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Short Review

Clean-in-place systems for industrial bioreactors: design, validation and operation

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SUMMARY

Guidelines for design, validation and operation of clean-in-place systems for industrial fermentation plant are presented. Design of vessels, surface finishes, materials of construction, types and locations of valves are some of the considerations addressed. Requisite levels of turbulence for cleaning of pipes and vessels are discussed as well as typical cleaning sequences. Recommendations for validation of cleaning are presented and the significance of design of cleaning systems in ensuring satisfactory validation is pointed out. To the extent possible, validation of cleaning should be carried out with real process soil or soil closely simulating actual fermentation broths.

INTRODUCTION

Exacting requirements of cleanliness, prevention of contamination and sterile operation of biopharmaceutical production plants place stringent demands on design, validation and operation of in-place cleaning systems. These demands are very different from those encountered in 'hygienic' processing of food and dairy products. Here we will evaluate the cleaning problems in bioreactors and discuss the design of clean-in-place (CIP) systems to handle those problems.

Bioreactors are the core of any biopharmaceutical production plant. Biocatalysts — microorganisms, animal or plant cells — are produced and maintained in bioreactors. A production facility typically has a train of bioreactors ranging over 0.02–250 m^3. In a great majority of processes, the reactors are operated in batch mode, under sterile or monoseptic conditions. The most common operational practice starts with culturing microorganisms or cells in the smallest bioreactor. After the batch time, the contents of this reactor are transferred to a larger, pre-sterilized, medium-filled, reactor and this process is repeated until the largest production reactor in the train is reached [5,6]. Further processing of the raw product, or downstream processing [6], is usually done under non-sterile, bioburden-controlled, conditions until the final few finishing operations. As seen in this general process description, at any given time a plant may have several bioreactors at different stages of processing and some empty reactors which need to be cleaned along with associated transfer piping. Thus, while some parts of the bioreactor train are being cleaned others are in processing. Except for the smallest reactors, cleaning is invariably done automatically using clean-in-place (CIP) techniques. A properly designed, validated and operated CIP system not only reduces downtime of bioreactors, but ensures consistency of the cleaning operation which is essential to achieving a contamination-free product with fewer batch losses.

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THE CLEANING PROBLEM

Irrespective of whether a stirred tank, bubble column, fluidized bed, external-loop airlift, or internal-loop airlift reactor is used, the general features of these reactors are similar. Some of the main features are illustrated in Fig. 1 using the stirred tank bioreactor configuration as an example [3]. These features must be understood to gain an appreciation of the cleaning problems. The reactor vessel is provided with a vertical sight glass, and side ports for pH, temperature and dissolved oxygen sensors are a minimum requirement [3]. Retractable sensors which can be replaced during operation have additional implications for the design of the CIP sequence. Connections for acid and alkali (for pH control), antifoam agents and inoculum are located above the liquid level in the reactor vessel. An air (or other gas mixture) sparger supplies oxygen (and sometimes CO_2 or ammonia for pH control) to the culture [3]. When mechanical agitation is used, either a top- or bottom-entering agitator may be employed. The bottom entry design is more common and it permits the use of a shorter agitator shaft, often eliminating the need for support bearings inside the vessel

