors: (a) external-loop airlift; (b) internal-loop airlift; (c) stirred tank; and (d) bubble column.*



■ *Figure 2. (bottom) A bioreactor with air inlet and exhaust groups arranged for in-place sterilization with steam.*



■ *Figure 3. A filter sterilization unit hardpiped to a bioreactor. The medium is pumped through the prefilter, the sterilization filter, and the transfer line to the bioreactor.*

opened. Steam now flows through the filters, and condensate drains away through the thermodynamic steam traps. The filters, valves, and associated pipework reach sterilization temperature (≈122 °C) very quickly (in ≈1 min), and are held at the temperature for the required time (25–30 min). Valves 14, 15, 2 and 9 are closed in order (17, 18, 19, 7 and 13); valves 1 and 8 may be opened. The air inlet and exhaust groups are now sterile, but isolated from the nonsterile bioreactor.

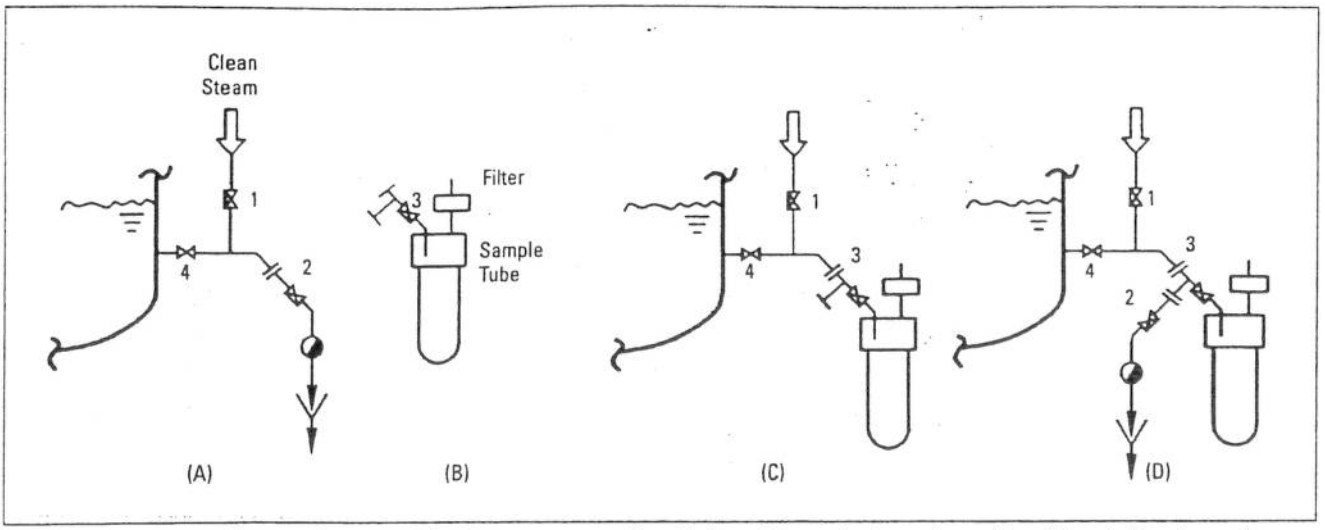
In some applications, a mechanical foam breaker or a condenser form part of the air exhaust group. When a foam breaker is used, it is located on the headplate of the bioreactor. A double mechanical seal is provided on the shaft of the foam breaker where it enters the bioreactor. In this case, additional systems are needed for steam sterilization of the seal, and for lubrication of the seal with cooled, clean steam condensate. (Foam breakers have been successfully used in several hybridoma suspension cultures in small and large bioreactors.)

A similar consideration applies to the mechanical shaft seal in mechanically stirred bioreactors. Mechanical seals make the system more complex, and seals do fail. Nevertheless, agitation through stirrers mounted at the bottom of the tank with mechanical shaft seals is in use for many cell culture processes. When a stirred-type bioreactor must be employed, a magnetic coupling of the stirrer to the motor is preferred to the mechanical seal. Bottom driven, magnetically coupled agitators have been used in cell culture reactors up to at least 800 L. In larger bioreactors, the torque on the larger impeller may uncouple a magnetic coupling particularly during sterilization. In microbial fermentations, the density and viscosity of the broth place limitations on the maximum scale at which a magnetically coupled impeller may be used.

