UNIT-II STERILIZATION AND DISINFECTION

Introduction:

Although microorganisms are very much beneficial to mankind, they are also harmful to (others) mankind *i.e.* they are popular enough in causing many diseases to man, animals even plants, spoilage and contamination. Because of the hazardous consequences, it is absolutely essential to kill such organisms or to inhibit their growth (this also includes prevention of contamination). It is known that, man is practicing many ways for disinfection and sterilization right from the start of civilization. This chapter is concerned with different physical and chemical methods used for control of microorganisms. Initially, let us find out some definitions related with control.

1. Sterilization (Latin-Sterilis - is unable to produce offspring or barren)

It is the process by which all living cells, viable spores, viruses, viroids are either destroyed or removed from an object or habitat.

An object is said to be sterile when it is totally or completely free of viable microorganisms. By sterilization, microbial population is reduced to level that is considered safe by Public Health Standards.

Sterilization refers to any process that removes, kills, or deactivates all forms of life and other biological agents such as prions present in or on a specific surface, object, or fluid. Sterilization can be achieved through various means, including heat, chemicals, irradiation, high pressure, and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization, in that those methods reduce rather than eliminate all forms of life and biological agents present. After sterilization, an object is referred to as being sterile or aseptic

2. Disinfection:

It is the process of killing or inhibition or removal of microorganisms that may cause disease. It kills growing forms but not resistant spore forms. Thus, it is not sterilization. Normally, it is done by using chemicals. It is applicable for inanimate objects.

The chemical agent used for disinfection is called as *disinfectant.*

3. Antisepsis (Greek - anti - against and sepsis - putrefaction): It is the prevention of infection or sepsis, achieved by killing or inhibiting growth of pathogen. It is applied for living tissues.

4. Antiseptic: It is the chemical agent used to kill or inhibit microbial growth and metabolism. It is applied to body or living tissue.

5. Sanitization: It is the process of reducing level of microbial population which is considered safe by public health standards.
It is a sort of disinfection. It is usually followed in restaurants and for inanimate objects.

6. Germicide: It is an agent that kills growing forms but not necessarily the resistant spore forms of germs. To denote the type of an antimicrobial agent a suffix - cide is used (cide - is to kill) e.g. Bactericide - an agent that kills bacteria. Similarly, fungicide, algaecide, viricide etc. are the different types of Germicide.

7. Microbiostasis: It is the condition in which growth of microorganisms is prevented. (Static is originally a Greek word statikas - causing to stand or stopping.) Agents that are used for this purpose are called bacteriostatic, fungistatic etc.

8. Disinfectant

A disinfectant is a chemical substance or compound used to inactivate or destroy microorganisms on inert surfaces (non-living material).

9. Antiseptic

Antiseptic is a chemical substance or compound used to inactivate or destroy microorganisms on surfaces or tissues of living bodies.

Disinfectants or antiseptics do not necessarily kill all microorganisms, especially resistant bacterial spores; they are less effective than sterilization. Disinfectants are frequently used in hospitals, dental surgeries, kitchens, and bathrooms to kill infectious organisms. Bacterial endospores are most resistant to disinfectants, but some fungi, viruses and bacteria also possess some resistance. In water treatment, a disinfection step with chlorine, ultra-violet

(UV) radiation or ozonation can be included as treatment to remove pathogens from water.

Disinfectants can act on microorganisms in two different ways: growth inhibition (bacteriostasis, fungistasis) or lethal (killing) action (bactericidal, fungicidal or virucidal effects).

Disinfectants

- **Phenol and Phenolics:**

Joseph Lister used it to prevent infection of surgical wounds. Phenols are coal-tar derivatives. They act as disinfectants at high concentration and as antiseptics at low concentrations. They are bactericidal, fungicidal, mycobactericidal but are inactive against spores and most viruses.

Phenolic compounds are chemically defined as compounds containing hydroxylated aromatic rings, the hydroxy group being attached directly to the phenyl, substituted phenyl, or other aryl group. Phenolics are used as disinfectants in household cleaners and can have an anti-inflammatory effect in mouthwash. Butylated hydroxytoluene is a phenol that is a common antioxidant in food, cosmetics and industrial fluids. The corrosive phenolics are used for disinfection of ward floors, in discarding jars in laboratories and disinfection of bedpans.

Chlorhexidine can be used in an isopropanol solution for skin disinfection, or as an aqueous solution for wound irrigation. It is often used as an antiseptic hand wash.

Chloroxylenols are less irritant and can be used for topical purposes and are more effective against gram positive bacteria than gram negative bacteria.

Hexachlorophene is chlorinated diphenyl and is much less irritant. It has marked effect over gram positive bacteria but poor effect over gram negative bacteria, mycobacteria, fungi and viruses.

