
6 Contamination Control Facilities for the Biotechnology Industry

P. J. TUBITO and T. J. LATHAM

INTRODUCTION

Modern biotechnology developed in the late 1970s, arising from advances made in molecular and cellular biology and their application to industrial processes. It built upon techniques developed in traditional biotechnology industries, such as antibiotic and vaccine manufacture, that exploited the properties of naturally occurring micro-organisms. Modern biotechnology could 'create' new organisms utilizing the techniques of genetic engineering. The potential escape of these new recombinant organisms from the laboratory to the outside environment was a concern both to scientists and to public administrations. From the very start, biocontainment was an issue that had to be addressed in the design and operation of modern biotechnology facilities.

Traditional biotechnology is used in a wide range of industries, including the manufacture of food and food additives, brewing, the production of bulk chemicals such as acetone, and the manufacture of pharmaceuticals, including antibiotics, steroids, vitamins, vaccines and others. Initially, it was expected that genetic engineering could be applied broadly across the range of these industries, but in the last 20 years the greatest and widest range of applications has been found in pharmaceutical manufacture. Since most 'biopharmaceuticals' are intended for parenteral administration and thus need to be sterile, this has led to the need to design manufacturing facilities that incorporate the dual requirements of biocontainment and asepsis. Such facilities must prevent the contamination of the outside environment by the recombinant organism, whilst also preventing contamination of the product itself by organisms or particulates. Often conflicting practical and regulatory requirements must be resolved to achieve a workable facility.

The aim of this chapter is to describe the contamination control facilities used in biopharmaceutical manufacturing plants. To do this, it is first necessary to describe the nature and typical features of bioprocess operations. Since the general influence of Good Manufacturing Practices (GMP) on pharmaceutical cleanrooms has already been described in Chapter 4, we concentrate largely on biocontainment and the integration of this with GMP requirements.

BIOTECHNOLOGY—THE INDUSTRY

It was only in 1973 that foreign DNA fragments were functionally incorporated into a cell structure. This breakthrough demonstrated that the selective, deliberate alteration of the hereditary code of a living cell was possible. Recombinant DNA makes it possible to modify cells to produce a desired product. As an example, rDNA techniques have been used to modify the genetic makeup of a bacterium to enable it to produce human growth hormone. Prior to this innovation, this product could only be obtained in limited quantities from the pituitary glands of human cadavers. The product could potentially be contaminated by human viruses that had infected the source—Creutzfeldt–Jacob virus being a particular concern. This illustrates one advantage of biopharmaceutical products: their freedom from contamination by viruses such as hepatitis and HIV. Another advantage is the ability to manufacture large quantities of human proteins unobtainable by other means.

Monoclonal antibody technology, which uses a fused product of an antibody-producing cell and a cancerous cell called a hybridoma, was developed in 1975. The hybridoma cell can be designed to produce large quantities of a specific antibody, and this technology is now exploited for the manufacture of diagnostics and therapeutic proteins.

The first products derived from these new technologies and approved by the regulatory agencies were human insulin (1982) and human growth hormone (1985). These were closely followed by an interferon (1986), hepatitis B vaccine (1986), tissue plasminogen activator (1987), interleukin 2 (1988) and erythropoietin (1989). All of these pharmaceutical products were supplied for use as injectables. In recent years, further products have been licensed. Currently, over 150 recombinant protein products have been developed to the stage of clinical trials or beyond.

BIOPROCESS OPERATIONS

Prior to discussing biocontainment it is necessary to describe the nature of the processing operations used in biopharmaceutical manufacture. These will, of course, vary according to the nature of the product and the producing organism, the scale of manufacture and the ultimate function of the product and its dosage form. However, there are typical methods and operations used across the broad spectrum of biopharmaceutical manufacturing that are different from those used for chemically synthesized pharmaceuticals.

Typically, a biopharmaceutical manufacturing process can be divided into four major steps: media preparation, fermentation, product recovery and purification, and finishing. These are illustrated in Figure 6.1.

Media Preparation

Media preparation involves the dispensing of raw materials from bulk supplies, which may be liquid or solid, the formulation of defined media solutions and their subsequent sterilization. When the organism is a yeast, fungus or bacterium, the media components will mostly be solids. Usually, they will include a carbohydrate such as dextrose or sucrose, a nitrogen source such as yeast extract and smaller quantities of materials

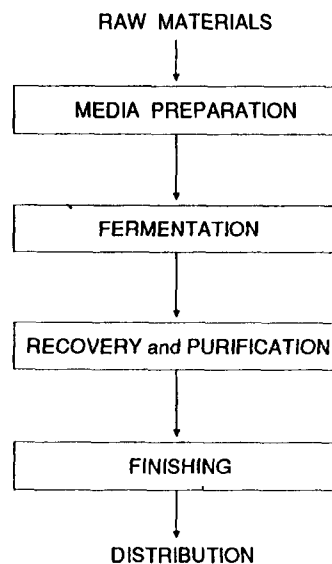


FIGURE 6.1. Bioprocessing steps.

supplying vitamins, minerals and essential growth factors. Processes utilizing animal cells or hybridomas have more complex nutritional requirements, the media being essentially a protein solution that is often based on bovine serum albumin. Such media may be supplied in either liquid or powdered form.

