**Programme: Bachelor of Science (First Year)**

**Subject: Microbiology**

**Semester : II**

**Paper Code: MIG 102**

**Paper Title: Industrial and Food Microbiology**

**Unit 2 : Maintenance of sterility**

**Module Name : Sterilisation of air: filtration, heat,electrostatic precipitation,uv light and chemical agents.**

**Module Number : 6**

**Name of the Presenter: Dr.Rasika Desai Gaokar**

**<u>Notes</u>**

## Sterilization of Air:

- In general, the industrial fermentations are carried out under vigorous and continuous aeration.
- For an effective fermentation, the air should be completely sterile, and free from all microorganisms and suspended particles.
- There is a wide variation in the quantity of suspended particles and microbes in the atmospheric outdoor air.
- On average, the microorganisms may range from 10-2,000/$m^3$ while the suspended particles may be 20-100,00/ $m^3$.
- Among the microorganisms present in the air, the fungal spores (50%) and Gram-negative bacteria (40%) dominate.
- Air or other gases can be sterilized by filtration, heat, UV radiation and gas scrubbing.
- Among these, heat and filtration are most commonly used.

### (a) Air sterilization by heat:

In the early years, air was passed over electrically heated elements and sterilized. But this is quite expensive, hence not in use these days.

**(b) Air sterilization by filtration:**

Filtration of air is the most commonly used sterilization in fermentation industries.

i) HEPA Filters:

HEPA filters or High Efficiency Particulate Air Filters are an essential part of pharmaceutical and health care industries where sterile environments are mandatory.

Particles that cannot be seen by the naked human eye are responsible for air borne diseases.

- HEPA filter removes particulates (generally called aerosols) such as micro-organisms from the air. The HEPA filter does not remove vapours or gases.
- HEPA filters used in Laminar Air flow benches and biosafety cabinets should have a minimum filtration efficiency of 99.99% against airborne particles 0.3 microns in size.

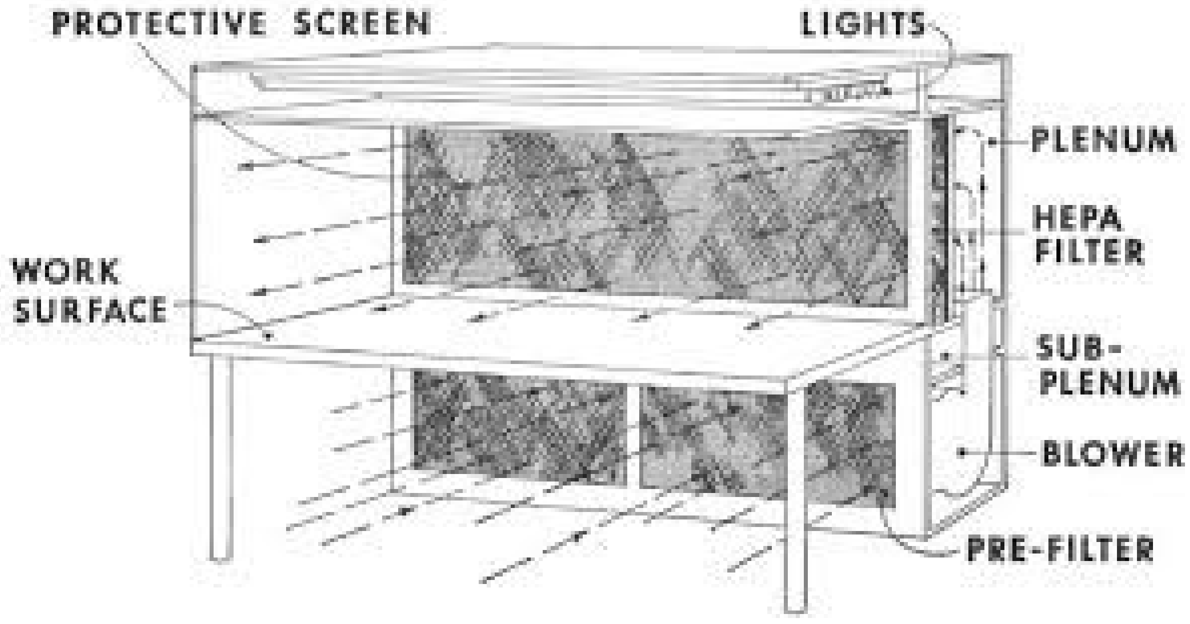


**Fig 1: Laminar Air Flow**

i) Depth filters:

When the air is passed through glass wool containing depth filters the particles are trapped and removed (Fig 2).