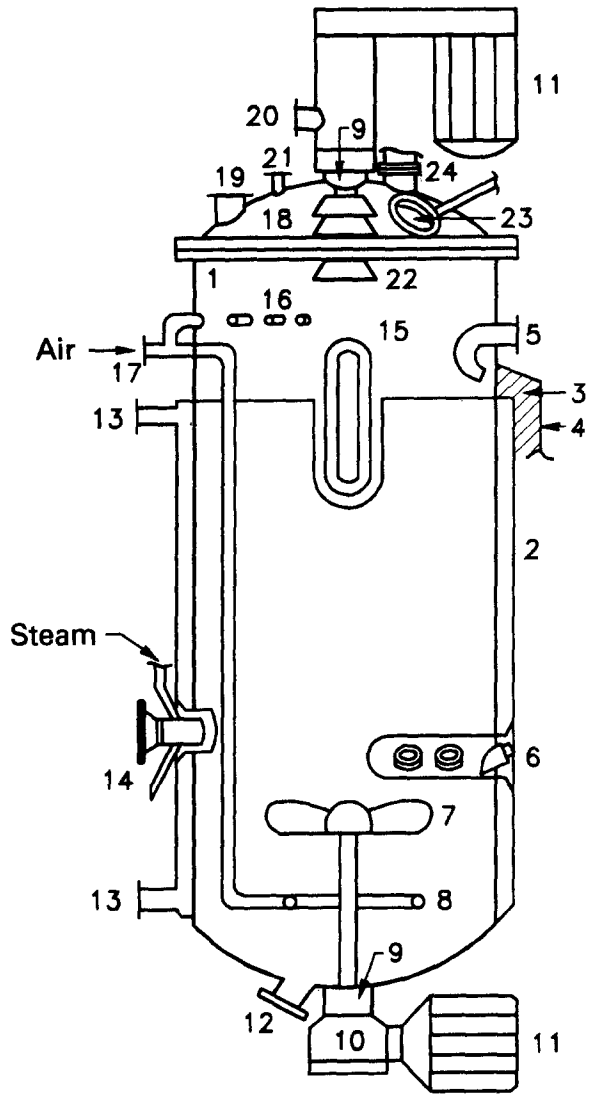

Fig. 1. A typical bioreactor: (1) reactor vessel; (2) jacket; (3) insulation; (4) shroud; (5) inoculum connection; (6) ports for pH, temperature and dissolved oxygen sensors; (7) agitator; (8) gas sparger; (9) mechanical seals; (10) reducing gearbox; (11) motor; (12) harvest nozzle; (13) jacket connections; (14) sample valve with steam connection; (15) sight glass; (16) connections for acid, alkali and antifoam chemicals; (17) air inlet; (18) removable top; (19) medium or feed nozzle; (20) air exhaust nozzle; (21) instrument ports (several); (22) foam breaker; (23) sight glass with light (not shown) and steam connection; (24) rupture disc nozzle.

[3]. The shaft of the agitator is provided with steam-sterilizable, single or double mechanical seals. Double seals are preferred, but they require lubrication with cooled clean steam condensate. Alternatively, when torque limitations allow, magnetically-coupled agitators may be used thereby eliminating the mechanical seals. The magnetic coupling assembly must be located within the vessel and the design of the coupling, as well as the design of the cleaning procedure, affects the cleanability of the vessel. A typical coupling is shown in Fig. 2.

The most practical method of oxygen supply to large scale culture of microbial as well as animal cells is through

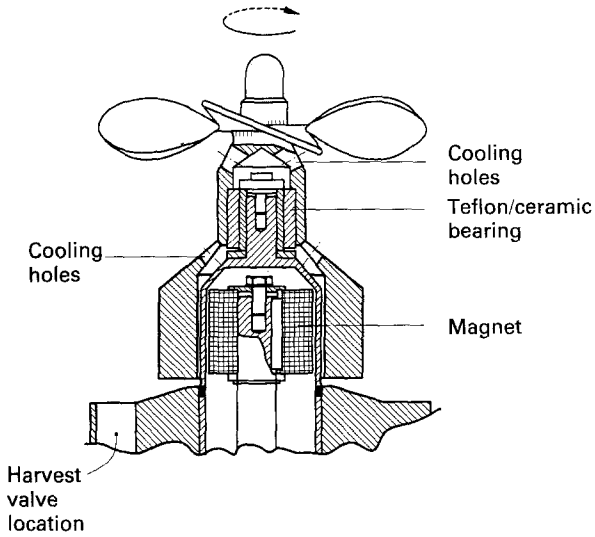

Fig. 2. A magnetically coupled agitator. The rotating element in the bioreactor is supported on a ceramic/teflon bearing. The rotor is provided with holes which allow circulation of culture fluid for cooling and lubrication.

submerged aeration [5]. Aeration inevitably produces foam which is controlled with a combination of chemical antifoam agents and mechanical foam breakers [3]. Foam breakers are used exclusively when the presence of antifoams in the product is not acceptable or if the antifoam interferes with such downstream processing operations as membrane-based separations or chromatography. The shaft of the high speed mechanical foam breaker, as shown in Fig. 1, must also be sealed using double mechanical seals as explained for the agitator. As discussed later in this article, the product-contacting surfaces of the foam breaker and part of the air exhaust pipe which may contact the foam must also be internally cleanable [3]. Details of a typical foam breaker are shown in Fig. 3.