The nature of the media and its components defines the requirements of media preparation areas. These will usually include means of weighing solids, metering liquids, mixing and dissolving these components. The area will be supplied with some form of purified water. The formulated media will be sterilized, nowadays most commonly by filtration but possibly by heat in the case of solids-containing fermentation media.

The presence of dusts from solids-batching operations means that some form of environmental control facility is required to protect operators and prevent cross-contamination. Conventional dust extract systems may be used, or fume cupboards. On a large scale, unidirectional downflow booths are quite popular. Where biopharmaceutical GMP standards apply, the operation will be carried out in a suitable environment, usually class 10 000, to prevent contamination of the media by extraneous material.

The culture organism has not yet been introduced into the process, so biocontainment is not an issue for media preparation areas.

Fermentation

Fermentation is the process by which a micro-organism grows and converts raw materials into products. The micro-organisms may be a yeast, fungus or bacterium. Where an animal cell or hybridoma is used as the production organism, the process is usually referred to as cell culture.

Fermentation requires that the micro-organism or cell be provided with an environment suitable for optimal growth and product formation. This is provided in the form of a bioreactor or fermenter. This is a closed vessel that can be sterilized and into which the sterile media is injected. The media is then aseptically inoculated with the production organism. The bioreactor allows control of the liquid environment in terms of temperature, pH, oxygen content and other parameters. It also allows aseptic operation such that a pure culture of the production organism is maintained.

There are various designs of bioreactor, but mostly they are agitated jacketed vessels fabricated from stainless steel. For biopharmaceutical manufacture they typically range from 1000 to 50 000 litres. They have sophisticated instrumentation and control systems. A typical example is shown in Figure 6.2. The maintenance of asepsis and the containment of the hazardous organisms requires special attention due to the large number of possible penetration points. These are primarily the exhaust air vent, the agitator seal and static seals at valve and pipe or flange joints.

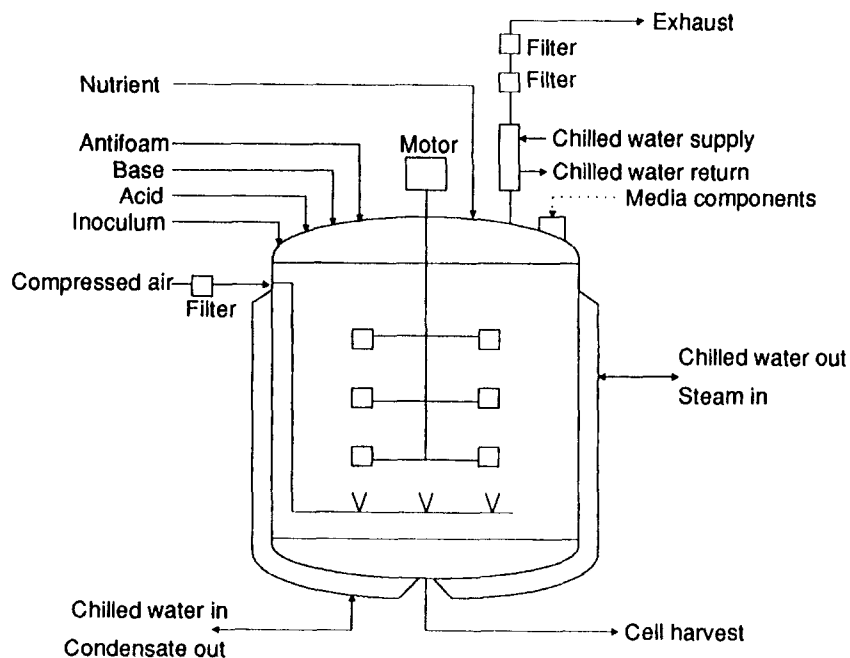


FIGURE 6.2. Suspension culture fermenter schematic.

Recovery and Purification

In many bioprocessing operations the living micro-organism has fulfilled its useful purpose by the end of fermentation, and so it is killed. This may be achieved by chemical or thermal treatment in the bioreactor, or by filtration of the culture fluid as it is discharged from the bioreactor. In such cases, biocontainment may not be required for subsequent processing operations.

It is always preferred that the micro-organism is contained within the bioreactor.

TABLE 6.1. Unit operations for the recovery and purification of proteins.

Function	Unit operation
Cell harvesting	Disk-stack centrifuges, cross-flow microfilters
Lysis	homogenizers, ball mills
Debris removal	Disk-stack centrifuges, cross-flow microfilters
Crude purification	Precipitation (with salts, solvent, pH shift)
High resolution	Chromatography
Product concentration	Ultrafilters (UF)
Desalting	UF, gel filtration chromatography
Precipitate recovery	Centrifuges (tubular bowl, disc-stack)
Sterilization	0.22 μm filters

There are many instances, however, where biocontainment must be extended to include recovery and purification processes. This may be because the cells cannot be killed because of potential damage to the product or disruption to the efficiency of the processing operation. It may also occur if even the killed cells have a potentially toxic effect. This situation often arises in vaccine manufacture, where antigenic toxins are being produced. Even relatively innocuous micro-organisms, such as those used for producing enzymes for biological washing powders, have been found to cause a potentially harmful immune reaction in some process operators.

