MICROBIAL BIOTECHNOLOGY

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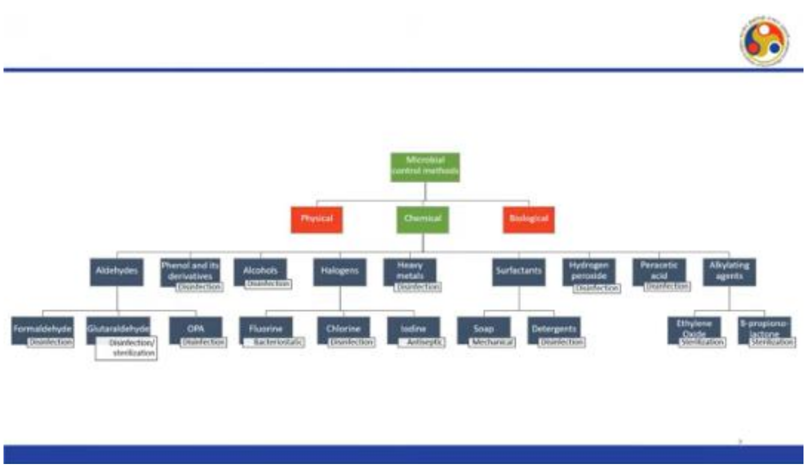
Lecture-17

Lec 17: Control methods: Chemical methods

Hello everyone, welcome back to my course on microbial biotechnology. We are in module 5 where we are discussing the control of microorganisms. Today we will be focusing on the chemical methods of microbial control. So, in the last lecture we have discussed about the physical methods of microbial control. So, today we will be discussing about the chemical methods of microbial control under which we have various agents like aldehydes,

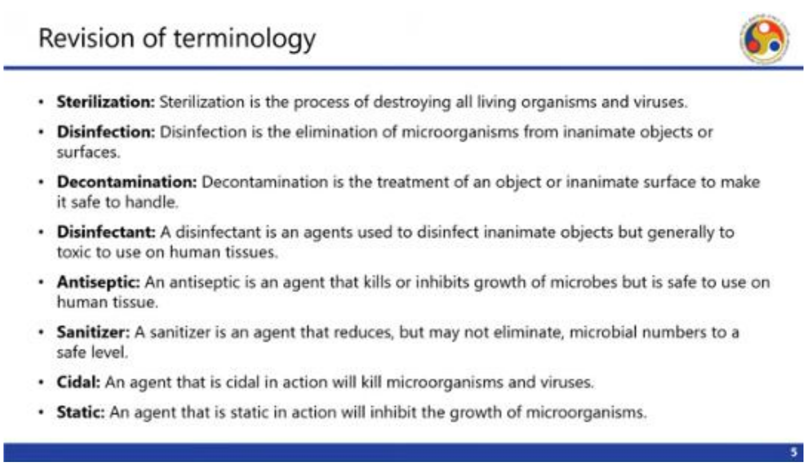
phenol and its derivatives. And then we have alcohols. We also use certain halogens like fluorine, chlorine, iodine. Then we have certain metals which we use for disinfection. And then we have surfactants like sorbs and detergents and then hydrogen peroxide, parasitic acid, and alkylating agents like ethylene oxide and beta-propionate electron.



In aldehydes, we have various compounds like formaldehyde, glutaraldehyde, etc. So, we'll be discussing these in detail one by one. Before that, let us have an overview of the chemical methods of microbial control. Chemical control of microorganisms refer to the use of various chemical substances which we have discussed in the earlier list or compounds which prevent, inhibit or eliminate growth and spread of microorganisms. Various chemicals exhibit varying degree of effectiveness against specific types of microorganisms.

Chemical agents used for this purpose are known as antimicrobial agents or disinfectants. These substances can function by disrupting the cell walls or membranes of microorganisms, interfering with their metabolism or damaging their genetic material. Antimicrobial chemicals are often utilized in various settings such as healthcare facilities, research laboratories, food processing industries and households to maintain hygiene, prevent infections and control the spread of disease. So, in this lecture briefly we will be discussing about some of the terminologies we learned in the last lecture and the various chemical agents which we have already briefed in the table earlier. So, let us briefly have a recap of the terminology that we learned in the last class.

So, sterilization is the process of destroying all living microorganisms and viruses. Disinfection is the elimination of microorganisms from inanimate objects or surfaces. Decontamination is the treatment of an object or inanimate surface to make it safe to handle. Disinfectant are agents used to disinfect inanimate objects that are generally toxic to use on human tissues. Antiseptic is an agent that kills or inhibits growth of microbes but is safe to use on a human tissue.



Sanitary is an agent that reduces but may not eliminate microbial numbers to a safe level and it can be used on humans or human tissue. Cydal are basically an agent which will kill microorganisms and viruses. So, we'll call them as fungicidal or bactericidal. And then we have certain agents which we call as static agents. These will inhibit the growth of microorganisms like bacteriostatic or fungistatic.