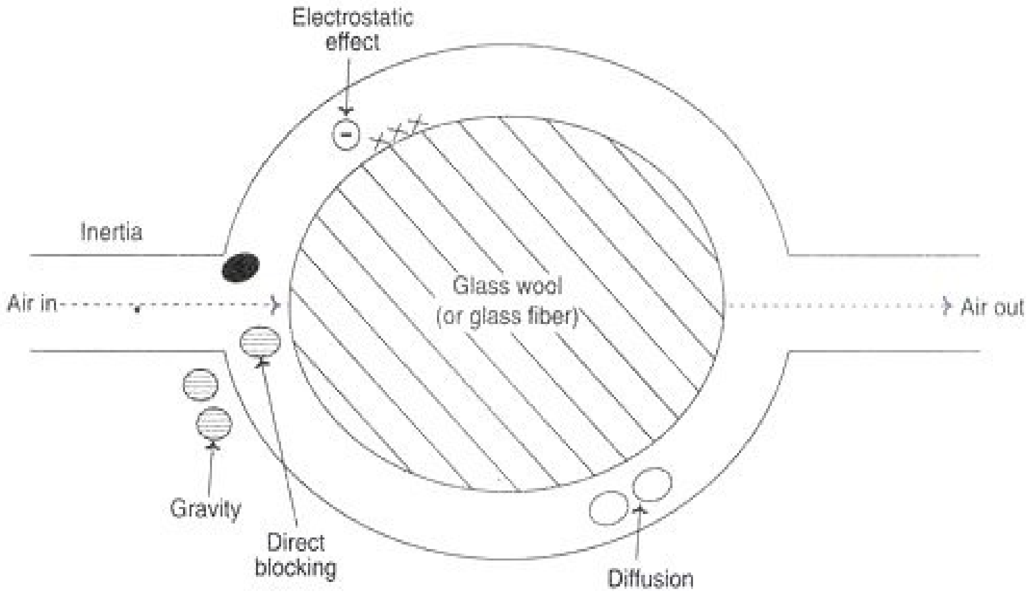
This filtration technique primarily involves physical effects such as inertia, blocking, gravity, electrostatic attraction, and diffusion.

Glass wool filters can be subjected to steam sterilization and reused.

But there is a limitation in their reuse since glass wool shrinks and solidifies on steam sterilization.

- In recent years, glass fiber filter cartridges (that do not have the limitations of glass wool filter) are being used.

- Fig. 1 Depth filter




*Use of depth filter in air sterilization.*

**Fig. 2 Depth filter**

ii) Membrane cartridge filters:

These are removable pleated membrane filters made up of cellulose ester, nylon or polysulfone.

Membrane cartridge filters are smaller in size, simpler for operation and replacement.

The most important limitation of air sterilization is that there is no filter that can remove bacteriophages.

Bacteriophages are capable of crippling the industrial fermentation.

e.g., bacteriophages interfere in the production of glutamic acid by Corynebacterium glutamicum.

(**c) Air sterilization using uv light:**

- Ultraviolet irradiation (UV) uses ultraviolet light in order to deactivate a wide range of microorganisms.
- UV light can also have the advantage of being effective against protozoa, including Cryptosporidium.
- The process relies on UV light being able to pass through the air requiring treatment with sufficient energy to inactivate the pathogens.
- Effectiveness is significantly impaired if air contains particulate material. UV does not have any residual disinfectant effect in the water.
- UV light will also photo-oxidise some organic compounds, breaking them down into smaller molecules. This has some application in the treatment of compounds causing tastes and odours, pesticides and algal toxins. The shorter wavelengths of UV, around 185 nm, tend to be more effective in this application.

***Strengths of UV –***

Cheap and effective disinfection

Chemical free

Relatively simple to install, operate and maintain

**Weaknesses of UV-**

No lasting disinfectant residual

Cannot operate without power

Is ineffective if the dose and contact time are not correct

Difficult to verify whether air has been adequately disinfected.