Triclosan is organic phenyl ether with good activity against gram positive bacteria and effective to some extent against many gram negative bacteria including Pseudomonas. It also has fair activity on fungi and viruses.

Examples: 5% phenol, 1-5% Cresol, 5% Lysol (a saponified cresol), hexachlorophene, chlorhexidine, chloroxylenol (Dettol). Nowadays many commercial preparations are available. The germicidal action depends upon concentration used.

Mode of action

Act by disruption of membranes, precipitation of proteins and inactivation of enzymes It causes denaturation or precipitation of proteins, inactivation of enzymes, leakage of amino acids, and disruption of cells. It is hypothesized that the lethal action of phenolic disinfectants is due to damage of permeability mechanisms, the repair of which is prevented by concomitant inhibition of energy-yielding metabolic reactions.Basically the lethal effect is because of damage to membrane.

Phenolics may be bacteriostatic or bactericidal. They are effective against vegetative cells than spores and viruses. Sometimes they are fungicidal. Effectiveness of phenol and phenolics is reduced in alkaline pH in presence of organic matter, in presence of soaps at low temperatures. They have unpleasant odour and they cause skin irritation. But they remain active for long time.

Use / application

These are used for disinfection of sputum, urine, faeces, contaminated equipments, commercial preparations.

e.g.Cresols, Xylenols, Lysol, Qrmophenylphenol, Hexachlorophene.

Phenol is quite toxic, however, and concentrated solutions cause severe but painless burns of the skin and mucous membranes. Less-toxic phenols, such as *n*-hexylresorcinol, have supplanted phenol itself in cough drops and other antiseptic applications. Butylated hydroxytoluene (BHT) has a much lower toxicity and is a common antioxidant in foods

Alcohols

Alcohol and alcohol plus Quaternary ammonium compounds comprise a class of proven surface sanitizers and disinfectants approved by the EPA (Environmental Protection Agency) and the Centres for Disease Control for use as a hospital grade disinfectant. Alcohols are most effective when combined with distilled water to facilitate diffusion through the cell membrane; 100% alcohol typically denatures only external membrane proteins. A mixture of 70%

ethanol or isopropanol diluted in water is effective against a wide spectrum of bacteria and viruses like Covid-19.

e. g. Ethyl alcohol, Isopropyl alcohol, Butyl Alcohol, Amyl Alcohol

Alcohols are most widely used disinfectants. Ethyl alcohol ($CH_3.CH_2.OH$) is effective against vegetative and non-sporeforming cells. It is bactericidal fungicidal and even viricidal. But it is not sporicidal. It is volatile *i.e.* can't remain for a longer time after its application. It is irritating. It is effective in 50-60% concentration. However 70% alcohol is most effective. It doesn't produce sterile conditions. Other higher alcohols are effective but as they are immiscible in water, not used widely.

Mode of action:

The primary mode of action is related to coagulation/denaturation of proteins and solubility of the alcohols in lipids. Without water, coagulation cannot occur, therefore a 70% solution of isopropyl alcohol or ethanol will be more effective than higher concentrations of alcohol. Ethyl alcohol denatures proteins. It also dissolves membrane lipids. It denatures cell wall and cell membrane proteins. Alcohols cause cell proteins to clump and lose their function. Specifically, the cell membranes lose their structure and collapse, thereby killing it.

Use and application:

Alcohol is often used to disinfect small surfaces (e.g. rubber stoppers of multiple-dose medication vials, and thermometers) and occasionally external surfaces of equipment (e.g. stethoscopes and ventilators). It is used to disinfect thermometers and other small instruments.

Alcohols are used as Antiseptic, Astringent, Deodorant, Evaporating water from the ear Liniment for muscle aches, Cleaning dry erase boards, Cleaning makeup brushes, Cleaning sinks and chrome, Deodorizing shoes, Disinfecting computer mouse and keyboard, Disinfecting mobile phone, Dissolving windshield frost, Getting rid of fruit flies, Creating homemade disinfectant, Cleaning jewellery, Preventing ring around the collar, Refreshing sponges, Removing hairspray from mirrors and tile, Removing ink and permanent marker stains, Removing stickers andCleaning stainless steel.

Limitations

- Alcohols evaporate quickly, leaving no residue.
- The efficacy of alcohol is limited if organic matter is present.
- Alcohols are highly flammable.
- Can damage rubber and plastic materials.
- Can cause skin irritation.
- Relatively poor sporicide
- Possesses unpleasant odour
- Skin and mucous membrane irritant.
- Toxic
- Materials incompatible (stains and odours)
- Relatively expensive.