In most instances, the bioreactor is designed for a maximum allowable working pressure of 40–45 p.s.i.g. at a design temperature of 150–180 °C [3]. The vessel is designed to withstand full vacuum [3]. Overpressure protection is provided by a rupture disc located on top of the bioreactor. Other items located on the head plate of the vessel are nozzles for media or feed addition and for sensors (e.g. foam electrode), and instruments (e.g. pressure gauge). A CIP system must adequately clean all these items.

The CIP devices and procedures must be matched to the specific configuration of the bioreactor and to the fermentation process to ensure satisfactory cleaning. For example, as a rule, a bioreactor which has processed hybridoma or other animal cell culture broth is far easier to clean than one which has processed broths of *Streptomyces* or such other mycelial fungi as *Penicillium chrysogenum* or *Tolypocladium inflatum*. Fermenters which process broths of yeasts and non-polymer producing, non-filamentous, bacteria represent cleaning problems of intermediate difficulty. Thus, for the same type and size of bioreactor vessel processing different types of cultures the cleaning demands

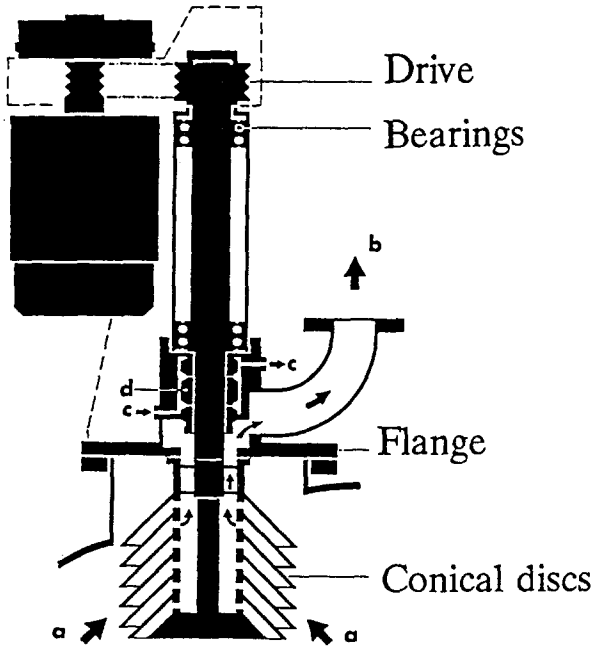

Fig. 3. A Chemap Fundafoam® mechanical foam breaker. The foam enters the rotating conical discs at (a) and is separated into gas and liquid by the centrifugal force. The liquid spins into the bioreactor and liquid-free gas exhausts through nozzle (b). The mechanical seal (d) is lubricated by sterile cooling water (c). During CIP, the cleaning fluids flow into the reactor through the air exhaust nozzle (b).

are different. In one case a 1-min pre-rinse may be sufficient to remove gross soil; in another case, a 5-min pre-rinse may leave behind a lot of adhering debris, thus affecting the cleaning time, temperature and the strength of cleaning agents needed for subsequent cleaning steps.

CIP SYSTEM DESIGN AND OPERATION

General design principles

Typically, SS304 construction of CIP systems is satisfactory although the non-CIP processing equipment is generally made of SS316L. For ease of cleaning, bioreactors with electropolished surface finish of $Ra \leq 0.3$ μm are preferred [3]; however, the components of the CIP system, tanks, pipes, valves and so forth, which do not come in contact with the product may have a lower level of finish at *Ra* of 0.4–0.5 μm [8], without electropolish. This level of finish allows a level of cleanability equivalent to that accepted in hygienically designed dairy product contact surfaces [8] and is quite satisfactory for the CIP system. Further lowering of surface finish is not recommended because the CIP system must be adequately self cleaning.

To ensure removal of gross soil and avoid its sedimentation, the minimum flow velocity through the CIP and transfer piping is considered to be 1.5 ms^{-1} [8], but a higher value of 2.0 ms^{-1} is recommended. In addition, the Reynolds number of the flow must be well into turbulent regime to ensure good radial mixing, heat transfer (uniform heating), mass transfer (of cleaning chemicals and soils) and momentum (scouring action of eddies) transfer. A minimum Reynolds number of 10 000 has been suggested [8], but a higher value of at least 30 000 is preferred. The CIP piping should be free of dead spaces as much as possible; if unavoidable, the depth of the dead zone must be less than two-pipe diameters to ensure adequate cleaning using CIP techniques. These recommendations are based on data reported by Grasshoff [8], but are generally more conservative.