The typical unit operations of protein recovery are described in Table 6.1. The initial steps are dependent upon whether the product is intracellular (produced and retained within the cell) or extracellular (excreted into the fermentation broth). If the recombinant organism has not been killed in the bioreactor, then the initial recovery steps may have to be performed under biocontainment.

For large-scale recombinant fermentations producing an intracellular product, the initial recovery of the cells from the fermentation broth is typically performed in a disk-stack centrifuge. The recovered cells are then broken apart (lysed) in an homogenizer or bead mill to release the product. The remaining unit operations are selected according to the properties of the product and the contaminating molecules, which are either left over from the fermentation medium or released from the cells during lysis. The aim of the initial recovery processing is the high efficiency recovery of the product protein with maximum rejection of unwanted material, preferably also reducing the volume of the process fluid.

A complete discussion of the various unit operations is beyond the scope of this chapter. It will, however, be useful to describe those operations in which biocontainment is frequently needed, and these are largely the initial recovery steps after the fermenter which may still contain live or dead cells, or substantial quantities of impurities.

Centrifugation Disk-stack centrifuges are most frequently used for separating cells from their fermentation medium. Usually machines are used that can continuously discharge the cell paste, and thus centrifugation of the contents of any particular fermenter is a continuous process. Machines are available for biocontainment operations, being provided with 0.22 μm filters to prevent aerosol release from vent pipes. They

have facilities for steam sterilization to enable the centrifuge to be decontaminated at the end of the operation.

Tubular bowl centrifuges are sometimes used for cell separation, but are more commonly used to recover protein inclusion bodies (solid protein aggregates formed within bacterial cells). Biocontainment is difficult because these machines release aerosols from their shaft seals during operation. Additionally, they retain the cells in the bowl, which has to be dismantled at the end of the operation to recover the product. They are therefore not recommended for biocontainment operations. If necessary, they may be housed in flexible film isolators to contain aerosol release, and their dismantling may be done by operators wearing breathing suits.

Homogenization Homogenizers break cells apart by pumping the broth at high pressure across a letdown valve. The broth may be circulated several times through the homogenizer, each pass releasing increasing quantities of protein. The broth must be cooled to remove the heat generated by the pump. Studies have shown that homogenizers release aerosols containing cell fragments, so for biocontainment the whole machine must be enclosed in an isolator. Methods have been developed for decontaminating the homogenizer by steam after use. The internal surfaces of the isolator must also be decontaminated prior to personnel entry, and this may be achieved by some form of vapourized disinfectant, either formaldehyde, peracetic acid or hydrogen peroxide.

Microfiltration Cell recovery can be accomplished using microfiltration systems, which employ membranes made from a variety of materials including ceramics and fluoropolymers. These membranes can be in spiral, flat or tubular cartridges. Filtration is achieved at relatively low pressures, a cell concentrate being retained by the membrane separated from a cell-free permeate. Only a few of the available membranes are steam-sterilizable, but chemical disinfection can be used for decontaminating the non-steamable membranes. The typical disinfectants used are peracetic acid, hydrogen peroxide and caustic solutions.

Finishing

The final formulation and packaging is performed in the finishing area. Sterile products are processed in a similar manner to conventional sterile pharmaceuticals (see Chapter 4). Some of the typical operations performed in the finishing area are described in Table 6.2.

TABLE 6.2. Unit operations for the finishing of proteins.

Function	Unit operation
Solution preparation	Stoppering/capping
Sterile filtration	Lyophilization (freeze drying)
Terminal sterilization	Inspection
Component preparation	Labelling/coding
Aseptic filling	Packaging

At this stage of processing there is rarely a requirement for biocontainment. However, some form of containment is usually necessary to prevent the exposure of operators to pharmacologically active materials. The need to protect the product from contamination usually requires features that also provide the necessary level of containment, and these will have a significant impact on the design of the facility, particularly in the usual case where the product is finished in a sterile dosage form.

The regulatory agencies prefer that aseptic filling be carried out in a dedicated building separate from that which houses a biocontainment area. If this is not feasible due to cost or space limitations, then the filling facility can be isolated from the biocontainment area in the same building. Thus, they are provided with separate access routes, separate air handling systems and dedicated equipment, utility services and glassware.

Utility Services

Utility services that may potentially contact the process fluid containing the biopharmaceutical product must be of such a quality that contamination is prevented or minimized. Therefore, those services that enter the processing equipment, such as process air, sterilizing steam and process water, are supplied in purified form.

It is not permissible under GMP standards to use non-purified water in biopharmaceutical manufacturing. At the very least, water must be under some form of chemical and microbiological control, and usually a pharmacopoeial grade of water is required. US and European Pharmacopoeias define standards for Purified Water (PW) and Water for Injection (WFI). The latter is the higher standard and is used in the later stages of the processing of parenteral products. PW is produced usually by a series of purification methods, which may include carbon filtration, softening, deionization and membrane processes. WFI is usually produced by distillation, although reverse osmosis is also used in the United States and ultrafiltration is permissible in Japan. PW may be distributed through stainless steel or plastic pipe systems, designed to be sanitizable. WFI is always distributed through stainless steel pipe systems that are designed to be steam-sterilizable. In both cases, the pipe systems are designed as a recirculating loop to reduce microbial proliferation should contamination occur. Often in the case of WFI, the loop is run hot (60–80°C) to decrease further the opportunity for microbial growth.

