BRITISH STANDARD

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12461:1998

Biotechnology — Large-scale process and production — Guidance for the handling, inactivating and testing of waste

The European Standard EN 12461:1998 has the status of a British Standard

ICS 07.080; 13.030.01

National foreword

This British Standard is the English language version of EN 12461:1998.

The UK participation in its preparation was entrusted to Technical Committee CII/58, Biotechnology, which has the responsibility to:

— aid enquirers to understand the text;

— present to the responsible European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;

— monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this committee can be obtained on request to its secretary.

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English version

Biotechnology — Large-scale process and production — Guidance for the handling, inactivating and testing of waste

Biotechnologie — Procédé à grande échelle et production — Guide pour la manipulation, l'inactivation et le contrôle des déchets

Biotechnik — Verfahren in Großmaßstab und Produktion — Leitfaden zur Handhabung, Inaktivierung und Prüfung von Abfall

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CEN

European Committee for Standardization
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Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

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Foreword

This European Standard has been prepared by Technical Committee CEN/TC 233, Biotechnology, the secretariat of which is held by AFNOR.

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Contents

Introduction

This European Standard supports industrial activities in the area of biotechnology covering operations both non-genetically modified and genetically modified microorganisms and with both non-pathogenic and pathogenic microorganisms (see annex C [1], [2]).

International, national and local rules, guidelines, safety regulations and instruction manuals that deal with the handling of microorganisms in all steps of fermentation and downstream processes, as well as those used in environmental biotechnology, should be considered.

1 Scope

This European Standard gives guidance on the assessment and the selection of procedures for treatment of waste process microorganisms from biotechnological plant to ensure the safety of people and the environment.

This European Standard applies to wastes and effluents (solid, liquid and gaseous) emitted from biotechnological processes which include traditional processes such as brewing or food processing, and fermentation for pharmaceutical and chemical products, as well as biotechnological processes for environmental and agricultural application.

This European Standard for biotechnological processes is only applicable until gas, liquids and solids are ready for safe transfer to normal industrial or municipal waste handling units.

This European Standard is not applicable to the waste from hospitals, nor to the treatment of chemical and physical hazardous waste.

NOTE Attention is drawn to relevant national regulations.

2 Definitions

For the purposes of this standard, the following definitions apply.

2.1
biohazardous waste
biological waste which can cause a hazard

2.2
cell culture
in vitro growth of cells derived from multicellular organisms

2.3
disinfectant
chemical agent which is able to reduce the number of viable microorganisms

2.4
disinfection
process of reducing the number of viable microorganisms by various physical and chemical methods

2.5
hazard
intrinsic potential property or ability of something (e.g. any agent, equipment, material or process) to cause harm
[EN 1620]

NOTE Harm is an injury or damage to health of people and/or to the environment.

2.6
microorganism
any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material
[EN 1619]

NOTE For the purposes of this standard, the term microorganism covers the term of biological agent, according to the directive 90/679/EEC: microorganisms, including those which have been genetically modified, cell cultures and human endoparasites which may be able to provoke any infection, allergy or toxicity.

2.7
inactivation
process used to reach a state free of a viable process microorganism

2.8
physical containment
system for confining a microorganism or organism or other entity within a defined space
[EN 1620]

2.9
process microorganism
microorganism used for production purposes in a biotechnological process or constituting (part of) the product itself

2.10
sterile
state of being free from viable microorganisms

NOTE 1 In practice, no such absolute statement regarding the absence of viable microorganisms can be proven. However, sterile conditions can be regarded as established by using an accepted or recognized method of sterilization.

NOTE 2 The process of inactivation of viable microorganisms during a sterilization procedure is usually described by an empirical mathematical function, commonly an exponential function. By their mathematical nature, such functions can be reduced to very low numbers, but not to zero. However, these empirical functions can be applied to control or assess the process parameters of a sterilization procedure to realize a desired degree of inactivation of viable microorganisms.