Chlorine and Chlorine Compounds

Chlorine has been used for applications, such as the deactivation of pathogens in drinking water, swimming pool water and wastewater, for the disinfection of household areas and for textile bleaching. Hypochlorites, the most widely used of the chlorine disinfectants, are available as liquid (e.g., sodium hypochlorite) or solid (e.g., calcium hypochlorite). Aqueous solution of 5 – 6% sodium hypochlorite is called as household bleach. They have a broad spectrum of antimicrobial activity, do not leave toxic residues, are unaffected by water hardness, are inexpensive and fast acting, remove dried or fixed organisms and biofilms from surfaces and have a low incidence of serious toxicity.

Mercuric chloride is used as a disinfectant.

Hypochlorous acid (HOCl) produced releases nascent oxygen (o-) which oxidizes the enzymes and proteins and thus kills microorganisms.e. g. Liquid (sodium hypochlorite) or solid (calcium hypochlorite) or gas (Chlorine gas)

Mode of action-

Oxidizes the enzymes and proteins and thus kills microorganisms.

Limitations

- Relatively low protection against protozoa.
- Lower disinfection effectiveness in turbid waters.
- Potential taste and odour objections.
- Potential long-term effects of chlorination by-products.

Iodine

Tincture of iodine (2% iodine in 70% alcohol) is an antiseptic. Iodine can be combined with neutral carrier polymers such as polyvinylpyrrolidone to prepare iodophores such as povidone-iodine. Iodophores permit slow release and reduce the irritation of the antiseptic. For hand washing iodophores are diluted in 50% alcohol. 10% Povidone Iodine is used undiluted in pre and postoperative skin disinfection.

Mode of action:

They are oxidizing agents and cause damage by oxidation of essential sulfydryl groups of enzymes.

Limitations

1. Concentrated iodine compounds can be irritating to the skin,
2. Can stain clothes or damage rubber and some metals.
3. Iodine agents are inactivated by QACs and organic debris.

Heavy Metals

The oligodynamic effect (from Greek *oligos*, "few", and *dynamis*, "force") is a biocidal effect of metals, especially heavy metals, that occurs even in low concentrations.

Scholarly texts from ancient India promoted the use of brass and silver in ritual cleansing practice as well as in consumption of food and drink. The ancient Indian medical text Sushruta Samhita promoted the use of specific metals in surgical procedures as a measure to prevent infection. Brass door-knobs and silverware both exhibit this effect to an extent.

The metals react with thiol (-SH) or amine (-NH) groups of proteins, a mode of action to which microorganisms may develop resistance.

USES

Antimony

Orthoesters of diarylstibinic acids are fungicides and bactericides, used in paints, plastics, and fibers. Trivalent organic antimony was used in therapy for schistosomiasis (Disease caused by parasitic worms).

Arsenic

For many decades, arsenic was used medicinally to treat syphilis. It is still used in sheep dips, rat poisons, wood preservatives, weed killers, and other pesticides. Arsenic is also still used for murder by poisoning, for which use it has a long and continuing history in both literature and fact.

Bismuth

Bismuth compounds have been used because of their astringent (shrink), anti-inflammatory, bacteriostatic and disinfecting actions. In dermatology bismuth subgallate is still used in vulnerary salves (something applied on skin to deodorize and heal wound) and powders as well as in antimycotics. In the past, bismuth has also been used to treat syphilis and malaria.

Boron

Boric acid can be used as an antiseptic for minor burns or cuts and is sometimes used in salves and dressings, such as boracic lint. Boric acid is applied in a very dilute solution as an eye wash. Dilute boric acid can be used as a vaginal douche to treat bacterial vaginitis due to excessive alkalinity.

Boric acid was first registered in the US as an insecticide in 1948 for control of cockroaches, termites, fire ants, fleas, silverfish, and many other insects. The product is generally considered to be safe to use in household kitchens to control cockroaches and ants.

Copper

Brass vessels release a small amount of copper ions into stored water, thus killing fecal bacterial counts as high as 1 million bacteria per milliliter.

Copper sulfate mixed with lime (Bordeaux mixture) is used as a fungicide and antihelminthic. Copper sulfate is used chiefly to destroy green algae (algicide) that grow in reservoirs, stock ponds, swimming pools, and fish tanks. Copper 8-hydroxyquinoline is sometimes included in paint to prevent mildew. Paint containing copper is used on boat bottoms to prevent barnacle growth (biofouling).

Gold

Many bacteria have the capability to form a three-dimensional, strongly adherent network called 'biofilm'. Biofilms provide adherence, resourcing nutrients and offer protection to bacterial cells. They are involved in pathogenesis, disease progression and resistance to almost all classical antibiotics. Gold and silver nanoparticles and their respective ions exert antimicrobial action by damaging the biofilm structure, biofilm components and hampering bacterial metabolism via various mechanisms. While exerting the antimicrobial activity, these nanoparticles approach the biofilm, penetrate it, migrate internally and interact with key components of biofilm such as polysaccharides, proteins, nucleic acids and lipids via electrostatic, hydrophobic, hydrogen-bonding, Van der Waals and ionic interactions. Gold is used in dental inlays and inhibits the growth of bacteria and in treatment of tuberculosis and rheumatoid arthritis.

