Sterilization techniques

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Although many microorganisms are beneficial and necessary for human well-being, microbial activities may have undesirable consequences, such as food spoilage and disease. Therefore it is essential to be able to kill a wide variety of microorganisms or inhibit their growth to minimize their destructive effects. The goal is:

(1) to destroy pathogens and prevent their transmission

(2) to reduce or eliminate microorganisms responsible for the contamination of water, food, and other substances.

(3) to possible the aseptic techniques used in microbiological research.

Sterilization [Latin sterilis, unable to produce offspring or barren] is the process by which all living cells, viable spores, viruses, and viroids are either destroyed or removed from an object or habitat. A sterile object is totally free of viable microorganisms, spores, and other infectious agents. When sterilization is achieved by a chemical agent, the chemical is called a sterilant.

PRINCIPLES OF DISINFECTION

The main objective of a cleaning programme is to control microbial activity. Although an adequate cleaning programme will eliminate nearly all the soil present, it will not destroy or remove all the microorganisms. This requires a second step - disinfection.

Disinfection is defined as: "the destruction of microorganisms but not usually bacterial spores. This does not necessarily involve killing all microorganisms, but reducing their number to a level not normally harmful to health.

Disinfection methods can be divided into two groups:

1.Non-chemical disinfection methods

2.Chemical disinfection methods

Non-chemical disinfection methods

Heat/steam

In many cases steam is very good for disinfection but it may be inconvenient or impractical due to the following reasons.

Providing steam is expensive and may cause materials to deteriorate and equipment to distort. If steam is used it will take considerable time to heat and cool equipment.

Chemical disinfection methods

The most essential for an effective chemical disinfection programme is a clean surface. Consequently, to achieve microbial control, the cleaning and disinfection programme must be thorough, compatible and totally effective.

The choice of chemical disinfectants is determined by the following considerations:

Public health regulations

Spectrum of effectiveness, i.e. ability to kill many types of microorganisms

Must be efficient under the conditions of use

Quick action

Presence or absence of organic matter

Corrosive properties

Type of processing area and type of surface to be disinfected

Non-toxic and gentle to the skin

Inexpensive

Must not affect the odour or flavour of the food processed on the equipment

Examples of chemical Disinfectants

Chlorine and chlorine-releasing compounds(sodium or calcium hypochlorite is a cheap disinfectant commonly in use)

Quarternary ammonium compounds

Amphoteric (ampholytic) compounds

Phenolic compounds

Peracetic acid

Antiseptics: An antiseptic is a substance that stops or slows down the growth of microorganisms. They're frequently used in hospitals and other medical settings to reduce the risk of infection during surgery and other procedures.

Antisepsis [Greek anti, against, and sepsis, putrefaction] is the prevention of infection or sepsis and is accomplished with antiseptics. These are chemical agents applied to tissue (animate or living) to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population. Because they must not destroy too much host tissue, antiseptics are generally not as toxic as disinfectants.

Examples are : -Furacin and soframycin and dettol

TYNDALLIZATION(Fractional steam sterilization):

A method developed and devised by John Tyndall was a physicist from County Carlow, Ireland. Tyndallization is done by **repeated exposure of substances to flowing steam** for sterilization and is also called fractional or discontinuous sterilization. A process sometimes used with the aim of sterilizing certain heat labile materials (e.g., culture media)

Principle

The method is affected by employing streaming steam (flowing steam) at temperature of approximately 100ºC for about 30 minutes on three consecutive days and being incubated at room temperature or 37ºC for the intervening period.After each heating, the resting period will allow spores that have survived to germinate into bacterial cells; these cells will be killed by the next day's heating.

Tyndallization is based on the assumption that the materials so treated are able to support the germination and out grwth of any spores they may contain. During the first day of exposure, it kills virtually all organisms except bacterial spores, and it stimulates spores to germinate ro vegetative cells. During overnight incubation, the cells multiply and are killed on the second day. Again the material is cooled and few remaining spores germinate, only to kill on the third day.

Arnold sterilizer is an instrument used to carryout Tyndallization process, which make use of flowing steam as the sterilizing agent.