Sampling carries with it a certain risk of introducing contamination into the reactor.

The bioreactor is sterilized either filled with the medium or empty. Empty sterilization is the norm in cell culture applications because the media are invariably heat sensitive. During empty sterilization, the harvest valve and valve 16 (Figure 2) are opened. Steam is let into the vessel through the gas sparger (valves 10, 15, 3 and 4 open), the air exhaust pipe (valves 13, 17, and 6 open), and the upper sight-glass steam connection (valve 12 open). Valves 4 and 5 open and shut out of phase to ensure that both are sterilized. During sterilization of the vessel, a steam jacket (not shown) surrounds the bioreactor.

The valve closing sequence is as important as the opening one. The harvest valve and valve 12 are closed first. The steam trap connections (valves 15 and 17) are closed next, followed by the steam supply valves (10 and 13). The bioreactor is immediately pressurized with sterile air through the air inlet filter (valves 1, 2, 3 and 5 open). Once the vessel has cooled, the exhaust valves (6, 7 and 8) are opened. The



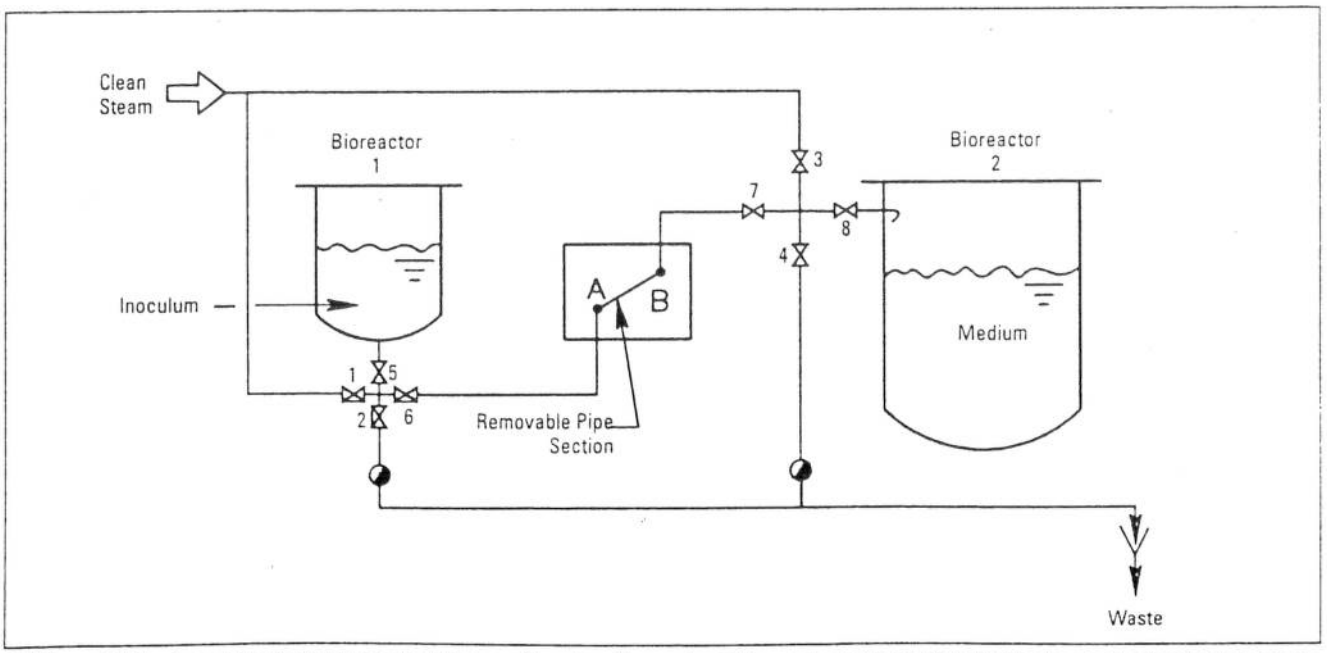
■ *Figure 4. For sterile sampling, a quick-disconnect sampling device with a submicron breathing filter is first heated in an autoclave.*

reactor is now sterile and ready to receive the nutrient medium.