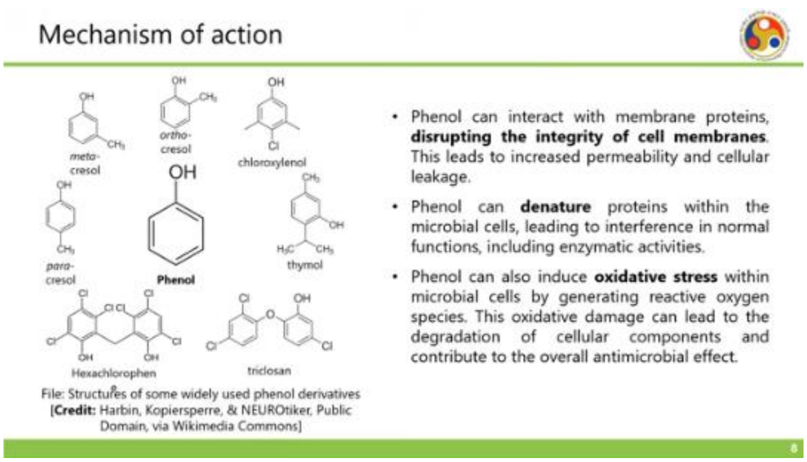
Now, all these chemicals that we are going to discuss today have certain properties which make them good chemical control agents. So, what are these properties? Number one, the chemicals should show a broad spectrum of antimicrobial activity even at low concentrations. For effective use, it should be soluble in water or other commonly used solvents if not water. This agent should have the capacity to effectively penetrate biological membranes for germicidal action.

In a solution, the chemicals must be stable and should not show loss in germicidal action. Chemicals should be selectively toxic towards the microbes and should not be toxic to humans and animals. Chemicals should be non-corroding and non-staining. These chemicals must be widely available and cost-effective to be the most preferred. Now let us discuss one of these chemicals, phenol, which is also very common in our household use.

So, this is a known carboxylic acid, and it is one of the earliest antiseptic substances. Here you can see a vial of carboxylic acid, then you can see a medallion commemorating Joseph Lister's development of carboxylic spray for antiseptic use during surgery. Phenol exhibits bacteriostatic properties within a concentration range of around 0.1% to 1% and demonstrates bactericidal and fungicidal effects at 1% to 2%. The concentration of phenol will actually also dictate its property, whether it is going to be static or it is going to be cidal. So, as already discussed, Joseph Lister pioneered antiseptic surgery by introducing carboxylic acid to reduce infections during operations in the late 19th century.

The mechanism of phenol is disinfectant and involves its ability to disrupt and denature proteins, leading to the inactivation of microorganisms. So, you have here various structures of some widely used phenol derivatives, and you can see metacresol, then orthocresol, and also triclosan over here. What are the mechanisms of action? As already briefly discussed, phenol can interact with membrane proteins, disrupt the integrity of the cell membranes, and this leads to increased permeability and cellular leakage. Phenol also affects DNA and proteins within the microbial cells,

leading to interference in normal functions, including enzymatic activities. Phenol can also induce oxidative stress within microbial cells by generating reactive oxygen species. This oxidative damage can lead to the degradation of cellular components and contribute to the overall antimicrobial effect. So, these are some of the phenol derivatives, like the cresols, including orthocresols, metacresols, and paracresols, which are effective microbial agents.



They are widely used in the disinfectant industry, antiseptics, and industrial settings for their broad-spectrum microbial activity. So, this is, for example, a formulation with triclosan at roughly around 0.75%. This triclosan, as we are discussing, is a chlorinated aromatic compound with broad-spectrum antimicrobial properties. It inhibits bacterial fatty acid synthesis.

Disrupting cell membranes, it is commonly used in consumer products like soaps, toothpastes, and deodorants as an antibacterial agent. Another phenol derivative is chloroxylenol, for example, which is marketed as Dettol. It is a widely used brand of antiseptic and disinfectant, with chloroxylenol as its active ingredient. Then we have thymol, which is produced by the alkylation of metacresol and propene, disrupts cell membranes, and exhibits antioxidant activities. These are found in some mouthwashes like Listerine and are used in natural antimicrobial formulations as disinfectants.

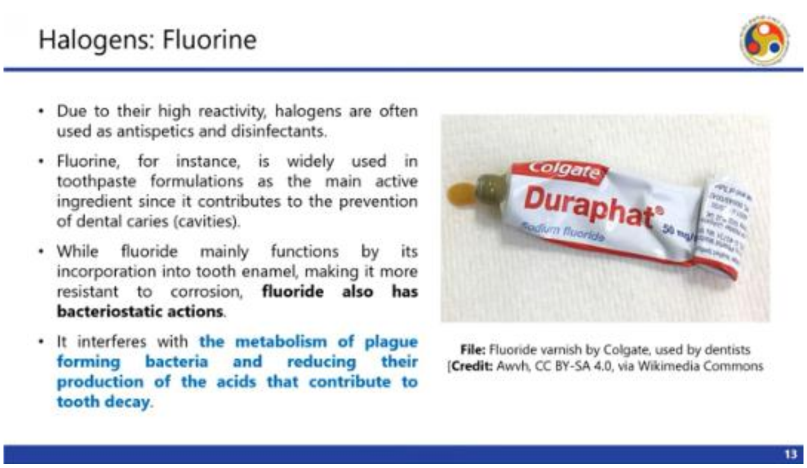
The next class of compounds or agents used as disinfectants are alcohols. Alcohols are inexpensive and easily available. These are widely used in disinfecting laboratory and chemical instruments and as hand sanitizers. The germicidal effect of alcohol increases with molecular weight. Methanol is the least germicidal.

