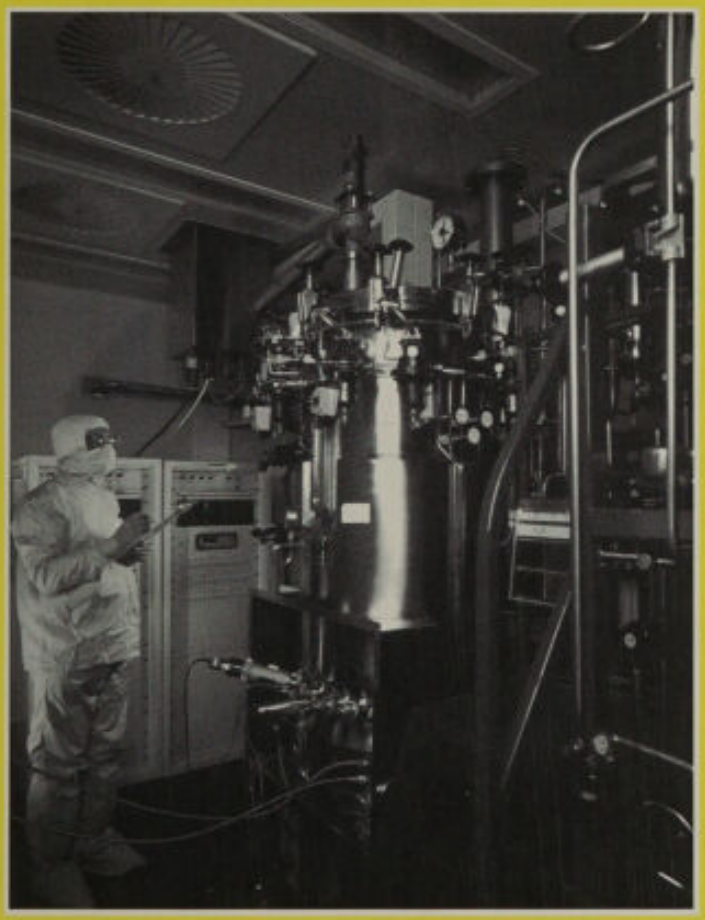
The large-scale contained use of biological agents / Advisory Committee on Dangerous Pathogens.

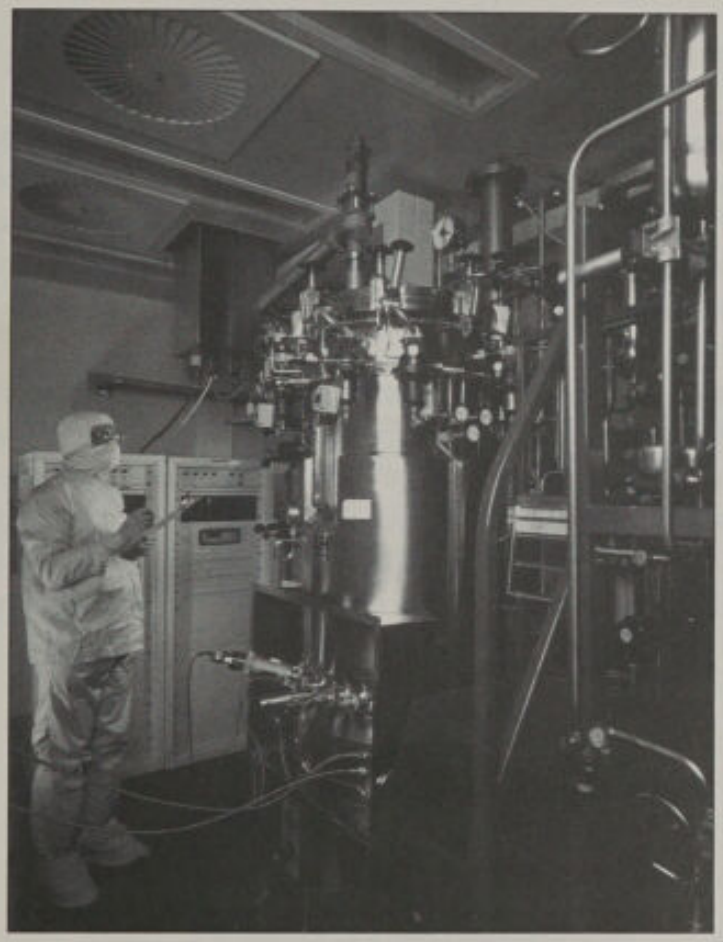
Advisory Committee on Dangerous Pathogens



The large-scale contained use of biological agents

HSE BOOKS

Advisory Committee on Dangerous Pathogens



The large-scale contained use of biological agents

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PREFACE

This publication is intended to provide advice and information on good practice in the large-scale use of micro-organisms. It is intended as a supplement to guidance on the requirements of the Control of Substances Hazardous to Health Regulations 1994 (COSHH), which can be found in the Advisory Committee on Dangerous Pathogen's publication *Categorisation of biological agents according to hazard and categories of containment.*[1]

The main focus of this publication is to provide advice on safe standards of operation for the large-scale use of biological agents in Hazard Groups 2, 3 and 4. It is not principally aimed at those who wish to use Hazard Group 1 agents. By definition these are still potentially hazardous, for example they may be allergenic and/or toxic and therefore, where appropriate, reference will be made to such uses. It also does not seek to provide advice on the use of low-risk organisms which fall outside the definition of a biological agent, although for the sake of completeness, references to such agents are made in the text and appendices.

Because of the great diversity of the industries which use biotechnology and their accepted modes of 'good practice', a publication such as this cannot cover in detail all the accepted processing standards. Appropriate standards are being gathered together for each sector in the form of good manufacturing practice (GMP) guidelines.* Some of these will be quoted but it must be remembered that they are primarily concerned with the maintenance of product quality rather than health and safety.

Appendix 1 of this guidance sets out definitions of the classification of biological agents into four hazard groups.

Appendices 2 and 3 are included with the guidance for information only. The tables were originally developed for the European Commission as part of a major survey of all the important industry sectors which use biotechnology.[2]

Many of the industry sectors do not use organisms which are biological agents within the meaning of the COSHH Regulations. The practices shown for each sector will therefore be appropriate for the organisms used and will not necessarily apply to the use of biological agents.

Appendix 2 gives a summary of the industry characteristics on a sector basis and shows a consensus view on subjects such as building construction, fermentation systems, personnel protection and restrictions.

Appendix 3 summarises engineering standards on an industry sector basis. It covers valve, pipework and building standards and shows industry norms.

* In the context of this guidance, the term GMP means good manufacturing practice, not good microbiological practice.

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INTRODUCTION

1 Modern biotechnology as we know it, like most other current technologies, has developed during the nineteenth and twentieth centuries. It is based in a wide range of traditional industries, many of which have existed in one form or another for thousands of years. These traditional processes enabled products liable to quick spoilage, which were often seasonal, to be converted into stable and storable products for use in the leaner months.

2 Non-pathogenic micro-organisms are widely used in a number of major industry sectors, foods, drinks, waste treatment and the large-scale production of antibiotics. They involve micro-organisms with long histories of safe use.

3 The use of micro-organisms with some known capacity to cause ill health, either by infection, allergic response or toxicity, has also developed in recent years, for example:

- the production of citric acid using Aspergillus species (for example *Aspergillus niger*) which have a known potential to cause allergic response and, more rarely, infection;

- the large-scale manufacture of industrial enzymes for enzymatic washing powders which can cause sensitisation and allergic responses.

4 The first successful vaccines, based on the use of pathogenic organisms, were made more than 100 years ago. Methods had to be devised for this work to be carried out safely. What we refer to as containment methodology has developed from this.

5 Developments in molecular biology have led to a number of new techniques, including genetic modification. This has allowed micro-organisms to be tailored to perform specific functions, rather than relying on traditional methods of mutation and strain selection.

Health and safety legislation

6 European Council Directive 90/679/EEC, now implemented in Schedule 9 of the COSHH Regulations 1994, is concerned with the protection of workers from risks related to exposure to biological agents at work. As such it encompasses micro-organisms which are naturally occurring, or result from a conventional strain selection programme or are genetically modified. The Directive also includes the use of relevant cell cultures and human endoparasites.

7 It should be noted that the Directive and the national legislation arising from it, do not differentiate between traditional and genetically modified organisms. Both are concerned only with the risks to workers which might arise due to infection or disease caused by exposure to biological agents including allergenic and toxic effects. All these factors must be taken into account when designing safe, large-scale plant, buildings and systems of work suitable for use with such agents.

8 The use of any biological agent which is also a genetically modified organism requires compliance with:

- COSHH 1994;
- Genetically Modified Organisms (GMO) (Contained Use) Regulations 1992;
- Genetically Modified Organisms (GMO) (Contained Use) (Amendment) Regulations 1996.

9 Management of Health and Safety at Work Regulations 1992 (MHSW) and other relevant legislation also apply to any work with biological agents.

Purpose of guidance

10 This guidance provides an amplification of Parts II and III in Schedule 9 of COSHH and the associated guidance from the Advisory Committee on Dangerous Pathogens (ACDP). It also draws on guidance produced on the advice of the Advisory Committee on Genetic Modification (ACGM).

11 It is divided into four main sections:

- management systems;
- primary containment - fermentation systems for use with biological agents;
- secondary containment - process rooms and buildings (contained areas);
- personal protection.

12 For the purposes of this publication the model used will relate to the pharmaceutical and allied industries' concept of sterile fermentation which, although it employs mainly low-risk organisms, does use higher containment level organisms in Hazard Groups 2, 3 and 4 for a very small proportion of its products. Containment at higher levels is also used by the enzyme industry which largely follows similar practice.

13 It is emphasised that the pharmaceutical industry concept is not the only way of carrying out large-scale fermentation at higher containment levels. Up to now, however, no other major industry sector applications have been developed and it is therefore not possible to give pragmatic and proven guidance using different concepts. This will not necessarily continue in the future and the guidance may be revised as necessary to incorporate new techniques and new applications.

14 The guidance is based primarily on Containment Level 2, where traditionally words such as 'minimise' and 'optional' have been used. Because this is the area of greatest uncertainty, the guidance will attempt to provide practical advice by expansion of the concepts behind the use of these terms in the legislation. As each section is discussed, the additional requirements for Containment Levels 3 and 4 will be introduced as necessary.

15 Where applications are being designed for the use of Hazard Group 4 biological agents the requirements are specialised. Although general guidance parameters can be indicated and attention drawn to the differences between Containment Level 3 facilities and those at Containment Level 4, this guidance cannot be regarded as offering exhaustive information on the design of a Level 4 installation. The information necessary for such a project can only be finalised after a detailed, in-depth engineering study and risk assessment carried out jointly by the prospective user and the regulatory authorities.

MANAGEMENT SYSTEMS

Health and safety management

16 The management of health and safety is a general requirement of the Health and Safety at Work etc Act 1974 (HSW Act) and, more recently, the Management of Health and Safety at Work Regulations 1992 (MHSW). It is very important, in situations where materials being handled can present a hazard to human health and safety, that sound management systems are in force and that they are monitored and audited regularly.

17 The requirement is to provide a healthy and safe place of work, *so far as is reasonably practicable*. This involves using systems of work which result from detailed examination of each task, identifying the hazards and assessing the risks, in order to define safe methods and to ensure that hazards are eliminated or risks minimised.

18 Employers must have a health and safety policy (Section 2(3) HSW Act) and when they have five or more employees they must record their arrangements for health and safety (regulation 4, MHSW). The organisation of health and safety should include details of the management structure and individual responsibilities, together with employee involvement and responsibilities. It should make provision for effective communication and documentation of health and safety matters and should ensure that all personnel are provided with suitable information, instruction and training.

19 The management system should ensure that there are adequate measures to assess safety performance and that the system is regularly audited, preferably by persons independent of the activity concerned.

20 Employers have a duty to consult employees on health and safety matters. The Safety Representatives and Safety Committees Regulations 1977, as amended, require employers to consult safety representatives appointed by any trade unions which they recognise and, if requested, set up a safety committee. Under the Health and Safety (Consultation with Employees) Regulations 1996, employers must consult any employees not covered by the 1977 Regulations. Further information and details of additional guidance can be found in the free leaflet *Consulting employees on health and safety: A guide to the law*.[3]

21 Depending on the type of operation and the circumstances of the use of a biological agent, it may be prudent to set up a biological safety committee. The precise way in which such a committee is integrated with other committees should be appropriate and be determined for the particular circumstances. Work using genetically modified agents normally requires oversight by a Genetic Modification Safety Committee (GMSC), in accordance with regulation 11 of the GMO (Contained Use) Regulations 1992, to advise on risk assessment. Further guidance on the establishment and running of a GMSC may be found in the ACGM *Compendium of guidance*, which is available from

the ACGM Secretariat, Health Directorate, Rose Court, 2 Southwark Bridge, London SE1 9HS.

22 Where the decision is taken to form a committee, it should be chaired preferably by someone who is competent in the work and who has access to the person with overall responsibility for health and safety within the organisation. This access should be available for the consideration of serious items and for any need to resolve outstanding issues where the decision cannot be made at committee level.

23 It is recommended that the committee should consist of representatives of management with responsibility for such work. There should also be an equal number of persons elected by the workers. It is also recommended that the agenda and minutes of the committee should be available for all employees to see.

Quality management

24 The British Standard 5750:1987 Part 0 has been superceded by:

- BS EN ISO 9000-1:1994, which deals with the principle objectives and characteristics of a quality management system.[4]

- BS EN ISO 9004-1:1994 goes on to consider organisational goals, meeting both company and customer needs, risks, costs and benefits, management responsibilities, the quality loop system, the structure and documentation of a quality system, provisions for audit and review, procurement and production procedures.[5]

25 These aspects of quality management should form part of the procedures which control the industrial application of biotechnology. Although not mandatory, it is recommended that where possible conformity with these standards should form part of the overall management system objectives for processes involving biotechnology.

Training

26 There is the need to ensure a clear understanding, by all employees, of any identifiable risks to their health and safety arising from work and the actions to be taken in dealing with situations in which exposure may occur. COSHH requires that all employees must receive suitable and sufficient information, instruction and training on the risks and precautions to be taken. Under the MHSW Regulations, employees must receive comprehensive and relevant information on the risks, their prevention and the preventative measures available. Employees must also receive adequate health and safety training at a level which will ensure competence in their work. Training should be given before the operative is allowed to commence duties in the contained area. It should be documented in the personal records of the individual and be signed off by both the trainer and the trainee.

Safe working procedures

27 While a health and safety policy statement may deal only in general terms with an employer's intent to develop and maintain a safe working environment, it could also make reference to more specific information on safe

day-to-day working. This should be contained in local codes of practice or safe working procedures. Employers have a responsibility to make the policy and codes freely accessible to any person (whether or not his employee) who carries out work in relation to the employers' duties, either by putting them on display or by individual issue. All operations should be carried out according to agreed safe working procedures. Information and instructions relating to safe practices should form part of such procedures. The steps to defining a safe system of work are:

- assess each task;
- identify the hazards and assess the risks;
- define safe working methods;
- implement the system;
- monitor the system.

Permit-to-work procedures

28 A formal permit-to-work procedure is a well tested and effective way of ensuring that a safe system of work is in place to carry out engineering maintenance and other non-production activities related to biological processes. The key features of such a procedure are as follows.

- A written permit-to-work, signed by a designated responsible person, who has carried out a risk assessment of the work area and the work proposed. This constitutes a formal authorisation for the engineering work, which it describes, to be carried out. The work should be completed in the manner described, using the safety precautions detailed, by the recipient or by persons under his control.
- Persons appointed to positions which involve them in permit-to-work systems should have adequate knowledge, experience and training before they are given the authority to issue or receive permits.

Plant and process modification procedures

29 When a large-scale plant is designed for use in operations which involve biological agents, two sets of design parameters should be observed:

- those demanded by various aspects of health and safety legislation, including any specific measures required for the satisfactory containment of the organism being used;
- any specific measures required as part of GMP for that product and in that industry. These measures may, in certain industries, form part of the manufacturing licence, the original design being part of the submission made to the regulatory authority.

30 From time-to-time, various plant modifications and process changes may be necessary. Such alterations should only be carried out after the risk assessment has been reviewed and amended as necessary.

31 Such procedures should include a competent and complete review of the technical design, which should be thoroughly documented and subject to a formal approval procedure by designated competent persons. This applies equally to both engineering modifications which often require detailed drawings and approval of specifications and components, and also to process changes which involve alteration of chemical or biological constituents and operating conditions.

32 Assessment of any perceived or identifiable change in risk should be carried out in the detail relative to the degree of change proposed. Normally this would be conducted in a step-wise fashion starting with a preliminary hazard and risk assessment. If such an assessment showed that a significant increase in risk might foreseeably occur, then more detailed assessment methodologies may be necessary. There is also a requirement to consult employees on any change that may substantially affect their health and safety at work (see leaflet *Consulting employees on health and Safety: A guide to the law*).[3]

33 The assessments and subsequent approvals should only be carried out by designated competent persons who have adequate qualifications and experience in the relevant technologies and have received appropriate training in the methods of assessment to be used.

34 Where such changes affect the containment of a biological agent or the GMP procedures which form part of a legal permit or licence, then the appropriate regulatory authority (which may not necessarily be the HSE) must be notified.

Emergency procedures

35 A set of emergency procedures for the site and the contained area should be available in an emergency manual. All operatives must be trained in emergency response. COSHH specifically requires:

- plans to deal with accidents involving biological agents;
- provision of written instructions and, if appropriate, notices to be displayed at the workplace covering what to do in the case of an accidental release of biological agents which could cause severe human disease (Hazard Group 3 and above);
- reporting by the employee of any accident or incident which caused or might have caused a release;
- action to be taken by the employer in informing employees or their representatives about any such incident.

36 The manual should specify all the major emergencies which can be foreseen for a particular site and should specify procedures for each of these situations. Safe methods should be specified for the shut down and evacuation of the contained area and subsequent re-entry at the end of the emergency. An emergency team should be nominated and trained to take command during an emergency situation.

37 Regulation 14 of the GMO (Contained Use) Regulations 1992 requires specific action to be taken in the event of an accident involving genetically modified micro-organisms. It should be understood that, for the purposes of these regulations, 'accident' has a special meaning, namely any incident involving a significant and unintended release of GMOs which presents an immediate or delayed hazard to human health or the environment. Further information can be found in *A guide to the Genetically Modified Organisms (Contained Use) Regulations 1992,*[6] as amended 1996 .

Emergency planning

38 Depending on the size and complexity of a site and the materials being handled, it may be a statutory requirement to have an emergency system and plan in operation. In all cases it is considered good practice to have such a system when handling Hazard Groups 2, 3 and 4 agents.