2.11
sterilization
process used to reach a sterile state

2.12
validation
documented procedure for obtaining, recording and interpreting the results needed to show that a process will constantly yield a product complying with predetermined specifications

2.13
verification assay
assay used to determine whether material meets the intended specifications

2.14
waste
by-product arising from a process, or unwanted substance or article derived from any activity

NOTE Examples of waste are scrap material, effluent, unwanted residue or surplus arising from any process or activity, or any substance or article which is discarded or to be disposed of as being broken, contaminated, spoiled, or worn out

3 Waste management policy

The production of waste should be minimized and, if possible, the recovery of materials should be attempted.

A documented waste management policy should be established, describing the measures for prevention, minimization, segregation, handling, storage, treatment, re-use, transportation and disposal of waste from a large-scale biotechnological process.

The waste management system and the responsibilities and duties allocated to managers, supervisors and employees should be specified. The arrangements for effective control of biohazardous waste should be integrated with general management and supervisory organization within the production process.

Documented operational procedures, describing the methods used for effective waste management should be established. These documents should be reviewed at regular intervals and updated, if necessary. Attention is drawn to international, European and national requirements for the control of waste.

A description should be given of the methods and procedures for handling, inactivating and treating waste for both normal conditions and deviations. It is also necessary to describe the commissioning, maintenance and use of plant and equipment in accordance with other appropriate biotechnological European Standards and guidelines.

Comprehensive information should be provided on the risks to health and safety arising from waste which contains pathogenic microorganisms, together with details of its treatment and the prevention and control measures which are used in normal and emergency procedures. This information should be understandable to technical and non-technical personnel alike.

The waste management plan, together with the practical arrangements for the control, treatment and disposal for waste, should be subject to a quality assurance and control programme or equivalent systematic monitoring and auditing programme. The quality of the waste management system should be assured by periodic checks and inspections of the various arrangements and procedures. These include operating conditions and control devices of plant and equipment, the composition and characterization of the waste loads, and adherence to approved standard operating procedures. Test and inspection results should be documented, together with details of any action taken to correct deviations from the intended operating conditions. The results and documentation of quality assurance or audit programmes should be submitted to the internal supervising office.

4 Characterization of waste stream

The following are essential elements, which should be included and documented in a waste management plan:

— definition of the physical and chemical parameters which can affect the choice of treatment and testing methods, such as the amount of suspended solids or pH;

— methods for the segregation of biohazardous waste from non-biohazardous waste at the point of origin, if possible;

— methods for the segregation of other categories of waste (such as hazardous chemical or radioactive products) which do not contain microorganisms when there is incompatibility with the biohazardous waste treatment methods.

A detailed statement should be given of the various activities, processes and the types of waste that are subject to the waste management plan (see Figure 1).

5 Establishment of procedures

The procedures for appropriate treatment of waste steam should be developed. Figure 2 shows the main steps to be conisdered to guide the choice of waste treatment procedures.