For a CIP system for bioreactors, either valves with metal bellows sealed stem [10], or diaphragm and pinch valves are the only ones recommended even through there is no requirement for sterile processing [4]. All other types of valves — even those commonly accepted in food processing plants — carry a significant risk of contaminating reactors with accumulated debris during the final rinse cycle. Accumulation of debris at gaskets and valve spindles has been clearly documented [8] for ball valves, butterfly valves, and gate and globe valves which are also difficult to clean using CIP methods.

Design of the CIP system should consider cleanability of the system itself and attention must be given to drainage, elimination of crevices and stagnant areas, minimization of internals, arrangement of valves and pumps, piping welds, sanitary couplings, instrumentation and instrument ports [4]. The system must have a splash-resistant exterior of clean design which is easily washable by hosing or wiping. Other general aspects of design of CIP systems are discussed by Adams and Agarwal [1].

A single CIP system usually services all the bioreactors in a production train and the transfer piping associated with the reactors. The CIP flows are directed to selected equipment by making appropriate pipe connections at a transfer flow plate which is a central location for all the transfer inlets and outlets from all the bioreactors in the plant [4]. A flow plate for a plant with three bioreactors is shown in Fig. 4. The CIP flow inlet and outlet shown on the plate in Fig. 4 are in addition to other cleaning flow supply points which connect the CIP system to the bioreactors [4]. These are discussed later in this article.

During cleaning, or transfers between bioreactors, the inlets and outlets on the transfer plate must be connected by removable pipe sections which provide positive assurance against accidental mixing of the contents of various bioreactors, or a bioreactor and CIP fluids [4]. In practice, the flow plate is so configured that different, non-interchangeable, pipe sections are needed to connect specific inlets and outlets, thus eliminating the possibility of erroneous connection. Usually, either a standard operating procedure (SOP) or a computer display instructs the operator to make the necessary connections on the flow plate. In automatic systems, the operator must acknowledge making the pipe connections and the controller unit verifies that the correct connections have indeed been made before proceeding further. Proximity switches located on the flow plate provide the necessary signals for the automatic checks. Such fail safe systems are highly recommended for CIP systems for multiple