Demerits of tyndallization

Although the method usually results in sterilization, occasions arise when several spores fails to germinate.The method also requires that spores to be in a suitable medium for germination

such as a broth.

Pasteurization.

Pasteurization is a process in which packaged and non-packaged foods (such as milk and fruit juice) are treated with mild heat, usually to less than 100 °C (212 °F), to eliminate pathogens and extend shelf life. ... Spoilage enzymes are also inactivated during pasteurization.

The process was named after the French microbiologist, Louis Pasteur, whose research in the 1860s demonstrated that thermal processing would deactivate unwanted microorganisms in wine. Spoilage enzymes are also inactivated during pasteurization. Today, pasteurization is used widely in the dairy industry and other food processing industries to achieve food preservation and food safety.

Two Kinds of Pasteurization

Low-Temperature Long Time (LTLT)

High-Temperature Short Time (HTST)

Benefits of Pasteurization

Eliminating harmful bacteria like Listeria, Salmonella, Listeria, Staphylococcus aureus, Yersinia, Campylobacter, and Escherichia coli O157:H7.

Preventing diseases like scarlet fever, tuberculosis, brucellosis, and diphtheria.

Sterilization

Sterilization may be defined as the statistically complete destruction of all microorganisms including the most resistant bacteria and spores.Methods of sterilization

1. Moist Heat Sterilization:

2. Dry Heat Sterilization:

3. Sterilization by Radiation:

4.Sterilization by Filtration

5. Gas Sterilization:

1. Moist Heat Sterilization:

Moist heat sterilization is one of the most effective methods of sterilization where the steam under pressure acts as a bactericidal agent.

Moist heat sterilization usually involves the use of steam at temperatures in the range 121–134°C.

High pressure increases the boiling point of water and thus helps to achieve a higher temperature for sterilization.

High pressure also facilitates the rapid penetration of heat into deeper parts of material and moisture present in the steam causes the coagulation of proteins , causing an irreversible loss of function and activity of microbes.

The most commonly used standard temperature-time cycles for clinical porous specimens (e.g. surgical dressings) and bottled fluids are 134°C for 3 minutes and 121°C for 15 minutes, respectively.

An autoclave is a device that works on the principle of moist heat sterilization through the generation of steam under pressure.

In the pharmaceutical and medical sectors, it is used in the sterilization of dressings, sheets, surgical and diagnostic , containers, and aqueous injections, ophthalmic preparations, and irrigation fluids, in addition to the processing of soiled and contaminated items.

Moist heat can be used in sterilization at different temperatures:

2. Dry Heat Sterilization:

Dry sterilization is the process of removing microorganisms by applying moisture-free heat which is appropriate for moisture-sensitive substances.

The dry heat sterilization process is based on the principle of conduction; that is the heat is absorbed by the outer surface of an item and then passed onward to the next layer. Ultimately, the entire item reaches the proper temperature needed to achieve sterilization.

Dry moisture-less heat destroys microorganisms by causing denaturation of proteins and also lyses the proteins in many organisms, causes oxidative free radical damage, causes drying of cells, and can even burn them to ashes, as in incineration

Dry heat sterilization is used for the sterilization of materials which are difficult to sterilize by moist heat sterilization for several reasons.

Substances like oil, powder, and related products cannot be sterilized by moist heat because moisture cannot penetrate into deeper parts of oily materials, and powders are destroyed by moisture.

Similarly, laboratory equipment like Petri dishes and pipettes are challenging to sterilize by moist heat due to the penetration problem.

Thus, in dry heat sterilization usually higher temperatures in the range 160–180°C are employed and also require exposure times of up to 2 hours depending upon the temperature employed.

This principle is used in instruments like hot air oven and incineration, which generates very hot moisture-free air.

In addition to the fact that this method achieves an adequate sterility assurance level, this method also destroys bacterial endotoxins

Instruments used for this are Hot air oven, 3.Sterilization by Radiation:

Radiations can be divided into two groups:

1.Electromagnetic waves ...infrared radiation, ultraviolet light, X-rays and gamma rays.