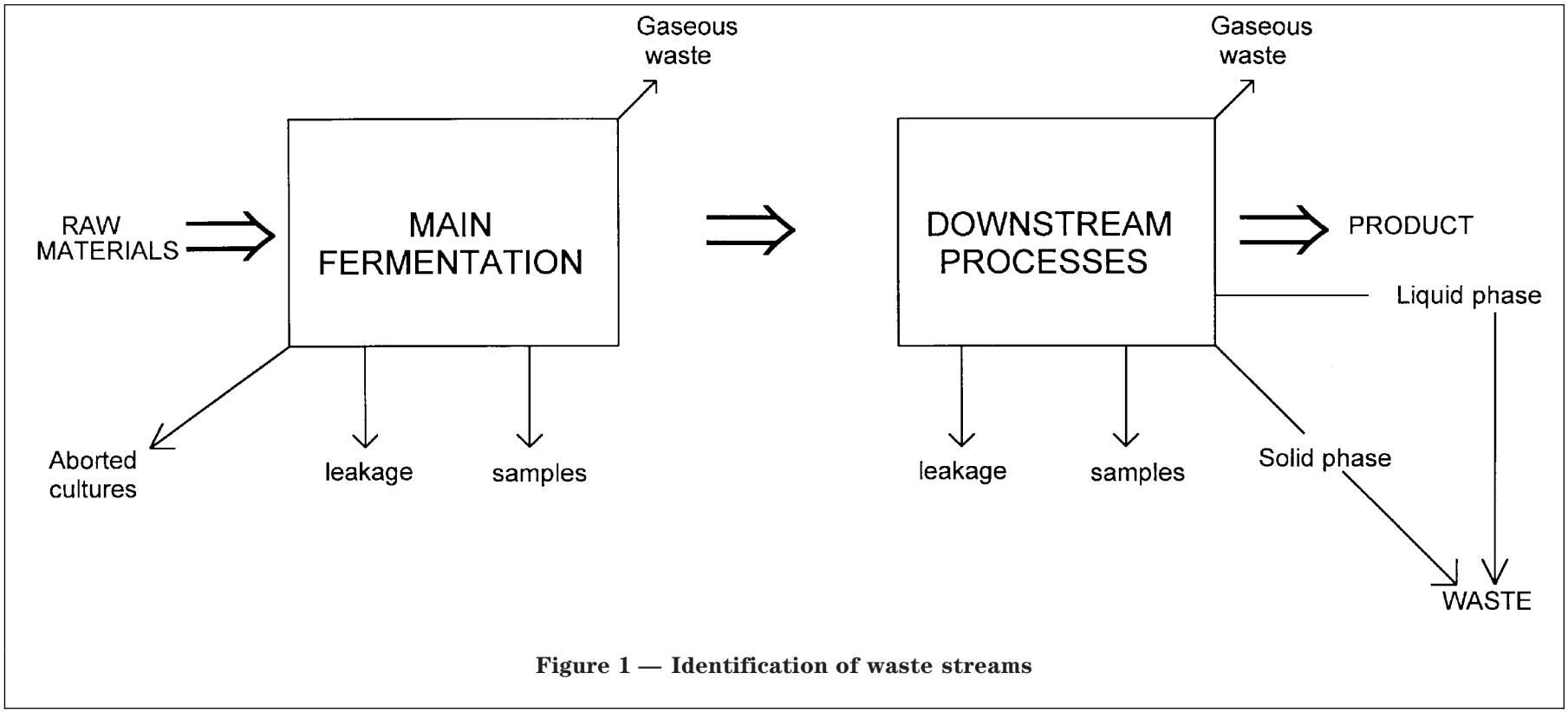


Figure 1 — Identification of waste streams

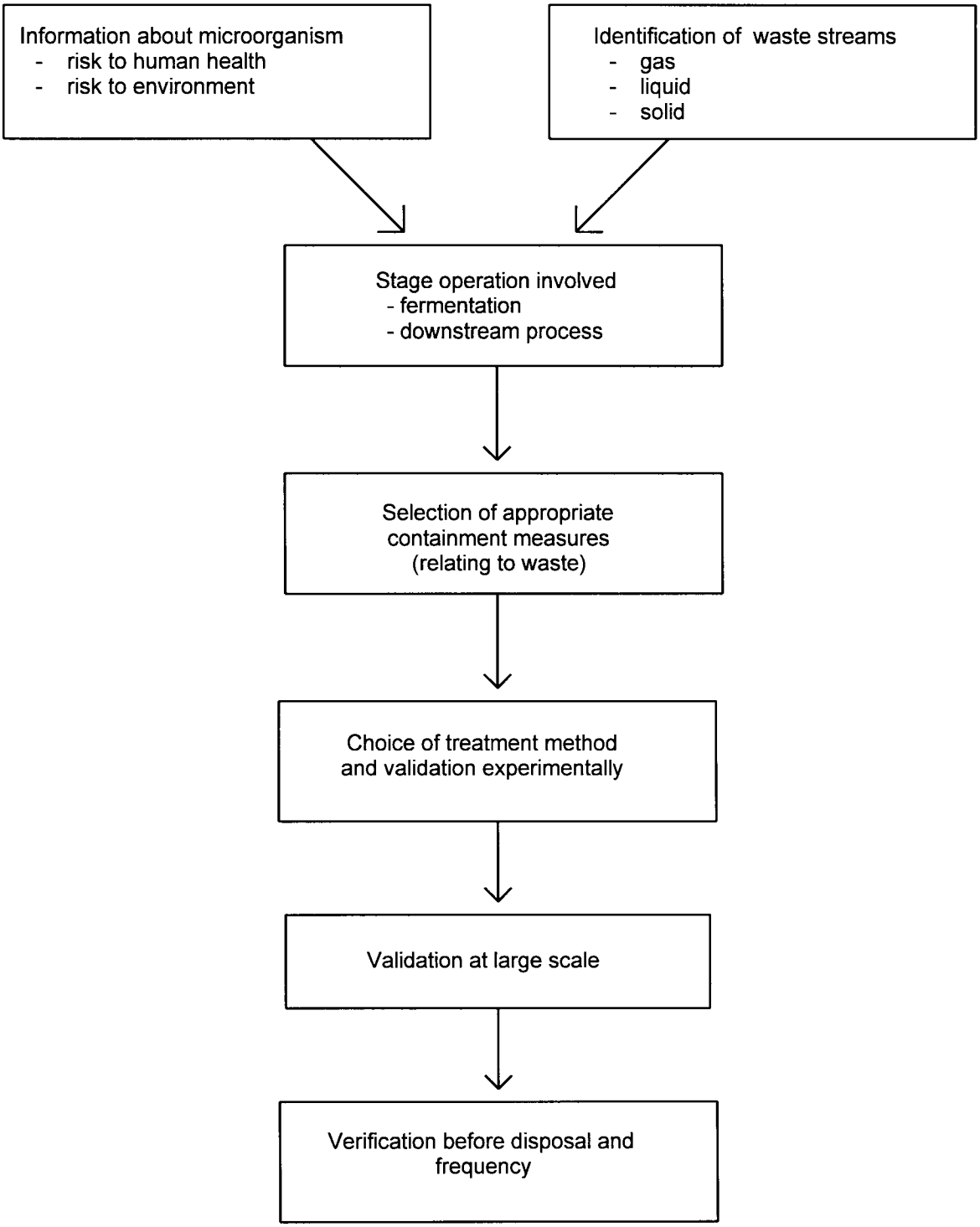


Figure 2 — Establishment of procedures

6 Waste treatment methods

6.1 General

The choice of methods should take into account the category of microorganism, determined in accordance with national, European or international classification rules, the objectives of the treatment (minimize or prevent) and the following criteria:

— efficiency of the method;

— process-specific operation conditions;

— interference with other process parameters;

— maintenance conditions.

Different methods can be used. The inactivation process, which can combine different methods of treatment, should be validated and documented. Any change relevant to safety in the process should be reviewed, validated as necessary and documented (see Table 1).

Table 1 — Example of waste treatment methods related to the type of waste

Treatment	Type of waste		
	Gas	Liquid	Solid
Thermal	X[1)]	XXX[3)]	XXX
Chemical	XX[2)]	XX	XX
Irradiation	X	X	X
Incineration	XX	X	XXX
Filtration	XXX	X	N/A[4)]
Biological	X	XX	X

[1)] X possible
[2)] XX appropriate
[3)] XXX recommended
[4)] N/A not applicable

6.2 Thermal treatment

The combination of temperature and time is critical for the effectiveness of the thermal treatment. A number of factors also influence the success of thermal treatment, such as the number and type of microorganisms present, the composition of the liquid and solid phases, the pH value and water activity.

NOTE Some examples are given as follows:

— for the majority of non-sporulating process microorganisms, temperatures from 60 °C to 70 °C for 10 min to 20 min are usually sufficient;

— for most thermoresistant microorganisms in wet conditions (for example sporulating microorganisms), batch procedure at 121 °C for 20 min or continuous flow at 140 °C for 30 s to 60 s are typical (see annex A);

— for gaseous effluent, heated gas mantle is possible.

6.3 Chemical treatment

There is a considerable diversity in the formulation and handling of chemical disinfectants (see annex C [5]). It is therefore essential to follow the recommendations of the manufacturer.

Examples of disinfectants are sulfuric acid, peracetic acid, sodium hydroxide solution, hypochlorite, formaldehyde, glutaraldehyde and alcohols (e.g. isopropanol, ethanol).

NOTE Examples of application are given in Table B.1.