The effect increases in order for ethanol, propanol, butanol, and so on. Ethanol and propanol are the most commonly used alcohols as disinfectant agents. For example, ethanol concentrations of around 60% to 80% also have a virucidal effect against lipophilic viruses and many hydrophilic viruses. Here you can see two brands of hand sanitizers. On the left, it has an isopropyl-based formulation.

Then, what is the mechanism of action of alcohol in controlling microbes? Alcohol, in appropriate concentrations, works by first solubilizing the lipid bilayer of the cell wall in gram-negative cells and membranes to create pores. Alcohol then enters the cytoplasm through these pores to denature cellular proteins, finally killing the bacteria. It must be mentioned that 70% alcohol is more effective than absolute alcohol.

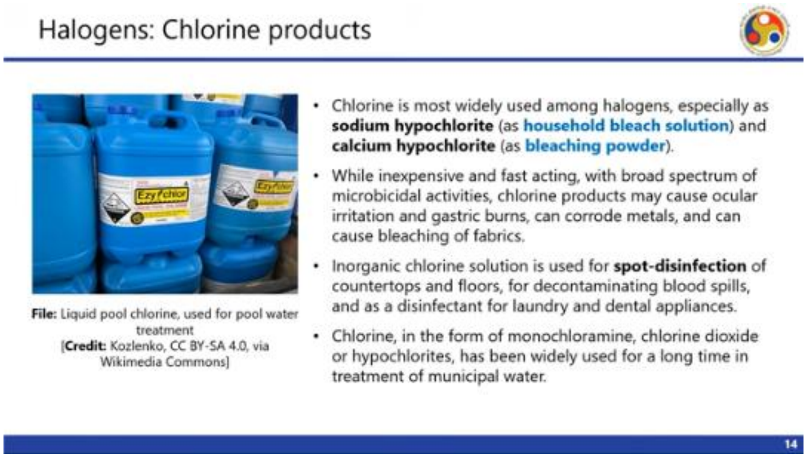
This is because absolute alcohol causes extreme dehydration, making cells shrink and thus preventing alcohol from entering the cell. This leads to bacteriostatic action but not bactericidal effects. At 70% concentration, both dehydrating and denaturing effects work almost in equilibrium, causing bactericidal effects. One major disadvantage of using alcohol is its volatility, which limits its effectiveness to immediate application.

Let us now discuss the halogens—for example, fluorine—and then you have these fluoride varnishes by Colgate, used by dentists in this toothpaste over here. So, due to their high reactivity, halogens are often used as antiseptics and disinfectants. Fluorine, for instance, is widely used in toothpaste formulations as the main active ingredient since it helps prevent dental caries. While fluoride mainly functions by incorporating into tooth enamel, making it more resistant to corrosion, it also has bacteriostatic effects. It interferes with the metabolism of plaque-forming bacteria, reducing their acid production, which contributes to decay.



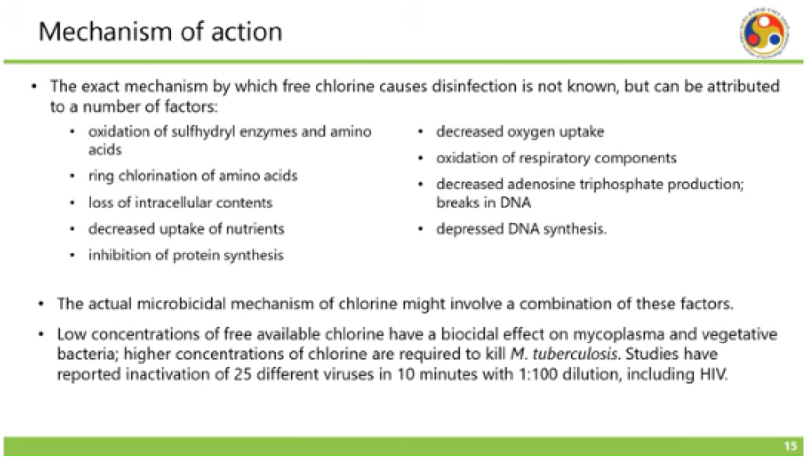
Chlorine is another halogen which is widely used among the halogens overall, especially as sodium hypochlorite in household bleach solutions and calcium hypochlorite as bleaching powder. These are inexpensive and very fast-acting. They have a broad spectrum of microbial activity. Chlorine products may cause ocular irritation and gastric burns, of course. They can corrode metals and bleach fabrics.

Inorganic chlorine solutions are used for spot disinfection of countertops and floors, for decontaminating blood spills, and as disinfectants for laundry and dental applications. Chlorine in the form of monochloramine, chlorine dioxide, or hypochlorite has been widely used for a long time in the treatment of municipal water. Now, what is the mechanism of action of chlorine? The exact mechanism by which free chlorine causes disinfection is not fully understood. But it can be attributed to a number of factors, such as oxidation of sulfhydryl enzymes and amino acids, ring chlorination of amino acids, loss of intracellular contents, decreased uptake of nutrients, inhibition of protein synthesis, and decreased oxygen uptake.