39 In the case of an emergency which involves the general population or is a major risk to the environment, it becomes a duty of the police to take overall control for the incident, and control is vested with the senior police officer in charge of the emergency.

40 However, it will take time for the police to arrive and assume control. During that period the emergency situation must be properly controlled by the site management and emergency team. Once the police have arrived at a major incident, they will still require technical knowledge and advice from the site personnel in order to carry out their responsibilities.

41 Disaster scenarios which require emergency action can arise from a number of sources. In setting up an emergency system it is necessary to plan for all these types of eventuality. Such happenings might be:

(a) PLANT MALFUNCTIONS
Vessel failure;
Pipework failure - steam or process;
Building failure;
Fire - solvent or electrical;
Explosion;
Toxic vapour emission;
Flammable vapours emission;
Gas emission;
Leakage of micro-organisms.

(b) NATURAL DISASTER
Severe electrical storm;
Flooding;
Earthquake.

(c) ACTIONS OF THIRD PARTIES
Aircraft crash on factory;
Railway crash;
Road crash;
Arson, sabotage, terrorism;
Large-scale food poisoning.

42 This list is not exhaustive and will vary from site to site. The potential risk scenarios must be assessed for each particular site.

43 Organisations can normally cope with minor emergency situations which may involve only one or a small number of employees or a small section of a plant. Specific plans will be needed in circumstances which may affect the health and safety of a large number of employees and/or the general public.

44 The emergency may cause damage to:

- company property;
- the ability to continue/resume normal operations;
- property outside the company site;
- the natural environment.

45 An emergency plan should include a number of proactive systems, which are designed to reduce the frequency and severity of any situation. Such systems should include:

- adherence to regulations;
- comprehensive company emergency system;
- an organisation for health and safety;
- regular auditing;
- regular checks that both systems and equipment work when required.

46 In addition to the proactive measures, it is also essential to have a reactive system which will respond if an emergency develops. The assumption cannot be made that the normal line management is best equipped to deal with an extraordinary emergency situation.

47 The reactive systems should include details of:

- who does what;
- names and telephone numbers;
- order of activities;
- individual responsibilities;
- job tasks.

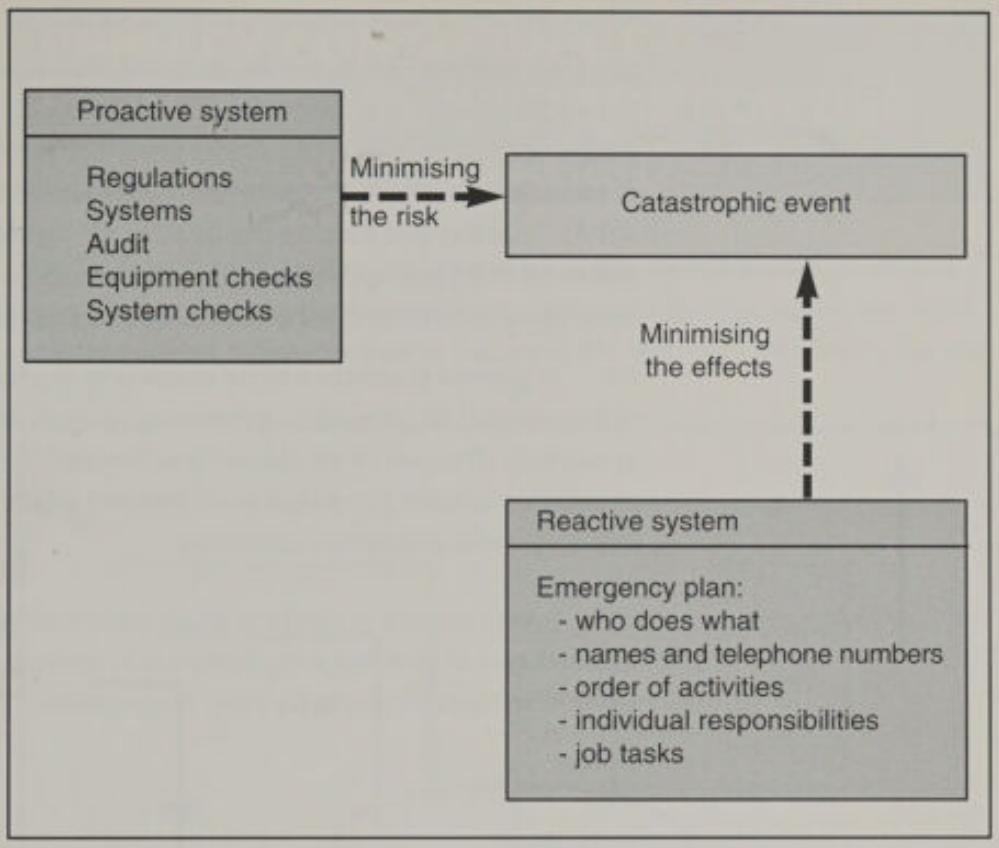


Figure 1 Emergency plan

48 The emergency system has to operate 365 days a year, 24 hours a day and should be divided into two systems: normal hours and out of hours.

49 A reactive emergency plan depends on a team, all of whom have specific responsibilities under emergency conditions.

50 A well tried method of stating these responsibilities is by each member of the team having an emergency manual, which consists of several sections labelled 'YOU AS A...'.

51 These sections summarise, in order, the individual's responsibilities. Each section relates to the activities of a single team member and it is not over-complicated or confused by references to the work of others.

52 Typical 'YOU AS A...' sections may relate to:

Site emergency officer;
Director;
Manager;
Supervisor;
Operator;
Security guard;
Doctor;
Nurse;
First aider;
Fire team member;
Personnel;
Public relations;
Maintenance team;
Health, safety and environmental specialist;
Safety representative;
Roll call marshall.

53 These 'YOU AS A...' sections should be based on the assumption that the whole site has to act together and that individuals may not have time or the proper opportunity to think for themselves. All the information, including that on communication, phone numbers, etc, which is required should be on their 'YOU AS A....' section and their actions listed in the logical operational sequence. In the event of an emergency all employees should go to their appointed emergency location and carry out the instructions on the card.

54 In general guidance it is not possible to illustrate effectively what the actions should be. The detail will depend on each site and its range of operations. This part of the guidance is intended to illustrate the scope of an emergency system and a type of system and organisation which will be effective under emergency conditions.

55 Part of the responsibility of those concerned with the organisation and maintenance of an emergency system is to develop good relationships with external individuals, authorities and organisations. These include:

Police;
Fire;
Ambulance;
Hospital:
- Accident and emergency unit;
- Burns unit;
- Eye unit;
- Microbiologist;

Water authority;
Public Health Department;
Health and Safety Executive;
Environment Agency;
Railways;
Airports;
Member of Parliament;
Local and national media.

Spillage procedure

56 Where the assessment shows it to be necessary, there should be a properly documented spillage plan for use in an emergency. It should show precisely what steps have to be taken and who is responsible for their implementation. The method adopted must be validated against the agents and the methods used.

57 It is recommended that three levels of spillage form the basis of such a policy:

- less than 1 litre;
- one litre to 100 litres;
- more than 100 litres.

58 All personnel must be trained in the actions to take, as part of the implementation of the spillage policy.

PRIMARY CONTAINMENT - FERMENTATION SYSTEMS FOR USE WITH BIOLOGICAL AGENTS

59 Although there is great variation in fermenter design, based on various concepts for powering gas/liquid mixing, the most frequently used is the sterile pressure vessel, which has been a feature of the antibiotics industry for the last 50 years.

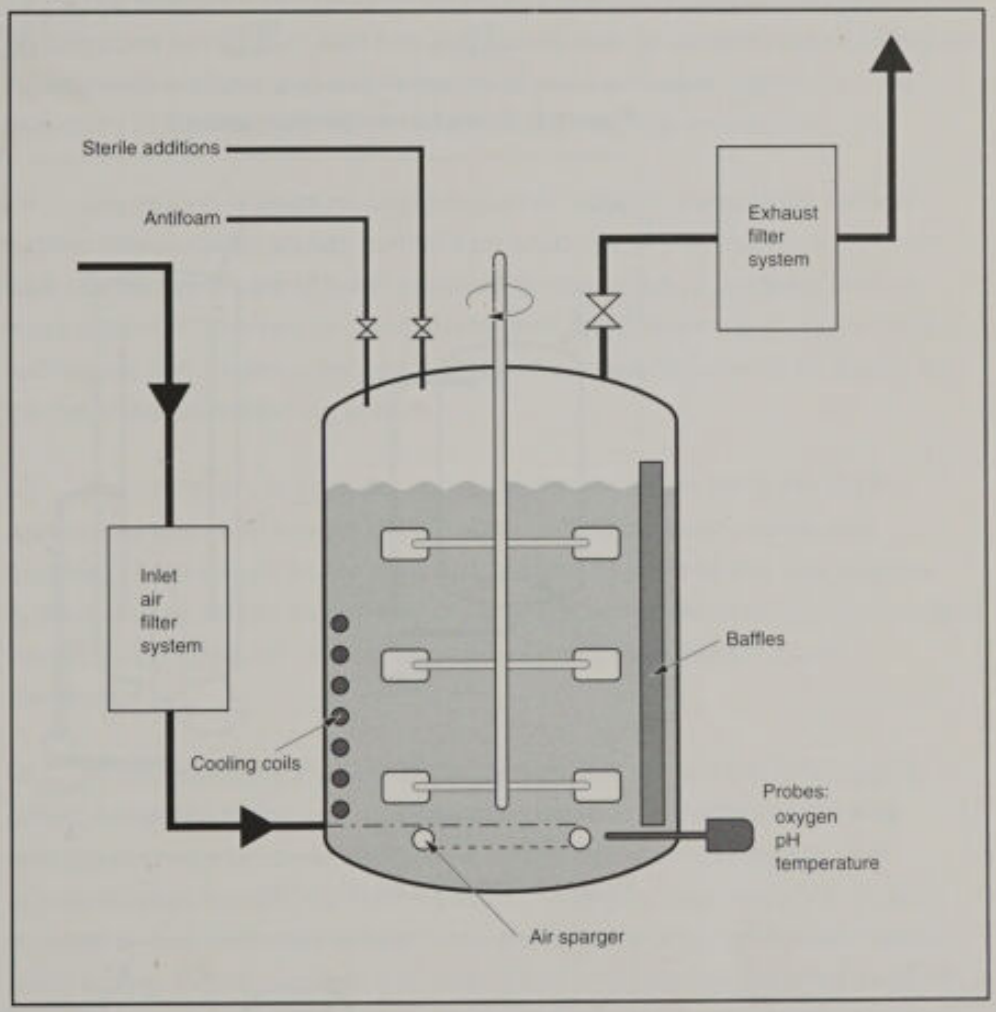


Figure 2 Diagram of typical sterile pressure vessel fermenter

60 This model, in its many forms, will be used as the basis for the guidance which follows. It should be recognised that many of the design features of the sterile pressure vessel are readily transposable to other types of fermenter.

61 Many submerged culture fermentations are aerobic; for example, the production of some antibiotics, enzymes, steroids, vitamins, amino acids, biomass and cell cultures of higher organisms. A wide range of contained fermentation equipment has been developed for these processes in which different methods are used to introduce the energy required to obtain good gas/liquid mixing in the fermenter. The following illustrations show the range of such applications.

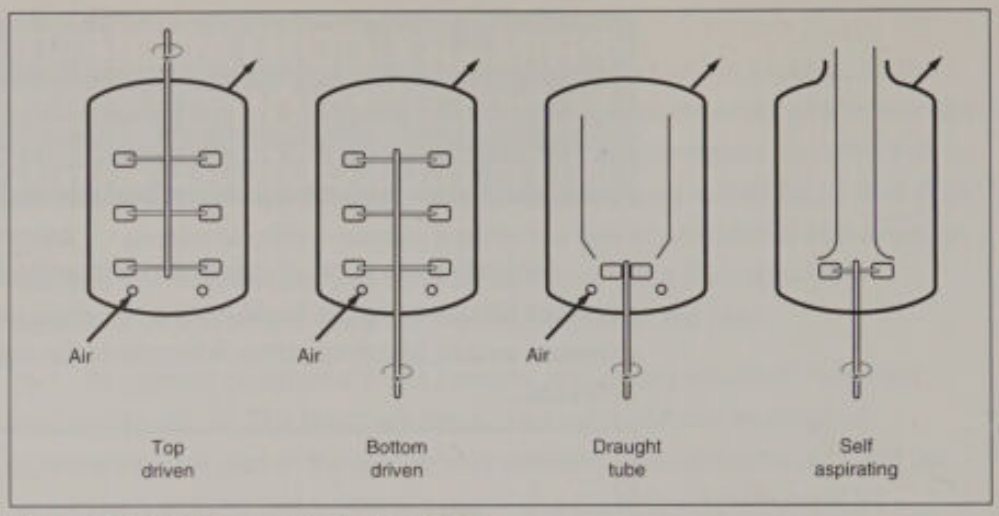


Figure 3 Drive by mechanical agitator

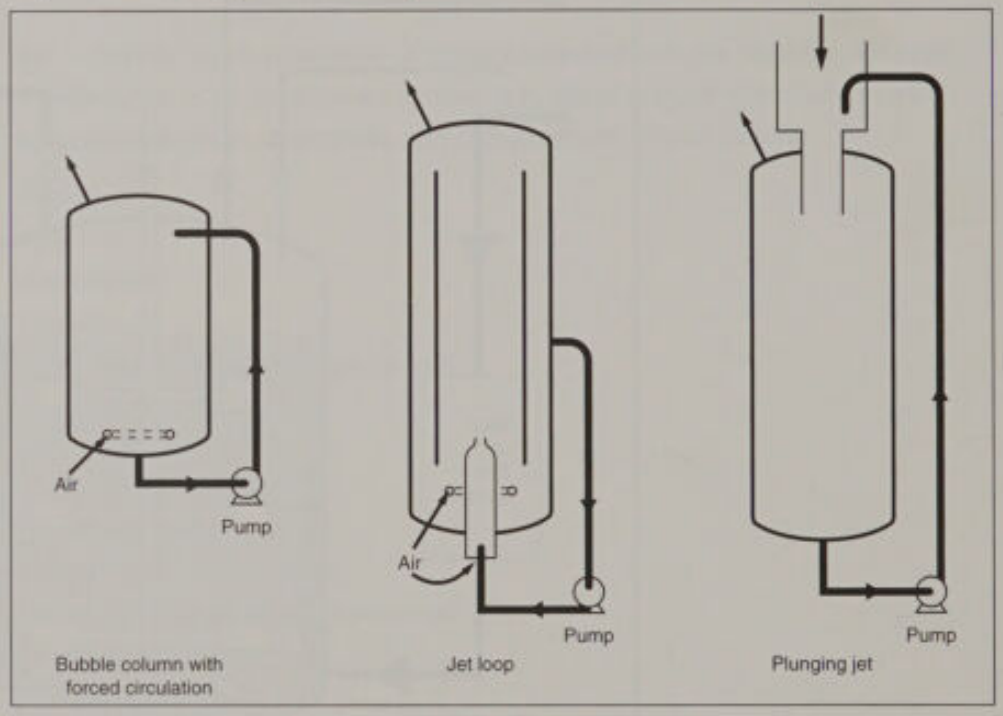


Figure 4 Drive by liquid pump

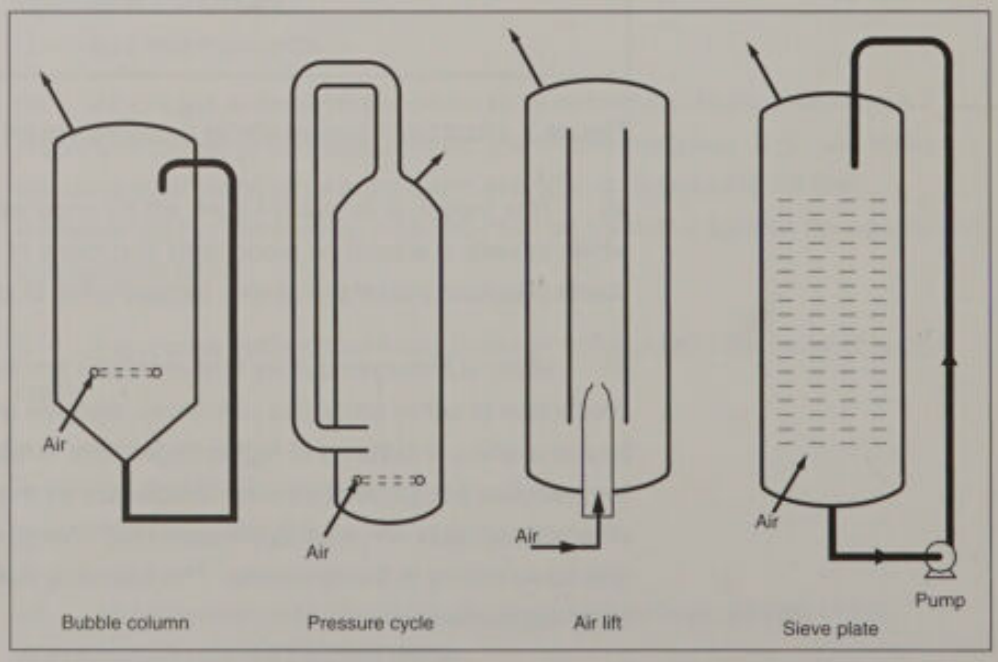


Figure 5 Drive by gas pressure

General layout design parameters

62 In planning a new or modifying an existing fermenter installation, great care should be taken to ensure that the general layout is suitable for the operation. It is important that there is sufficient space around all vessels and other equipment for normal operations and also enough room for routine maintenance work, for example valve replacement, motor and gearbox removal and the provision of the necessary headroom to permit agitator shaft withdrawal. The space should also be adequate to ensure that spillages can be managed without endangering the safety of the operatives carrying out emergency work.

Fermenter system containment

63 The fermenter system is regarded as the primary containment and should be subjected to validated leak test procedures with all connections including the agitator seal in place, and before the introduction of viable organisms. The frequency of such testing will be determined by the risk assessment.

Pipework

64 Gas testing techniques using helium or halogen compounds such as sulphur hexafluoride, should normally be used to test that pipework does not leak. Alternatively, rate of fall of pressure or rate of rise of pressure from an evacuated condition can be used and is cheaper. The choice of one or more techniques and the frequency with which each is applied should be based on the risk assessment for the system.

65 It is necessary to consider various factors in determining the most appropriate choice of system of pipework, jointing methods, valves and gaskets. There should be the minimum number of joints of any type and the system chosen should be capable of withstanding repeated temperature cycling from ambient up to 121 °C during cycles of production, cleaning and sterilisation.

