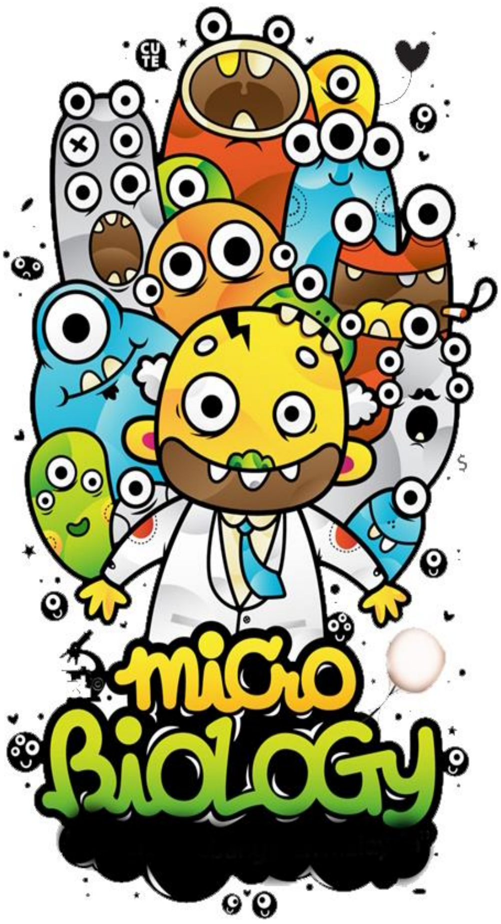

<u>Subject:</u> Chemical Biocides

<u>Lecture No.</u>: 9

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Factors Affecting the Activity of Chemical Biocides

• **Exposure time:** increasing the exposure time to a biocide will result in higher extent of microbial death.

• **Concentration:** the more concentrated a disinfectant, the greater its efficacy and the shorter the time necessary to completely kill a certain number of m.o.

• **Temperature:** increasing temperature by 10°C roughly doubles the rate of chemical reactions and thereby increases the potency of chemical agents.

• **pH:** pH can affect the potency of chemical agents by affecting their degree of ionization and hence their permeability into microbial cells. pH can also alter the properties of the microbial cell itself.

• **Presence of organic matter:** organic matter like faeces, blood and pus may reduce the activity of different chemical agents to variable degrees.

Chemical biocides:

1- Organic acids:

Weak and strong acids both have the ability to lower the optimal pH, which as a result will lead to either survival or growth-rate effects. The drop in pH usually reduces the growth rate of the micro-organisms. At a certain point these micro-organisms can no longer cope with this drop in the pH causing them to die.

Organic acids are mainly **used as preservatives** for food and pharmaceutical products. Usually because strong acids (e.g. Nitric acid\Sulphuric acid) can't be used as disinfectants or antiseptics due to their harmful effects on the living tissue (high toxicity), and incompatibility with the non-living things.

This type of chemical biocide is considered an oxidative phosphorylation uncoupler; in respiration the oxidation of certain molecules (e.g. Acetyl-CoA) will cause the accumulation of protons at one side of the mitochondrial membrane. As protons flow down their gradient and back into the matrix, they pass through an enzyme called ATP synthase, which harnesses the flow of protons to synthesize ATP. In case of uncoupling, oxidation continues to function, leading to oxygen consumption but phosphorylation of ADP is inhibited because the membrane is now permeable to these protons and they can move freely without having to pass through ATP-synthase.

Main examples of organic acids are:

- Benzoic acid (pKa 4.2): Mainly used as preservative for Drugs\ Can be used as an Antiseptic as a treatment for Athlete's foot (a fungal infection).
- Sorbic acid (pKa 4.8): Found in Drugs.
- Lactic acid (pKa 3.9): Found in yoghurt, pickles and some drugs.
    - These acids are low level to intermediate level in activity, mainly intermediate level.
    - The added salt in pickles inhibits many types of bacteria, but it also selects and encourages the growth of one type of bacteria (*Lactobacilli*) and these bacteria produce lactic acid which then works as a preservative. Many companies now put acetic acid in pickles because it's cheaper and works almost the same as lactic acid.

The equilibrium of the acid with its conjugate base is mainly dependent on the pH of the media and the pKa of that acid.
The undissociated form (HA) is usually the active antimicrobial agent. For example, if we use benzoic acid which has a low pKa (4.2) on a high pH we will have a higher rate of dissociation leading to insufficient activity (loss of the active form). Due to their relatively low pKa, these agents are only useful for acidic products (i.e. with pH less than 5).
Esters of these acids such as Parabens have higher pKa (between 8-8.5) and hence can be used for neutral and products with higher pH (up to 7-8).

2- Alcohols

Alcohols are aliphatic chains attached to hydroxyl groups. Phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (—OH) bonded directly to an aromatic hydrocarbon group.