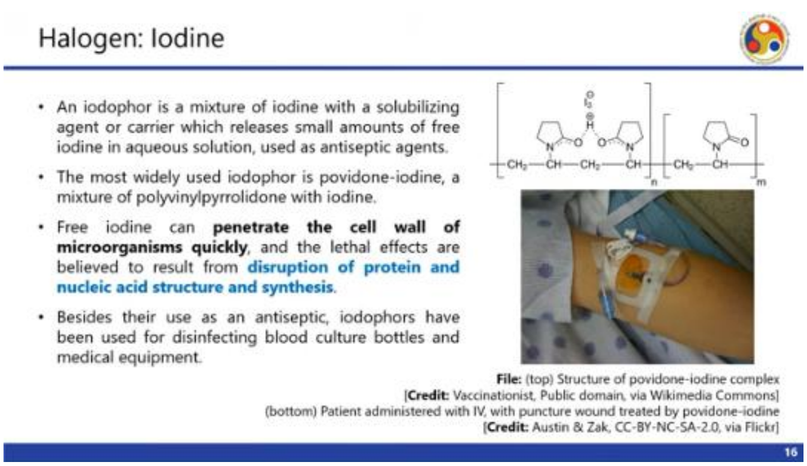


Oxidation of respiratory components, decreased adenosine triphosphate production, breaks in DNA, and depressed DNA synthesis. The actual microbiocidal mechanism of chlorine might involve a combination of many of these factors. Low concentrations of freely available chlorine have a biocidal effect on mycoplasma and vegetative bacteria. Higher concentrations of chlorine are required to kill M. tuberculosis or Mycobacterium tuberculosis.

Studies have reported inactivation of 25 different viruses in 10 minutes with a 1:100 dilution, including HIV. Another halogen that is used as a disinfectant agent is iodine. An iodophore is a mixture of iodine with a solubilizing agent or carrier, which releases small amounts of free iodine in aqueous solution, used as an antiseptic agent. So, you can see here the structure of a povidone-iodine complex. In this particular figure, it is being used as a disinfectant in medical clinics.



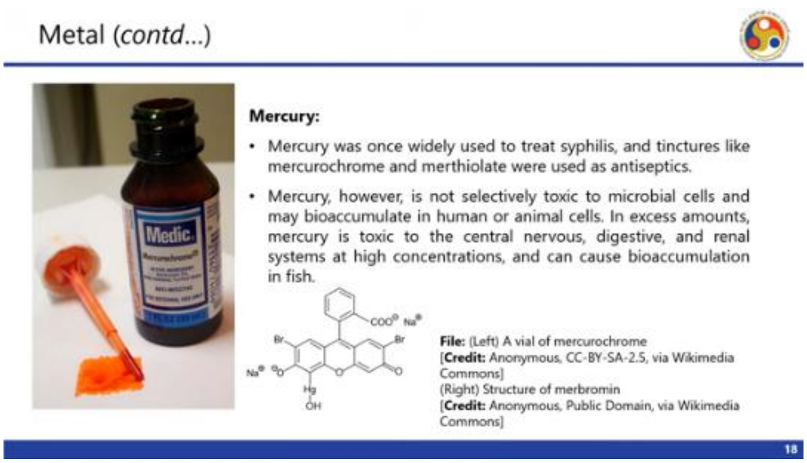
The most widely used, IDO4, as we have already discussed, the povidone-iodine, is a mixture of polyvinylpyrrolidone with iodine. Free iodine can penetrate the cell wall of microorganisms quickly, and little effect is believed to result from disruption of protein and nucleic acid structure and synthesis. Besides its use as an antiseptic, iodophores have been used for disinfecting blood culture bottles and medical equipment. Let us now discuss certain metals which are used as chemical control agents. Metals have been used as disinfectants for a very long time, but their use has now been limited due to their toxicity.



Metals bind to sulfur-containing amino acids of cellular proteins and can inhibit enzymatic activity. Let's discuss copper. Copper sulfate is a common algaecide, used to control algae growth in swimming pools and fish tanks. Then we have zinc.

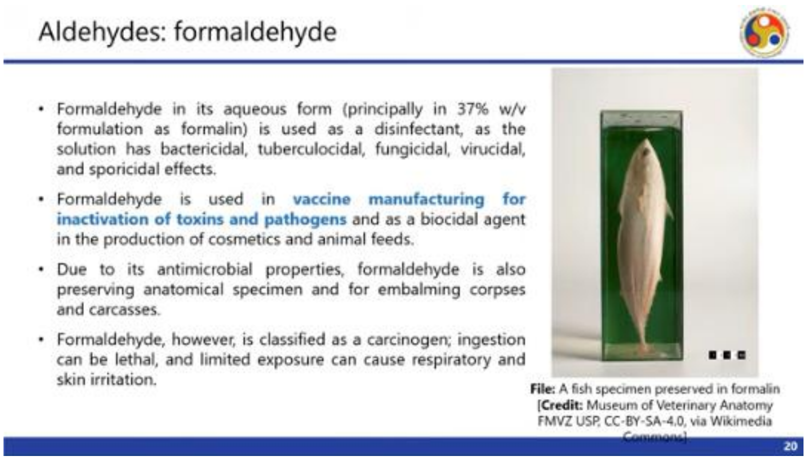
For example, zinc chloride is an ingredient in mouthwashes. While zinc oxide is found in a variety of products, including topical antiseptic creams such as calamine lotion, diaper ointments, baby powder, and dandruff shampoos. Then we have, of course, toxic metals like mercury. It was once widely used to treat syphilis, and tinctures like mercurochrome and merthiolate were used as antiseptics.