- **Principle of UV Disinfection:**
- UV radiation inactivates microorganisms by penetrating cell walls and disrupting vital cell functions.
- If sufficient energy reaches the cell it results in the death or impairment of that cell, and consequently the organism itself.

- The most effective wavelength of UV radiation for damaging cell DNA is 254nm, although in practice wavelengths between approximately 200 – 300 nm are generally considered biocidal.
- Bacteria are more susceptible than larger protozoan parasites such as Cryptosporidium and Giardia, although with a sufficient dose and contact time UV can be considered effective at deactivating these. There is less agreement on vulnerability of viruses, although UV undoubtedly has some effect. Sometimes UV is used in combination with other treatments such as ozone or hydrogen peroxide.

**Generation of UV Radiation:**

- Special lamps are used to generate UV radiation, and these are enclosed in a reaction chamber made of stainless steel or, less commonly, plastics. Low pressure mercury lamps, which generate 85% of their energy at a wavelength of 254 nm, are most commonly used; their wavelength is in the optimum germicidal range of 250 to 265 nm.
- These lamps are similar in design, construction and operation to fluorescent light tubes except that they are constructed of UV-transparent quartz instead of phosphor-coated glass.
- The intensity of UV radiation emitted decreases with lamp age; typical lamp life is about 10 to 12 months.
- **(c) Air sterilization using electrostatic precipitation:**
- An **electrostatic precipitator** (ESP) is a filtration device that removes fine particles, like dust and smoke, from a flowing gas using the force of an induced **electrostatic** charge minimally impeding the flow of gases through the unit.
- The most basic precipitator contains a row of thin vertical wires, and followed by a stack of large flat metal plates oriented vertically, with the plates typically spaced about 1 cm to 18 cm apart, depending on the application. The air

stream flows horizontally through the spaces between the wires, and then passes through the stack of plates.

- A negative voltage of several thousand volts is applied between wire and plate. If the applied voltage is high enough, an electric corona discharge ionizes the air around the electrodes, which then ionizes the particles in the air stream.