66 When examining the cost of the system, it is not just the initial capital outlay, but also running and maintenance costs which determine the total balance of choice of components for a contained system. Stainless steel scheduled pipe is attractive on the basis of initial purchase cost, but on-site fabrication may increase the total costs above those of an installation carried out in tubing. Tubing requires a smaller space and also has the advantage of improved appearance, particularly when jointed using inert gas orbital welding techniques.

67 Above 80 mm (3 inches) diameter, valves suitable for use with tubing become increasingly expensive, and above this diameter it is recommended that pipe is used. Pipe rather than tubing should be used for the fabrication of connections from vessels because of its greater strength.

68 Wherever possible, stainless steel pipework should be used for drainage systems. Copper is not sufficiently robust and the joints will eventually leak. Mild steel pipework will initially give adequate containment, but corrosion can lead to early, sometimes undiagnosed, failure of the system.

69 When joints in tubing or pipes are necessary, welding is the preferred option. X-ray testing allows examination of the mechanical quality of a weld but does not guarantee that it is leak-tight. The use of gas testing is therefore preferred and should be applied to each joint and then to the system as a whole. Welds should also be subjected to a hydrostatic test of one-and-a-half times the normal working pressure for two hours. Where possible, inspection of welds should take place during fabrication, rather than after delivery of the complete plant to the site of the contained area.

Couplings

70 It is recommended that pipework for use at Containment Level 3 should be of all welded construction unless another form of joint is justified. It is strongly recommended that pipework for Containment Level 4 be welded and it is recommended that all welds are subjected to both X-ray and gas testing before use.

71 For work at Containment Level 2, a variety of clamped and flanged joints may be used. There are a number of differing types of clamp available, all of which have their own specific advantages. In practice it is the correct fitting, support and testing of gaskets which is the key factor, rather than the type of clamp or flange system. In each case, it is important that the user is able to show that the coupling/gasket system chosen is performing to the design requirement. Of the following, the ring joint type coupling, the international dairy federation and the in-line cleaning coupling are not recommended for use in biopharmaceutical plants. Triclover clamp type joints have become the standard in the UK.

- *The ring joint type (RJT) coupling* - consists of a male part which carries an external thread and sealing groove, a liner, a seal and a hexagonal nut. One problem with this type of fitting is that the gap between the male part and the liner makes cleaning in place and sterilisation difficult. The problem does not arise in those industries, for example dairy and brewing, which favour this type of coupling because pipework is normally dismantled and cleaned manually.

- *The international dairy federation (IDF) coupling* - consists of the same four basic components as the RJT but the ends of the male part and the liner are specially shaped to give a smooth, crevice-free joint. It may be used with a support ring for the seal. This is optional at the smaller sizes, but should be used for couplings of more than 60 mm (2.5 inches) diameter at pressures greater than 10 bar gauge. One disadvantage is that these couplings are only available in sizes of 25 mm (1 inch) and above.

- *The in-line cleaning (ILC) coupling* - this is similar to the IDF coupling but the seal has rounded edges. It also has the advantage that it is available in all sizes and can therefore be used throughout an installation.

72 The above couplings all rely on a hexagonal nut which is screwed onto the matching thread on the male part, in order to apply compression to the seal.

- *Triclover clamp type joints* - an alternative method is to use a clamp to force together two liners, each of which is grooved to locate a shaped seal. The seal, which is usually flanged and contoured, can be made to give a crevice-free joint in the pipework. Such couplings can be dismantled easily, particularly if the clamp is tightened by a quick-release clip. These clamps cannot be accidentally over-tightened unless they are designed to be tightened using a locking bolt, in which case the use of excessive force can result in a cut seal. It is essential to use a torque wrench for such applications.

73 Alternatively a pair of flat flanges can be made perfectly gas-tight when used with a suitable seal, the dimensions of which are arranged to produce a crevice-free joint. Care should be taken to ensure that the flange faces are smoothly machined and do not contain any radial scratches which could cause leakage paths. It is also preferable that the bolts which join the flanges are tightened with a torque wrench to avoid distortion to the flanges.

74 The use of screwed pipe is not recommended at Containment Level 2, because of difficulty in sterilisation and decontamination. The use of screwed pipe is not acceptable at Containment Levels 3 or 4.

75 Whatever method of coupling is used, it is essential that the pipework is adequately supported to prevent distortion of the couplings and gives good pipe alignment. This support should also take into account the dimensional changes introduced by thermal expansion during sterilisation cycles. Failure to make such provision can result in distortion or cut seals.

Static seals

76 The choice of materials for seals and gaskets depends on the specific process conditions and factors such as temperature, exposure to steam, temperature cycles, chemical resistance and other factors. Some of the typical materials commonly used are:

		General temperature range (°C)
Polytetrafluoroethylene	PTFE	–40 to 150
Ethylene propylene diene modified	EPDM	–50 to 150
Isobutylene isoprene	IIR Butyl	–30 to 130
Butadiene acrylonitrile	NBR	–40 to 100
Fluoroelastomer	FPM	–30 to 175
Silicone		–60 to 230 static (175 dynamic)

77 *Polytetrafluoroethylene (PTFE)* This exhibits a very broad range of chemical and thermal serviceability. However, the effects of temperature, pressure and absorption of chemicals on PTFE and their interactions should be considered. A modified PTFE, developed for use where intermittent steam sterilisation is used, provides improved resistance to creep and elevated steam temperatures.

78 Care must be taken in the application of PTFE as a sealing material because it is a thermoplastic rather than an elastomer, and as such does not have the resilient properties desirable in a static seal. To overcome this, it is recommended that the PTFE static seal be designed to utilise a relatively thin layer of PTFE as the product contact material, and a reinforcement or backing material that has the desired resilient properties, such as EPDM or FPM.

79 One example of this is the 'envelope' or 'sandwich' gasket. In this design, a PTFE outer layer surrounds an inner core of FPM or EPDM. The PTFE provides for product compatibility while the FPM inner core provides heat resistance and resilience.

80 Another example of this is for a diaphragm seal in a diaphragm valve. A thin layer of PTFE is in contact with the product and is backed by a thicker layer of FPM or EPDM. Similar to the clamp union gasket, the PTFE provides for product compatibility while the FPM inner core provides heat resistance and resilience.

81 It is important in both cases that the PTFE is not bonded to the elastomer reinforcement but should be allowed to float freely. Bonding can cause additional stress and premature failure.

82 *Ethylene propylene diene modified (EPDM)* This is a cured organic peroxide which provides the maximum heat stability and the best compression set which can be achieved using a polymeric gasket material. EPDM does not contain sulphur, which can be poisonous in some biotechnology processes.

83 EPDM has a good resistance to steam but a very poor resistance to oil-based solutions.

84 *Isobutylene isoprene (IIR) BUTYL* This is resin-cured and provides excellent resistance to water-based media, salts, dilute acids and alkalis. Lowest permeability of all elastomers.

85 *Butadiene acrylonitrile (NBR)* This is resistant to aqueous media, paraffinic hydrocarbons and alcohols, suitable for use with antifoams based on animal fats or any water-based media containing hydrocarbons.

86 *Fluoroelastomer (FPM)* This is resistant to strong oxidising agents, chlorinated solvents, ozone and UV light. Poor in hot water and steam.

87 *Silicone* This is resistant to water, ozone and UV light. It does not support bacterial life and can be used over a good temperature range. Poor tear strength and poor in high-pressure steam and oils.

88 Storage conditions for elastomers are also important to ensure best performance and need to take into account:

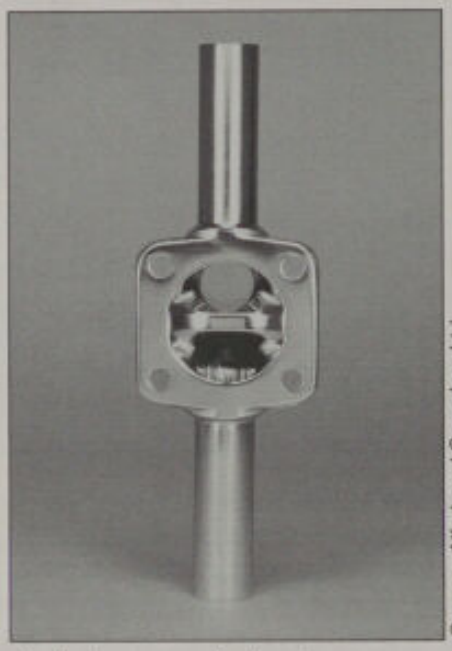
Condition	Storage requirement
Temperature	below 25 °C
Humidity	dry environment
Light	avoid direct sunlight or artifical light with a high violet content
Oxygen/ozone	ozone is aggressive to rubber and ages the material
Oil/greases	avoid contact during storage

89 Some identification to show the age of the material being used will avoid the use of ageing materials and the subsequent reduction in performance.

90 O-rings can be made in various sections or profiles. Profiled O-rings are generally easier to fit than round rings. They are not necessarily satisfactory at low pressures and care must be taken in installations of this type when designing the dimensions of the seal groove. At higher pressures, some deformation of the O-ring takes place and a good seal can be obtained. With shaped O-rings which involve a flat part in the cross-section, care must be taken to ensure that a smooth face, in line with the pipe inner wall, is obtained in order to avoid a crevice which may harbour contamination. There is little evidence to suggest that in a static seal, two O-rings are better than one. The disadvantages are that the coupling is more difficult to assemble, it is difficult to detect failure of one of the seals as the seals cannot be tested independently and the space between them may give rise to contamination. The application of a steam seal between two O-rings has been used but this should generally be regarded as an unnecessary complication.

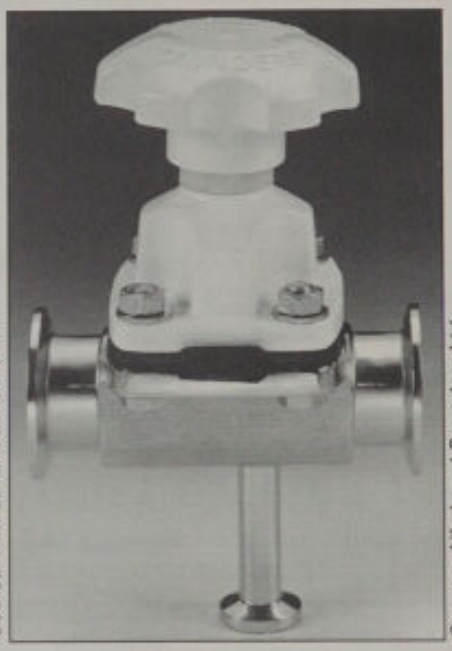
Valves

91 The diaphragm valve is generally the valve of choice for process biotechnology applications at all containment levels, although ball valves can be used at Containment Levels 1 and 2. The choice of seal material is important in a diaphragm valve. Either a PTFE envelope, EPDM or EPDM coated with PTFE will give good results.



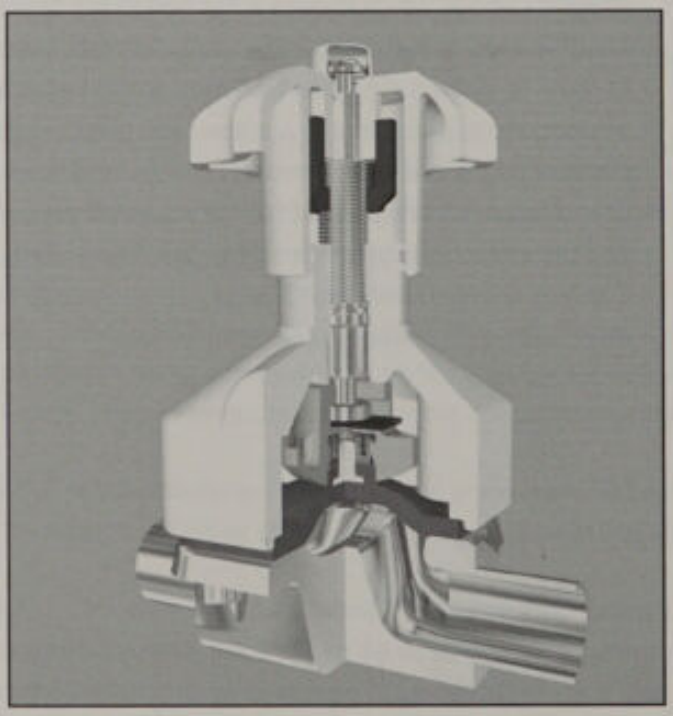
Source: Alfa Laval Saunders Ltd

A diaphragm valve body



Source: Alfa Laval Saunders Ltd

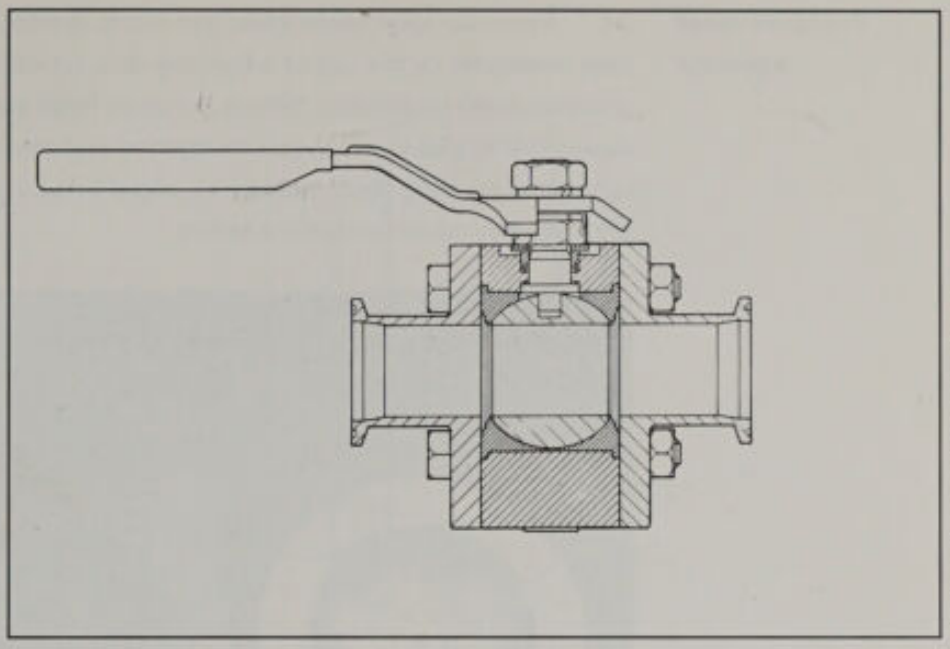
The body of a diaphragm valve with steam connection



Source: Alfa Laval Saunders Ltd

Cross section of a diaphragm valve

92 Ball valves are preferable for use on steam systems. Where ball valves are fitted on process applications at Containment Levels 1 or 2, they are likely to require more maintenance than diaphragm valves. One disadvantage of the diaphragm valve is that it is not always clear whether the valve is open or closed; in the case of a ball valve this is obvious from the position of the handle.



Source: Worcester Controls UK Ltd

Figure 6 Cross section of a ball valve

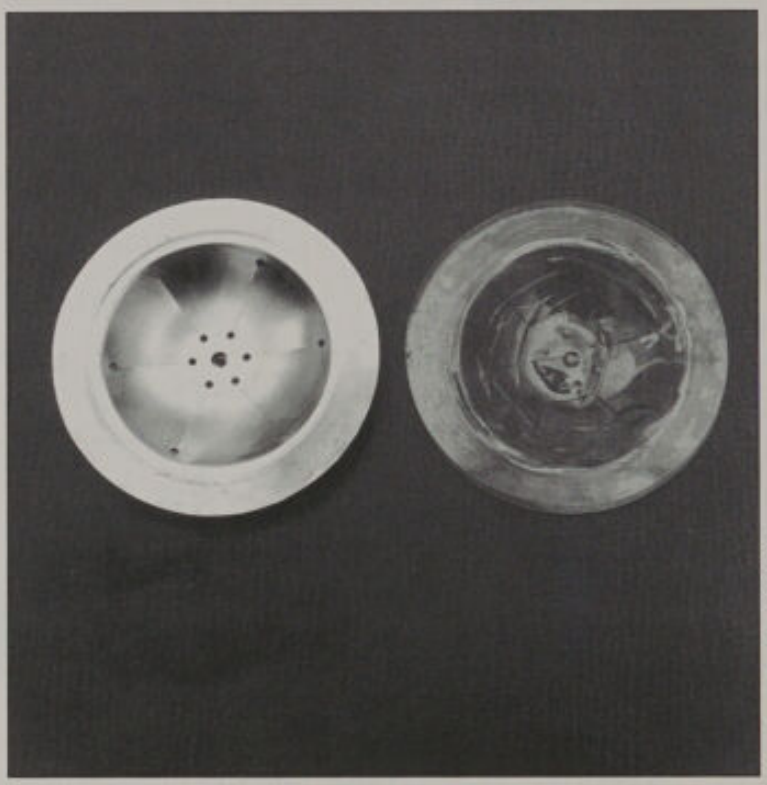
Source: Worcester Controls UK Ltd

Cut away view of a ball valve

93 Butterfly valves may be considered to be similar to ball valves but their assembly causes difficulties for biotechnology applications. Such valves may therefore be used for applications at Containment Levels 1 or 2, but are generally not used at Containment Levels 3 or 4 unless it can be demonstrated that they are contained.

Pressure relief systems

94 Pressure relief assemblies can cause sterilisation problems which are best overcome by the use of a bursting disc, typically stainless steel, but other materials may be suitable. Where pressure relief valves are used, cleanable valves are available which can be opened and steamed through. It should be noted that bursting discs must be changed frequently because they do fatigue and this may cause premature failure.



Photographed at CAMR

Bursting disc components for assembly in a fermenter

95 An alternative approach is to regulate the utilities so that the maximum pressures which can be developed are lower than the safe working pressure of the vessels and pipework. This practice should be agreed with HSE inspectors and the insurance company concerned, before proceeding with the design and installation.

Agitator seals

96 Agitators may be either top or bottom drive or may be driven by external non-contact sources such as magnetism. Magnetic drives have the advantage that fermenter containment is not breached and mechanical seals are therefore not required. In the case of agitators which use an externally protruding driven shaft, an effective seal is required. For Containment Level 1 applications, either a stuffing box or a single-face mechanical seal will suffice.