So, here you can see a vial of mercurochrome in this picture, and you can see the compound in this diagram. Mercury, however, is not selectively toxic to microbial cells and may bioaccumulate in human or animal cells. In excess amounts, mercury is toxic to the central nervous, digestive, and renal systems at high concentrations and can cause bioaccumulation in fish. So, another metal that is used as a chemical control agent is silver.



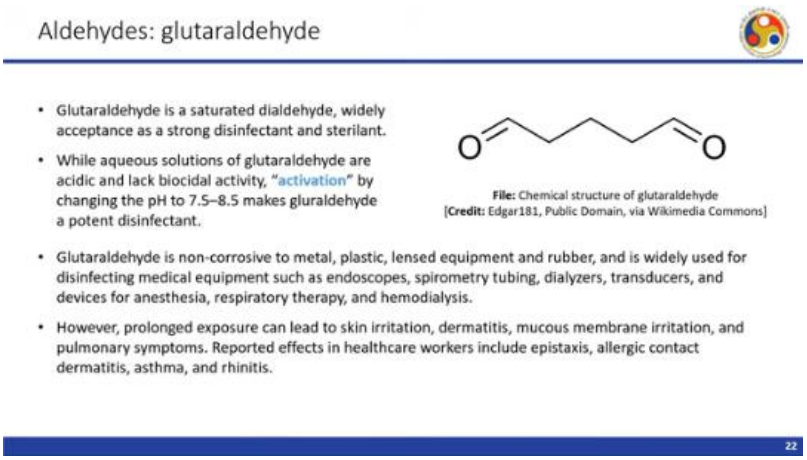
Silver sulfadiazine, which is commercially sold as Silvadene cream, is widely used for treatment, for example, of topical wounds, especially for the prevention of infection in burn wounds. Silver nitrate drops were used for the prevention of ophthalmia neonatorum, eye infections that can occur due to exposure to pathogens in the birth canal in newborns. Let us now discuss another class of compounds: the aldehydes. Let us begin with formaldehyde, which in its aqueous form—principally in a 37% weight-by-volume formulation—is used as a disinfectant, as the solution has bactericidal, tuberculocidal, fungicidal, virucidal, and sporicidal effects. Here you can see a fish specimen preserved in formalin in a museum, and so it can increase the shelf life by many, many years and decades.

Formaldehyde is used in vaccine manufacturing for the inactivation of toxins and pathogens and as a biocidal agent in the production of cosmetics and animal feeds due to its antimicrobial properties. Formaldehyde is also used in preserving anatomical specimens and for embalming corpses and carcasses. Formaldehyde, however, is classified as a carcinogen. Injection can be minimal, and limited exposure can cause respiratory and skin irritation.



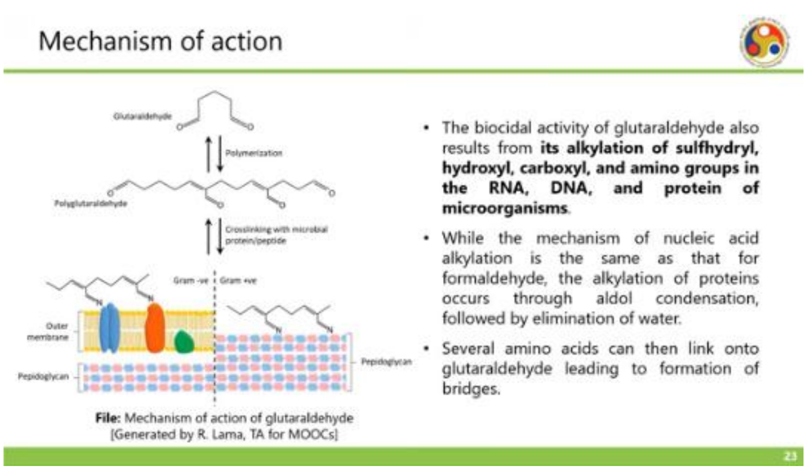
What is the mechanism of action of formaldehyde? It brings about the monohydroxymethylation of adenine on both DNA and RNA, which blocks genome reading. Formaldehyde can also cause monohydroxymethylation of the N-terminal amino acid residue or amino acids containing nitrogen and sulfur in the side chain. By reacting with the amino or sulfhydryl groups, such monohydroxymethylation reactions result in the formation of skip bases, which can cross-link with arginine and tyrosine and, to a lesser extent, with glutamine, asparagine, tryptophan, and histidine residues. These cross-links form inter- and intramolecular methylene bridges, causing the proteins to coagulate.

Another class of aldehyde, for example, is glutaraldehyde, which is a saturated dialdehyde widely accepted as a strong disinfectant and sterilant, while aqueous solutions of glutaraldehyde are acidic and lack biocidal activity. Activation by changing the pH to 7.5 to 8.5 makes glutaraldehyde a potent disinfectant. Glutaraldehyde is non-corrosive to metal, plastic, lanced equipment, and rubber and is widely used for disinfecting medical equipment such as endoscopes, spirometer tubing, dialyzers, transducers, and devices for anesthesia, respiratory therapy, and hemodialysis. However, prolonged exposure can lead to skin irritation, dermatitis, mucous membrane irritation, and pulmonary symptoms. Reported effects in healthcare workers include epistaxis, allergic contact dermatitis, asthma, and rhinitis.



