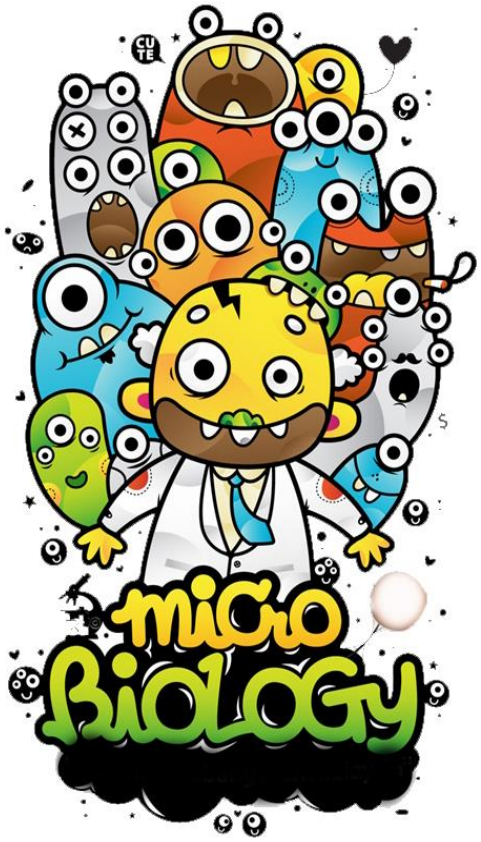

Subject: Chemical Biocides 2

Lecture No.: 10

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Last time we talked about iodine, which covers wide range microorganisms.

We have two ways for preparation of iodine:

- Lugol's solution:
    - G-ve bacteria has been reported to be resistant this way of preparation
- Iodine tincture:
    - Composed of 90% ethanol (hydro alcoholic solution).
    - Considered as a high-level disinfectant as the ethanol in it covers G-bacteria.
    - High level disinfectant

  In this type of preparation there's some problems:

    - Allergic reactions in some people
    - Stains skin and fabric

  We solve these two problems by using I**odophors,** in which iodine is found in physical complex with another substance (polymer or surfactant).

  Iodophors:

    - 'Povidone- iodine'; which contains PVP polymer (polyphenvenylpyrrolidone) complexes with iodine.
    - The iodine in the complex cannot react with bacteria nor it can cause allergy to the skin.
    - Before we use iodophors, we dilute it, so the iodine molecules are slowly released from the complex and can react with microorganisms.

      This way not a large number of iodine molecules are released in the free form at once, so we reduce skin staining and sensitization.
    - When using Povidone- iodine(Betadine), we should leave it on the surface for enough time to exert its effect as the iodine is slowly released, and what is good is that it gives action for an along time.

6- Oxidizing agents

- Any substance that has high reduction potential
- Not all oxidizing agent are used as Biocides, but most of them are.
- One example is Hydrogen peroxide:
    - It's an oxidizing agent derived from a group of compounds called 'Reactive Oxygen Species'(ROS).
    - Considered as a high-level disinfectant, with sporicidal activity. (chemical sterilent).
    - Used by macrophages inside our bodies to kill micro organisms specially bacteria; after phagocytosis of the bacteria, destruction of bacteria by one of two ways:
        - Degradation by enzymes
        - Destruction by ROS:
          Most of ROS are free radicals, superoxide, hydrogen peroxide, hydroxyl radical.
          They are highly reactive.
          For example, hydroxyl radical has something called 'diffusion limited reactivity';
          Which means it moves only 10-20 nm and reacts with everything in its way oxidizing them.
          They are not stable and we cannot prepare aqueous solution from them, because their half-lives are always in nano – micro seconds.
    - Hydrogen peroxide is less reactive, stable enough, with a relatively long half-life(months). So, it's the most used ROS as an oxidizing agent because it can be tolerated by the skin.
    - It covers all microorganism as it's a strong oxidizing agent that can oxidize most of cellular micro molecules specially proteins (remember most of the enzymes are proteins), nucleic acids, and phospholipids.

- Uses of H_2O_2:
  - Skin antiseptic specially for infected wounds, but not for pre-operative skin treatment nor surgical scarping because there are better agents for that.
  - Used for disinfection of contact lenses as it is active against (acanthamoeba) which causes keratitis in contact lens wearers.
  - At low concentration (3-6% solution) it can be used as a general disinfectant.
  - Vapour-phase hydrogen peroxides are used for sterilization of inclosed places and small areas such as glove boxes and isolators. Its more efficient in vapour-phase than liquid. (tools used in isolated chambers need to be sterilized, we can either use formaldehyde [alkylating agent], or by vapour-phase H_2O_2[oxidizing agent].