Lead

Physicians prescribed various forms of lead to heal ailments ranging from constipation to infectious diseases such as the plague. Lead was also used to preserve or sweeten wine.[15] Lead arsenate is used in insecticides and herbicides.[16] Some organic lead compounds are used as industrial biocides: thiomethyl triphenyllead is used as an antifungal agent, cotton preservative, and lubricant additive; thiopropyl triphenyllead as a rodent repellant; tributyllead acetate as a wood and cotton preservative; tributyllead imidazole as a lubricant additive and cotton preservative.

Mercury (1:100 / 1:1000)

Phenylmercuric borate and acetate were used for disinfecting mucous membranes at an effective concentration of 0.07% in aqueous solutions. Due to toxicological and ecotoxicological reasons phenylmercury salts are no longer in use. However, some surgeons use mercurochrome despite toxicological objections. Mercurochrome is still available to purchase in Australia to use on minor wounds.

Dental amalgam used in fillings inhibits bacterial reproduction. Organic mercury compounds have been used as topical disinfectants (thimerosal, nitromersol, and merbromin) and preservatives in medical preparations (thimerosal) and grain products (both methyl and ethyl mercurials). Mercury was used in the treatment of syphilis. Calomel was commonly used in infant teething powders in the 1930s and 1940s. Mercurials are also used agriculturally as insecticides and fungicides.

Silver (1:1000)

The metabolism of bacteria is adversely affected by silver ions at concentrations of 0.01–0.1 mg/L. Therefore, even less soluble silver compounds, such as silver chloride, also act as bactericides or germicides, but not the much less soluble silver sulfide. In the presence of atmospheric oxygen, metallic silver also has a bactericidal effect due to the formation of silver oxide, which is soluble enough to cause it. Even objects with a solid silver surface (e.g., table silver, silver coins, or silver foil) have a bactericidal effect. Silver drinking vessels were carried by military commanders on expeditions for protection against disease. It was once common to place silver foil or even silver coins on wounds for the same reason.

Silver sulfadiazine is used as an antiseptic ointment for extensive burns. An equilibrium dispersion of colloidal silver with dissolved silver ions can be used to purify drinking water at sea. Silver is incorporated into medical implants and devices such as catheters. Surfacine (silver iodide) is a relatively new antimicrobial for application to surfaces. Silver-impregnated wound dressings have proven especially useful against antibiotic-resistant bacteria. Silver nitrate is used as a hemostatic, antiseptic and astringent. At one time, many states required that the eyes of newborns be treated with a few drops of silver nitrate to guard against an infection of the eyes called gonorrheal neonatal ophthalmia, which the infants might have contracted as they passed through the birth canal. Silver ions are increasingly incorporated into many hard surfaces, such as plastics and steel, as a way to control microbial growth on items such as toilet seats, stethoscopes, and even refrigerator doors. Among the newer products being sold are plastic food containers infused with silver nanoparticles, which are intended to keep food fresher, and silver-infused athletic shirts and socks, which claim to minimize odors

Thallium

Thallium compounds such as thallium sulfate have been used for impregnating wood and leather to kill fungal spores and bacteria, and for the protection of textiles from attack by moths. Thallium sulfate has been used as a depilatory and in the treatment of venereal disease, skin fungal infections, and tuberculosis.[22]

Tin

Tetrabutyltin is used as an antifouling paint for ships, for the prevention of slimes in industrial recirculating water systems, for combating freshwater snails that cause bilharzia, as a wood and textile preservative, and as a disinfectant. Tricyclohexyltin hydroxide is used as an acaricide. Triphenyltin hydroxide and triphenyltin acetate are used as fungicides.[23]

Zinc

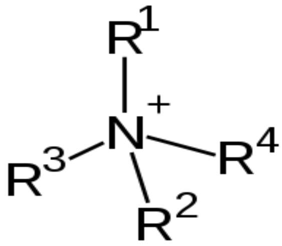
Zinc oxide is used as a weak antiseptic (and sunscreen), and in paints as a white pigment and mold-growth inhibitor. Zinc chloride is a common ingredient in mouthwashes and deodorants, and zinc pyrithione is an ingredient in antidandruff shampoos. Galvanized (zinc-coated) fittings on roofs impede the growth of algae. Copper- and zinc-treated shingles are available. Zinc iodide and zinc sulfate are used as topical antiseptics.[25]

Safety

Besides the individual toxic effects of each metal, a wide range of metals are nephrotoxic in humans and/or in animals. Some metals and their compounds are carcinogenic to humans. A few metals, such as lead and mercury, can cross the placental barrier and adversely affect fetal development. Several (cadmium, zinc, copper, and mercury) can induce special protein complexes called metallothioneins.