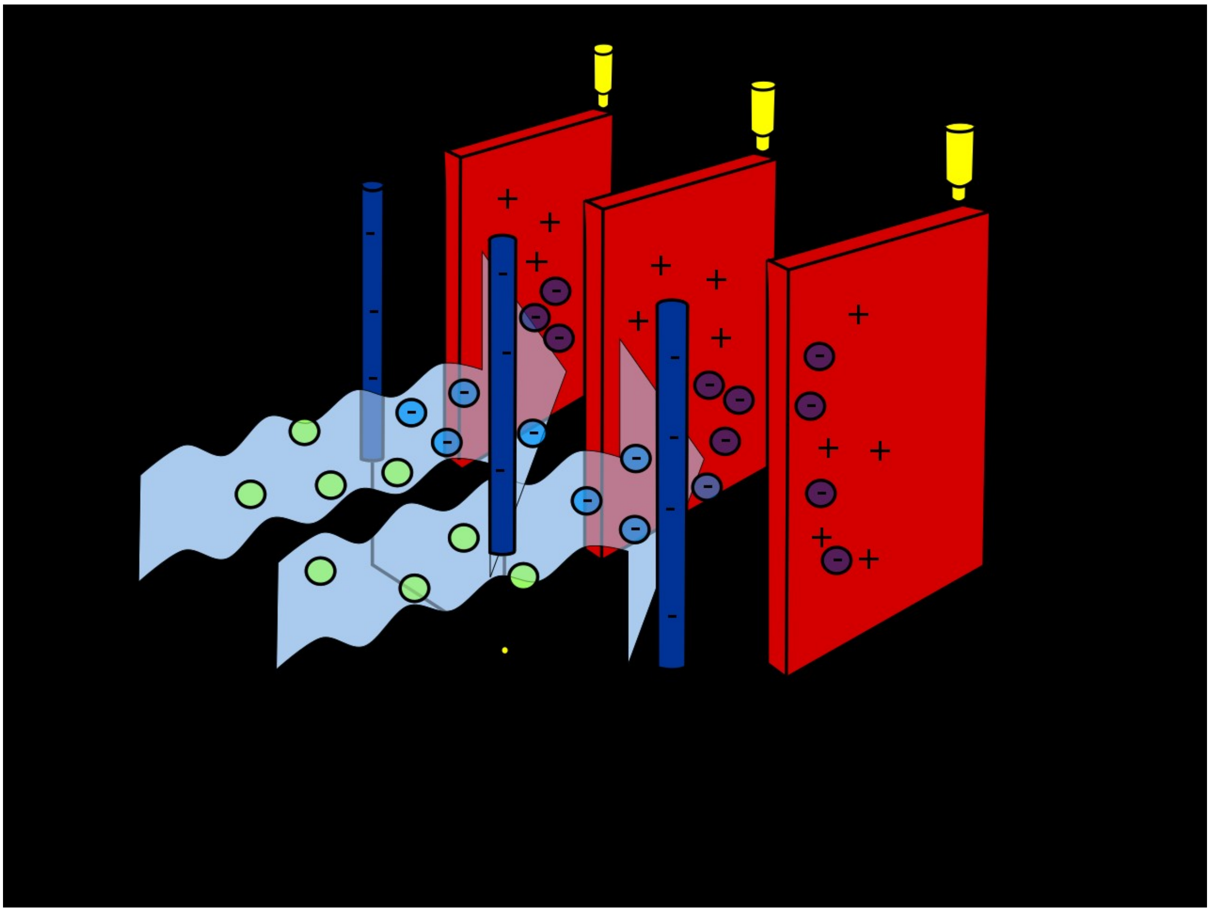


**Fig.2 Electrostatic precipitator**

**e) Air sterilisation using chemical agents:**

Air disinfectants are typically chemical substances capable of disinfecting microorganisms suspended in the air.Disinfectants are often assumed to be limited to use on surfaces, but that is not the case. In 1928, a study found that airborne microorganisms could be killed using mists of dilute bleach.

An air disinfectant must be dispersed either as an aerosol or vapour at a sufficient concentration in the air to cause the number of viable infectious microorganisms to be significantly reduced.

**a) Alcohol Disinfectants**

- Alcohols, usually ethanol or isopropanol, are sometimes used as a disinfectant, but more often as an antiseptic, the distinction being that alcohol tends to be used on living tissue rather than nonliving surfaces. These alcohols are non-corrosive but can be a fire hazard. They also have limited residual activity due to evaporation, which results in brief contact times unless the surface is submerged. They also have a limited activity in the presence of organic material.
- Alcohols are most effective when combined with purified water to facilitate diffusion through the cell membrane; 100% alcohol typically denatures only external membrane proteins of microorganisms. A mixture of 70% ethanol or isopropanol diluted in water is effective against a wide spectrum of bacteria, though higher concentrations are often needed to disinfect wet surfaces. Additionally, high-concentration mixtures (such as 80% ethanol + 5% isopropanol) are required to effectively inactivate lipid-enveloped viruses (such as HIV, hepatitis B, and hepatitis C). Alcohol is only partly effective against most non-enveloped viruses (such as hepatitis A), and is not at all effective against fungal and bacterial spores.

b) Aldehydes**:** such as formaldehyde and glutaraldehyde, have a wide microbiocidal activity and are sporocidal and fungicidal. They are partly inactivated by organic matter and have slight residual activity.

Some bacteria have developed resistance to glutaraldehyde; it has also been found that glutaraldehyde can cause asthma and other health hazards, hence ortho-phthalaldehyde is replacing glutaraldehyde.

c) Ethylene oxide appears as a clear colorless gas with an ethereal odor with a flash point below 0°F.

Its vapours are  heavier than air. May polymerize exothermically if heated or contaminated. If the polymerization takes place inside a container, the container may rupture violently. Vapors very toxic. Vapors irritate the eyes, skin, and respiratory system. Prolonged skin contact may result in delayed burns. Used to make other chemicals, as a fumigant and industrial sterilant.

d) Hydrogen peroxide: is a peroxide and oxidizing agent with disinfectant, antiviral and anti-bacterial activities. Hydrogen peroxide exerts its oxidizing activity and produces free radicals which leads to oxidative damage to proteins and membrane lipids of microorganisms. This may inactivate and destroy pathogens and may prevent spreading of infection..