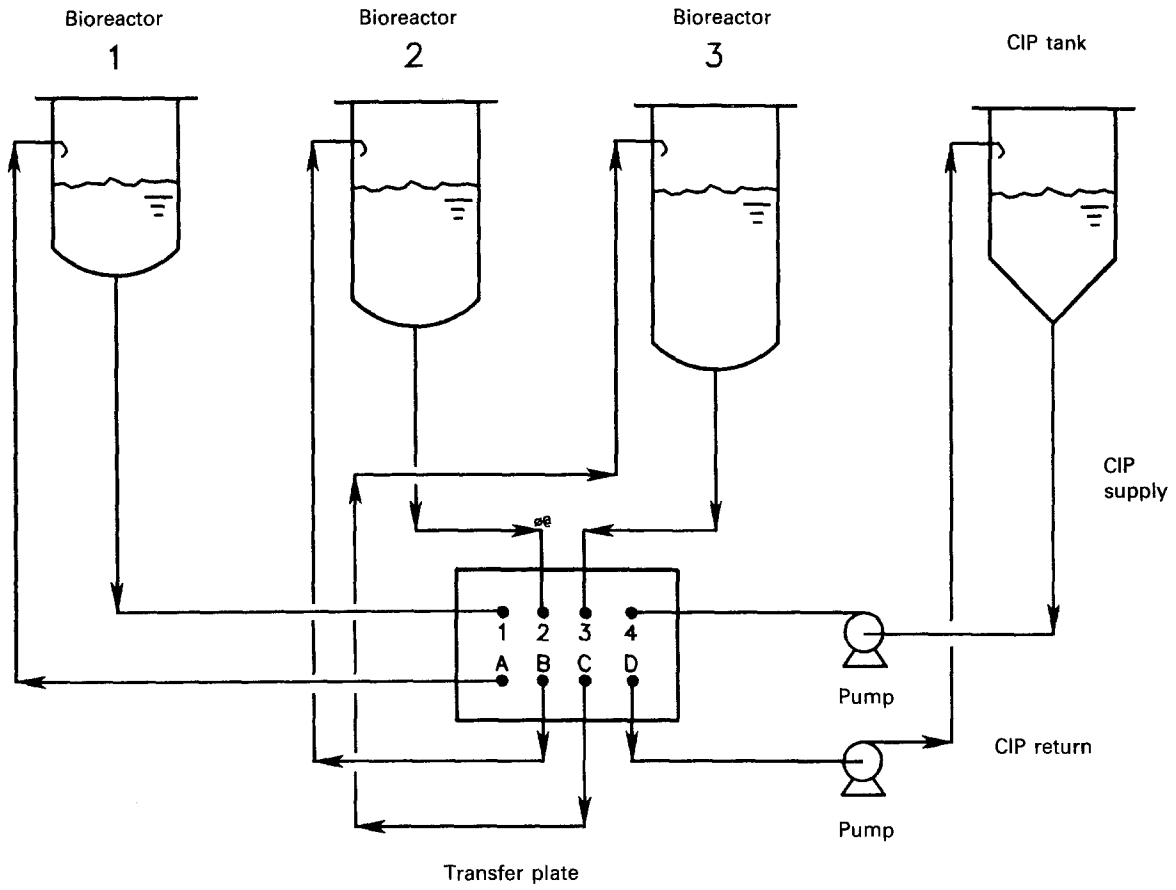

Fig. 4. Connection of a train of bioreactors through the transfer flow plate for cleaning-in-place and other transfer operations.

bioreactors. The connections on the flow plate are made using easy-to-install sanitary couplings.

The simple scheme shown in Fig. 4 allows for transfers between various bioreactors (e.g. connection of 1 and B on the flow plate allows transfer of reactor 1 to reactor 2) and provides a means of circulating the CIP fluids through any of the bioreactors [4]. Thus, during cleaning of bioreactor 2 (Fig. 4), the CIP supply point 4 is connected to the transfer inlet B and the outlet of the reactor (point 2) is connected to the CIP return line at point D. Depending on the process, the size of reactors at any stage of the production sequence is 6 to 20-fold the size of the preceding bioreactors in the train [6]. Thus, the volume requirements of cleaning solutions may vary tremendously, imposing difficult demands on the design of the CIP system.

For adequate cleaning, the CIP solutions must also be supplied to the reactor through removable, static or dynamic spray balls, or dynamic spray nozzles [4]. In addition, the air exhaust piping up-stream of the exhaust gas filter and the air inlet piping should also receive the cleaning solutions (Fig. 5). For cleaning with jet spray, pressures of 30–40 p.s.i.g. are optimal. Permanently installed spray heads are not recommended for bioreactors because of potential difficulties with sterilization. These devices must be inserted in the reactor through one of the ports on the head plate. Because spray balls are often drilled to provide spray

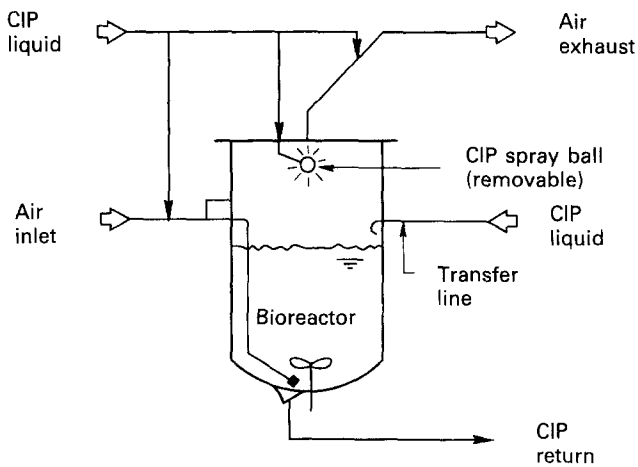

Fig. 5. Delivery of the CIP liquids to the bioreactor. The flow of CIP solutions is sequenced through the transfer line, the air inlet and exhaust groups and the spray ball of the bioreactor.

patterns suited to cleaning of a specific vessel, proper installation of these balls is essential to ensure that the cleaning solutions reach all areas [13]. Spray balls are designed to be self draining [13] and self cleaning. Better coverage of the tanks is possible with removable rotating spray nozzles [4]. Spray balls typically require a flow of

4–12 L min^{-1} m^{-2} of the internal surface [7]. Often, the balls are designed to spray the upper one-third of the tank [7] and the remaining surface is irrigated by the falling liquid film. Design should ensure that the region directly above the spray ball is also cleaned [13].