Steam is very often injected into biopharmaceutical processing equipment as part of the sterilization process, and any contaminant in the steam could remain in the equipment and contaminate the pharmaceutical product. Therefore, contaminant-free steam must be supplied, and is commonly known as clean steam. Conventional plant steam, used for HVAC heating, has additives to prevent boiler scaling and corrosion. These cannot be added to the water used for raising clean steam, because they would be carried over into the steam. The feedwater is usually treated to remove dissolved chemicals that cause scaling and corrosion, and special stainless steel steam generators are used to provide further corrosion resistance. The feedwater may be softened, deionized or purified water, of good microbiological quality. The clean steam quality is usually defined such that the condensate is equivalent to the pharmacopoeial grade of water used in the facility. Clean steam that produces condensate equal to WFI quality is often referred to as pure steam.

Air, and sometimes other gases (nitrogen, CO₂, oxygen), are sometimes injected into process equipment, to feed growing micro-organisms, to effect pressure transfers or to blow dry equipment after cleaning. The gas is usually sterilized by filtering through 0.22 µm filters prior to entering the facility, and is then distributed through steam-sterilizable stainless steel piping to the user points. If the air is supplied from a compressor rather than cylinders, precautions may be required to prevent contamination from the compressor. An oil-free compressor may be used, but this does not deal with hydrocarbon contaminants from the atmosphere. Perhaps the better solution is to use a conventional oil-lubricated compressor with adequate downstream purification filters. This is a more reliable, more energy efficient and less costly system than oil-free systems, and can be designed to deal adequately with atmospheric contaminants.

There may be certain instances where other services must be treated to prevent contamination of the cleanroom environment. For instance, lubricated air used for powering air diaphragm pumps must be piped outside the cleanroom, otherwise an oil film can quickly arise in the area around the pump.

BIOCONTAINMENT

Biocontainment was an issue of concern for microbiologists for many years preceding the development of recombinant DNA techniques. It was required for use with potentially hazardous natural organisms that might be encountered in general research, medical diagnosis and the manufacturing of vaccines, amongst other things. The advent of genetic engineering was accompanied by a fear that new 'unnatural' micro-organisms might be released into the environment, with unpredictable effect. Biocontainment of recombinant micro-organisms was addressed very early in their development, and the first guidelines were published by the National Institutes of Health in the United States in 1976. These have subsequently been revised, and to them has been added a range of international and national guidelines.

Biocontainment Legislation

In the United States US requirements for biocontainment of Genetically Modified Organisms (GMOs) are given in the revised NIH Guidelines, published in the Federal Register of 18 July 1991 (56 FR 33174) and in force since then. These guidelines offer four standards of laboratory containment, Biosafety Levels 1 to 4 (BL1 to 4). For experiments at greater than 10 litres scale, four additional biocontainment categories are offered. For non-pathogenic, non-toxigenic recombinant organisms, or those that are unable to survive outside controlled laboratory conditions, the Good Industrial Large Scale Practice (GLSP) level is recommended. Above this are Biosafety Levels—Large-Scale (BL1-LS, BL2-LS and BL3-LS) each the equivalent to BL1 to 3. There is no standard provision for the large-scale culture of the very hazardous organisms that require BL4 biocontainment.

Table 6.3 shows the equivalent biocontainment standards for the US, European and UK legislation. The specific requirements of the US regulations are given in Table 6.4.

In Europe In the countries of the European Union, biocontainment of GMOs is required to meet the standards of the EC Directive 90/219/EEC of 1990. In the United Kingdom, these have been formulated into the Genetically Modified Organisms

Table 6.3. Equivalent biocontainment standards for large-scale operations.

	US NIH	EC Directive	UKGMO
No biohazard	GLSP		
Low biohazard	BL1-LS	LSCC-1	B2
Medium biohazard	BL2-LS	LSCC-2	B3
High biohazard	BL3-LS	LSCC-3	B4

Table 6.4. Facility design requirements of US biocontainment measures for large-scale operations with potentially hazardous genetically manipulated micro-organisms.

Specifications	NIH containment levels			
	GLSP	BL1-LS	BL2-LS	BL3-LS
1. Airlocks at suite entrances				Required
2. Biohazard signs at suite entrances			Required	Required
3. Negative room air pressure				Required
4. Room finishes to suit easy surface decontamination				Required
5. Decontamination facilities for personnel				Required
6. HEPA filtration of exhaust air from the suite				
7. Closed systems for all operations with viable organisms		Required	Required	Required
8. Treatment of exhaust process gases from closed systems		Minimize release	Prevent release	Prevent release
9. Closed system to be operated at as low a pressure as possible				Required
10. Containment of rotating seals and other penetrations of the closed system			Prevent release	Prevent Release
11. Instrument monitoring of the integrity of the closed system			Required	Required
12. Validated integrity testing of the closed system			Required	Required
13. Validated inactivation of organisms prior to removal from the closed system		Required	Required	Required
14. Control of aerosols during process operations	Minimize release with procedural controls	Minimize release with engineering controls	Prevent release	Prevent release
15. Inactivation of waste materials		Required	Required	Required
16. Emergency systems/procedures for handling accidental spillages	Recommended	Required	Required	Required

(Contained Use) Regulations, issued as a Statutory Instrument by the Health and Safety Executive and in force since February 1993. This offers methods for classifying organisms according to their potential hazard, and three levels of biocontainment (B2, B3 and B4) for large-scale production of those recombinant organisms that are pathogenic or toxigenic and are capable of surviving outside the controlled environment. These are identical to Large-Scale Containment Categories (LSCC) 1, 2 and 3 of the EC Directive.