7- Phenols

- Any aromatic group with OH
- Usually slightly acidic, Pka = 7-8, so we can use them in neutral or acidic environments (we can't use acidic compounds in PKa higher or lower than their PKa).
- Phenols are one of the oldest used biocides, but no longer plays any significant role as an antibacterial agent due to its caustic effect, and it doesn't cover some viruses and fungi and bacterial spores.
- It cannot be used as anti-septic, or disinfectant. As we have better, safer intermediate level disinfectant.
- Derivatives of phenols have lower caustic effect and more used nowadays:
  - Tar-acids:
    Naturally occurring derived from petrol or oil-rock صخر زيتي

Ex: cresols and xylenols, less expensive and irritant than phenol and used as typical intermediate disinfectant but not as antiseptics as they have irritant smell and tissue toxicity.

- Chlorinated phenols:
  by chlorination of cresols and xylenols we get chlorocresols and chloroxylenols (active ingredient of dettol).
  these are much less toxic from any other phenol compounds so they can be used as disinfectant and antiseptic. At the same time the activity of these compounds is lower than phenols and considered as low level disinfectant.

- Bisphenole:
  the same thing, better safety profile but lower activity.
  The most popular example of this group is triclosan.
  The triclosan is not used any more in medical filed because its widely used in consumer products such as antibacterial soaps, hand lotions and shampoo.
  Because of the widely used small concentrations many types of bacteria are resistant to this type of disinfectant.
  (the lower the activity of the disinfectant, the more chance of having resistance by many types of bacteria)

<u>Student question:</u> How does the resistance of bacteria against antibiotics or Biocides occurs (specially with low concentration)?
Because of a genetic reason, mutation that makes a new enzyme that degrade the target, or by getting a plasmid. The process of resistance is called selection in which antibiotics will kill other bacteria and give the chance for the resistant or mutant one to grow and make a new population (all of it is resistant to the antibiotic)
(the faster the antibacterial agent works, the lower chance of selection to occur)

Hydroxyl radicals kill the microorganism within seconds, so no time for selection to occur but when we talk about triclosan especially with low concentration this make astatic effect (very weak action) the growth in the population stop and any mutation or getting plasmid will lead to selection and the biocides will not work.

8-Surface active agents, surfactants:

- Any amphiphilic compound (has hydrophilic head and hydrophobic tail)
- Not all surfactant has antimicrobial activity usually cationic surfactants are the main surfactants that have good antimicrobial activity
- When we talk about cationic surfactants we mainly are talking about a group called **Quaternary ammonium compounds (quats)** this group has a good antimicrobial activity against wide range of vegetative microorganism, but not bacterial spores.
    - We consider it as intermediate level disinfectant
    - Ex: cetrimide and benzalkonium chloride
- As the biguanide, quats are one of the best biocides in term of safety profile so they are used mainly as antiseptics and can be used as preservative and disinfectant.
- Some G-ve bacteria such as pseudomonas have developed resistance to quats.
- Usually most active in PH=7-8 and incompatible with many anionic compounds so we can't use hard water for dilution and it has to be with soft water, because anion such as carbonate and phosphate make with quats insoluble complex.
- Highly affected by organic matter so we have to make good cleaning before disinfection or antiseptic. (quats activity is reduced by the presence of organic matter and anionic compound)

2nd part of anti-microbial methods: **Physical methods**

- ✓ Reminder: usually for decontamination sterilization, we prefer physical methods Rather than chemical methods.

❖ Physical methods for sterilization:

- Heat
- Radiation

} Destruction (killing of microorganisms) …

Killing of all forms of microbial life; where bacterial endospores are known to be most resistant and hence are used as 'biological indicators' to test the efficiency of these methods.

- Filtration } No destruction, just mechanical removal using special filters.

❖ That's why we defined sterilization as: mechanical **destruction** or **removal** of all microorganisms.

A. Heat

- Done by exposure to high temperatures.
- Mainly for sterilization, could also be used for disinfection:
  - Like pasteurization; we heat up milk to 63 Celsius for 30 minutes, Or 72 Celsius for 30 seconds……. this doesn't kill bacterial spores or even some viruses and so it's not sterilization.
  - For sterilization 63,72 Celsius is no longer enough, and much higher degrees are needed.

- The heat temperatures needed for complete destruction of everything depends whether the heating process is in the presence of water molecules or not:
    - Moist heat sterilization
        - heating in presence of water steam (saturated water vapor) (gaseous state)
        - Temperatures required: 121 – 234
    - Dry (ambient) air sterilization
        - Temperatures required: 160 – 180

- How do high temperatures destroy microorganisms? It damages the macromolecules, mainly proteins. By different Mechanisms:
    - Oxidative damage
    - Denaturation and hydrolysis.... this is the more efficient mechanism
        - Hydrolysis needs water and thus this mechanism exists in moist heat sterilization.
- As a result of having the more efficient mechanism predominant in moist heat sterilization, we consider moist heat sterilization to be more efficient than dry heat sterilization **at the same temperatures.**
    - To achieve the same activity; <u>lower temperatures</u> and <u>shorter exposure</u> time is required for moist heat sterilization.