❖ Quaternary ammonium compounds

Quaternary ammonium cations, also known as **quats**, are positively charged polyatomic ions of the structure NR_4^+, R being an alkyl group or an aryl group. Unlike the ammonium ion (NH_4^+) and the primary, secondary, or tertiary ammonium cations, the quaternary ammonium cations are permanently charged, independent of the pH of their solution. **Quaternary ammonium salts** or **quaternary ammonium compounds** are salts of quaternary ammonium cations with an anion.



Quaternary ammonium compounds have antimicrobial activity. Certain quaternary ammonium compounds, especially those containing long alkyl chains, are used as antimicrobials and disinfectants. Examples are benzalkonium chloride, benzethonium chloride, methylbenzethonium chloride, cetalkonium chloride, cetylpyridinium chloride, cetrimonium, cetrimide, dofanium chloride, tetraethylammonium bromide, didecyldimethylammonium chloride and domiphen bromide. Quats are good against fungi, amoeba, and enveloped viruses. Quats act by disrupting the cell membrane
These are a large group of related compounds. Some concentrated formulations have been shown to be effective low-level disinfectants. Quaternary ammonium compounds at or above 200 ppm plus alcohol solutions exhibit efficacy against difficult to kill non-enveloped viruses such as norovirus, rotavirus, or polio virus.

Mode of action:

It increases the permeability of the cell and coagulation of cell

Limitations

Some QACs can cause allergic skin rashes even with very limited exposure. Eye contact with QACs can cause burning of the eyes. Splashing concentrated QAC solution in your eye can cause severe injury including blindness. Breathing in QACs can cause irritation of the nose and throat.

The disadvantage is when quaternary ammonium is mixed with organic matter it loses its effectiveness. This makes it an ineffective disinfectant in situations where blood, urine, fecal matter or soil may be present.

❖ Gaseous Sterilization

- Gaseous sterilization involves the process of exposing equipment or devices to different gases in a closed heated or pressurized chamber.
- Gaseous sterilization is a more effective technique as gases can pass through a tiny orifice and provide more effective results.
- Besides, gases are commonly used along with heat treatment which also facilitates the functioning of the gases.
- However, there is an issue of release of some toxic gases during the process which needs to be removed regularly from the system.
- The mechanism of action is different for different types of gases.
- Some of the common gases used for gaseous sterilization are explained below:

❖ Aldehydes

Aldehydes have antibacterial, sporicidal, antifungal and antiviral activity. 40% Formaldehyde (formalin) is used for surface disinfection and fumigation of rooms, chambers, operation theatres, biological safety cabinets, wards, sick rooms etc. It also sterilizes bedding, furniture and books. 10% formalin with 0.5% tetraborate sterilizes clean metal instruments. 2% gluteraldehyde is used to sterilize thermometers, cystoscopes, bronchoscopes, centrifuges, anasethetic equipments etc.

e. g. formaldehyde and glutaraldehyde

Mode of action-

Acts through alkylation of amino-, carboxyl- or hydroxyl group, and probably damages nucleic acids. It kills all microorganisms, including spores.

Denatures cell wall and cell membrane proteins, inhibit the activity of enzymes in the cell.

Limitations

Formaldehyde is colourless but has a pungent odor.When formaldehyde is present in the air at levels exceeding 0.1 ppm, some individuals may experience adverse effects such as watery eyes; burning sensations in the eyes, nose, and throat; coughing; wheezing; nausea; and skin irritation.

- **Ethylene oxide**

Ethylene oxide (EO, EtO) gas treatment is one of the common methods used to sterilize, pasteurize, or disinfect items because of its wide range of material compatibility. It is also used to process items that are sensitive to processing with other methods, such as radiation (gamma, electron beam, X-ray), heat (moist or dry), or other chemicals. Ethylene oxide treatment is the most common chemical sterilization method, used for approximately 70% of total sterilizations, and for over 50% of all disposable medical devices.

Ethylene oxide treatment is generally carried out between 30 and 60 °C (86 and 140 °F) with relative humidity above 30% and a gas concentration between 200 and 800 mg/l. typically, the process lasts for several hours. Ethylene oxide is highly effective, as it penetrates all porous materials, and it can penetrate through some plastic materials and films. Ethylene oxide kills all known microorganisms, such as bacteria, viruses, and fungi, and is compatible with almost all materials even when repeatedly applied. It is flammable, toxic, and carcinogenic; however, only with a reported potential for some adverse health effects when not used in compliance with published requirements. Ethylene oxide sterilizers and processes require biological validation after sterilizer installation, significant repairs or process changes.