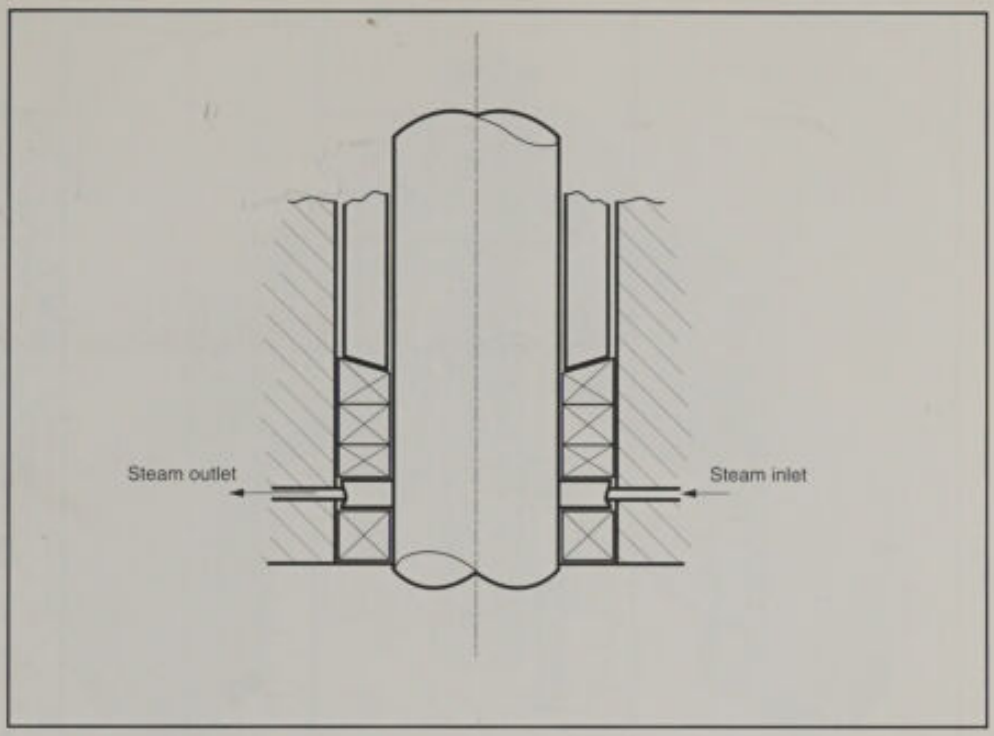
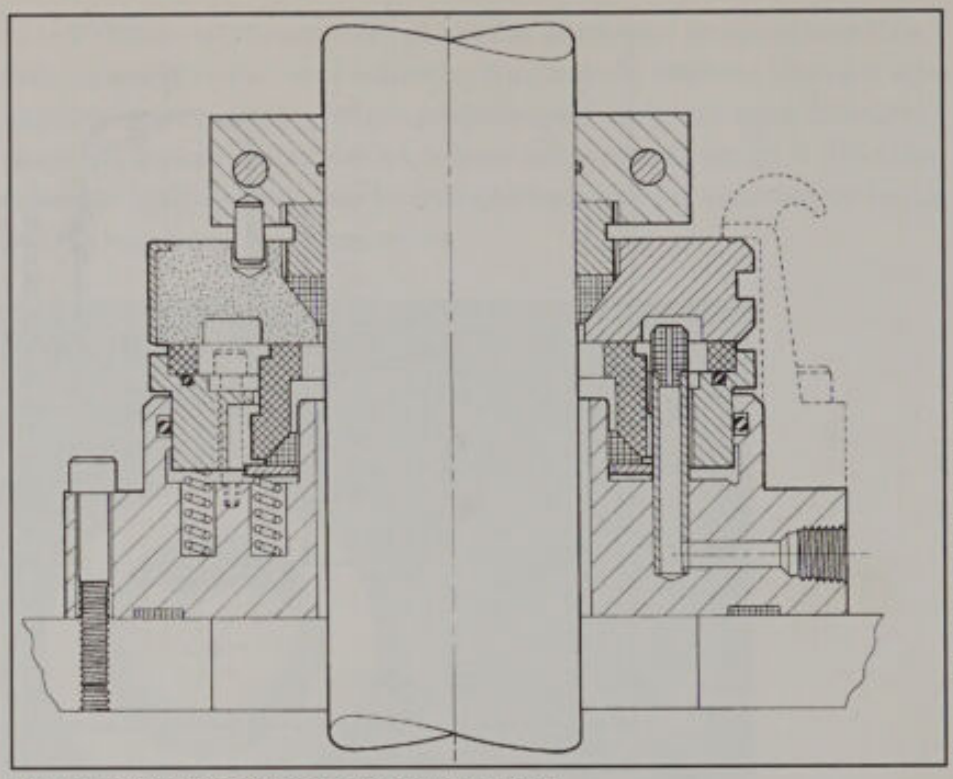


Figure 7 Stuffing box with steam barrier

97 Single-or double-face mechanical seals are suitable for fermenter agitator shafts working at Containment Level 2. These will minimise leakage and the escape of organisms. Although the legislation allows the use of single seals at Containment Level 1 and 2, engineering considerations concerning the life and effectiveness of the seal face may determine that a double seal represents a more practical solution. Single and double seals are available with either liquid or gas rubbing lubrication. The 'dry' contact seal faces, which are traditionally used, utilise carbon/graphite against a ceramic carbide, typically silicon carbide in Europe and tungsten carbide in the US. These seals really require the presence of some water vapour if they are to function in an optimum manner. Usually this need is met in the atmosphere inside a fermenter vessel but it becomes important in agitated downstream precessing vessels which may be blanketed with dry nitrogen or double seals which use a dry nitrogen barrier. Under these conditions fast deterioration of the seal faces may occur. Specialised materials are now available for contacting seals which use dry nitrogen services.

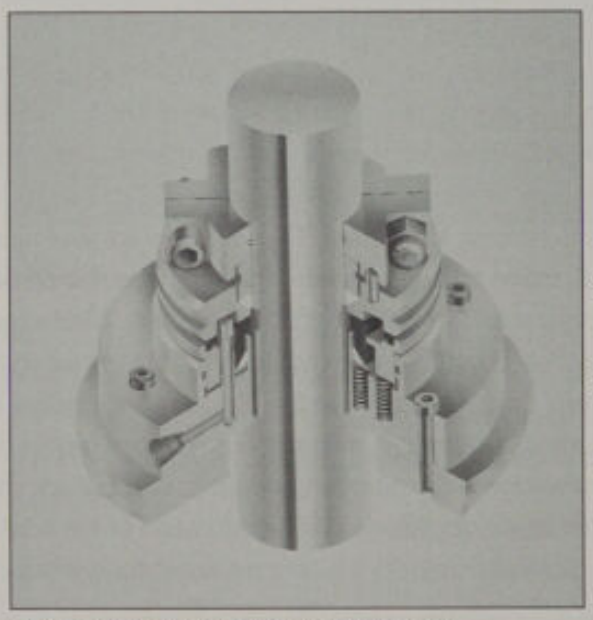
98 An innovation in the industry has been the development of double non-contacting seals with gas lubrication, which benefit in having no wear of the rubbing faces. Gas consumption levels are higher and the seal supplier will generally specify a minimum gas barrier pressure differential above the vessel pressure.

99 For Containment Levels 3 and 4 it is essential to use a double-face mechanical seal with either steam or sterile condensate under pressure, fed to the interspace. It is essential that the steam or condensate pressure be greater than the vessel pressure. Steam or condensate temperature should be high enough to sterilise the interspace in the seal. Condensate should be piped to kill tanks, or steam treated prior to discharge.



Source: John Crane Mechanical Seals

Figure 8 Cross-section of a mechanical seal



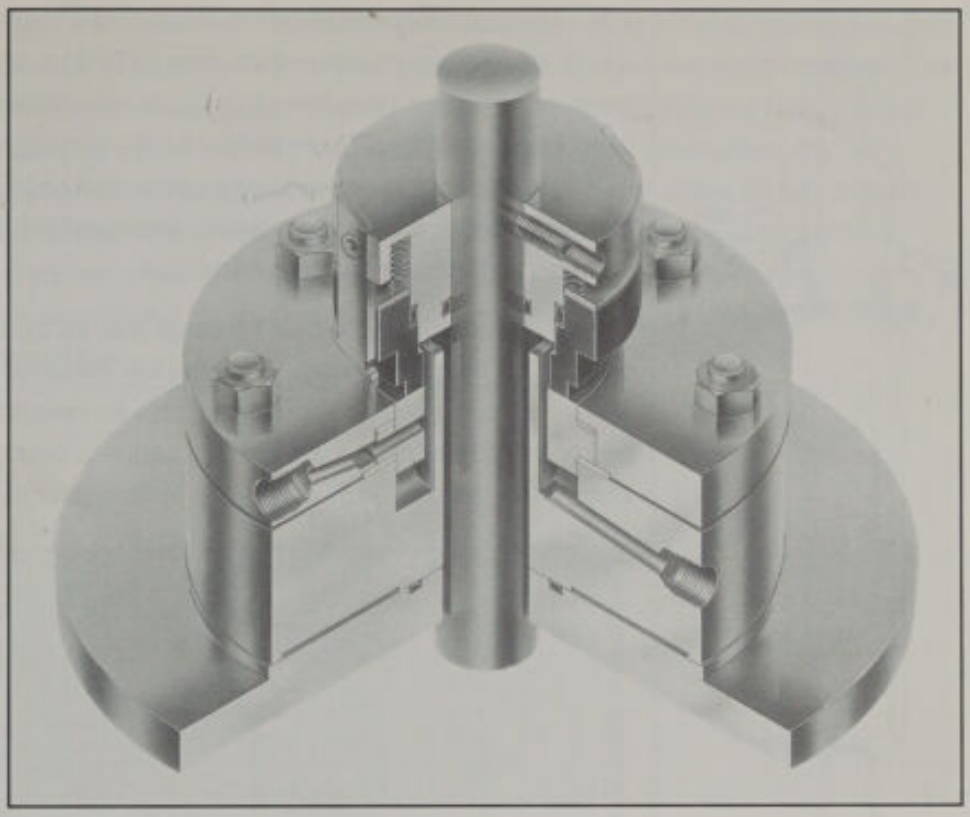
Source: John Crane Mechanical Seals

Cut-away view of a mechanical seal

100 One of the problems associated with the use of steam in double mechanical seals is that if the steam pressure fails, the seals may leak.

101 Condensate pressure should be continuously monitored and alarmed for Containment Level 3 and 4 applications.

102 Seal face changes should be the subject of a preventive maintenance programme. As wear takes place on the seal faces of a mechanical seal (an inevitable occurrence with gas-lubricated contact seals) wear debris is formed and would normally fall into the vessel below the seal. Seals are now available in which a ring is present to collect this debris and so prevent contamination of the process material.



Source: John Crane Mechanical Seals

Mechanical seal with debris well

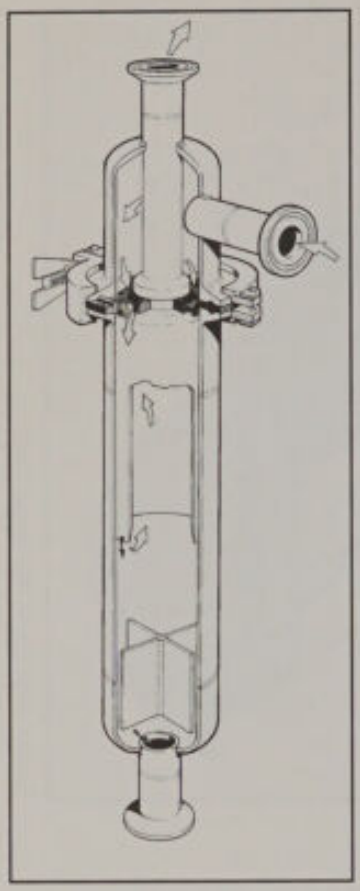
Inlet air treatment

103 Most aerobic fermentations require sterile air. This should be generated using an oil-free means and be filtered through a 0.22 µm cartridge filter. Some users at large scale achieve satisfactory results using beds packed with cotton wool or glass fibre to achieve sterilisation of the inlet air stream. This is an acceptable treatment for Hazard Group 2 organisms where it can be shown to be effective and appropriate. Packed beds should not be used in applications using organisms in Hazard Groups 3 or 4, which use sterilisable filter housings similar to those used for exhaust gases, the performance of which can be validated.

Exhaust air treatment

104 For Hazard Group 2 organisms, the legislative requirement is to minimise the release of viable organisms.

105 In practice, this is an area of considerable variation in standard. For example, in some applications commercial confidentiality is of the utmost importance and users prefer to prevent the release of organisms either by the use of a 0.22 µm filter system or by incineration. It should, however, be stressed that this is an optional, process-based decision. The minimisation required, ie acceptable levels of release, should be calculated from the risk base of the work being undertaken.

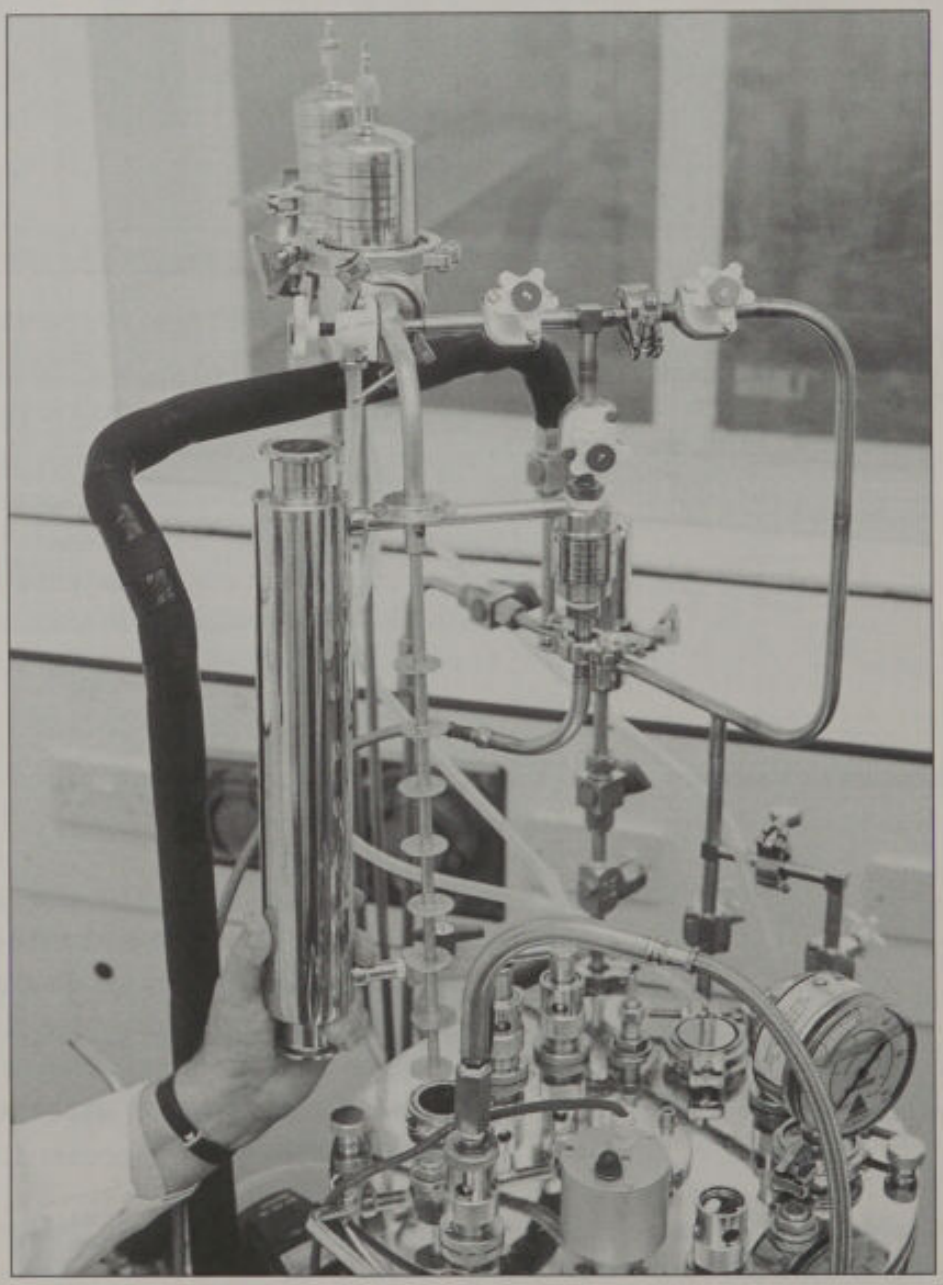


Source: Domnick Hunter Ltd

Figure 9
A centrifugal separator with tangential entry of air

106 Users would normally be recommended to use a centrifugal separator or a high efficiency particulate absorption (HEPA) grade cartridge filter but without the need to have a parallel installation to ensure complete sterility at times of filter changing. Where users decide to use cartridge filters, consideration should be given, depending on the foaming characteristics of the fermenter medium, to the use of cyclone separators and/or impingement filters as a pre-stage in order to preserve the integrity of the cartridges.

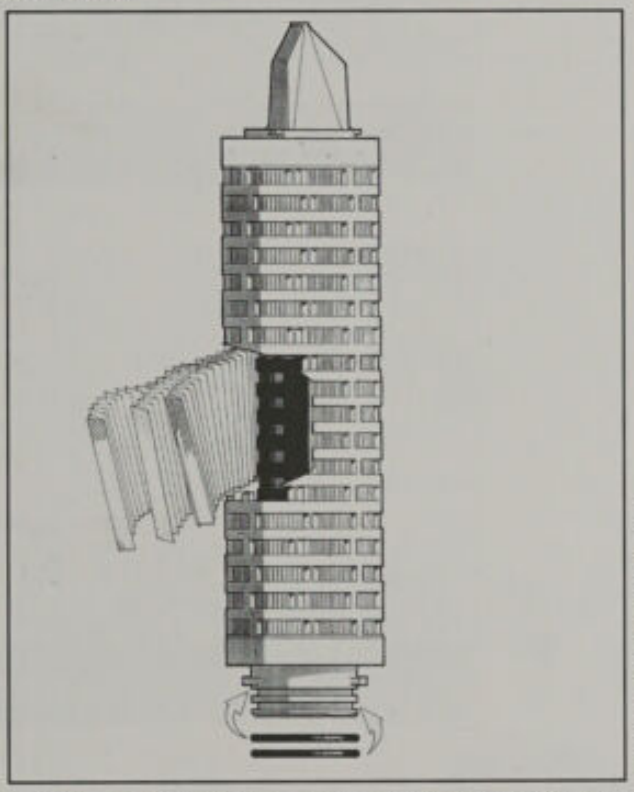
107 Alternatively, a pre-heating stage may be placed in the exhaust airline to ensure that any water vapour droplets are evaporated, thus only presenting the filter with the gaseous phase. It may also be considered good practice to install hydrophobic and hydrophilic filters in series in order to present an effective filtration surface under all conditions of fermenter air discharge.