Cleaning is achieved by physical action of high velocity flow, jet sprays, agitation and chemical action of cleaning agents enhanced by heat [4]. While mechanical forces are necessary to remove gross soil and to ensure adequate penetration of cleaning solutions to all areas, most of the cleaning action is provided by chemicals — surfactants, acids, alkalis and sanitizers. The generally applicable cleaning scheme for bioreactors utilizes a water pre-rinse to remove gross soil; a hot alkali recirculation step to digest and dissolve away the remaining soil; a water wash to remove residual alkali; and a possible hot water-for-injection (WFI) wash [4]. Optional acid wash and sanitization steps may be added in some applications.

For bioreactors for parenteral products and other biopharmaceuticals, potable quality deionized water is recommended for all pre-rinsing and detergent formulations. Pre-rinse should be on a once-through basis without recirculation. This ensures that the gross soil does not recirculate through the CIP system, thus reducing potential contamination. The pre-rinse liquid should be allowed to drain fully. The time saving practice of chasing pre-rinse with subsequent wash solutions is acceptable for pipes [13], but not for bioreactors where the possibility of dilution of cleaning chemicals with residual pre-rinse can be significant. A 5- or 6-min pre-rinse is usually sufficient for bacterial, yeast and animal cell culture reactors. Following pre-rinse, a 1% (w/v) solution of sodium hydroxide at 75–80 °C should be circulated through the equipment so that all product contact surfaces are exposed to this solution for 15–20 min. Alkali should be discarded after use; re-use for the next cleaning is not recommended in bioreactor applications. Dilution, contamination with soil and microbial spores which can survive for long periods [14] and loss of quality definition of the starting material for the next cleaning, are some of the arguments against re-use of cleaning chemicals. A deionized or reverse osmosis (RO) water rinse at 25–35 °C is used to remove all alkali from the system.

Sanitization washes, for example, with solutions of quaternary ammonium salts (QATs), commonly practised in non-sterile food processing plants are not needed for sterile bioreactors. For parenteral products, a hot WFI wash ensures that all residual water complies with quality standards.

Routine acid washes of bioreactors are not necessary if, as is the norm, deionized water is used in production and cleaning, and the peculiarities of production (e.g. media high in Ca^{2+} and Mg^{2+}) do not favor build-up of acid soluble deposits. An occasional acid wash, every 6 months, with 5-min recirculation of 0.5% (w/v) nitric acid at 60 °C is sufficient. The acid wash should be done after the alkali cleaning and rinsing steps.

In mechanically agitated bioreactors, the spray of cleaning solutions may be unable to achieve proper cleaning of the agitators, magnetic couplings, mechanical seals and the lower portions of baffles [6]. Therefore, filling of the vessel to at least above the level of the lowermost impeller and agitation at impeller Reynolds numbers of 10^8–$10^{8.5}$ is recommended during pre-rinse, alkali recirculation and the final rinse. Agitation for 2–3 min is sufficient to dislodge adhering soil. For bioreactors which process fungal broths, the pre-rinse procedure may have to include filling of the tank to above normal working level and intense agitation for several minutes. These recommendations assume that reactors are being CIP'd soon after use and caking of soil has not occurred.

The air exhaust group and the foam breaker as shown in Fig. 1, are cleaned together by sequencing CIP liquids through the air exhaust pipe into the reactor vessel. During cleaning, the foam breaker should be switched on for short periods (approximately 5 s) in pre-rinse, alkali recirculation and final rinse cycles. This ensures complete cleaning of all internal surfaces. Those parts of the foam breaker (Fig. 1) which extend into the reactor can be cleaned only by ensuring that the spray balls or nozzles within the reactor provide good coverage of these surfaces without leaving any dead zones.

Any cleaning scheme should consider manual or automatic cleaning of bioreactor sample valve (Fig. 1) within an otherwise automated CIP scheme. Operations such as filling of tank, agitation, switching of foam breaker during cleaning, etc., can all be automated within the CIP program; however, such an integrated CIP system requires communication between the controllers of bioreactors and the CIP system.