The most common EO processing method is the gas chamber method. To benefit from economies of scale, EO has traditionally been delivered by filling a large chamber with a combination of gaseous EO either as pure EO, or with other gases used as diluents; diluents include chlorofluorocarbons (CFCs), hydrochlorofluorocarbons (HCFCs), and carbon dioxide.

Ethylene oxide is still widely used by medical device manufacturers. Since EO is explosive at concentrations above 3%, EO was traditionally supplied with an inert carrier gas, such as a CFC or HCFC. The use of CFCs or HCFCs as the carrier gas was banned because of concerns of ozone depletion. These halogenated hydrocarbons are being replaced by systems using 100% EO, because of regulations and the high cost of the blends. In hospitals, most EO sterilizers use single-use cartridges because of the convenience and ease of use compared to the former plumbed gas cylinders of EO blends.

Mode of action

Ethylene oxide. EO's microbicidal activity is a result of alkylation of proteins, DNA, and RNA in microorganisms, which prevents normal cellular metabolism and replication and thus renders affected microbes nonviable.

Limitations

EPA has concluded that ethylene oxide is carcinogenic to humans by the inhalation route of exposure. Evidence in humans indicates that exposure to ethylene oxide increases the risk of lymphoid cancer and, for females, breast cancer.

Acute exposures to EtO gas may result in respiratory irritation and lung injury, headache, nausea, vomiting, diarrhoea, shortness of breath, and cyanosis. Chronic exposure has been associated with the occurrence of cancer, reproductive effects, mutagenic changes, neurotoxicity, and sensitization.

❖ **Sulfur dioxide**

Sulfur dioxide or sulphur dioxide is the chemical compound with the formula SO_2. It is a toxic gas responsible for the smell of burnt matches. It is released naturally by volcanic activity and is produced as a by-product of copper extraction and the burning of sulfur-bearing fossil fuels. Sulfur dioxide has a pungent smell like nitric acid

As Food preservative

Sulfur dioxide is sometimes used as a preservative for dried apricots, dried figs, and other dried fruits, owing to its antimicrobial properties and ability to prevent oxidation. As a preservative, it maintains the colorful appearance of the fruit and prevents rotting. It is also added to sulfured molasses. Sublimed sulfite is ignited and burned in an enclosed space with the fruits. This is usually done outdoors. Fruits may be sulfured by dipping them into either sodium bisulfite, sodium sulfite or sodium metabisulfite.

In wine making

Sulfur dioxide was first used in winemaking by the Romans, when they discovered that burning sulfur candles inside empty wine vessels keeps them fresh and free from vinegar smell.

As a fumigant

In the beginning of the 20th century, sulfur dioxide was used in Buenos Aires as a fumigant to kill rats that carried the Yersinia pestis bacterium, which causes bubonic plague. The application was successful, and the application of this method was extended to other areas in South America. In Buenos Aires, where these apparatuses were known as Sulfurozador, but later also in Rio de Janeiro, New Orleans and San Francisco, the sulfur dioxide treatment machines were brought into the streets to enable extensive disinfection campaigns, with effective results.

Limitations

Sulfur dioxide affects the respiratory system, particularly lung function, and can irritate the eyes. Sulfur dioxide irritates the respiratory tract and increases the risk of tract infections. It causes coughing, mucus secretion and aggravates conditions such as asthma and chronic bronchitis.

Sulfur dioxide's contribution to acid rain can cause direct harm to trees and plants by damaging exposed tissues and, subsequently, decreasing plant growth. Other sensitive ecosystems and waterways are also impacted by acid rain

Sulphur dioxide is widely used in the food and drinks industries for its properties as a preservative and antioxidant. Whilst harmless to healthy persons when used in recommended concentrations, it can induce asthma when inhaled or ingested by sensitive subjects, even in high dilution.

Large volumes of SO_2 erupted frequently appear to overdrive the oxidizing capacity of the atmosphere resulting in very rapid Warming.

❖ Beta propiolactone

β-Propiolactone is an organic compound of the lactone family, with a four-membered ring. It is a colourless liquid with a slightly sweet odour.

Propiolactone is a disinfectant used for the sterilization of blood plasma, vaccines, tissue grafts, surgical instruments, and enzymes. It has been used against bacteria, fungi, and virus. It is currently FDA approved for its use as an indirect additive used in food contact substances. Propiolactone was first commercially available in the United States in 1958.

Beta-Propiolactone is used for vaccines, tissue grafts, surgical instruments, and enzymes, as a sterilant of blood plasma, water, milk, and nutrient broth, and as a vapor-phase disinfectant in enclosed spaces.