Alcohols with microbial activity are divided into:

1- Short aliphatic alcohols

Methanol: Can’t be used as biocide due of its high toxicity and temporal loss of sight.
Ethanol and Isopropanol (both have the same properties and are used alternatively): are commonly used as disinfectants and antiseptics. Firstly, because they’re safe to use, usually they’re well tolerated by the human body (skin, mucous membranes etc.). Secondly, they show an intermediate level of disinfection meaning they can destroy all vegetative bacteria, most viruses & fungi, yet don’t kill bacterial spores, fungal spores and small naked viruses.

2 - Highly substituted alcohols (mainly used as preservatives)

Factors to be considered while using alcohols:

1- **Contact time:** alcohols are volatile compounds. To achieve full activity of the biocide, contact time must be for 5 minutes. For example, soaking a medical instrument in ethanol for five minutes will be enough to achieve the wanted results. But wiping hands with ethanol will not be beneficial because we need at least 5 minutes of contact. Many commercially available products are modified to decrease their volatility.

2- **Concentration:** Ethanol and Isopropanol are not effective at their pure form and can't function without water (at least 5% water). The major mechanism of their action is denaturation of proteins and solubilization of phospholipids. Denaturation is a process in which proteins lose their tertiary structure and in the presence of water this change will be permanent. (How? The ethanol\ isopropanol will cause the unfolding of the protein and water will form bonds preventing the protein from going back to its folded form after alcohol is evaporated.) Presence of water is essential for their activity but their activity drops sharply at concentrations below 50%. Therefore, in order for them to perform the desired activity as biocides water concentration should be 60-95%.
*75% is the most commercially available concentration may be found.

3- **Presence of organic matter** significantly reduces the antimicrobial activity of aliphatic alcohols and hence proper cleaning is required prior to their use as disinfectants.

00:00-14:15

<u>3- Alkylating agents</u> (used as biocides and anti-cancer group)

Strong electrophiles which have chemical groups that can form permanent covalent bonds (hence causing an <u>irreversible</u> damage) with hydroxyl, amino, imino and sulfhydrl groups in proteins and nucleic acids in a process called electrophilic addition. Technically because all micro-organisms have proteins and nucleic acids these biocides cover all the micro-organism in sufficient concentrations and exposure time. They are high-level disinfectants with sporicidal activity (chemical sterilants).

They're considered as carcinogens causing a change in the genetic material due to its capability to react with both human and bacterial DNA. Therefore, they're only used for sterilization in a controlled manner, we don't use them for general purposes (i.e. antisepsis or disinfection). Sterilization is usually preformed using physical factors but if the item was heat sensitive (e.g. optics, electrics, and plastics) we can't expose it to high temperatures and radiation is not usually available so we go for alkylating agents.

***Ethylene oxide** (not an aldehyde)**:** Highly explosive and can't be detected by smell (that's why its level must be monitored). It is mainly used in its gaseous form for the sterilization of re-usable instruments in addition to thermolabile disposable medical equipment (e.g. gloves, syringes, cannulas and IV sets). **Only** used industrially and it has higher efficacy than formaldehyde.

***Formaldehyde:** Volatile and should be used carefully (remember it's carcinogenic). It's commonly used as a preservative for products that won't be used by humans or any living animal. For example, formalin is used chiefly as a preservative for biological specimens.
Formalin: a colorless saturated solution of formaldehyde (38-40%), in water. 1:10 diluted formalin (~4% formaldehyde) is also used as a general purpose <u>disinfectant.</u> It can't be used as antiseptic and any possible exposure between formaldehyde and the user should be avoided because it's carcinogenic.

***Gluteraldehyde:** Commonly used in hospitals.

All alkylating agents' maximum efficacy is achieved at alkaline pH (neutral or about neutral). Aldehyde is not stable at alkaline pH. In commercial products its usually prepared in acidic solution and in order to activate it before using we add Sodium Hydroxide solution. Now they use orthophthaldehyde because it's stable at alkaline pH and can be prepared at the pH that it is most active on, but it's more expensive.

14:15-23:50

4- Biguanides

Biguanides are basic compounds with amino group. They're are most active in their di-cation form (ionized nitrogen atoms) which exists at pH 7-8. In addition of their use as biocides, biguanides are used in wide drug groups such as anti-hyperglycemic and anti-malarial groups.

They are one of the safest biocides that can be used on the human tissue, therefore they're mainly used as antiseptics, but can also be used as disinfectants and preservatives.
Biguanides (e.g. chlorhexidine and alexidine) show intermediate level of disinfection (less than alcohols) with little or no activity against mycobacteria and spores. They also can't kill some of the gram -ve bacilli (e.g. pseudomonas aeruginosa, Salmonella). They can serve a survivals media for this bacteria and that's why we don't commonly use them as disinfectants (They can be combined with other agents such as alcohols to cover the killing of gram -ve bacteria).

<u>The activity of these biocides is reduced by the presence of:</u>
1- Organic matter
If you have blood on your hand for example and you tried to clean it up using these agents it won't be effective at all.