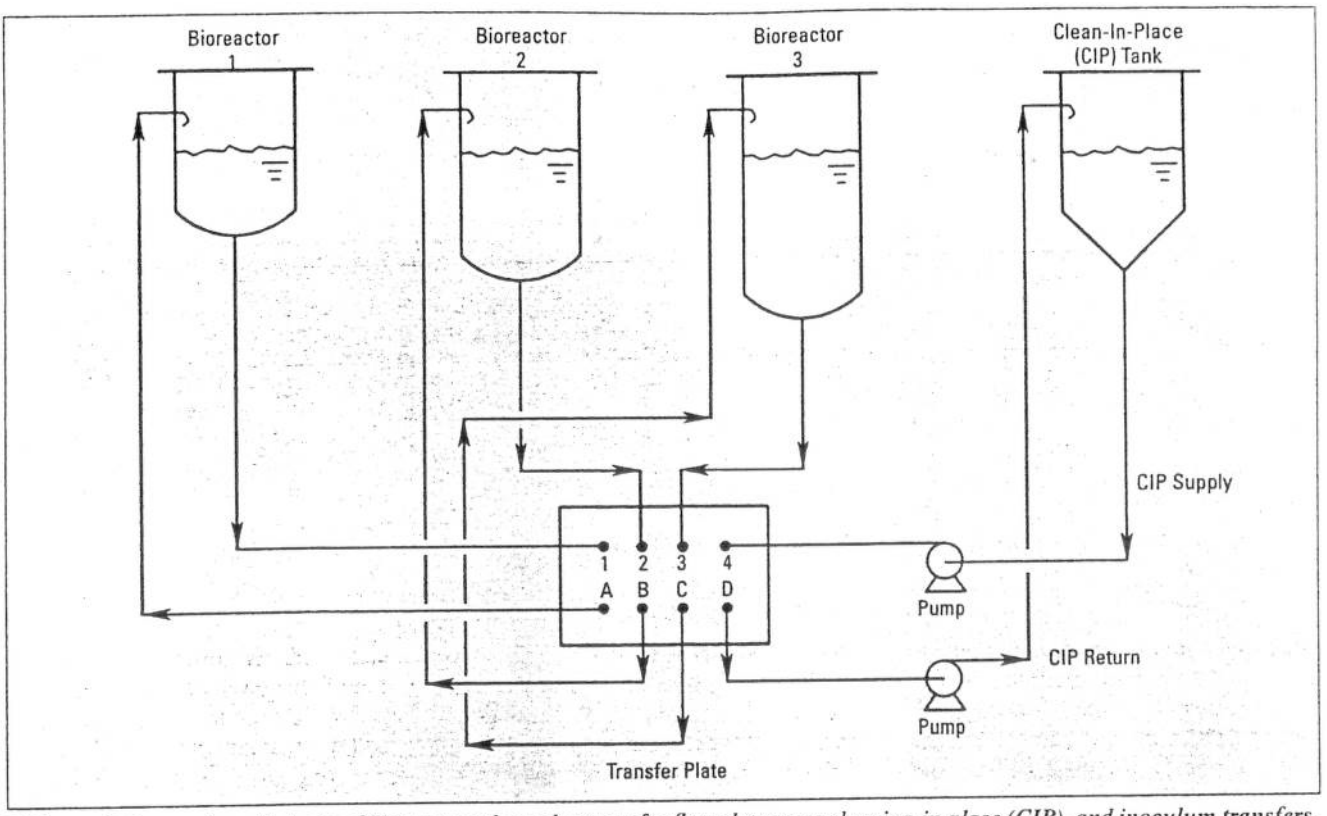
Frequently, the medium is passed through a steam sterilized filter directly into the bioreactor. The filter is hardpiped to the bioreactor as shown in Figure 3. The pipe section between the steam cross and the bioreactor in this case must be sterilized along with the bioreactor. Flexible transfer piping should be avoided because of potential difficulties in eliminating air pockets and condensate pools.