UK requirements for the containment of naturally occurring micro-organisms are given in *Categorization of Pathogens According to Hazard and Categories of Containment*, 4th edn, 1995, published by the Advisory Committee on Dangerous Pathogens (ACDP). This categorizes micro-organisms according to four levels of potential hazard, from Group 1 (unlikely to cause human disease) to Group 4 (capable of causing an untreatable, frequently fatal disease, which may spread through the community). Four containment levels are defined, CL 1 to 4, each appropriate for the equivalent hazard group. They are aimed primarily at laboratories and animal houses rather than process equipment. They are therefore of less use than GMO regulations for the design of biopharmaceutical manufacturing facilities.

Recently, a British Standard/EuroNorm has been issued for biotechnology containment facilities, and provides a common standard required to be met in all the member states of the European Community. This is BS/EN 1620 (1997), 'Biotechnology—Large-scale process and production—Plant building according to the degree of hazard'. This lists four containment categories similar to those of the ACDP listed above. Additionally, it provides for classification of micro-organisms according to their potential hazard to humans, animals or plants.

Primary Containment

Items 1–5 in Table 6.4 are measures of primary containment in that they aim to contain the hazard within a 'closed system' of processing equipment. It is impossible to create a completely closed system due to the need to supply feedstocks, remove samples, fill and vent the system, etc. Therefore, provisions must be made to minimize or prevent the release of micro-organisms during these operations.

The following is a list of features that should be considered for incorporation into the equipment design of a contained system:

- All vessels and equipment containing live organisms should be suitable for steam sterilization. Preferably, they should be designed for sterilization-in-place (SIP) rather than relying on dismantling for sterilization in an autoclave.
- Exhaust gases from contained processing vessels such as bioreactors must be passed through 0.2 μm sterilizing filters. Under certain circumstances, the exhaust air may also require incineration.
- Special consideration must be given to the design of seals on penetrations into the closed system. Flange joints, instrument probes and other static joints usually require a single O-ring at the lower levels of containment. Vessels used for B4 containment may require double O-rings or double O-rings with steam flushed in the annulus.
- Dynamic joints, such as agitator shaft seals, may require a double mechanical seal, flushed with a sterilizing agent such as steam or condensate.

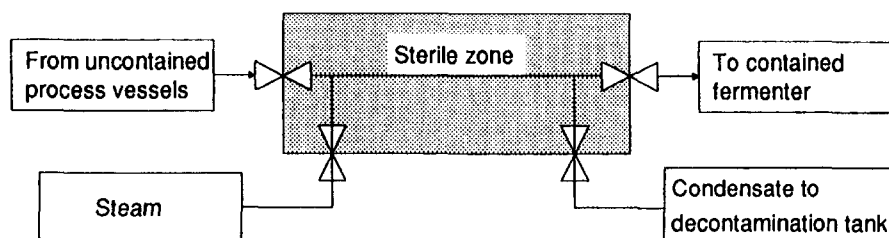


FIGURE 6.3. Steam barrier.

- During operation, a steam barrier can be maintained on fixed piping leading to the contained vessel. Figure 6.3 illustrates the operation of a steam barrier. During a transfer, the steam and condensate valves are closed and the process fluid can be transferred from the uncontained vessel to the fermenter. During fermentation, the two valves in the process transfer line are closed and steam is introduced in the zone between the two valves. This steam barrier is maintained throughout the fermentation to ensure that the bioreactor will not be contaminated and that the contained organism will not escape.
- Potentially contaminated waste streams such as sampling waste and steam condensate from equipment sterilization must be routed to a decontamination tank for sterilization prior to discharge. Contaminated process waste streams such as centrifuge supernatant must also be routed to the decontamination tank.

In cases where the equipment cannot be designed as a closed system, such as when removing cell paste from a tubular bowl centrifuge, the room itself may be regarded as the primary containment. In such cases, the operators within the room must be protected by breathing suits.

Secondary Containment

The primary containment of a bioprocessing operation may be breached by various means, such as leakage, equipment failure and maloperation. It is necessary, therefore, to provide secondary containment, which is largely facility-orientated. It is designed to prevent any organisms released from the contained process plant from passing outside the facility to the outside environment.

The major implication of secondary containment is that the facility actually becomes part of the process operation. A great deal of co-ordination is therefore needed between the various engineering disciplines during the design of a facility to ensure that the overall containment concepts are applied consistently to both the process equipment and the building.

The design implications of secondary containment to the architectural discipline range from room layout to the selection of room finishes. The following identifies some of the established design practices:

- It is desirable to separate physically the biocontainment area from other building functions. The entry should not be from an unrestricted corridor. Containment categories BL1-LS and BL2-LS may require an area for gowning and washing.