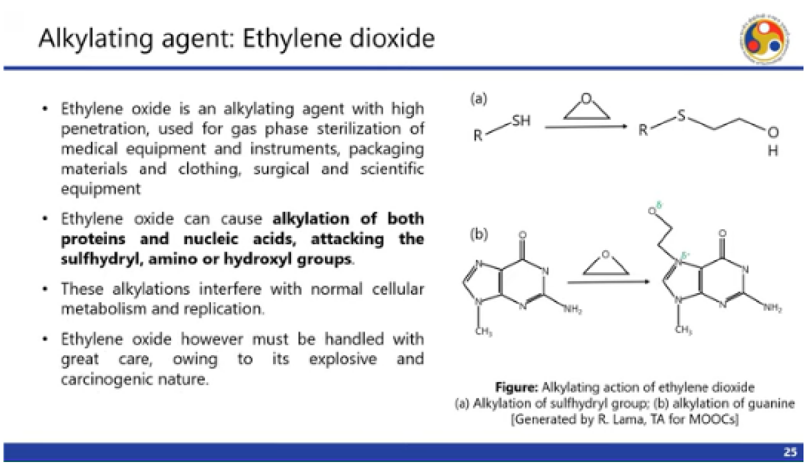
What is the mechanism action of glutaraldehyde? So, you can see here glutaraldehyde undergoing polymerization to form polyglutaraldehyde and it leads to cross-linking with microbial and protein in the peptides. And then you can see here the reaction in the gram-negative in the outer membrane and in the gram-positive in the peptidoglycan. So, briefly the biocidal activity of glucohaldehyde also results from its alkylation of sulfahydryl, hydroxyl, carboxyl and amino groups in the RNA, DNA and protein of microorganisms. The mechanisms of nucleic acid alkylation is the same as that for formaldehyde.

The alkylation of proteins occurs through aldol concentration followed by elimination of water. Several amino acids can then link onto glutaraldehyde leading to formation of bridges. Another type of aldehyde is the ortho-thalaldehyde or OPA like glutaraldehyde. Ortho-telaldehyde also interacts with nucleic acids, amino acids and proteins of microorganisms. However, compared to glutaraldehyde, OPA is a less potent cross-linking agent but has numerous advantages over glutaraldehyde.



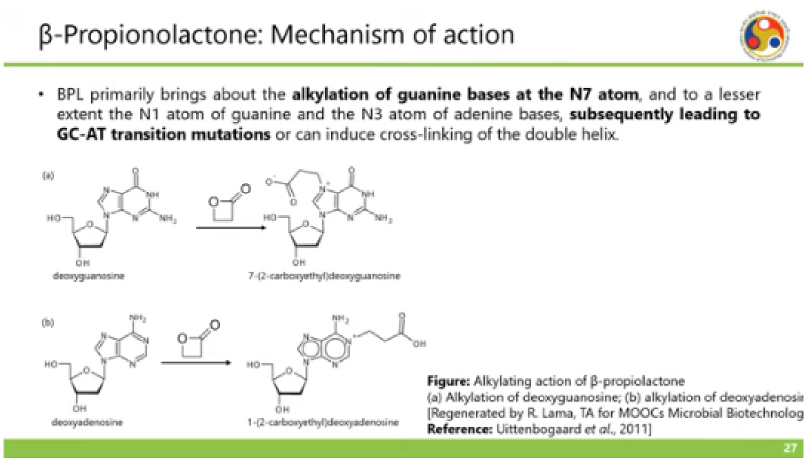
OPA shows excellent stability over a wide range of pH ranging from 3 to 9. Opiate does not cause irritation to the eyes and nasal passages and has a barely perceptible odor and does not require exposure monitoring. Lipophilic aromatic nature of opiate can assist its uptake through the outer layers of micro bacteria and gram negative bacteria, OPA shows very strong bioactivity against glutaraldehyde resistant mycobacteria and a potent sporicidal effect against bacillus atropius spores. A potential disadvantage of OPA is that it stains proteins gray including unprotected skin and thus must be handled with caution.

Let us now discuss another class of chemicals used in controlling microbes: the alkylating agents like ethylene dioxide. It is an alkylating agent with high penetration, used for gas-phase sterilization of medical equipment and instruments, packaging material, and surgical and scientific equipment. So, here in this figure, we can see the alkylating action of ethylene dioxide: alkylation of the sulfur hydryl group on the top and then the alkylation of guanine in the figure below (B). Ethylene oxide can cause alkylation of both proteins and nucleases, attacking the sulfur hydryl, amino, or hydroxyl groups. These alkylations interfere with normal cellular metabolism and replication. Ethylene oxide must be handled with great care owing to its explosive and carcinogenic nature.



Let us now discuss another alkylating agent, briefly known as BPL or beta-propiolactone. BPL is widely known for its virucidal and sporicidal effects. It is used in vaccines for virus inactivation, but its usage as a microbial control agent is limited due to its carcinogenicity. You can see here the skeletal formula of BPL in this picture.