Photographed at CAMR

Condenser/impingement separator in fermenter exhaust gas stream. The photograph shows the outer tube removed from the airline to illustrate the impingement discs which are mounted non-concentric to improve separation

108 For some work it is sufficient to pass fermenter exhaust gases through a cyclone separator. This may be followed by a spray tower where the gases will be in contact with a disinfectant hypochlorite or phenolic spray before being released. While this can, based on an adequate risk assessment, be acceptable for a large-scale installation, smaller-scale users should consider the overall cost of such a system compared with the use of cartridge filters which will provide at least the same reduction in organisms. Consideration must be given to the disposal of the disinfectant in accordance with appropriate legislation.

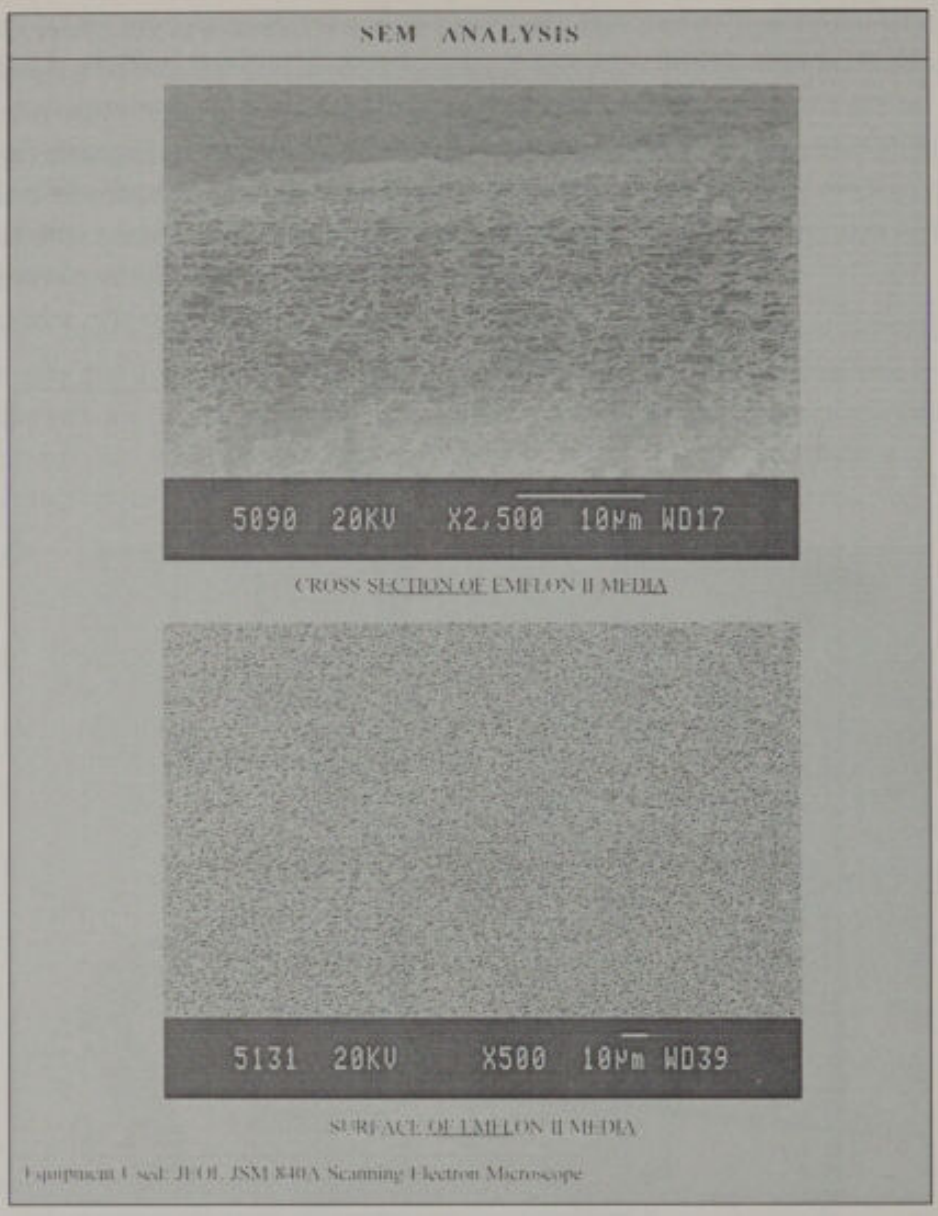


Source: Domnick Hunter Ltd

Figure 10 Construction of an exhaust gas filter element

Source: Pall Ultrafine Group

Cut away view of an air filter housing



Source: Pall Ultrafine Group

Scanning electron micrographs showing the cross-section x 2500 and the surface x 500 of exhaust gas filter medium

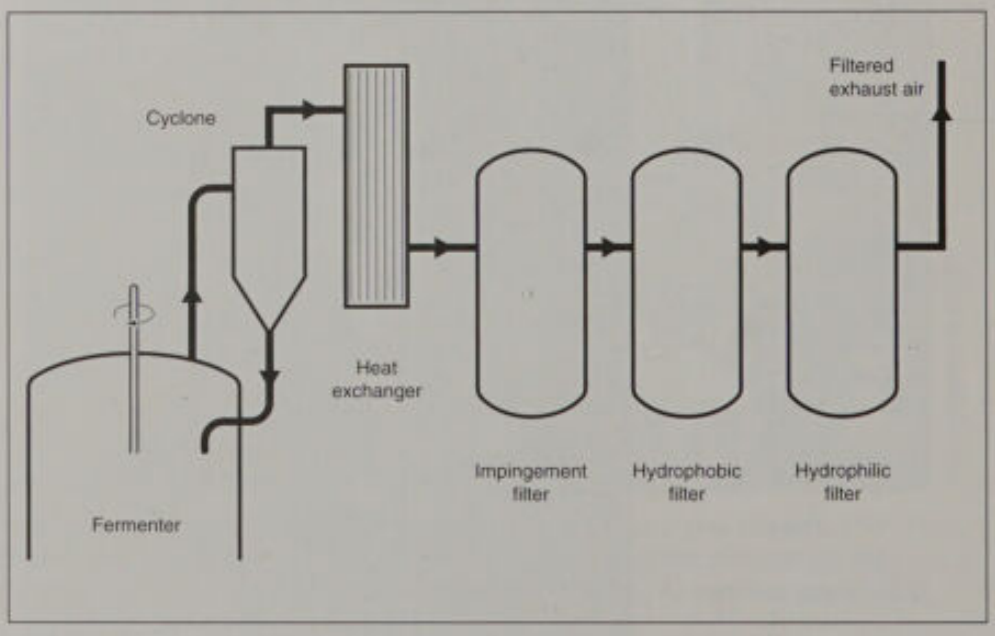
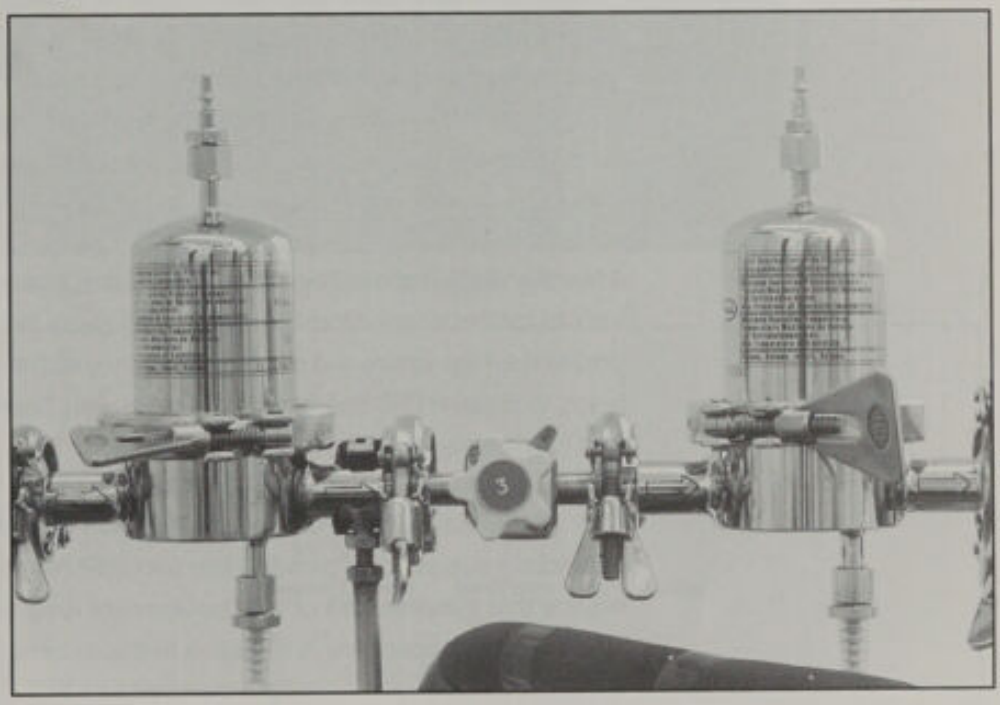


Figure 11 Typical arrangement of cyclone, heater and exhaust filters

109 At Containment Levels 3 and 4, the requirement is to treat exhaust gases so as to prevent escape. Prevention is normally achieved by HEPA-filtration cartridges in a parallel installation to ensure complete downstream sterility at all times. The specification of the cartridges must be suitable to provide complete retention of the smallest particles which may be generated in the fermentation process. For the safety of engineering personnel, the filter must be capable of being steam sterilised before being changed. The operation of the steam sterilisation process should be via a validated technique, as should the re-installation process of a new filter. At Containment Level 4, the use of two HEPA filters in series in each leg is required to reduce the risk of a breach of containment due to filter failure. Inlet/exhaust filter testing is a vital aspect of safety assurance.



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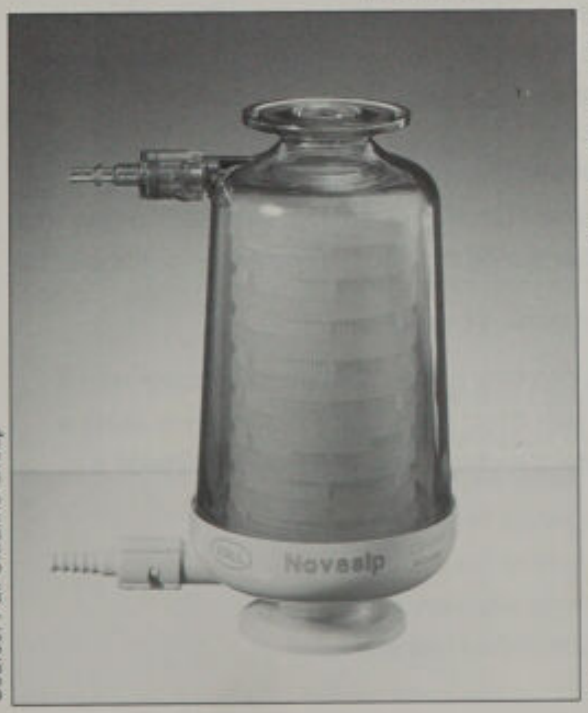
Two exhaust filters in series using triclover clamp fittings in a Containment Level 3 installation



Source: Pall Ultrafine Group

110 A new development consisting of a fully disposable filter assembly made from advanced polymers and which is steam sterilisable *in situ* is now available. This not only provides a lower cost installation than a traditional stainless steel housing but also enables the plant to be cleaned in place.

Sterilisable in situ *disposable filter*

Fermenter operating pressure

111 For reasons of process sterility it is normal practice to operate fermenters at a pressure which is positive to their immediate surroundings. Normally a differential of up to 0.5 bar is accepted practice. The pressure differential should be monitored and alarmed.

112 It is important to ensure that emergency venting arrangements installed to deal with accidental over-pressurisation, are suitably designed to contain emissions of viable micro-organisms. For applications at Containment Level 2, provisions are needed to minimise or prevent the escape of viable organisms into the workplace. In the cases of applications at Containment Levels 3 or 4, containment is of critical importance and equipment should be designed to resist any foreseeable over-pressure and must prevent loss of containment.

113 Particular attention should be directed to small fermenters where addition lines may be of plastic rather than stainless steel. These lines, which may contain acid or alkali for pH adjustment, could constitute a hazard if they or their connections were subjected to a positive pressure. In these cases fermentation should be carried out at, or as near as possible to, atmospheric conditions.

114 Similarly it should be remembered that it can sometimes be difficult to secure rubber connections firmly to small glass fermenters. A particular problem arises where the foaming tendency of the fermenter medium in use leads to small PTFE line filters becoming wet. This increases their resistance and can cause lines to blow off.

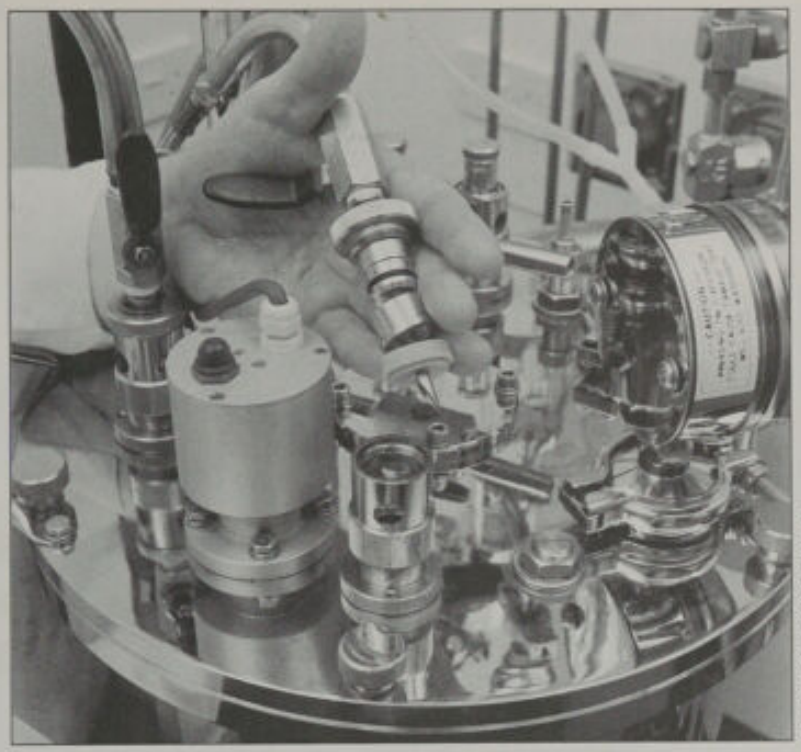
115 While it may be necessary to maintain fermenter pressure above the immediate surrounding atmosphere for GMP reasons, it is also necessary to ensure that the pressure of the containment area and its immediate surrounding atmosphere is negative to the external atmosphere.

Seed systems and inoculation

116 In order to achieve optimum biomass levels, single or double seed stages are universally used. These seed stages should be maintained sterile for process reasons and transfer to the fermenter should be through a validated system of flexible, pressure-rated tubing (for example, mesh-encased silicone tubing) or solid sterilisable stainless steel lines.

117 The process requirement should also satisfy the health and safety requirement to minimise (Containment Level 2) or prevent release of organisms (Containment Levels 3 and 4) either during the seed fermentation or transfer to the fermenter vessel.

118 Inoculation of seed vessels can take place using direct injection with a sterile needle/septum technique, with direct flaming of the septum or using a closed system with a stainless steel transfer vessel which is filled inside a microbiological safety cabinet.



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Component parts of a septum inoculation system

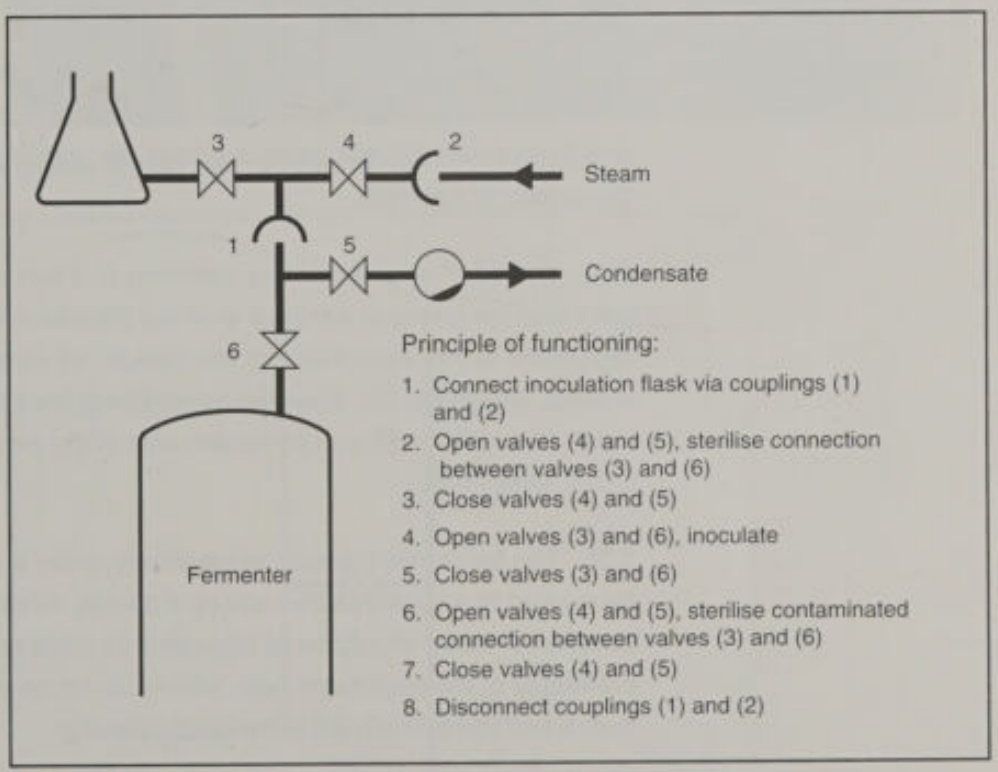
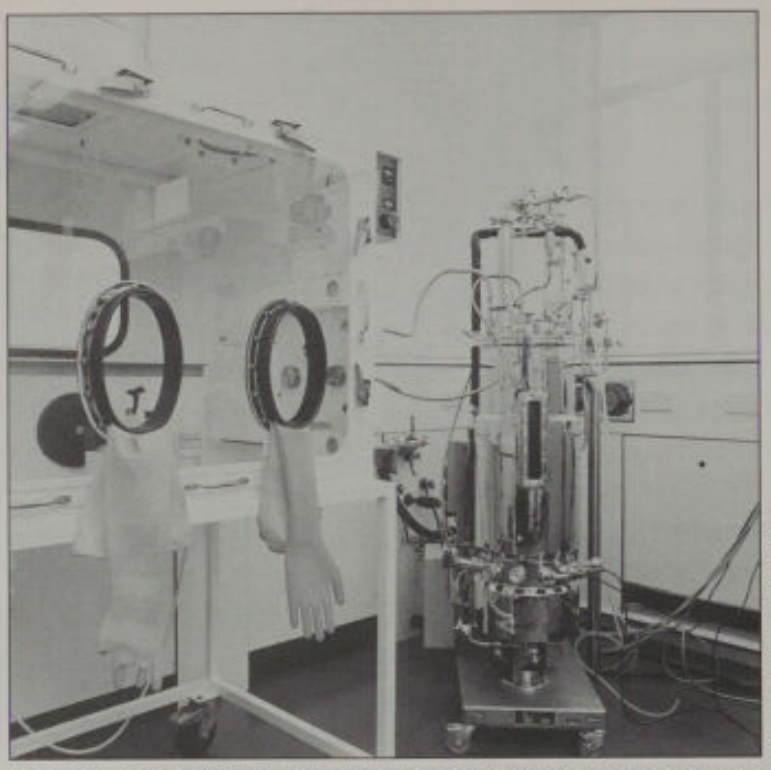


Figure 12 Diagrammatic layout of the inoculation procedure

119 In this case, the transfer inoculation container will be connected to the seed fermenter by screwed or quick release connections, the transfer pipework and valves steam sterilised and the contents transferred either by gravity or by the use of sterile air pressure. There is a need to re-sterilise connection lines before removal of inoculation container. Sterile needle/septum techniques are not normally used for applications at Containment Levels 3 and 4, but under certain well-controlled conditions they can be considered adequate.



