BOTANY [Hons.]
Sixth Semester
DSE3P (Practical)

Industrial and Environmental Microbiology

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Study 2: Sterilization techniques

Methods of Sterilization:

According to World health organization [WHO, 2019], sterilization is indispensable for the absolute obliteration or elimination of all microbes (spore-forming and non-spore forming bacteria, viruses, fungi, and protozoa) that could infect materials/ pharmaceuticals and in this manner amount to health risks.

Conventional sterilization methods utilize saturated steam under pressure or hot air. These techniques are the most consistent and must be used every time if probable. Other sterilization techniques consist of filtration, ionizing radiation (gamma and electron-beam radiation), and gas (ethylene oxide, formaldehyde).

Heating in an autoclave (steam sterilization)

When microorganisms are exposed to saturated steam under pressure in an autoclave, they are destroyed by the irreversible denaturation of enzymes and structural proteins. The temperature at which denaturation occurs varies inversely with the amount of water present. The preferred specifications for sterilization in an autoclave are 15 minutes at 121-124 °C (200 kPa).

Dry-heat sterilization

In dry-heat processes, there is oxidation of cell constituents. It requires a temperature greater than moist heat as well as an extended time interval. This technique can be applied to heat-stable, non-aqueous materials. Moist heat/ steam cannot sterilize these materials as it fails to penetrate. Such materials comprise of glassware, powders, oils, and other oil-based substances.

The materials to be sterilized by dry heat must be kept in containers which are either sealed or for the time being closed for sterilization. The whole content of each such container must be maintained in the oven for the given specific time and temperature as summarized in the table below. The hot air oven must be equipped with a forced air system to guarantee equal distribution of heat all the way through all the substances tat are being sterilized. This is done by monitoring the temperature. Containers which were temporarily closed during the sterilization procedure must be are sealed aseptically afterwards to avoid microbial re-contamination

TEMPERATURE (°C)	MINIMUM STERIZATION TIME (MIN)
160	180
170	60
180	30

Filtration

This technique is used generally for thermolabile solutions. These may be sterilized by passage through sterile bacteria-retaining filters, e.g. membrane filters (cellulose derivatives, etc.), plastic, porous ceramic, or suitable sintered glass filters, or combinations of these. However, asbestos-containing filters should not be used.

Suitable measures must be taken to avoid loss of solute by adsorption onto the filter and prevent the release of contaminants from the filter. Usually, membranes of less than 0.22 µm nominal pore size should be used Filters capable of withstanding heat may be sterilized by autoclaving at 121 °C for 15 - 45 minutes before filtration.

Subsequently, after filtration proper care must be taken for aseptic transfer of the sterilized solution to the final containers which are then to be immediately sealed with great care to exclude any recontamination. All filters, tubes, and equipment used "downstream" must be sterile. For filtration of a liquid in which microbial growth is possible, the same filter must not be used for procedures lasting longer than one working day

Exposure to ionizing radiation

Ionizing radiation in the form of gamma radiation can be used to sterilize certain active ingredients, drug products, and medical devices in their final container or package. It is accomplished by exposure to a suitable radioisotopic source such as 60Co (cobalt 60) orof electrons energized by a suitable electron accelerator. However, strict adherence to laws and regulations for protection against radiation must be carried out.

Gamma radiation and electron beams effectively ionize the molecules in microorganisms. As such, mutations occur in the DNA which in turn modify replication. These processes are very hazardous and only compliant and skilled staff should take part in such sterilization protocols. An absorbed radiation level of 25 kGy1 (2.5 Mrad)2 is usually selected. Radiation doses should be monitored with specific dosimeters during the entire process. Dosimeters should be calibrated against a standard source after receiving from the supplier and at regular intervals thereafter.

Gas sterilization

This method applies a chemical in a gaseous state to destroy microbes specially bacteria. The most widely used active agent of the gas sterilization process is ethylene oxide. These gases are mixed with suitable inert gases to minimize their highly toxic properties and the possibility of toxic residues. It is very difficult to control the whole process. It should only be taken into account if no other sterilization procedure can be used. It must only be carried out under the supervision of highly skilled staff.

The sterilizing efficiency of ethylene oxide is proportional to the concentration of the gas, the humidity, the time of exposure, the temperature, and the nature of the load. The gas concentration, temperature and humidity should be recorded for each cycle. After sterilization, time should be allowed for the elimination of residual sterilizing agents and other volatile residues. This must be confirmed by specific tests.

Students please Note:

The classification of different types of sterilization techniques will be sent to all of your email-IDs.

References/sources used:

1. https://apps.who.int/phint/pdf/b/7.5.9.5.8-Methods-of-sterilization.pdf

Further reading:

1. https://www.in.gov/isdh/files/Tab_1_Resource_CD.pdf
2. Yoo JH. Review of Disinfection and Sterilization - Back to the Basics. *Infect Chemother*. 2018;50(2):101–109. doi:10.3947/ic.2018.50.2.101
3. https://pubchem.ncbi.nlm.nih.gov/compound/Ethylene-oxide
4. Medical Sterilization Methods – White Paper, Dec 2003