Sterile sampling

Typically, bioreactors are sampled up to 5 times daily. Sampling carries with it a certain risk of introducing contamination into the reactor. The sampling assembly shown in Figure 4 reduces the risk of contamination and ensures a sterile sample. The device works as follows: In its initial state (Figure 4a) the sample valve (4 in Figure 4) on the bioreactor is connected to the steam trap via a sanitary quick connection; valves 1 and 2 are open to maintain a steam barrier between the bioreactor and the environment. Valves 2 and 1 are closed in order. A presterilized (autoclaved with valve 3 closed) sampling device (Figure 4b) is attached to the bioreactor as shown in Figure 4c. Valve 2 is attached to the sampling device (Figure 4d). Valves 1 and 2 are opened in order, and steam runs through the trap for ≈ 30 min. Valves 2 and 1 then are closed in sequence. The sample is collected by opening valves 3 and 4. Before removal of the sampling



■ *Figure 5. The inoculum from bioreactor 1 is transferred to bioreactor 2 through a transfer line that is steam sterilized prior to use.*



■ *Figure 6. Connection of a train of bioreactors through a transfer flow plate eases cleaning-in-place (CIP), and inoculum transfers.*

device, steam is run through the cross, which is then allowed to cool. The system then is returned to its initial position.

Inoculation

To initiate production, a sterile, medium-filled bioreactor must be inoculated with the desired microorganism, hybridoma, or other cell line. The inoculum usually constitutes between 5–25% of the working volume (liquid volume) of the bioreactor. The inoculum comes from a smaller bioreactor that is connected to the larger vessel (Figure 5) through a transfer connection plate. Because of the need for inoculum, a production plant may have a battery of bioreactors connected in series — each providing inoculum for the next larger reactor in the train. The transfer operations between bioreactors are sterile operations.

This plate provides an ingenious means of cleaning in place the bioreactors and the associated transfer pipes. It allows a single CIP system to serve the whole plant.

The inoculum transfer between bioreactors 1 and 2 (Figure 5) occurs as follows: Initially all valves are in a closed position. A pipe section is installed at the transfer plate between points A and B. Valves pairs 1 and 2, and 3 and 4 are opened. (Normally, steam supply valves are opened before the valves leading to steam traps.) After a preheat period, valves 2, 4 and 1 are closed. Valves 7, 6 and 2 are now opened to sterilize the transfer line. (Steam should enter at all high points in the line to displace air pockets.) Valve 4 is opened from time to time to prevent condensate accumulation. When the line has been held at the sterilization temperature for the required time, valves 2, 4 and 3 are closed. The transfer line is allowed to cool. Bioreactor 1 is pressurized to ≈ 0.6 bar positive sterile air pressure relative to reactor 2, and valves 8 and 5 are opened. Transfer takes place. The line is freed of most residual liquid by blowing through with sterile air.

In-place cleaning

Upon completion of a production run, and before the next sterilization, the bioreactor must be thoroughly cleaned. A clean-in-place (CIP) sys-