6.4 Irradiation

In special cases, the use of irradiation (e.g. UV radiation) is possible. Combination of time and energy should be considered.

6.5 Incineration

Incineration can be used as a method of waste treatment.

NOTE Incineration can be heating to more than 600 °C or direct burning.

6.6 Filtration

To remove microorganisms from gaseous effluent, filtration is appropriate and can also be used for liquid effluent.

NOTE The filter will have to be treated to inactivate microorganisms.

6.7 Biological treatment

Biological treatment can be used to minimize the number of process microorganisms in waste from containment level 1 facilities. For higher containment levels, only physical and chemical treatments are recommended.

NOTE Biological methods can be an anaerobic or aerobic treatment.

7 Risk management

7.1 Risk assessment

Microorganisms are classified with respect to human health and safety, and to hazard to the environment, according to the national, European (see annex C [1], [3]) or international classification rules.

A documented risk assessment should be made for the microorganisms and waste treatment process with regard to the general hazards identified. This should be reviewed and revised, if necessary, at the different stages of process design, process implementation and significant process change, and at periodic intervals.

In the case of activities involving exposure to several categories of microorganism, the hazard to environment and human health presented by each microorganism should be considered in preparing the assessment.

7.2 Selection of containment measures

7.2.1 *General*

Assessment of the microorganism and characterization of the waste stream should allow the appropriate containment measures to be applied (see Table 2). Attention is drawn to relevant national, European and international regulations.

7.2.2 *Containment level 1*

For waste issued from facilities of containment level 1, the principles of good occupational safety and hygiene should be observed. There are no special requirements for dealing with microorganisms at this level.

NOTE 1 Inactivation can be necessary and carried out for purposes other than human health or environment protection; for example, patent confidentiality or quality assurance.

NOTE 2 Waste can be recovered and used as a new product.

7.2.3 *Containment level 2*

Waste, gaseous, liquid and solid, from facilities of containment level 2 should be treated so as to minimize the release of viable microorganisms. Other national health regulations and environmental protection rules should also be followed.

7.2.4 *Containment level 3*

Waste from facilities of containment of level 3 (including fermentation broth, spillage, waste basins, steam condensate, exhaust gas and biomass) should be treated so as to prevent the release of any viable microorganism.

Effluents from sinks and showers should be inactivated before release if appropriate.

7.2.5 *Containment level 4*

There is currently little experience of procedures performed at this level. Special safety measures should be set case-by-case.

7.3 Verification assays of the treatment

Verification assays should be carried out to ensure that the number of viable microorganisms in a treated waste stream does not exceed specified acceptable levels established by the environmental risk assessment. Appropriate statistical methods should be used to make inferences from these tests, because it is impossible to verify the complete absence of microorganisms. For containment levels 3 and 4, when it is necessary to ensure absence of process microorganisms in the waste stream, this cannot be proven by testing of samples. Instead, validation is crucial.

Validation of any waste treatment process can also involve the periodic checking by verification test for the presence of viable microorganisms in the waste.

Where the characteristics of a waste stream can vary, the treatment method should be validated for effectiveness under "worst case" load conditions. The method chosen should indicate the concentration of viable microorganisms remaining in the waste stream after treatment.

NOTE Different complementary testing methods are available, including the following:

— growth-related methods:
  — plaque count;
  — colony culture most probable number;
— non-growth-related methods:
  — microscopy;
  — biochemical techniques;
  — immunoassay;
  — molecular biology techniques.

The choice of methods is not only influenced by the type of waste composition, concentration and kind of microorganism, but also by the methods of treatment.

To monitor the effectiveness of the treatment process, biological, chemical or other usable indicators should be used within a standard or "worst case" waste load.

Table 2 — Physical containment levels for waste from biotechnological processes

Requirements[1)]	Physical containment level			
	1	2	3	4
Treatment of exhaust gases from closed system	No[2)]	Minimize release	Prevent release	Prevent release
Containment of spillage with the entire contents of the largest vessel	No	Optional[3)]	Yes[4)]	Yes
Effluent from sinks and showers collected and inactivated before release	No	No	Optional	Yes
Treatment of contaminated and/or aborted culture and waste	No	Minimize release	Prevent release	Prevent release
Treatment of effluent before final discharge	No	Minimize release, case-by-case	Prevent release by inactivation by validated means	Prevent release by inactivation by validated means

1) When using this table, attention is drawn to existing national regulations concerning the requirements within a biotechnological area. See also annex C [1] and [3].