2. Streams of particulate matter...alpha and beta radiations.

Most commonly infrared radiation, ultraviolet light, gamma radiation and high-velocity electrons are used for sterilization.

Ultraviolet (non-ionizing) radiation

A narrow range of UV wavelength (220-280 nm) is effective in killing the microorganism. The most serious disadvantage of UV radiation as a sterilizing agent is its poor penetrating power. This is the result of strong absorption by many substances. The application of UV radiation is limited.

UV radiation owing to its poor penetrability of conventional packaging materials is unsuitable for sterilization of pharmaceutical dosage forms.

It is, however, applied in the sterilization of air, for the surface sterilization of aseptic work areas, and the treatment of manufacturing-grade water.

UV lamps are sometimes placed on the ceilings of rooms or in biological safety cabinets to sterilize the air and any exposed surfaces. Because UV radiation burns the skin and damages eyes, people working in such areas must be certain the UV lamps are off when the areas are in use.

(ii) Ionizing Radiations:

Ionizing radiations (X-rays, Gamma rays and Cosmic rays) are an excellent sterilizing agents and they penetrate deep into the objects.

These radiations do not produce heat on the surface of materials. Hence, sterilization using ionizing radiations is referred as cold sterilization.

It will destroy bacterial endospores and vegetative cells, both Prokaryotic and Eukaryotic; however ionizing radiation is not always effective against viruses.

X-ray and gamma rays are the commonly used ionizing radiation for sterilization.

These are high energy radiation which causes ionization of various substances along with water.

The ionization results in the formation of a large number of toxic O2 metabolites like hydroxyl radical, superoxide ion, and H2O2 through ionization of water.

These metabolites are highly oxidizing agents and kill microorganisms by oxidizing various cellular components.

With ionizing radiation, microbial resistance decreases with the presence of moisture or dissolved oxygen and also with elevated temperatures.

Radiation sterilization is generally exposed to items in the dried state which include surgical instruments, sutures, prostheses, unit-dose ointments, plastic syringes, and dry

pharmaceutical products.

4. Sterilization by Filtration:

The process of filtration is unique among sterilization techniques in that it removes, rather than destroys, microorganisms.

Further, it is capable of preventing the passage of both viable and nonviable particles and can thus be used for both the clarification and sterilization of liquids and gases.

The primary mechanisms involved in filtration are sieving, adsorption, and trapping within the matrix of the filter material.

Filtration uses membranous filters that have tiny pores that let the liquid pass through but prevent bigger particles such as bacteria from passing through the filter. Therefore, the smaller the pore, the more likely the filter is to stop more things from going through it.

Certain types of filter (membrane filters) also have an essential role in sterility testing, where they can be employed to trap and concentrate contaminating organisms from solutions under test.

These filters are then placed in a liquid nutrient medium and incubated to encourage growth and turbidity.

The principal application of sterilizing-grade filters is the treatment of heat-sensitive injections and ophthalmic solutions, biological products, air, and other gases for supply to aseptic areas.

They may also be required in industrial applications where they become part of venting systems on fermenters, centrifuges, autoclaves, and freeze dryers.

Chemicals Used for Sterilization or Disinfection

Ethylene Oxide,Ozone.,Bleach.,Glutaraldehyde and Formaldehyde.,

Phthalaldehyde.,Hydrogen Peroxide.,Peracetic Acid.,

Silver.,Phenols .

Phenolic compounds

Phenol was the first widely used antiseptic and disinfectant.

In 1867 Joseph Lister employed it to reduce the risk of infection during operations.

Today phenol and phenolics (phenol derivatives) such as cresols, xylenols, and orthophenylphenol are used as disinfectants in laboratories and hospitals.

Antimicrobial activity in phenol derivatives is increased by substitution of other components into the phenol ring structure.

Phenol is readily soluble in water while most of its derivatives are partially soluble.

Cresols of four different types are used as disinfectants.

These are o-cresol, m-cresol and hexylresorcinol.

Chemically cresols have a phenolic ring with methyl group attached in different positions.

Cresols forms suspensions in water and are very effective disinfectants.