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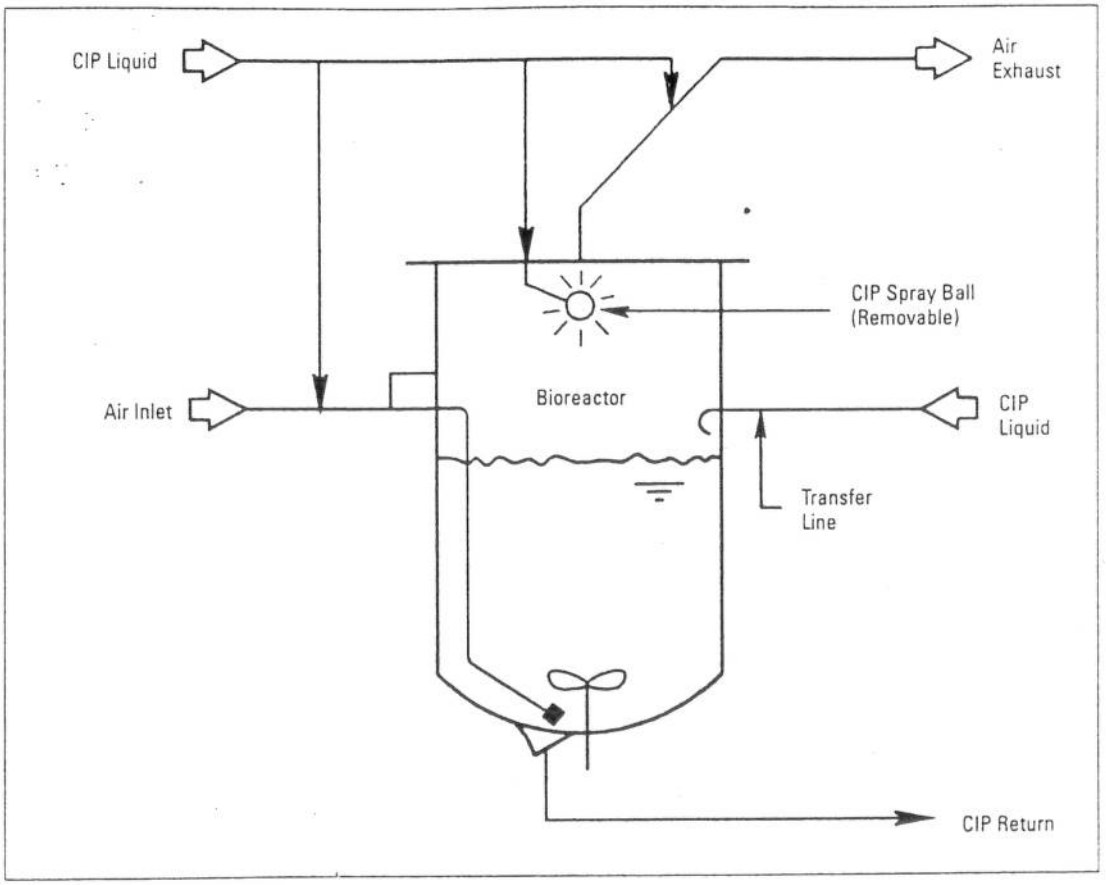

■ *Figure 7. To clean the reactor, the flow of CIP solutions is sequenced through the transfer line, the air inlet and exhaust groups, and the spray ball.*

tem and a transfer flow plate ease cleaning. A transfer flow plate contains all the transfer inlets and outlets from all the bioreactors in a production train (see Figure 6). This plate provides an ingenious means of cleaning in place the bioreactors and the associated transfer pipes. It allows a single CIP system to serve the whole plant. The removable pipe sections on the transfer plate are positive assurance against accidental mixing of the contents of various bioreactors, or a bioreactor and the cleaning solution tank. The simple transfer scheme of Figure 6 allows for transfers from bioreactor 1 to 2 (1 and B connected on the flow plate), and bioreactor 2 to 3 (2 and C connected). It also allows for CIP of either bioreactor 2 (4 and B, and 2 and D connected), or bioreactor 3 (4 and C, and 3 and D connected), with the transfer lines of the reactors being cleaned as well.

In practice, as illustrated in simplified form in Figure 7, the CIP liquid needs to be supplied to the bioreactor air inlet and exhaust groups, and to the reactor vessel through a spray ball (in addition to the CIP flow through the medium transfer lines). Cleaning is achieved by physical action of high velocity flow, jet sprays, agitation, and chemical action (such as by nonfoaming alkaline detergents, and sanitizing agents) enhanced by heat (60–80°C). Cleaning solutions are completely removed from the equipment by water rinse. A final rinse using high quality (reverse-osmosis) water is often employed for cell culture bioreactors; in some cases (such as for parenteral products) a hot, water for injection (WFI) rinse may be necessary.

Just the starting point

The several examples of sterile processing in bioreactors given here provide some insight into design and operation of bioreactors to meet the stringent sterility criteria. The examples, however, do not tell the complete story. A bioprocess engineer needs to consider many other design aspects to ensure that the bioreactor system does function in a sterile manner. Attention to selection and installation of sterile valves, ports, and instrumentation is important. So are the specifications of materials of construction *(2)*. The finish on surfaces that come in contact with the bioproduct, the quality of welds, proper drainage of the many pipes, the physical design of the bioreactor vessels, and design of the peripheral units are some of the equally critical areas of sterile engineering. A discussion of those aspects is left to another occasion. CEP

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