2) No: No special requirement for safety.

3) Optional: The extent to which these measures are to be applied should be decided on a case-by-case basis, subject to risk assessment.

4) Yes: Requirement.

Annex A (informative)

Example of a continuous effluent sterilization by a thermal inactivation system

Continuous sterilization enables:

— killing of microorganisms;

— reduction of cost of effluent treatment when compared to batch sterilization, because of the recovery of the heat in the sterilized effluent.

The principle of continuous sterilization is shown in Figure A.1. The following operations can be required:

— storage of contaminated effluent in a small buffer tank, where pH adjustment can take place or other additives can be added;

— pumping of this fluid towards and through the sterilization unit and the energy recovery system;

— sterilization performed at temperatures above 120 °C, depending on the holding time in the sterilization unit (for example, 140 °C for 2 min for most microorganisms).

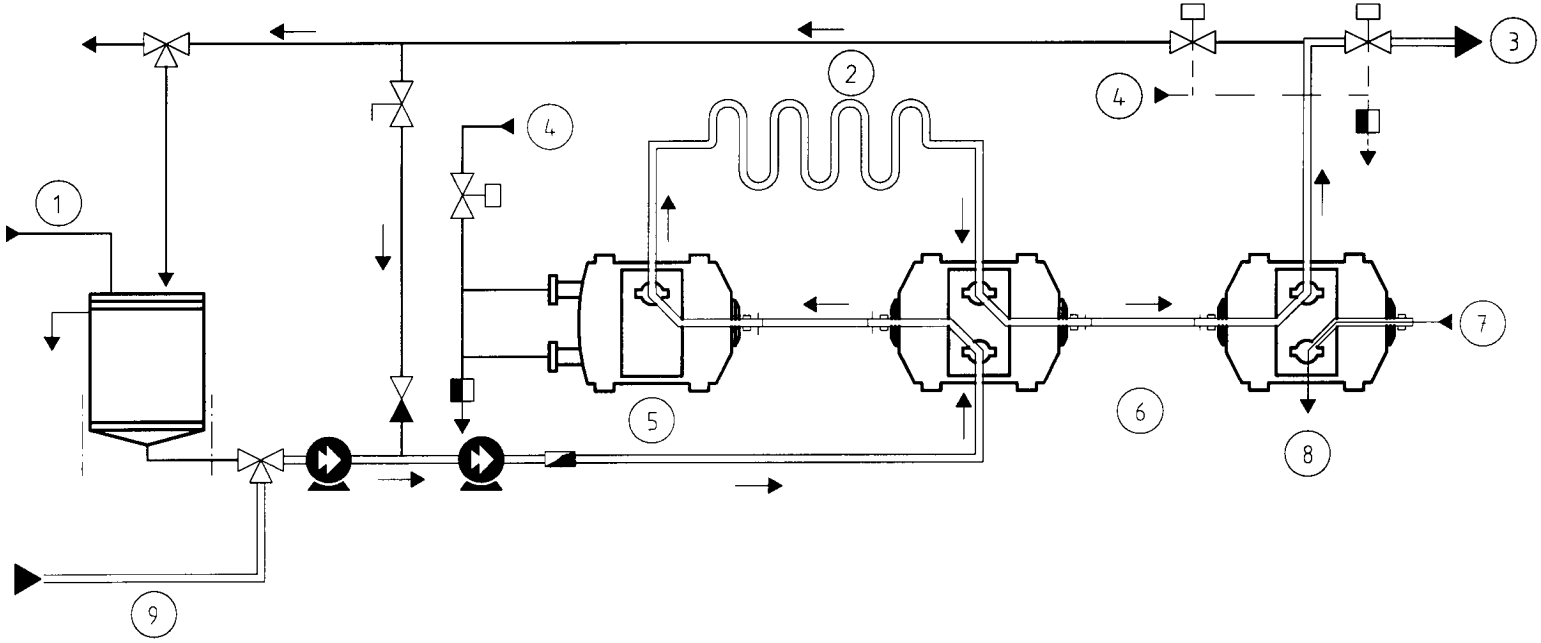
The holding time at sterilization temperature depends on the flow rate through the system and the fixed volume of the holding tube. In the design, this volume has to be calculated to ensure the holding time necessary to kill the microorganism at the chosen sterilization temperature for the maximum flow rate. The contaminated effluent is brought to the sterilization temperature by means of:

a) a heat exchanger where recovery of the heat of the sterilized effluent takes place through heat exchange with the incoming stream of contaminated effluent;

b) a heater where steam is used to bring the temperature of the preheated fluid further up to the chosen sterilization temperature.

The final cooling is only necessary to ensure an acceptable temperature of the rejected sterile effluent, which very often goes into a wastewater station. At this position, an in-line pH control can be required.

To avoid corrosion problems (for example due to the presence of chlorine in the effluent), the installation is partially constructed in Uranus B6 stainless steel for the equipment which operates at high temperature, and in polyethylene for the equipment which operates at temperatures below 60 °C.



1 Water additives
2 Holding tube
3 Sterilized fluid
4 Steam
5 Heater
6 Heat exchanger
7 Cooling water
8 Cooler
9 Contaminated fluid

Figure A.1 — Example of continuous effluent sterilization by a thermal inactivation system (see annex C [6])

Annex B (informative)
Examples of application of disinfectants

Table B.1 — Properties of some disinfectants (see annex C [7])

Disinfectant type	Active against*							Negative interference by		
	Fungi	Bacteria		Myco-bacteria	Spores	Lipid viruses	Non-lipid viruses	Protein	Hard water	Detergent
		Gram-positive	Gram-negative							
Phenolic compounds	xxx	xxx	xxx	xx	—	x	v	+	+	C
Hypochlorites	x	xxx	xxx	xx	xx	x	x	+++	+	C
Alcohols	—	xxx	xxx	xxx	—	x	v	+	+	—
Formaldehyde	xxx	xxx	xxx	xxx	xxx[a]	x	x	+	+	—
Glutaraldehyde	xxx	xxx	xxx	xxx	xxx[b]	x	x	+	+	—
Iodophors	xxx	xxx	xxx	xxx	x	x	x	+++	+	A

xxx: good v: depends on virus
xx: fair *a*: above 40 °C
x: slight *b*: above 20 °C
—: nil
* If the manufacturer's data or the respective data of technical literature are observed.

+++: very C: cationic
++: partly A: anionic
+: weakly
—: nil

NOTE Attention is drawn to the toxicity and/or allergenicity of disinfectants and their environmental impact.

Annex C (informative)
Bibliography

[1] Council Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified microorganisms. OJEC 08.05.1990, no. L 117, p 1.

[2] Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. OJEC 08.05.1990, no. L 117, p 15.

[3] Council Directive 90/679/EEC of 26 November 1990 on the protection of workers from risks related to exposure to biological agents at work (seventh individual Directive within the meaning of Article 16 of Directive 89/391/EEC). OJEC 31.12.1990, no. L 374, p 1.

[4] Council Directive 93/88/EEC of 12 October 1993 amending Directive 90/679/EEC on the protection of workers from risks related to exposure to biological agents at work (seventh individual Directive within the meaning of Article 16 (1) of Directive 89/391/EEC). OJEC 29.10.1993, no. L 268, p 71.

[5] *Laboratory biosafety manual*. WHO (1993). Second edition. ISBN 92 4 154450 3.

[6] *Safety in industrial microbiology and biotechnology*. Edited by C.H. COLLINS and A.J. BEALE. Butterworth-Heinemann, 1992.

[7] COLLINS, C.H. *Laboratory acquired infections: history, incidence, causes and prevention*. Second edition. London: Butterworths, 1988.

[8] EN 1619, *Biotechnology — Large-scale process and production — General requirements for management and organization for strain conservation procedures*

[9] EN 1620, *Biotechnology — Large-scale process and production — Plant building according to the degree of hazard*

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