Propiolactone was once widely produced as an intermediate in the production of acrylic acid and its esters. That application has been largely displaced in favour of safer and less expensive alternatives. β-Propiolactone is an excellent sterilizing and sporicidal agent, but its carcinogenicity precludes that use. It is used to inactivate a wide variety of viruses, for example as a step in vaccine production.

Mechanism of action

Propiolactone is an alkylating agent that acts through alkylation of carboxyl- and hydroxyl- groups. The lactone ring splits either at the first or third carbon. Propiolactone reacts with polynucleotides and DNA, mainly at N7 of guanine and N1 of adenine to form carboxyethyl derivatives. It also forms adducts with N3 of cytosine and thymine.

Limitatons

Beta-Propiolactone can severely irritate and burn the eyes with possible permanent damage (corneal opacities). Contact can irritate and burn the skin. Breathing beta-Propiolactone can irritate the nose, throat and lungs causing coughing, wheezing and/or shortness of breath.

Beta-Propiolactone may be a carcinogen in humans since it has been shown to cause skin and stomach cancers in animals. * Many scientists believe there is no safe level of exposure to a carcinogen.

❖ PHENOL COEFFICIENT TEST

Determining the Phenol Coefficient of Test Disinfectant

Phenol coefficient test is one of the methods used to estimate the disinfecting power of any disinfectant.

In phenol co-efficient method, we test the efficiency of chemical disinfectants. Disinfectant is an agent that inhibits or kills disease causing microorganisms. Disinfectant can be physical (heat or radiation) or chemical.

The efficiency of any disinfectant could be tested at two levels. The first one is how many microorganisms it can kill, and second one is how fast it can kill. In this way, the disinfectants are tested by observing how many microorganisms are being killed in given time.

In phenol co-efficient method, the test disinfectant is compared with phenol efficiency and indicated by its co-efficient. Phenol is a 6 carbon aromatic organic compound containing hydroxyl group. Phenol is known to kill or inactivate microbes by inhibiting microbial enzymes and shutting down the biochemical and molecular pathways. This eventually leads to the death of the microorganism. Phenol is also known to disrupt cytoplasmic membrane and leading to leakage of cellular content.

Surgeon Joseph Lister used phenol as disinfectant for the first time while operating his patient to avoid the infection. In 1903, Rideal and Walker developed the phenol co-efficient protocol to test the efficiency of diverse chemical disinfectants with that of the phenol. And hence, this test is also known by Rideal and Walker method.

Principle:

The antimicrobial activity of test disinfectant is determined by dividing the highest dilution of the chemical being tested that destroyed the microorganisms in 10 minutes but not in 5 minutes by the highest dilution of phenol that destroyed the microorganisms in 10 minutes but not in 5 minutes. When phenol co-efficient of test disinfectant is equal to 1, then it means that the test disinfectant efficiency is equivalent to phenol. When phenol co-efficient is more than 1, it means the test disinfectant is better than phenol.

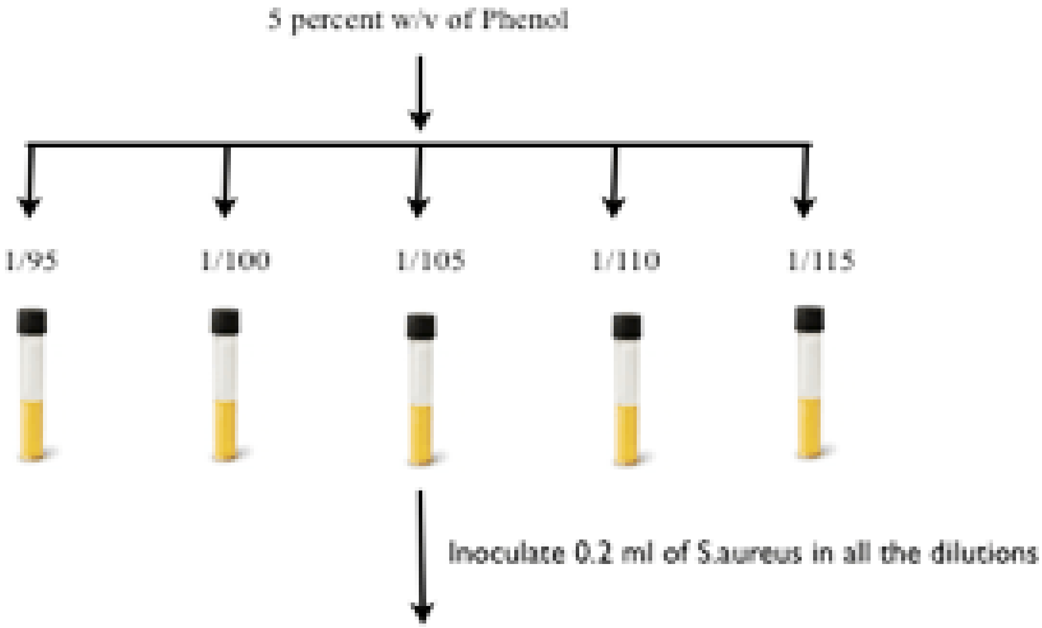
However, if it is less than one, then it indicates that the test disinfectant is inefficient than phenol. Hence, the value 1 or less than 1 or more than 1, indicates the efficiency of disinfectant with respect to phenol.