BL3-LS containment requires a change area with a personnel shower that provides access via an airlock.

- The biocontainment area must also be designed to contain an accidental spillage. This may be accomplished by having the floor recessed slightly below adjacent uncontained areas. The floor should be seamless, coved at the borders and sloped to the drains. Materials that are typically used include welded sheet vinyl, trowelled-on epoxy or terrazzo.
- The floor drains should be routed to the waste collection vessel for decontamination.
- The materials selected for walls and ceilings must be water resistant so they can be easily cleaned. Walls must also be chip resistant if mobile tanks are to be wheeled around. Typical wall finishes include gypsum board with either an epoxy paint or PVC coating and an epoxy paint over a block wall with a plaster filler.
- BL3-LS containment areas must be completely sealable to allow fumigation by a vapour-phase sterilant such as formaldehyde.

Some of the facility design concepts described above are demonstrated in the room layout presented as Figure 6.4.

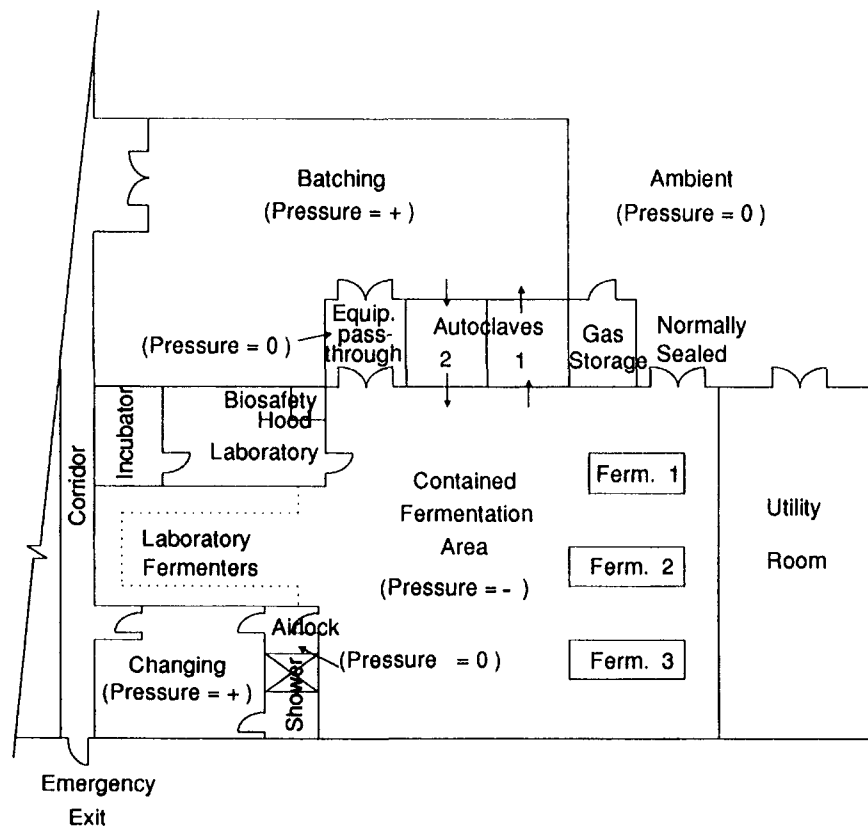


FIGURE 6.4. Hypothetical layout for a BL3-LS biocontainment facility.

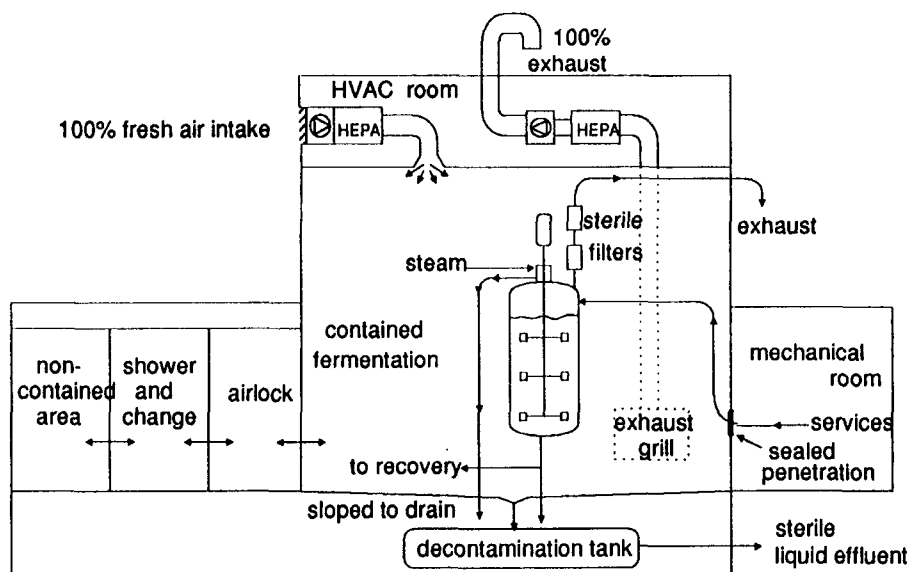


FIGURE 6.5. Section through a BL3-LS biocontainment facility.