BPL shows potent virucidal effects against several pathogens like equine encephalitis, rabies, poliomyelitis, coccidia, herpes simplex virus, measles, hog cholera, influenza, hepatitis, and even E. coli and tetanus. What is the mechanism of action of BPL or beta-propiolactone? BPL primarily brings about alkylation of guanine bases at the N7 atom and, to a lesser extent, the N1 atom of guanine and the N3 atom of adenine bases, subsequently leading to GC-to-AT transition mutations or cross-linking of the double helix. And you can see here in this picture the alkylating actions of BPL. In figure A, you can see the alkylation of deoxyguanosine.



In B, you can see the alkylation of deoxyadenosine. Let us now discuss another class of chemical agents that control microbial growth, which we briefly call surfactants. These are surface-active agents. They are a group of chemical compounds that lower the surface tension of water. Surfactants form the major components in soaps and detergents.

Most surfactants do not effectively destroy microorganisms but act by mechanically removing the organisms from surfaces. An important class of surfactants, however, called quaternary ammonium compounds, exhibit antimicrobial properties. Let us discuss the soaps. We regularly use soaps. Even if you are not a clinician or a researcher in a biological lab, this type of agent is known to you.

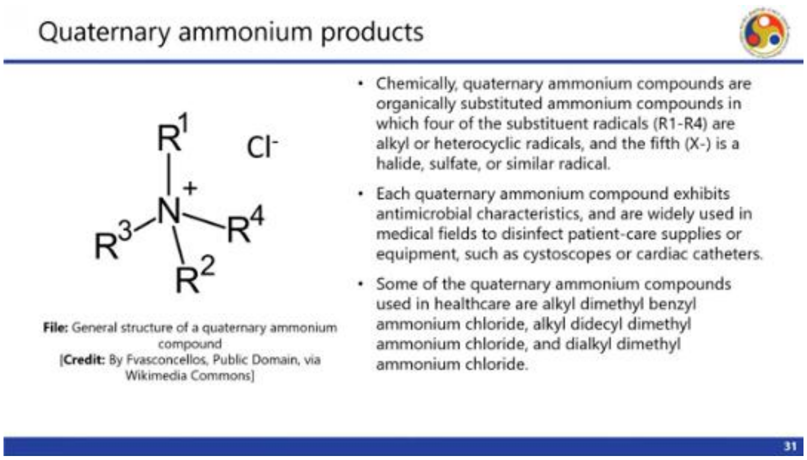
Here is an advertisement or awareness program, for example, by the World Health Organization, which tells about protecting yourself and others from getting sick by just washing your hands regularly after coughing or sneezing, when caring for the sick, because the sick person may have contamination before, during, and after you prepare food, before eating. After toilet use, when hands are visibly dirty, after handling animals or animal waste, you can use soaps and surfactants in this entire cleaning process. Soaps are salts of long-chain fatty acids and have both polar and non-polar regions, allowing them to interact with polar and non-polar regions in other molecules. Soaps do not kill or inhibit microbial growth and so are not considered antiseptics or disinfectants. However, they can interact with non-polar oils and grease to create emulsions in water, loosening and lifting away dirt and microbes from surfaces and skin.

Some swabs contain added bactericidal acids such as trichlorocarbon, trichlorocarbon, or chlorofluorocarbon, which introduce antiseptic or disinfectant properties to the swabs. Another surfactant is detergent. Detergent is a surfactant or a mixture of surfactant molecules with cleansing properties in dilute form. Based on their charges, detergents may be classified as anionic detergents. These are widely used for laundry.

They can mechanically remove microorganisms. Examples include sodium alkyl sulfate, such as sodium lauryl sulfate, and sodium alkyl benzene sulfonate, such as sodium 4,1-dodecyl benzene sulfonate. Then we have cationic detergents, which include an important class of disinfectants and antiseptics called quaternary ammonium salts; for example, we have benzalkonium chloride, alkyl dimethyl benzyl ammonium chloride, and cetylpyridinium chloride. Then we have non-ionic detergents, which are neither cationic nor anionic, like polyoxyethylene or glycoside-based detergents.

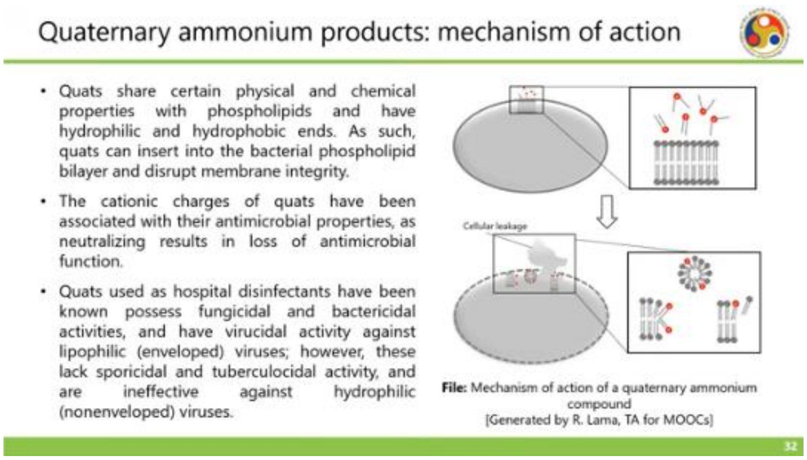
Some examples include Tween, Triton, and the Brij series. Then we have zwitterionic detergents. These exist as zwitterions within a particular pH range. Examples include CHAPS. Let us now discuss quaternary ammonium products, briefly called quats.