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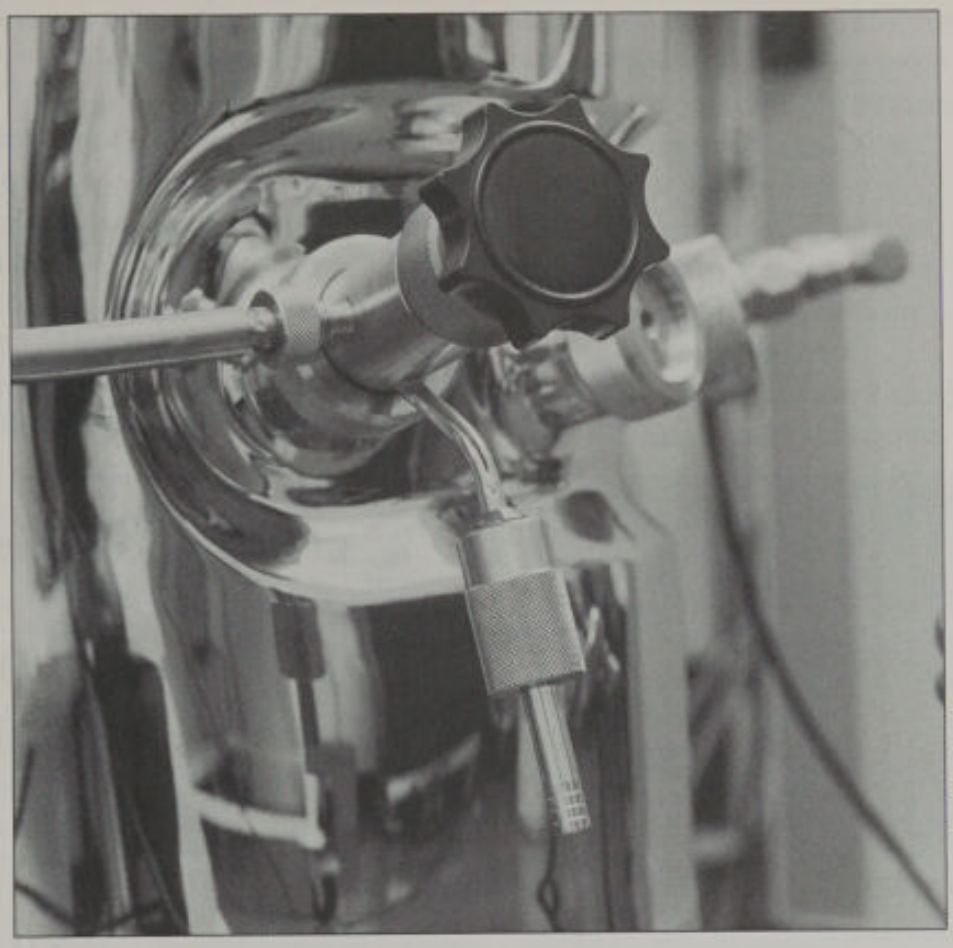
Inoculation of a fermenter using the septum technique operating at Containment Level 3

Sampling systems

120 For Containment Level 2 applications, sampling should be designed to minimise the unintentional release of organisms from the fermenter. Care should be taken that the receiving container is designed to control the generation of aerosols.

121 The GMP requirement for sampling of a fermenter is that the sample point and the taking of samples shall not introduce any contaminating organisms to the fermenter. For this reason, all sampling techniques must be aseptic. This is usually arranged by steaming the sampling connection when not in use, either on the downstream side of the sample valve or into the body of the valve itself.

122 The fermenter contents are normally under a positive pressure and can be ejected at a considerable velocity if a large valve is suddenly opened. The sample container should be of adequate size and the valve should be preferably of the diaphragm type, which can be opened gradually, rather than a ball valve which will allow rapid opening.



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Steam sealed sampling valve

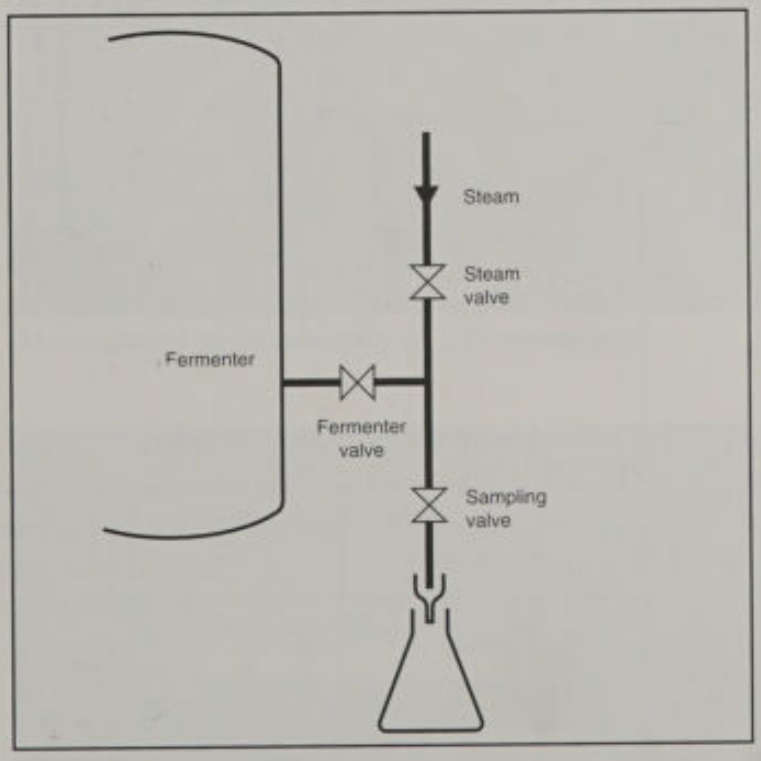


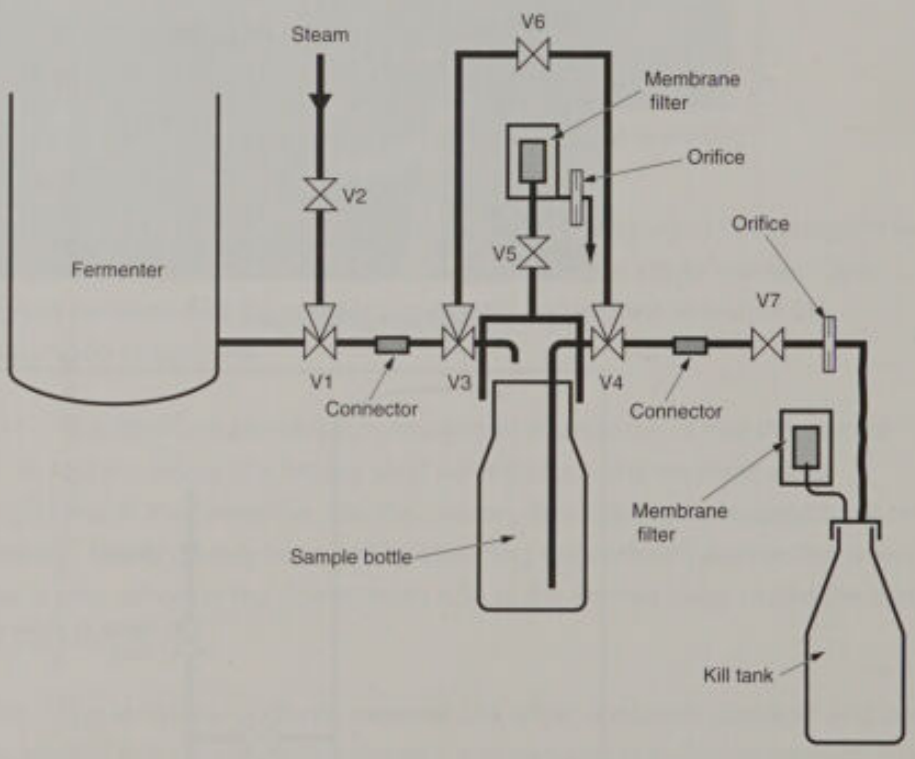
Figure 13 Valve combination for sampling

123 The choice of sample container depends on the type of sample being obtained. For example, a sterile sample requires a fully sterilisable closed container which can be attached to the sample point, the sample taken, then all connections re-steamed after closing the inlet valve to the sample container and finally detaching the sample container in a sterile manner. It may be

necessary to filter the displaced air from the container, depending on the risk assessment.

124 Samples of Hazard Groups 3 and 4 organisms, regardless of their intended use, must be taken in such a way as to prevent release of the organisms. For example, samples should be taken using a closed, aseptic technique and HEPA-filtering the displaced air from sample containers.

125 The use of localised containment should be considered as part of the regime of sample removal. The containment measures in the area of the sample point must correspond with the hazard classification of the organisms in the fermenter.



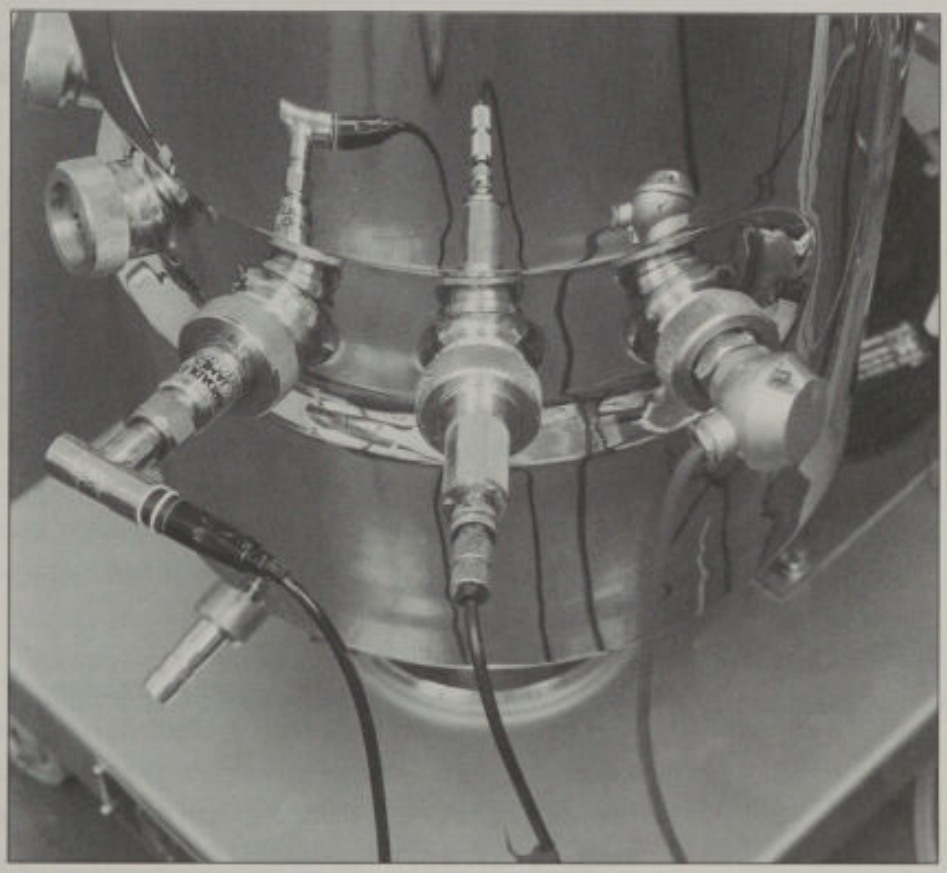
Valve positions for contained sampling

	Sterilisation of bottle	Sampling	Sterilisation after sampling	Disconnection of bottle
V1	closed	open	closed	closed
V2	open	closed	open	closed
V3	open	open	closed	closed
V4	open	closed	closed	closed
V5	open	open	open	closed
V6	closed	closed	open	closed
V7	open	open	open	closed

Figure 14 Layout of valves and valve sequencing for contained sampling

Measurement probes

126 Probes inserted into fermenter systems are normally of two types, those that are fixed, such as temperature or pressure transducers, or those which are retractable, such as those for measuring dissolved oxygen and pH, the design of which allows their replacement during a fermentation. While this latter practice has considerable merit from the viewpoint of the technical management of the fermenter, it is only acceptable in the case of applications at Containment Levels 1 and 2 and should not take place in applications at higher containment levels. Normally, synthetic elastomer O-ring seals are used in these applications. Double O-rings are sometimes considered for applications at Containment Levels 3 and 4, but the general comment about the use of O-rings, which is made in the section on couplings, also applies here.



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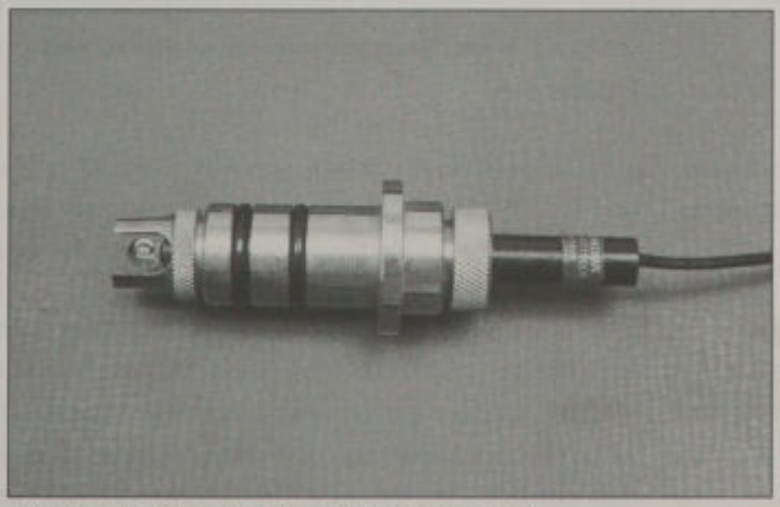
Measurement probe assembly on a fermenter wall



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pH electrode for insertion in fermenter wall

127 For high-risk fermentations, duplicate transducers represent a safe procedure when there is doubt about the reliability of the probe under process conditions.

Addition systems

128 Addition systems fall into two categories:

- those which are used for pH adjustment and are normally self-sterilising, for example by the use of concentrated acids or ammonia;
- antifoam and nutrient additions which normally require sterilisation of the lines and connections and the addition materials.

129 All additions to the fermenter must be sterile for GMP purposes and the addition system should be designed to minimise release at Containment Level 2 and prevent release of any organisms from the fermenter at Containment Levels 3 and 4.

130 Cartridge filter installations used for critical applications such as addition systems or product sterilisation present particular difficulties when filter replacement is required. This is made more difficult when the demands for cleaning and cleaning validation are taken into account. Such filters are typically polymeric and are installed in a stainless steel housing to enable them to be steam sterilised *in situ*. Filters must be changed after each batch is processed and the housings cleaned ready for re-use. Filter housings do not readily lend themselves to be cleaned 'in place' because of their geometry. Traditionally, housings have been removed and cleaned separately from the main plant.

131 Fully disposable filter capsules consisting of a filter permanently bonded into a plastic casing have been available for some time but, although they can be autoclaved or in some cases gamma-sterilised, they cannot be steam-sterilised in place and therefore must be installed aseptically. This type of filter removes the need for cleaning of the filter housing and makes containment easier to manage but the need to sterilise off-line creates other difficulties.

132 Note should be taken of the fully disposable filters which may be steam-sterilised *in situ*. These are also available for use in liquid sterilisation applications.

Downstream processing

133 In defining the criteria and requirements for Containment Levels 2, 3 and 4, COSHH specifies that bulk culture fluids should not be removed from the closed system unless the viable organisms have been inactivated.

134 This requirement has been the subject of considerable variation in interpretation. Some users consider that the closed system only includes the fermenter. There are some applications where to kill the organisms within the fermenter would damage or destroy the product or the viable organisms which may constitute the product. The requirement can be better understood if the closed system is extended to include the preliminary stages of downstream processing. This could include, as an example, centrifugation in a solid-liquid

separator, cell rupture in an homogeniser and re-centrifugation in a second solid-liquid stage centrifuge.

135 In those cases where it is desirable to retain viable organisms or if the product itself is toxic, it is necessary to maintain correct containment at all times. The important factor is the removal of the hazard before there is loss of containment. The method of hazard removal depends on the product and whether this is contained within the cells or in the liquid components of the downstream processing stage.

136 A 0.22 µm hydrophilic filter may be regarded as the outer barrier of the contained system. Alternatively, if acceptable from the process viewpoint, a chemical or heat-kill method of inactivation may be used. In this case, the materials may be inactivated either by return to the fermentation vessel or by processing in a kill tank, which must also be regarded as within the contained system. Whichever method is used, validation of the system will be required.

137 If they are part of the containment system, desludging centrifuges must be adapted to minimise release at Containment Level 2. At Containment Levels 3 or 4 they should prevent release. The vents from such machines, which should be totally enclosed, should be exhausted through HEPA filters.

138 Most industrial homogenisers are, in normal operation, completely closed systems. They operate at high pressure and should a fault develop, which results in a leak, they become a source of aerosols. Under these conditions, viable organisms or protein fragments, which although they may not be biologically viable can be potent allergens, may be released into the atmosphere. Homogenisers therefore need to be located in a flexible film isolator, safety cabinet or equivalent enclosure which is vented through a HEPA filter.