Requirements:

1. Phenol
2. Test Disinfectant
3. Gram positive bacteria – *S.aureus*
4. Nutrient broth

Procedure:

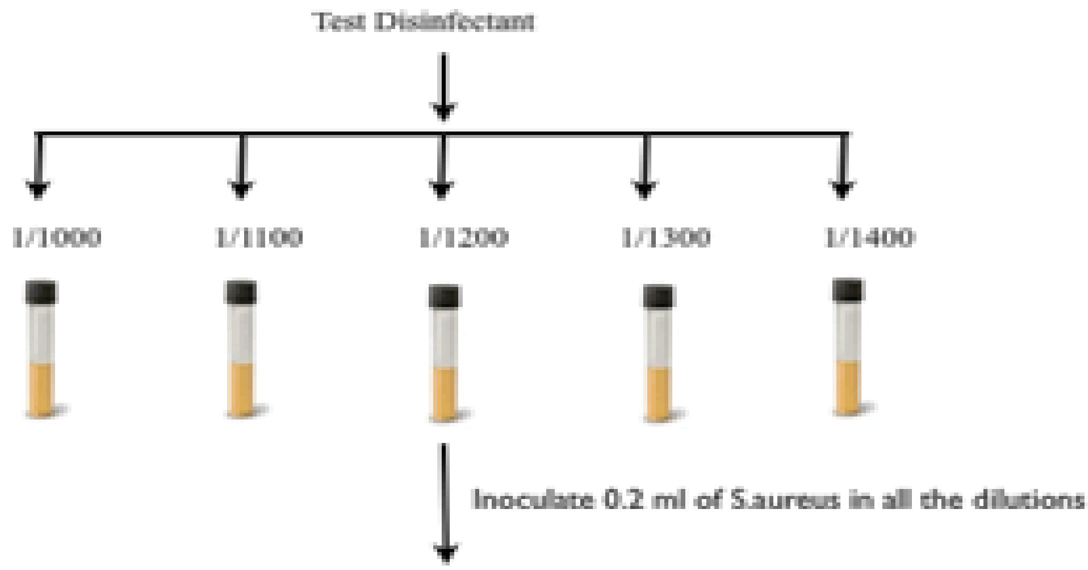
1. Standard Phenol: The stock solution (5 % w/v) of phenol is prepared. It is prepared by adding 5 gm. of phenol in 100 ml of sterile distil water. From this stock solution, test dilutions are prepared of 1/95, 1/100, 1/105, 1/110, 1/115. it means one part of phenol in 95, 100,105, 110 or 115 parts of water respectively.
2. Test Disinfectant dilution: The test disinfectant is diluted in dilution of 1/1000, 1100, 1200, 1300 and 1400.
3. 5 ml of these dilutions of phenol and disinfectant are dispensed in different test tubes.
4. Then 0.2 ml of 24 hours incubated *S.aureus* are inoculated in all the dilution test tubes. Note the time of your inoculation.
5. Stir the test tube, so that *S. aureus* comes into the contact of the disinfectant.
6. After the interval of 5, 10 and 15 minutes, transfer the loopful of culture from all different dilution of disinfectant in which *S. aureus* is inoculated to Nutrient broth.
7. Incubate the test tubes for 48 hours at 37 C.



1/95	1/100	1/105	1/110	1/115	
-	-	+	+	+	5 min
-	-	-	+	+	10 min
-	-	-	-	-	15 min

+

Indicates – growth – Indicates no growth



I/1000	I/1100	I/1200	I/1300	I/1400	
-	-	+	+	+	5 min
-	-	-	+	+	10 min
-	-	-	-	-	15 min

+ Indicates – growth – Indicates no growth

Results:

$$\text{Phenol Co-efficient} = \frac{\text{Highest Dilution of test disinfectant that destroyed microorganisms in 10 min and not in 5 min}}{\text{Highest Dilution of Phenol that destroyed microorganisms in 10 min and not in 5 min}}$$

Phenol Co-efficient for given disinfectant = 1200/105 = 11.42

Conclusion: The test Disinfectant is 11.42 times better then phenol.