The design implications of secondary containment that must be considered with respect to building services are the two methods used to isolate the environment within the secondary containment area from the outside environment. These are High Efficiency Particular Air (HEPA) filters and room pressure differentials. HEPA filters are required for air being exhausted from the higher containment categories. Supply air to the room may also be HEPA filtered in order to decrease generally the bio-burden within the room and increase the life of the exhaust air HEPA filters. Those HEPA filters that can be potentially contaminated should be installed in 'safe-change' housings so that the person changing the filter will not be exposed to the organisms.

BL3-LS containment requires that the area be maintained at a negative pressure relative to the adjacent area. Airlocks located between those areas will be maintained at approximately one-half of the pressure differential. This encourages airflow from the uncontained area to the contained area. The design concepts of primary and secondary containment as they apply to a typical BL3-LS fermentation area are illustrated in the building section shown in Figure 6.5.

Decontamination of Liquid Wastes

All the NIH large-scale biocontainment levels require that liquid waste be inactivated prior to release from the facility (Table 6.4, items 13 and 15). This is generally accomplished by collecting the waste in a contained vessel, then treating it either with a disinfectant or by heat to kill all recombinant micro-organisms present. The collected waste will include not only the process effluent, but also any liquid that may have been contaminated by the micro-organisms within the facility. This could include seal water, condensate from sterilization operations, wash water and other fluids.

Two main forms of waste inactivation are used. Batch inactivation requires a mini-

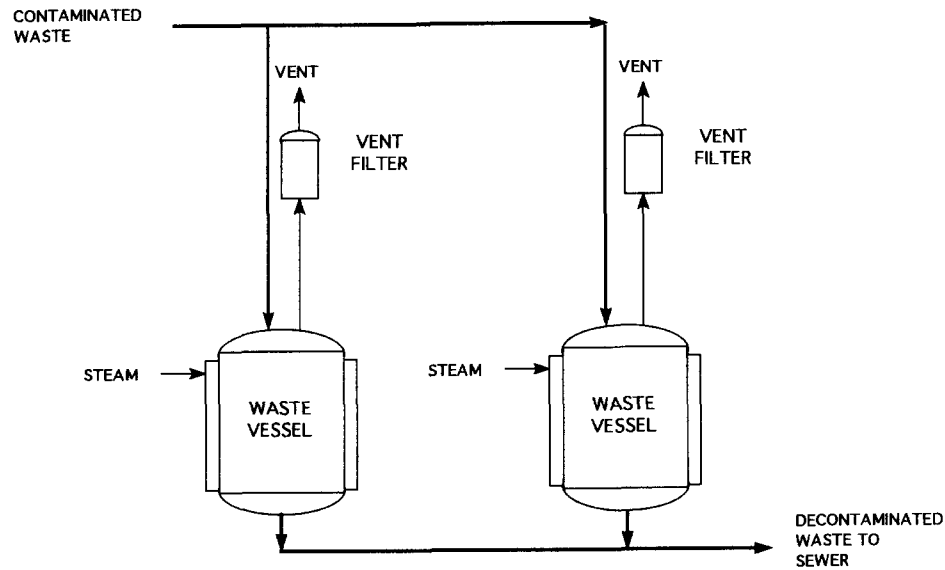


FIGURE 6.6. Batch decontamination of biohazardous waste.

mum of two waste collection vessels (Figure 6.6). These are filled alternately, and as each becomes full it is closed off and the waste collection function is transferred to the other vessel. The full vessel is then subjected to the inactivation procedure. Chemical disinfection requires that a chemical be added to the vessel, and then a relatively long holding time is required for this to take effect. Some micro-organisms are inactivated by high pH, so sodium hydroxide solution can be added to the vessel. For more resilient micro-organisms, proprietary disinfectants may be used.

Thermal disinfection is perhaps more common, and requires that the vessel contents be heated and held at a high temperature. Micro-organisms differ in their ability to withstand high temperatures and an appropriate temperature and holding time must be established by prior experimentation. Usually, temperatures between 80° and 100°C are used, with holding times of several minutes to one hour.

Continuous inactivation is a second form of waste inactivation. It usually involves a thermal method of inactivation, and requires only a single waste collection vessel (Figure 6.7). As this vessel fills, its contents are pumped through a heater and then through a holding coil or vessel, this system being designed to bring the waste to the appropriate temperature and hold it there for the required time. Usually, the temperatures used are higher than those of batch disinfection, but the holding times are consequently shorter.

BIOPHARMACEUTICAL MANUFACTURING FACILITIES

The biopharmaceutical manufacturing facility will include the main processing areas described previously. Many modern facilities are designed for multiproduct operation, such that there will be a separate suite of manufacturing areas for each product. There