2- Anionic compounds.

Some of anions (negatively charged species such as phosphate\carbonate) are found in hard water (tap water). If a product was diluted with the use of hard water these anions will form insoluble salts causing inactivation of the biocide. Instead, soft water (filtered or distilled) is usually used.

5- Halogens

- Halogens are any of the elements fluorine, chlorine, bromine, iodine, and astatine, occupying group VIIA (17) of the periodic table.
- High electronegativity —> strong oxidizing agents.
- Negatively charged halogens (Chloride, Iodide) have no microbial activity.
- Chlorine and iodine are mainly used as disinfectant biocides.

23:50-30:10

Chlorine Cl_2 (a disinfectant) is gaseous at room temperature, so in medical applications we use other chlorine compounds that release chlorine when used such as hypochlorite, the active ingredient in household bleaches. How? When sodium hypochlorite or calcium hypochlorite, is added to water, hypochlorous acid (HOCl) is formed. Hypochlorous acid exists in an equilibrium state in water; which means it partially dissociates to form a hydrogen ion, H+, and a hypochlorite ion, OCl-. The amount of each species depends on the pH pf the media and on the pKa of the acid.
This is shown in the following chemical reaction:

$$HOCl \rightleftharpoons H^+ + OCl^-$$

- When the media becomes more acidic (less than pH 7), the hydrogen ion concentration increases, they then combine with the hypochlorite ions and drive the equation to the left to form more hypochlorous acid.
- When the media becomes more alkaline (towards pH 8) the hydrogen ion concentration decreases. To make up for the shortage, hydrogen ions split from the hypochlorous acid and the equation is driven to the right to form more hypochlorite ions. The concentration of hypochlorous acid will therefore decrease, and the disinfecting power is reduced because while hypochlorous acid is a strong disinfectant (it dissociates spontaneously and gives Cl_2), the hypochlorite ion is only a weak disinfectant.

Commercially available hypochlorites (e.g. household bleach) are prepared in alkaline solutions (high pH) because in acidic solutions hypochlorous acid will dissociate spontaneously and give Cl_2 gas which is highly toxic and irritant to the mucous membrane in the respiratory tract. Therefore, should be diluted in water before their use to give hypochlorous acid; the more active form of hypochlorite.

Note: Decreasing the pH by dilution will cause a slow shift towards hypochlorous acid so the amount Cl_2 gas produced won't be high. That's why we mustn't add any acid to household bleach because it will rapidly decrease the pH producing high amounts of Cl_2 gas.

Halogens are strong oxidizing agents, can oxidize nuclide acids, proteins and phospholipids of the plasma membrane. Therefore, can cover most of the micro-organisms. Hypochlorite is considered a high level disinfectant with sporicidal activity (at relatively high concentrations). Whereas commercially available hypochlorites (e.g. household bleach) can't be used as chemical sterilants because at high concentration it won't work (because of the alkaline media) and at low concentrations (after dilution) they will have no activity against spores.

Hypochlorite's activity is highly affected by organic matter. It is not used in antisepsis because it is highly irritant and corrosive. It usually used as a disinfectant. Organic chlorine compounds such as chloramine and halazone are much less irritant than hypochlorite and hence can be used for wound cleansing and treatment of drinking water.

30:10-38:45

Iodine I_2, unlike chlorine, is solid at room temperature (not volatile). So we can use iodine directly without any complicated derivatives. If it was liquid, we can use it easily but as a solid it is hard to deal with. A solution is made from solid iodine, but I_2 is poorly soluble in water as it is. So we must use a co-solvent/ solubilizing agent to solubilize it in water. There are two ways to do so:

1. **The use of I- :** making a complex with I_2 forming I_3- (triiodide anion) which is soluble in water because it is negatively charged (hydrophilic). This reaction is called physical complexation and I_3- is in equilibrium with

I_2 and I^-. When I_3^- is consumed, it can be replaced by the forward reaction:

$$I_2 + I^- \rightleftharpoons I_3^-$$

This solution is called ***<u>Lugol's solution</u>***; iodine solubilized by I^-. The active agent in this solution is I_2.

2. **Using ethanol as a co-solvent:** Iodine is solubilized in water by the aid of ethanol, and the solution is called *Iodine tincture*. Iodine tincture has ≅90% ethanol, 10% water, and 2.5-5% I_2. The active agent (the biocide) in this solution is I_2 in addition to ethanol.

Iodine, like chlorine, is a high-level disinfectant with sporicidal activity. However, in most books it is not written under high-level disinfectants because it has a problem with gram -ve bacilli even though it can kill bacterial spores.
**According to this, can Lugol's solution be used as a high-level disinfectant? NO.
What about iodine tincture? YES, because ethanol can cover any gram -ve bacteria. That's why it is more commonly used as a biocide than Lugol's solution.

38:45-43:0

THE END