So chemically these quaternary ammonium compounds are organically substituted ammonium compounds in which four of the substituting radicals R1 to R4 as you can see in this image are alkyl or heterocyclic radicals and there is a fifth X is a halide sulfate or similar radical. Each quaternary ammonium chloride exhibits antimicrobial characteristics and are widely used in medical fields to disinfect patient care supplies or equipment such as cytoscopes or cardiac catheters. Some of the quaternary ammonium compounds used in health care are alkyl dimethylbenzyl ammonium chloride, alkyl didacyl dimethyl ammonium chloride, And dialkyl, dimethyl, ammonium chloride. What is the mechanism action of quads?

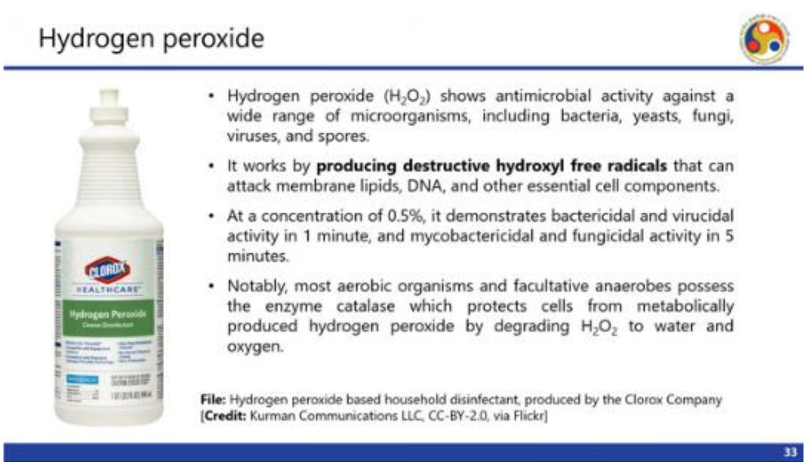


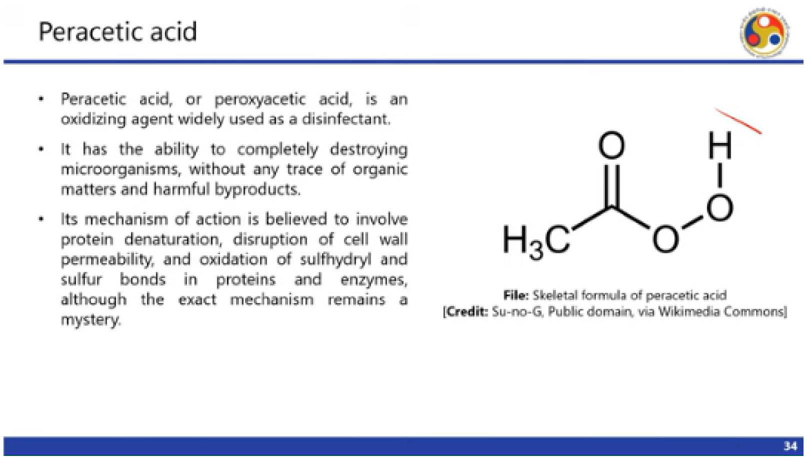
Quads share certain physical and chemical properties with phospholipids and have hydrophilic and hydrophobic ends. as such quads can insert into the bacterial phospholipid bilayer and disrupt membrane integrity. So, you can see here the mechanism action of this quaternary ammonium compound which is disrupting the membrane integrity. The cationic charges of quads have been associated with antimicrobial properties as neutralizing results in loss of antimicrobial function. Quads used as hospital disinfectants have been known to possess fungicidal and bactericidal activities and have virucidal activity against

lipophilic viruses however these lack sporicidal and tuberculosis activities and are ineffective against hydrophilic non-enveloped viruses another compound that is used as chemical control agent is the hydrogen peroxide which shows antimicrobial activity against a wide range of microorganisms including bacteria yeast fungi viruses and spores So, here you can see hydrogen peroxide-based household disinfectant. It works by producing destructive hydroxyl free radicals that can attack membrane, lipids, DNA and other essential cell components at a concentration of around 0.5%. It demonstrates bactericidal and virucidal activity in one minute and mycobactericidal and fungicidal activity in 5 minutes.



Notably, most aerobic organisms and facultative anaerobes possess the enzyme catalase, which protects cells from metabolically produced hydrogen peroxide by degrading hydrogen peroxide to water and oxygen. Let us now discuss a parasitic acid. Parasitic acid, or peroxyacetic acid, is an oxidizing agent widely used as a disinfectant, and you can see the skeletal formula of parasitic acid in this diagram. It has the ability to completely destroy microorganisms without leaving any trace of organic matter or harmful byproducts. Its mechanism of action is believed to involve protein denaturation, disruption of cell wall permeability, and oxidation of sulfhydryl and sulfone groups in proteins and enzymes, although the exact mechanism remains a mystery.





Parasitic acid can bring about the removal of microorganisms even at very low concentrations and also shows sporicidal and virucidal effects. So, we come to the end of this discussion on chemical control agents. Thank you for your patient hearing. Amen.