In some bioreactors, sensors such as pH and dissolved oxygen probes can be manually or automatically retracted during processing. The cleaning program should ensure — usually by a standard operating procedure — that such sensors are in the correct position in the fermenter during cleaning.

System configuration

A typical CIP system is shown in Fig. 6. For acid or alkali recirculation, concentrated solutions are metered (pumps P1 and P2 in Fig. 6) into deionized water-filled alkali/acid tanks. The contents of the tank are mixed by recirculation to the tank through the CIP supply pump (P3) while the CIP supply valves (1 and 2) are closed. A high efficiency, easy-to-keep-clean, plate heat exchanger (E1) heats the solutions to desired temperature. The solutions now flow through the exchanger, strainer (S1) and sight glass (SG) to either the flow plate or directly to the spray head on the bioreactor. Dry running of the supply pump is prevented by the no-flow sensor (FS). The CIP return line (and often the supply lines) have a sample point (valve 3) and the return pump, too, has no-flow protection (FS). A sight glass is provided also on the CIP return line. The return flow goes into one of the CIP tanks or to drain. During final water wash, the conductivity sensor (CS) diverts the return flow to drain until a pre-set low conductivity value has been reached indicating complete removal of acid or alkali from the system. Usually, the entire CIP sequence is automated, with the system stopping for the few steps

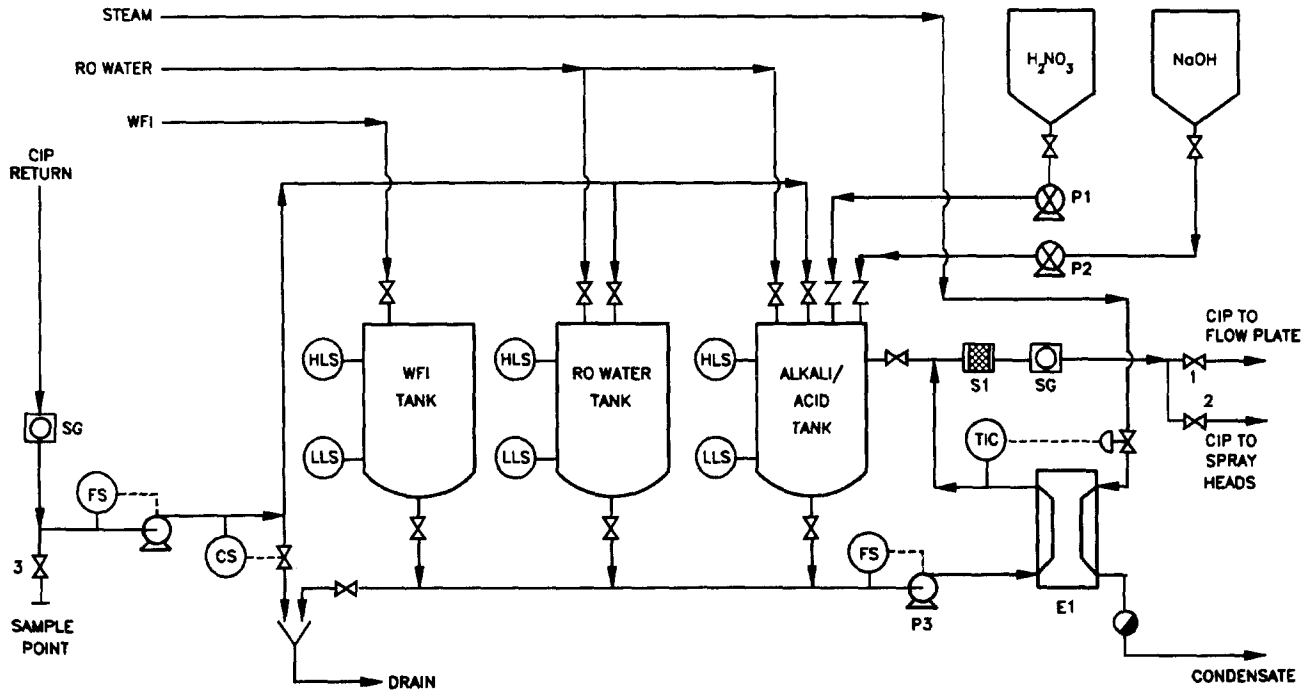

Fig. 6. A typical multi-tank CIP system with pumped return. One or two tank systems can also be used.

which may be arranged to require operator intervention. Several equipment-specific cleaning schemes with different durations of cleaning steps, sequences, flow volumes, temperatures, etc., can be programmed to provide the flexibility needed for a multi-reactor plant. Of course, the design of hardware — tanks, pumps, pipe sizes, etc. — must take into account the flexibility requirements.