139 It is important to consider the pressures developed in the pipework configuration of a homogeniser installation. Care should be taken to ensure that any joints, seals and couplings in high-pressure lines are suitable for their duty. Many of the couplings and seals normally used in biotechnology applications are not intended to be used at the pressures developed in parts of homogeniser installations.

SECONDARY CONTAINMENT - PROCESS ROOMS AND BUILDINGS (CONTAINED AREAS)

Separation of organisms from the environment

140 Biotechnology processes may be divided into two classes, those where the fermentation equipment is closed to prevent the leakage of organisms or the ingress of contamination and those where the fermentation is open to the external air. This guidance is only concerned with processes which use closed and contained equipment.

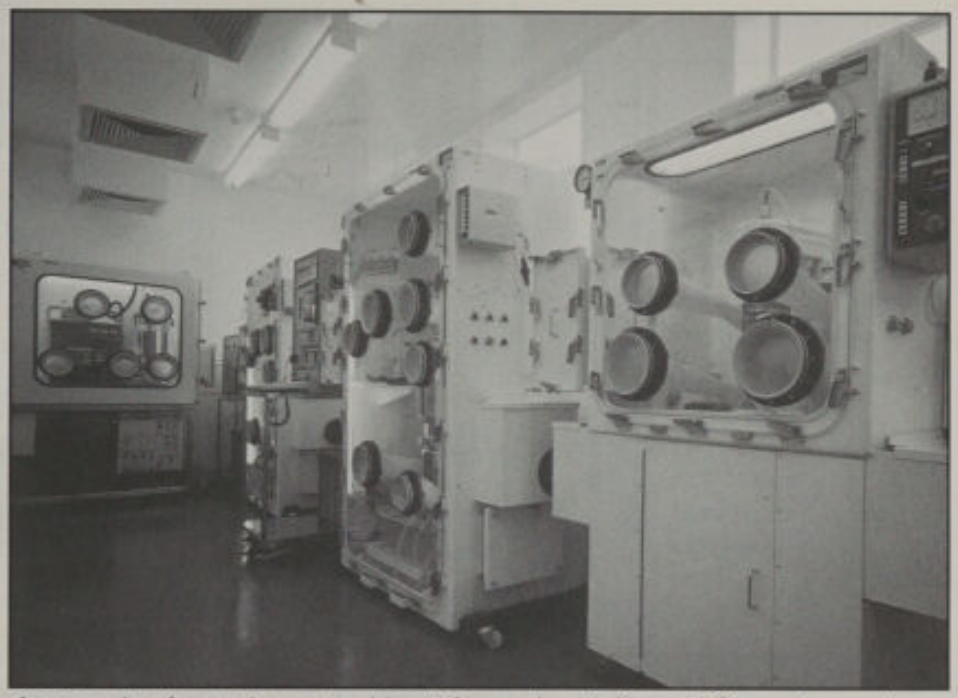
141 All processes should be carried out in equipment which is designed to minimise, in the case of Containment Level 2, or prevent, in the case of Containment Levels 3 and 4, the release of organisms. The system should be such that the fermentation equipment acts as the primary containment and that in addition the equipment is housed inside a closed building - a contained area, which may or may not be equipped with ventilation and air filtration, depending on the hazard arising from the agent being used.

142 Such a building may be the only one on a site, or alternatively it may be a designated building within an existing site where other compatible work is carried out. A contained area may also be a separate part of an existing building, within which other industrial processes are carried out. In this third case particular care should be exercised to ensure that it is not possible, in the event of an accident, to contaminate another part of the building which is not equipped to deal with the agent being used.

Photographed at CAMR

143 Ideally, the air inside the process buildings would be expected to be completely free from contamination by any process biological agent as it would be retained by the primary fermentation containment system. Care must be exercised in the design and positioning of both inlet and exhaust air stacks to ensure that exhaust air cannot be drawn into the ventilation inlets of the contained area or another installation.

Fermenter installed in a contained cabinet for work with Hazard Group 3 agents



Photographed at CAMR

A cascade of containment cabinets for work with Hazard Group 4 agents

Restricted access

144 It is important that access to work areas processing Hazard Groups 2, 3 and 4 agents should, for health and safety reasons, be restricted to those workers who have specific work to do in the area and who have received training in the operation, engineering and procedures to adopt when in the area.

145 GMP considerations require restricted access to all installations involving biotechnology. Such access should be limited to those persons who have a legitimate reason for being in the area, such as management, process personnel, engineers and approved visitors.

146 Primary restriction by means of entry to a production site through the normal factory entrance is not sufficient because of the presence of unauthorised people who are not aware of the precise nature of the work. It is recommended that entry is restricted to only those people who have direct business by use of a swipe card, card key or digital lock entry system or equivalent security access system. The rigour with which access is restricted will increase with increasing containment level. Where access is restricted, signs should be displayed on all entrances. At Containment Levels 3 and 4 it is often appropriate to have a signing in and out system in operation, to log facility usage.

Biohazard signs

147 Biohazard signs must be displayed on the external sides of all doorways of buildings handling Hazard Group 3 or 4 agents (optional for Hazard Group 2). The approved design appears in Schedule 9 of COSHH.

Internal room operating pressure

148 For rooms handling agents in Hazard Group 2, there is no specific need to operate process rooms at negative pressure. Although there is no requirement, many users choose to operate such areas at negative pressure. It is also accepted that the demands of GMP often require that rooms are under positive pressure to minimise/eliminate cross contamination.

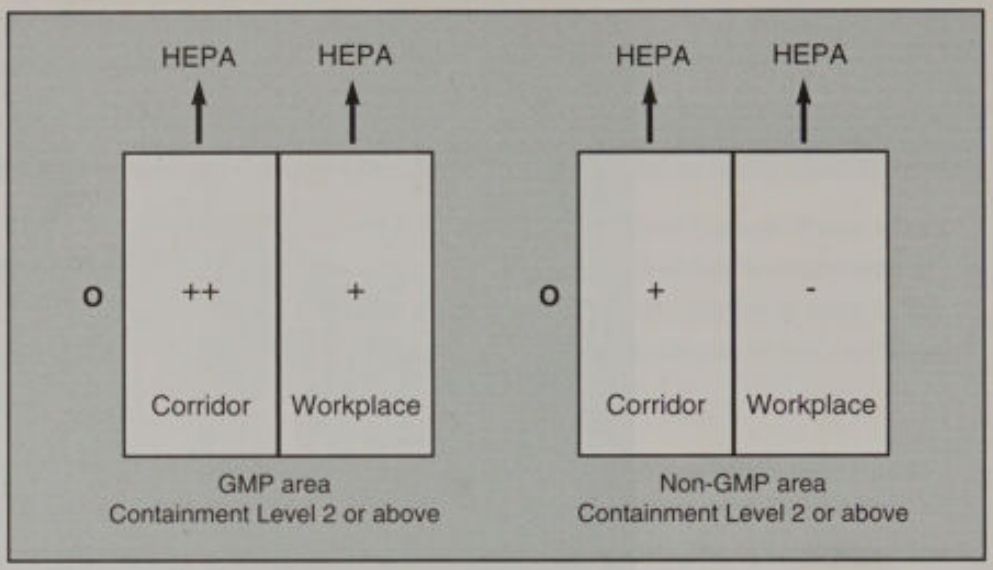


Figure 15 Air pressure requirements for GMP and non-GMP areas

149 In order to balance both the health and safety and GMP requirements of processes using Hazard Group 2 agents, consideration can be given to having a negative pressure cascade system of the order of 30 Pa, 60 Pa and 90 Pa relative to the outside atmosphere. The minimum recommended differential between rooms is 15 Pa (1 Bar = 100 000 Pa).

150 It is necessary under these conditions to have a balanced air flow using dampers and alarms to counteract the effects of normal events such as opening doors, as well as abnormal events such as a room leak or system failure.

151 Alternatively, consideration can be given to the use of a corridor or lobby with a HEPA-filtered positive air flow. The air pressure in the lobby should be positive to both the plant side to prevent or minimise escape of airborne organisms, as required by the Containment Level in question, and also to the outside to prevent or minimise contamination.

152 In such a system the corridor would be at positive pressure to the processing area, through a cascade, consisting of first the changing lobby, then the containment area and thirdly any contained equipment. The fermenter would be at positive pressure to the contained area, but negative to the outside atmosphere.

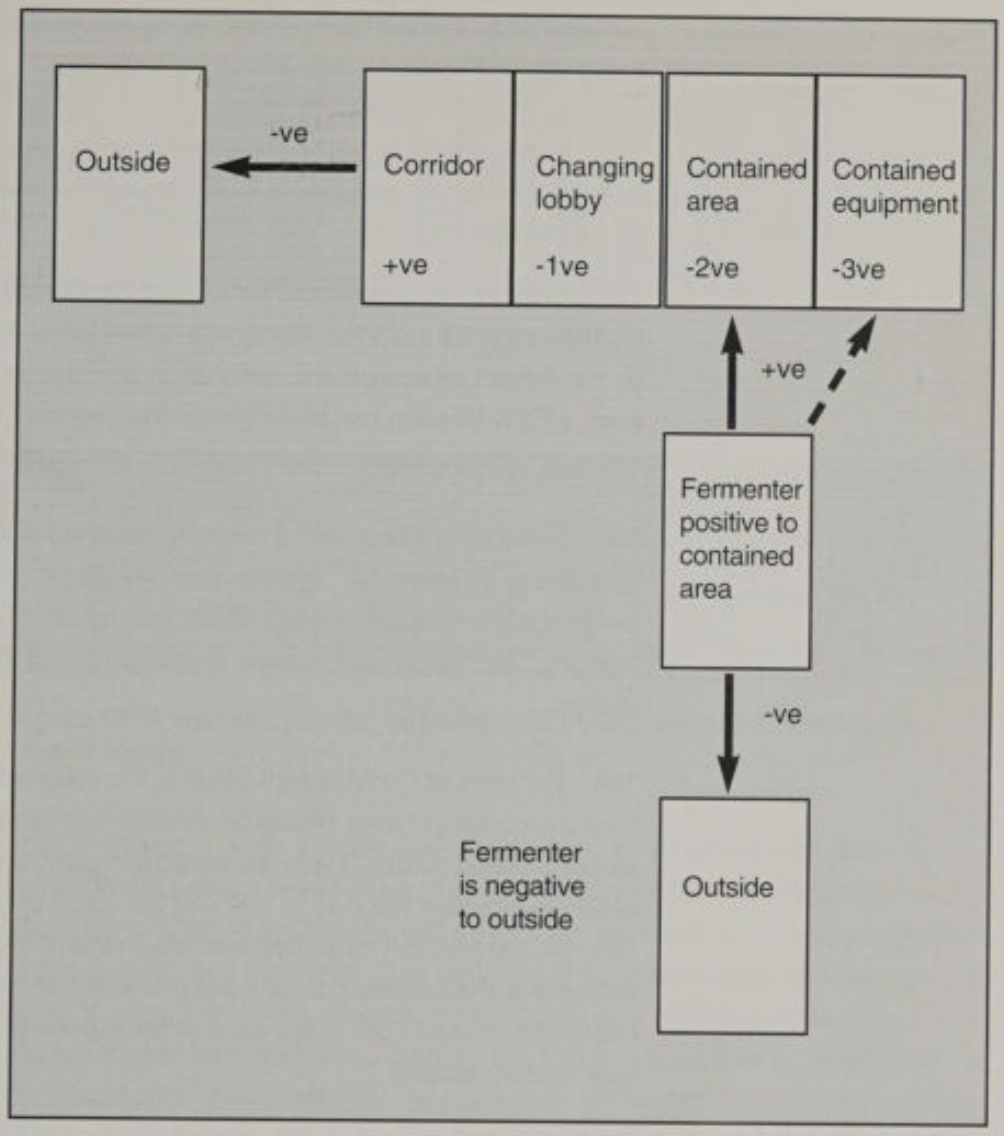


Figure 16 Typical air pressure cascade

153 The plant would still require secondary containment cabinets for any aerosol generating activities. It is important to use HEPA-filtered air for this application to prevent contaminated air from being drawn in.

154 For activities that are carried out in laboratory settings, users should refer to the more detailed advice on Containment Level 3 and 4 laboratories and the use of microbiological safety cabinets in the ACDP guidance.[1]

Ventilation and room air filtration

155 The room air in an area operating at Containment Levels 2, 3 and 4 should be free from contamination by the biological agents which will be retained within the primary containment. In the case of operations at Containment Level 2 there is no requirement for mechanical ventilation and therefore only a need to ventilate where necessary for comfort of operating personnel. Ten air changes/hour is a recommended circulation rate, although twenty changes per hour may be required for GMP operations. There is no specific requirement to filter the exhaust air from a Containment Level 2 facility. If HEPA filters are installed in the outlet or in the inlet and outlet for GMP reasons then it is recommended that to conserve filter life a coarse filter should be installed before the HEPA filters. Supply and extract air handling units should be interlocked to prevent over-pressurisation and release of possible

contaminants. Extract filters should be of safe design if the area is not fumigable or if routine fumigation is not proposed.

156 In the case of process areas designed to operate at Containment Levels 3 and 4, ventilation of the room space should be provided at between 10 and 25 air changes/hour.

157 In these circumstances, for work at Containment Levels 3 and 4, HEPA-filtration must be provided. For Containment Level 3 such filtration is mandatory on the extract air stream and optional on the inlet air. For Containment Level 4 work, HEPA-filtration can be single for the inlet side but must be double for the outlet air stream. The ventilation systems should be alarmed and indicated.

158 Fixed speed fans with a properly designed and reliable fail-safe alarm system can be used, but consideration should be given to the use of dynamically controlled variable speed fan motors to compensate for filter clogging. Alarms on manometers should be linked to indicate at the control station.

159 For work at Containment Level 4, it is recommended that the air exhaust from the contained area should be through twin fans, each driven by two variable-speed motors. Each fan would normally run at 50% load, but with the option to increase the load to 100% in the event of failure of one fan. If both fans fail, the inlet fan must stop and any extraction should be via the safety cabinets or the primary containment unit which should be sized to remove between 50% and 70% of the air in order to prevent over-pressurisation and loss of containment.

Entrance, exit and changing areas

160 Apart from the correct design of the process area and all of its service requirements, it is also necessary to ensure that the correct facilities are designed for the entry and exit of personnel, materials, samples, equipment and waste. Correctly sequenced changing facilities must also be provided. No air locks are required for Containment Level 2 facilities, unless this is for GMP purposes. The design of a changing area for Containment Level 2 should follow the advice on internal room operating pressure.

161 Occasionally it may be necessary to use personal protective equipment such as positive pressure air suits. This method of working creates additional requirements within the exit side of the changing area. The air suit must be decontaminated before it is removed. One of the specific requirements relates to airline couplings. It is essential that these can be cleaned without contamination and thereafter protected in preparation for future use. With a system such as this, discarded work clothes would be removed from the area via the autoclave.

162 Before commencing any work at Containment Levels 3 or 4, in which full containment at source is not designed into the system of work, the application must be discussed thoroughly and agreed with the regulatory authority.

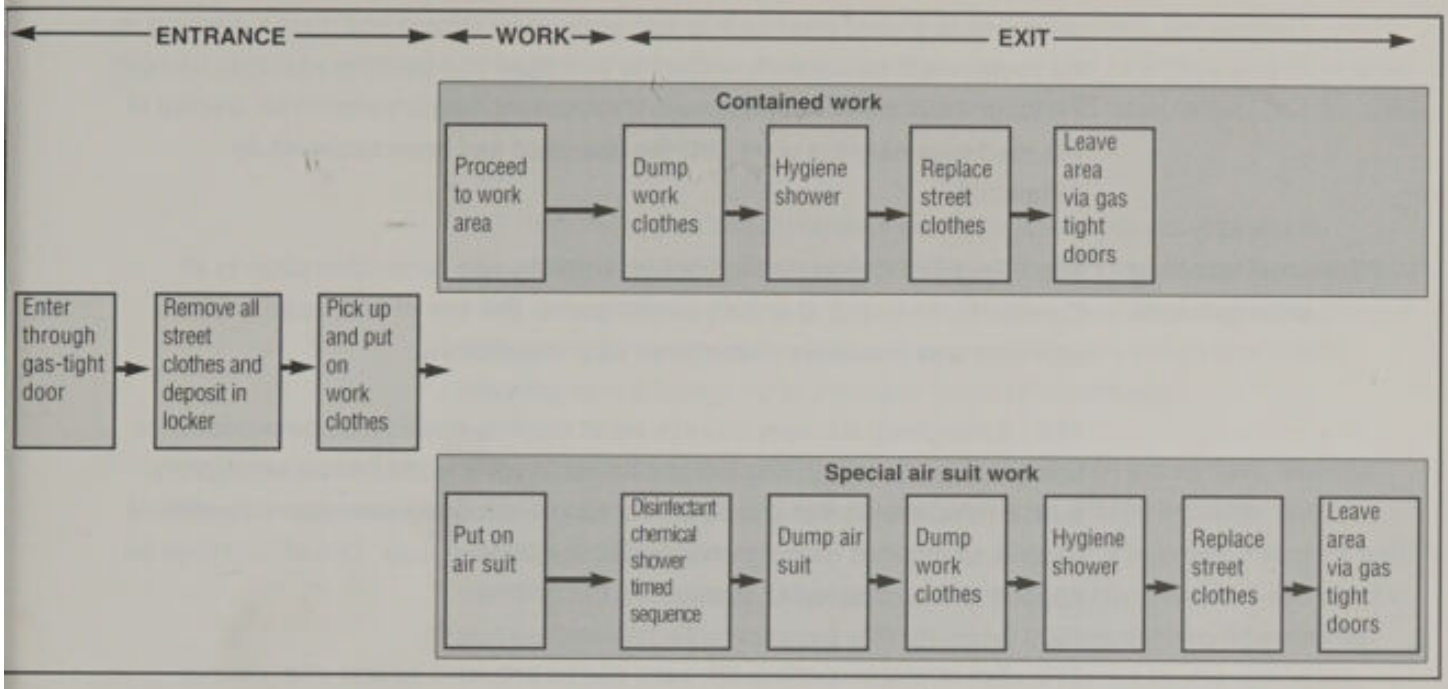


Figure 17 A typical layout for sequencing entry and exit of a Containment Level 4 facility

163 Although it is not a regulatory requirement, air locks are often fitted in Containment Level 3 installations. It is also recommended that installations designed to operate at Containment Level 4 should have a separate air lock for goods inwards and outwards. This would be fitted at both ends with gas-tight doors to allow fumigation. The exit route should also contain a dunk tank (double-ended tank containing disinfectant, into which an item is completely immersed prior to removal from the other side) which would enable the outside of sample containers, etc, to be disinfected before exit .