The commercial disinfectant Lysol is made of a mixture of phenolics (cresol). This preparation have a sustaining odour and contain a surface tension reducer in addition to the microbicidal. They find wide applications in disinfecting floors, furniture, barns, sheds, etc in 1-5% concentration.

Hexylresorcinol is another cresol marketed in the form of a solution mixed with glycerine and water. It is a surface tension reductant having a very high microbicidal property and an antiseptic in mouthwash, cough drops etc.

Bis-phenols (hexachlorophenes) are phenol derivatives having two phenol rings. Hexachlorophene is a combination of phenol with chlorine. It is a good skin antiseptic as it is a surfactant. Antiseptic soaps and hand wash contain hexachlorophenes.

Hexachlorophene has been one of the most popular antiseptics because it persists on the skin once applied and reduces skin bacteria for long periods. However, it can cause brain damage and is now used in hospital nurseries only in response to a staphylococcal outbreak.

Phenolics act by denaturing proteins and disrupting cell membranes. As a result cell contents leak out. Precipitation of proteins, inactivation of enzymes are other damages caused to the microbial cell due to the influence of phenols.

They have some real advantages as disinfectants: phenolics are tuberculocidal, effective in the presence of organic material, and remain active on surfaces long after application. However, they do have a phenol

DETERGENTS

Detergents (L.detergere = to wipe off or away) are organic molecules that serve as wetting agents and emulsifiers because they possess both polar hydrophilic and nonpolar hydrophobic ends.

Detergents solubilize otherwise insoluble residues and are very effective cleaning agents. They are different than soaps, which are derived from fats. Since soaps are not very efficient in hard water, new synthetic detergents or surfactants have been developed that prove superior to soaps.

Synthetic detergents do not form precipitates in alkaline or acid water or with minerals occurring in hard water. These detergents are used in laundry and dishwashing powders, sampoos and other washing preparations, and some of them are highly bactericidal. Chemically, synthetic detergents may be classified into following categories.

(i) Anionic. Anionic Detergents are sodium salts of sulphonated long chain alcohols or hydrocarbons. They have anions at the soluble ends of the chains. E.g: Sodium Lauryl sulphate and sodium n-dodecyl benzene sulphonate.

Mainly anionic detergents such as sodium or ammonium lauryl sulfate, sodium dodecylbenzene sulfonate, etc. are used in toothpaste to clean the teeth and to provide a foam that helps to carry away the debris. In anionic detergents, the anionic part of the molecule is involved in the cleansing action

Anionic surfactants work best to remove dirt, clay, and some oily stains. These surfactants work following ionization. When added to water, the anionic surfactants ionize and have a negative charge.

Anionic surfactants are widely used for industrial as well as household cleaning and for pesticide formulations. Of the anionic surfactants, biodegradable linear alkylbenzenesulfonates (LAS) are the most common and can be found in waste water systems and river water.

(ii) Cationic. Cationic detergents are quaternary ammonium salts of amines with acetate, chlorides or Bromides as anions. They have cations at the soluble ends of the chain. Cations are long-chain hydrocarbons having a positive charge on N atom. Eg: Cetyl trimethyl ammonium bromide.

Example of cationic detergents are quaternary ammonium compounds, benzalkonium chloride and cetyltrimethyl ammonium bromide. They will precipitate when mixed with an anionic detergent (soap)

Cationic detergents are more strongly absorbed on fabrics that are anionic or nonionic surfactants. They are used as wetting agents, fabric softeners, bacteriostats and emulsifiers. Example of cationic detergents are quaternary ammonium compounds, benzalkonium chloride and cetyltrimethyl ammonium bromide

Although anionic detergents possess some degree of antimicrobial properties, only cationic detergents are effective disinfectants. The most popular of cationic disinfectants are quaternary anmonium compounds.

Mode of action. Detergents mechanically remove the microorganisms from the surfaces (e.g., skin, dirty cloths) on which they are applied. They reduce surface tension and thereby enhance the wetting power of the water in which they are dissolved. Soapy water emulsifies and disperses oils and dirts and, as a result, the microorganisms become enmeshed in the detergent's lather and are removed by the rinse water. However, a number of compounds have been incorporated into detergents to increase their microbicidal activity.