Usage of cleaning chemicals, water and energy are the main factors which contribute to the cost of cleaning [11]. Reduced volume CIP systems, based on eductor-assisted recirculation of CIP fluids [12] can significantly lower the costs; however, as recommended in this article, when the cleaning scheme specifies part filling of bioreactor and agitation, eductor return CIP systems are of little advantage. In any case, the cost of CIP is usually a minor proportion of the cost of production of biopharmaceuticals, whereas the expense associated with inadequate cleaning can be substantial.

VALIDATION OF CLEAN-IN-PLACE OPERATIONS

Validation of a CIP system is a demonstration, to a reasonable degree of assurance, that cleaning according to a specified SOP will actually attain the required level of cleanliness, including removal of cleaning agents, in a reproducible manner.

Validation can begin after the pre-validation steps — installation qualification, performance qualification and operational qualification — have been successfully accomplished and suitable standard operating procedures have been developed. Validation should be carried out according to an internally reviewed and approved validation protocol specifying objectives, exact methods for achieving those objectives, and acceptance or rejection criteria. All validation and pre-validation must be clearly documented with piping and instrumentation diagrams, equipment check lists, calibration records, performance test results, etc.

Suitably placed sampling points for the CIP flows, sensors (e.g. conductivity sensor), sight glasses, etc., are essential to acceptable validation of the cleaning process. In addition, manual overrides on supply and return pumps and positive feedback switches for indication of valve positions are useful in validating.

Validation must document the correct sequencing of various valves and pumps. Specified temperatures and flow rates should be attained for the specified times. The pre-rinse, alkali circulation and other steps must take place in correct order. There should be checks on identity, strength and purity of the cleaning chemicals and on quality of water. Any CIP control hardware and software also need to be validated using methods previously described for other computer-based control systems [2,16].

Of the two main purposes of cleaning — prevention of such malfunctions as failures of sterilization and prevention of contamination — the latter may be the more difficult to attain. A realistic definition of 'acceptable level' of cleanliness can be considered to be that level which would eliminate such contamination as would alter the 'safety, identity, strength, quality, or purity' of the product.

After CIP, the equipment should be visibly clean with no sign of adhering soil. Filling of bioreactor with clean water and intense agitation (or aeration in pneumatic

reactors) for a few minutes should not release suspended solids into water. Residues of the CIP chemicals can be monitored by such methods as fluorometry [15], conductimetry [15] and pH measurements. Measurements of total organic carbon, proteins, carbohydrates or some specific component such as an enzyme or antibody may be an indicator of residual soil. Of course, validated analytical procedures must be employed in these measurements. When the residual concentrations in the final wash water are below the level of detection, concentrated samples may be used to prove reduction of soil to low levels. Among the methods applicable to CIP validation of bioreactors is swab testing. Willig and Stocker [17] describe this procedure: 'Representative and measured areas of surface are swabbed with cotton or other appropriate material. The swab is extracted with appropriate solvent and the level of the extractive quanitified.' The total residue may be calculated based on the surface area of the entire equipment.

Sometimes, simulated contaminants such as dyes are used to validate the CIP process. This approach may not give meaningful data because different substances have different rinsing kinetics [15]. Under identical conditions, dyes such as sodium fluoresceinate may take significantly longer to rinse than a more realistic soil such as casein. Therefore, as far as possible, the CIP process should be validated with actual fermentation runs. Rinsing kinetics should be considered in designing and validating the CIP schemes. Surfactants are generally more difficult to rinse from stainless steel equipment than sodium hydroxide, nitric acid and phosphoric acid [15]. Among surfactants, non-ionic ones are relatively easily rinsed [15], but many of these tend to foam a lot which is undesired in CIP systems. Further discussion of validation of cleaning is presented by Harder [9].

CONCLUSION

Guidelines for design, validation and operation of CIP systems for bioreactors have been presented. Successful CIP of bioreactors depends on correct identification of the cleaning demands, development of suitable cleaning schemes and attention to validation during design.

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