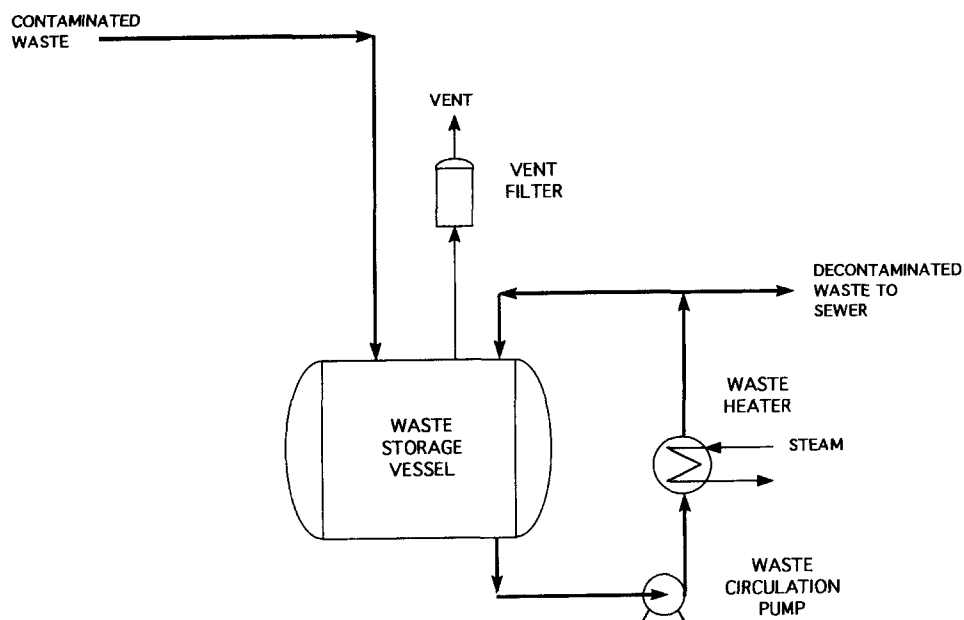


FIGURE 6.7. Continuous decontamination of biohazardous waste.

will be access and communication routes within the facility, together with the support areas required. These may include:

- Laboratories for process analysis, quality control, inoculum development.
- Utility and mechanical service rooms, service corridors for the distribution of piped services and interstitial floors for ducted services.
- Stores for equipment and materials. These may include cold rooms.
- Warehouses for raw materials and product.
- Locker rooms, changing rooms and toilets.
- Administrative facilities such as offices, archive stores, conference rooms.

The facility must be designed specifically to protect the worker, product and environment. Particular features that must be incorporated into the facility design include the integration of biocontainment and product isolation, security from fire and explosion hazards and precautions against operator exposure to radioactivity.

Integration of Biocontainment and Product Isolation

The GMP requirements relating to cleanrooms are described in Chapter 4. One of the key requirements is the use of positive pressure differentials in cleanrooms to prevent contamination from the external environment. This directly contradicts the biocontainment requirement for negative pressure differentials. Means have to be found to

satisfy both requirements. Generally, the requirement for the area around the process equipment to be at negative pressure is overriding. This need not necessarily be the process room itself, but can be a flexible film isolator, separately ventilated. Where equipment is too large or complex for such a solution, then the biocontainment suite (at negative pressure) must be contained within a surrounding building envelope at positive pressure. Air flow within the facility must be designed such that it is exhausted at an intermediate zone, usually an airlock, between the two areas.

Fire and Explosion

The design of biopharmaceutical facilities must include the normal building regulation requirements for limiting fire spread and assisting personnel escape. Some facilities must incorporate additional specialized requirements for flammable operations. Such operations are normally associated with the use of flammable solvents. These are not widely used in bioprocessing, but ethanol, isopropanol, acetone and other solvents are sometimes used as precipitating agents, for selective dissolution and other activities during recovery and purification operations.

The following are some of the design considerations:

- A segregated storage and dispensing area should be provided, incorporating drainage systems to contain any liquid spillage.
- Structures should be fire resistant.
- Mechanical exhaust ventilation should be provided, complete with controls and safeguards, to prevent build-up of flammable atmospheres.
- A high conductivity (anti-static) non-slip solvent-resistant floor should be installed.
- It may be a requirement under some national guidelines (e.g. NFPA in the United States) and some insurance company codes (e.g. Factory Mutual) to provide explosion relief in the building's walls or roof.
- Electrical installations are required.
- Process equipment may be provided with inert gas purging and blanketing.

Radioactivity

The production of some medical diagnostic products requires the use of radioactive tracers. The concentrated solutions of radio-isotopes must be handled in an area of the facility specifically designed for radioactive materials. The major design considerations include the following:

- Concentrated solutions of radio-isotopes should be handled within specially designed gloveboxes and hoods.
- Access to the room should be restricted with the entry via an airlock.
- The room must be maintained at negative pressure with respect to the adjacent areas.

- Mechanical ventilation of the room is required, utilizing once-through air (no recirculation).
- Exhaust air from the area should be filtered with activated carbon followed by HEPA filters to ensure that there is no radioactive emission to the outside environment.

CONCLUDING REMARKS

In the years since the publication of the first edition of this book, biocontainment has become a less emotive subject amongst scientists, engineers and the regulatory authorities at least. Most genetically engineered organisms are produced from non-hazardous natural organisms and agents. Additionally, they are either purposely weakened such that they cannot survive outside the controlled environment of the fermenter, or their metabolism is so overburdened by the changes made to it that they cannot compete with natural organisms. In either case, the hazard to the environment is reduced and this has been recognized by the regulatory authorities. Probably most biopharmaceutical facilities being built today are classified at the lowest level of containment. Nevertheless, stringent biosafety is still an issue in many facilities, and the use of recombinant bacteria and yeasts for vaccine manufacture, in particular, will ensure that it will continue to be so.