Fumigation

164 The need for fumigation depends on the particular type of organism being used. No general rule can be established. The need will be assessed on a case-by-case basis. As a general rule, the provision of fumigation capability is not required for Containment Level 2 applications. With Containment Level 3 applications fumigation is optional, but it is normal practice and would be expected unless there are good reasons against. Fumigation is a requirement for applications involving Containment Level 4.

165 More detailed advice on the application of fumigation techniques can be found in the ACDP publication *Categorisation of biological agents according to hazard and categories of containment.*[1]

166 Users are reminded of the toxic dangers of using formaldehyde as a fumigant. Rooms fumigated using this material should be carefully exhausted to the atmosphere and tests should be carried out before re-entry to ensure that the level of formaldehyde is below the maximum exposure level (MEL) of 2 ppm.

167 Following a fumigation operation consideration should be given to the use of permanganate filtration of the exhaust air which can reduce the formaldehyde level to 15 ppm before emission to atmosphere. Workers carrying

out the tests should wear appropriate protective clothing and masks. Venting of the rooms must be carefully controlled to ensure that persons who may be near the installation are not endangered. If necessary, barriers should be erected in site roadways near the plant until the operation has been successfully completed.

Personnel washing arrangements

168 Facilities should be provided for washing and decontamination in all Containment Level 2, 3 and 4 process areas. The use of foot, knee or elbow operated taps is recommended in all such installations.

169 Emergency showers and eye wash stations should also be provided for use in an accident involving the contamination of a worker. In an emergency such as this, the worker should call for help in decontamination and should remove all affected clothing while inside the working area. This should then be bagged and autoclaved or incinerated.

170 For operator comfort, showers can be provided for use after normal operations. The requirement for showers as part of the safety and hygiene programme is only mandatory when working at Containment Level 4, though optional at Containment Level 3.

171 The effluents from the sinks and showers must be collected and inactivated before being sent to drain in Containment Level 4 facilities, and preferable for Containment Level 3.

Spillage containment

172 While there is no specific requirement for an installation operating with agents in Hazard Group 2 to be designed to contain the entire contents of a fermenter in the event of catastrophic failure, it is considered good practice. If incorporated into the initial building design, this requirement can often be satisfied at little or no additional cost.

173 Containment may be arranged in several ways, either as a bunded area below the fermenter, or as enlarged drain channels under the operating floor or by drainage to a kill tank.

174 In all cases it is important that not only can the spilled contents be satisfactorily inactivated by chemical or heat treatment, but that it is also not possible to move the spillage from the containment area without the deliberate performance of an action designed to do so, for example the starting of a pump. Lines which drain from the bottom of the containment area should be avoided as the isolation valves may be inadvertently left open in which case spillage could be discharged to an effluent system without treatment. Good practice would be to empty the spillage containment area by pumping from above.

175 Besides gross spillage, provision also should be made for minor spillages resulting from, for example, the breakage of glass equipment, an operating error or leakage from a pipe flange or a valve.

176 In the event of a spillage or breach of containment, the normal arrangement should be to evacuate the area and for operators only to re-enter when any aerosols which may have been generated have settled and the area has been fumigated.

177 Sealed drains should be provided for the subsequent disposal of decontaminated spills. For installations operating at Containment Levels 3 and 4 this is a necessity. Safeguards in such facilities must be designed to prevent effluent contaminated with viable organisms in Hazard Group 3 or 4 from reaching land drains or to foul sewers under all conditions.

Materials used in building construction

178 In recent years, building finishes in fermentation plants have become more standardised, particularly in small plants. In a large industrial plant operating at Containment Level 2, concrete floors and standard industrial wall cladding are acceptable. Floors are usually of concrete, often of a special non-porous type and either sprayed with an epoxy coating or covered in welded vinyl. In either case the coating should be radius-curved up the wall some 300 mm to give a continuous corner effect.

179 Walls and ceilings are either epoxy or glass fibre/epoxy sprayed, or of welded vinyl or resin-bonded fibre. Welded vinyl may be used as a wall 'covering' in some installations but is not suitable for negative pressure operations where the pressure differential may cause the vinyl to lift from the support surface.

180 Particular attention should be paid to sealing plates where pipes enter and leave the plant.

181 The floor, wall and ceiling surfaces for Containment Level 3 should be the same as for Containment Level 2, except that the use of industrial cladding is not acceptable because of the difficulty of achieving containment with this material.

182 It is recommended that for installations operating at Containment Levels 3 or 4, the ceilings should consist of two layers arranged so that the centres of the lower sheets are over the joints between the sheets of the upper layer. This arrangement will reduce the possibility of leakage through the joints.

183 Light fittings should be splash-proof and in conformity with BS EN 60529 where possible.[8] Consideration should be given to access for maintenance purposes from above the contained area, particularly in the case of facilities for work at Containment Levels 3 and 4.

184 Floors, walls, ceilings, doors, windows and fixtures should be flush-fitting to allow easy cleaning. No ledges should be permitted. Pipework should be enclosed as much as possible. All corners and angles should be radiused for easy cleaning.

PERSONAL PROTECTION

185 Correct personal protective clothing and equipment must be provided for all personnel who enter an installation designed for operation with Hazard Groups 2, 3 or 4 agents.

Lockers

186 All operatives should be issued with a personal locker in which to store personal clothing. For Hazard Group 2, either a separate locker should be provided for work clothing which is used on more than one occasion or adequate individual storage space should be provided external to the contained area for the storage of work clothes.

Overalls, gowns and hats

187 A single-piece overall or two-piece suit consisting of jacket and trousers, and a hat, should be provided on an individual issue basis for each operator. Provision must be made for the safe storage of these clothes either adjacent to the entrance of the area or in a second locker specially provided for this purpose. Reference should be made to the Approved Code of Practice for the COSHH Regulations 1994, Schedule 9, Part I, paragraph 9, which gives specific information about requirements for storage, cleaning and segregation of clean clothing and equipment.[7]

Frequency of change

188 Frequency of change of protective clothing varies with the nature of the work and the rate at which the clothing becomes soiled in normal use. GMP requirements will also considerably influence the frequency of changes.

189 Change frequency should be more than once a week and once a day is preferred. For work at Containment Level 3, soiled clothing should be autoclaved after use and prior to washing.

190 For work at Containment Level 4, all personnel should remove all street clothing, including underclothes, and be provided with all the necessary work clothes. For these operations, a change of clothing should be provided on each entry into the area.

191 Work involving micro-organisms in Hazard Group 4 requires that after use the clothing is autoclaved before removal from the containment area and then incinerated.

192 Where clothing is known to have been contaminated, special care must be taken to ensure that it is placed in a sealed container prior to autoclaving and incineration.

Personal protective equipment (PPE)

193 While PPE should not normally be used as an alternative to rendering a work system inherently safe by eliminating the hazard, it may be required to protect workers from risks not associated with exposure to the organism. Consideration should be given to the personal issue of the following items of PPE:

- gloves;
- goggles, safety spectacles, visors and helmets;
- safety shoes;
- safety hat.

194 The need for specific items of PPE will vary with the nature of the work. Its selection should form part of the risk assessment under COSHH.

Smoking, eating, drinking, cosmetics and medicines

195 No eating, drinking, smoking or application of cosmetics should be allowed in any workplace which processes biological agents or in the downstream processing plant associated with such work. Rest areas should be specifically provided for such purposes. If the site rules permit smoking on site a special separated area should be provided for such purpose. Medicines for personal use should not be brought into the work areas.

Respiratory protective equipment (RPE)

196 At Containment Level 2, RPE will not normally be needed unless risk assessment shows that it is necessary. Under these conditions, RPE will need to be available in case of a breach of containment caused by a leak, spillage or escape of culture, to help in dealing with the emergency and subsequent clean up. In routine fermentation operations the use of RPE will not normally be required, since exposure to biological agents should be adequately controlled at source by the use of totally contained plant or local exhaust ventilation such as microbiological safety cabinets or isolators.

197 There could well be a requirement for the use of RPE in routine activities other than fermentation, such as when dispensing materials used in making culture media, handling dry active materials derived from downstream processing or in animal work. Working practices and measures which control exposure should preferably be used but may not always be reasonably practicable.

198 If formaldehyde fumigations are performed there may be a need for suitable RPE to be kept available in case of emergency, although systems and procedures which eliminate the need to enter a hazardous atmosphere should be used.

199 For operations involving the cleaning up of spills of culture fluids at Containment Level 2 or above, a greater level of protection will be required than can be offered by disposable masks or negative pressure RPE. It is likely that sufficient protection will only be provided by a powered positive pressure respirator or independent air supply device. The options available are:

- hoods;
- blouses;
- full face-piece masks;
- full suit.

200 Nominal protection factors (NPFs) in the range 500 to 2000 should be aimed at, having regard to the nature of the biological agent being handled.

201 The RPE chosen can either be a powered respirator, which will probably have a belt-mounted assembly consisting of a battery-powered pump and filters supplying purified air to the face via a hose. Alternatively, the respirator face piece may have an independent pure air supply from a compressed air cylinder or airline.

202 Respirators fitted with particulate filters are not suitable for use in atmospheres deficient in oxygen or containing hazardous concentrations of gases or vapours. The use of canister respirators is not recommended because of the concentrations and the wide variety of gases and vapours which may be encountered in a fermentation facility.

203 Equipment supplied with compressed air either from a piped supply via a hose, or from cylinders in the case of self-contained breathing apparatus, will be more suitable. In either case appropriate procedures must be in place to ensure that personnel are not exposed to biological hazards before, during or after use of the equipment. Where RPE is required under COSHH, attention is drawn to the need for training, maintenance, testing and record keeping.

APPENDIX 1 CLASSIFICATION OF BIOLOGICAL AGENTS

In regulation 2(1) of the Control of Substances Hazardous to Health Regulations 1994, 'biological agent' means any micro-organism, cell culture or human endoparasite, including any which have been genetically modified, which may cause any infection, allergy, toxicity or otherwise create a risk to human health.

Schedule 9, paragraph 3, sub-section 4, provides the following criteria for hazard classification of biological agents:

- Hazard Group 1 - unlikely to cause human disease;
- Hazard Group 2 - can cause human disease and may be a hazard to employees; it is unlikely to spread to the community and there is usually effective prophylaxis or treatment available;
- Hazard Group 3 - can cause severe human disease and may be a serious hazard to employees; it may spread to the community, but there is usually effective prophylaxis or treatment available;
- Hazard Group 4 - causes severe human disease and is a serious hazard to employees; it is likely to spread to the community and there is usually no effective prophylaxis or treatment available.

Appendix 2 Summary of industry characteristics

System feature	Anti-biotics	Baker's yeast	Beer	Bread	Cheese	Cider	Edible protein	Food enzymes & flav-ourings	Food grade enzymes	Wastes-open system	Wastes-closed system	Wine	Yoghurt
Buildings													
(a) Viable organisms handled inside buildings in closed systems	Yes	Yes	Partly	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
(b) Plant ventilated	Comfort only	No	No	No	Yes	No	No	Comfort only	Comfort only	Open air	Open air	No	No
(c) Buildings:													
• negative pressure	No	No	No	No	No	No	No	No	Yes	-	-	No	No
• positive pressure	No	No	Yeast	No rooms	Yes	No	No	No	No	-	-	No	No
(d) Building filtration:													
• HEPA	No	No	No	No	No	No	No	No	No	-	-	No	No
• Inlet	No	No	No	Yes	Yes	No	No	Yes	No	-	-	No	No
• Outlet	No	No	No	No	No	No	No	Yes	No	-	-	No	No
(e) Contain spillage	No	No	No	No	No	No	No	No	Yes	No	No	No	
(f) Suitable for fumigation	No	No	No	No	In	No certain areas	No	No	No	No	No	No	No

Summary of industry characteristics

System feature	Anti-biotics	Baker's yeast	Beer	Bread	Cheese	Cider	Edible protein	Food enzymes & flav-ourings	Food grade enzymes	Wastes-open system	Wastes-closed system	Wine	Yoghurt
Fermentation systems (a) Inoculation:													
• Sterile	Yes	Yes	-	No	No	No	Yes	Yes	Yes	No	No	No	No
• Clean	-	-	Yes	Yes	Yes	Yes	No	-	-	No	No	Yes	Yes
• Direct	-	-	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes
(b) Seed stages	Two stage	Three stage	No	No	No	No	?	Single	Single	No	No	No	No
(c) Agitation:													
• Agitation used	Yes	No	No	Yes	Yes/No	No	Yes	Yes	Yes	No	No	No	Yes
• Packed glands	Yes	-	-	No	No	-	No	No	No	-	-	-	-
• Single seals	Yes	-	-	Yes	Yes	-	No	No	No	-	-	-	Yes
• Double seals	Yes	-	-	No	No	-	Yes	Yes	Yes	-	-	-	-
• Steam sealed-mechanical	Yes	-	-	No	No	-	Yes	No	Yes	-	-	-	-
(d) Air filtration:													
• Sterile	Yes	Prelim stages	Not used	Not used	Not used	Not used	Yes	Yes	Yes	No	No	Not used	Yes
• Clean	-	-	-	-	-	-	-	-	-	No	No		

Summary of industry characteristics

System feature	Anti-biotics	Baker's yeast	Beer	Bread	Cheese	Cider	Edible protein	Food enzymes & flav-ourings	Food grade enzymes	Wastes-open system	Wastes-closed system	Wine	Yoghurt
(e) Fermenter pressure:													
● Negative	No	No	No		No	No	No	Yes	No	No	No	No	No
● Atmospheric	No	No	Yes		Yes	Yes	No	No	No	Yes	Yes	Yes	No
● Positive	Yes	Yes	No		No	No	Yes	No	Yes	No	No	No	Yes
● Open	No	No	Yes		Yes	No	No	No	No	Yes	No	No	No
● Closed	Yes	Yes	No		No	No	Yes	Yes	Yes	No	No	No	Yes
● Covered	No	No	Yes		Yes	Yes	No	No	No	No	Yes	Yes	No
(f) Sampling:													
● Sterile	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	No	No	No
● Clean	-	-	Yes	No	No	Yes	-	-	-	No	No	Yes	Yes
(g) Exhaust/air:													
● Filtered	No	No	No	No	No	No	No	Yes	No	No	No	No	No
● Treated	Yes	No	No	No	No	No	Yes	-	Yes	No	No	No	No
(h) Addition systems:													
● Sterile	Yes	No	Not used	Not used	Not used	Not used	Yes	Yes	Yes	Not used	Not used	Not used	Not used
● Clean	No	Yes	-	-	-	-	-	-	-	-	-	-	-
(i) Wastes:													
● Sterile	Optional	No	No	No	No	No	No	No	No	No	No	No	No
● Treated	Optional	No	No	No	No	No	No	Yes	No	No	No	No	No

Summary of industry characteristics

System feature	Anti-biotics	Baker's yeast	Beer	Bread	Cheese	Cider	Edible protein	Food enzymes & flav-ourings	Food grade enzymes	Wastes-open system	Wastes-closed system	Wine	Yoghurt
Biohazard signs	No	No	No	No	No	No	No	Yes	No	No	No	No	No
Access:													
• Semi-restricted	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	-	Yes	Yes
• Restricted	-	-	Yeast areas	-	Yes	-	Yes	-	-	-	Yes	-	-
Personnel protection & restrictions													
Clothing provided	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Changes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Equipment	Yes	Yes	Yes	Yes	Yes	Not	Yes used	Yes	Yes	Yes	Yes	Not	Yes used
1 Locker	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	-	Yes	Yes
2 Lockers	Optional	No	No	No	Yes	No	No	No	Yes	-	Yes	No	-
Smoking allowed	No	No	No	No	No	No	No	No	No	No	No	No	No
Eating allowed	No	No	No	No	No	No	No	No	No	No	No	No	No
Drinking allowed	No	No	No	No	No	No	No	No	No	No	No	No	No
Jewellery & cosmetics allowed	Optional	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Hairnets	No	Yes	No	No	No	No	No	No	No	No	No	No	No
Wash hand basins	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Disinfectant soap	No	No	Yes	Yes	Yes	No	No	No	No	No	No	No	No
Foot baths	No	No	Yes	No	Yes	No	No	No	No	No	No	No	No
Showers optional	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No	Yes
Showers compulsory	Yes	No	No	No	No	No	No	No	No	No	No	No	No
Emergency shower	Yes	No	No	No	No	No	Yes	No	Yes	No	Yes	No	No
Eye wash	Yes	No	No	No	No	No	Yes	No	No	No	No	No	Yes

Summary of industry characteristics

System feature	Anti-biotics	Baker's yeast	Beer	Bread	Cheese	Cider	Edible protein	Food enzymes & flav-ourings	Food grade enzymes	Wastes-open system	Wastes-closed system	Wine	Yoghurt
Medical examination	Yes	Yes	No	No	Yes	No	?	No	No *check	No *check	No	No	No
Training	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Standard procedures	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Spillage policy:	Yes	No	No	No	No	No	?	Yes	Yes	No	No	No	No
Containment	Optional	No	No	No	No	No	No	No	Yes	No	No	No	No
Environmental monitoring	Yes	No	No	Yes	Yes internal	No internal	?	Yes	No	No	No	No	No

Appendix 3 Engineering standards in biotechnology industries

System feature	Anti-biotics	Baker's yeast	Beer	Bread	Cheese	Cider	Edible protein	Food enzymes & flav-ourings	Food grade enzymes	Wastes-open system	Wastes-closed system	Wine	Yoghurt
Valve standards													
Normal industrial	■	■				■			■	■	■		
Clean	■			■			■	■				■	
Super clean			■	■	■			■				■	■
Pipework standards													
Normal industrial	■	■				■			■	■	■		
Clean	■		■	■			■	■				■	
Polished sanitary			■		■			■				■	■
Building standards													
Floors	Concrete	Concrete	Concrete or tiled	Concrete	Concrete or tiled	Concrete	Concrete	Concrete or tiled	Concrete	Concrete	Concrete	Concrete or tiled	Concrete or tiled
Walls	Normal industry	Normal industry	Mostly tiled	Normal industry	Washable	Normal industry	Normal industry	Washable	Normal industry	Normal industry	Normal industry	Normal industry	Normal industry
Ceilings	Normal industry	Normal industry	Washable painted	Normal industry	Washable	Normal industry	Normal industry	Washable	Normal industry	Normal industry	Normal industry	Normal industry	Normal industry
Special areas	Tiled		Tiled		Tiled							Tiled	Tiled

Key

■ Apply

□ Don't apply

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