- Most common sterilization cycles for:
    - Moist heat: 121 Celsius for 15 minutes
      134 Celsius for 3 minutes

    - Dry hear 160 Celsius for 2 hours
      180 Celsius for 30 minutes

  notice the time difference…. Even though 160(dry) is higher than 134(moist), it needs more time

- Penetration through packaging material and throughout the sterilization chamber: steam has got better penetration power than dry heat

- For all the reasons, above, heat sterilization in general is the most preferred method for sterilization in any **thermostable** substance.
    - Thermostable: the substance ability to withstand heat without any damage. For example, can we use plastic? No, because there will be product damage.

For any substance if it can undergo heat sterilization, we do not consider any other way.

So how do we choose moist or dry?

- We choose moist heat sterilization if:
    - Substance not water soluble
    - Substance not sensitive to moisture
    - Thermostable

For example, do we use moist heat sterilization:

- for iron? No, although it can stand heat it doesn't stand humidity as it gets rusty specially at high temperatures.
- For a Powder of water soluble drug? No, because it will soak, and transform to a solution.

Practical applications:

- Sterilization of growth media, specially that there's no ingredient that can be degraded by temperature (not thermolabile).
- Dressing, sheets in hospitals
- Aqueous preparations that are not thermolabile, pharmaceutical products, eye dopes, ophthalmic preparations in general, injectable.
- Microbiological waste: autoclaving always for waste because we are not concerned with any damage that occurs to the waste.
  For example:
  we're working on pathogenic bacteria in lab and we're done, we must sterilize it before putting it to waste.
  Or Changing a band of a patients infected wound, we sterilize before putting it to waste

The machine used for heat sterilization is called **autoclave.** in principle, it's like 'pressure pucker' but more controlled (steam at high temperatures + pressure).

we use Autclaving for:

- fabrics
- aqueous preperations if they were thermostable and resistant to moisture.
- decontamination for waste always (even if not thermostable). with one exception: if the waste was **explosive**.

✓ Moist heat sterilization:
- More efficient
- Lower temperatures
- Shorter exposure time
- More penetrative

- We choose dry heat sterilization if:
  - Thermostable
  - Sensitive to moisture

Practical applications:

- Surgical instruments, because most of it is metal with iron or even stainless steel (it can go rusty at high pressure + high moisture content).
- Powder
- Non-aqueous preparations
- Glass; in principle, we can use moist sterilization for glass, but as an exception we prefer dry heat here because dry heat does sterilization + **depyrogination** (destruction of endotoxins).
  (moist heat doesn't destroy endotoxins)

- Some bacteria called 'thermophile' live at extreme temperatures (around 100), they also die in heat sterilization. (those bacteria are not pathogenic; because they don't live in our bodies temperatures).

B. Radiation

Many items can't be subjected to heat, such as disposable medical equipment (injections, IV sets...)

- Firstly, what is radiation? There's many types of radiation:
  - Electromagnetic radiation: light with its wavelength range, gamma-rays to radio waves. (photons). Its energy depends on wavelength.
  - Particular radiation: it's particles like protons, alpha, electrons (beta). Its energy depends on momentum.
- Radiation sterilization can be achieved using:
  - ionizing radiation which has higher energy (e.g. gamma-rays and X-rays). Here the electron is loosed so that the bond will break." remember: bond is composed of 2 electrons ". That will affect macromolecules especially DNA – nucleic acids- that will cause multi strand break and so it will lose its sequence. It's highly risk and it may cause cancers by producing free radicals.

  - non-ionizing radiation (e.g. UV light). It may cause excitation only (electron isn't loosed). It is lower risk than ionizing one but it can make damage by another mechanism "*dimerization*"

- Penetration power is one of our concerns because sterilization is done after packaging. It is for ionizing radiation much higher than that for non-ionizing,

Note: UV is absorbed by packaging materials, so it won't affect the items inside.

E.g. for rays that use in radiation sterilization" ionizing ": gamma rays, x-rays which has lesser energy than gamma, electron beams which is produced by machines rather than isotopes for gamma.

- Notes:
  - ✓ Remember that we firstly try to use heat sterilization but if can't then we use radiation like in sensitive items. Also, if we don't have radiation like in hospital, we use chemicals which is mentioned earlier.

  - ✓ Ionizing radiation is the preferred method for the sterilization of thermolabile disposable medical equipment (e.g. gloves, syringes, cannulas and IV sets). It can also be used for powders and non-aqueous liquid and semi- solid preparations (e.g. ointments)

  - ✓ The major target for radiation is microbial DNA, with damage occurring as a consequence of ionization and free radical production by ionizing radiation or thymine dimer formation (dimerization) by non-ionizing radiation

  - ✓ Ionizing radiation is more damaging to microorganisms and has better penetrating power so it is more reliable and has wider range of applications in sterilization.

  - ✓ UV light can be used for air and surface sterilization in controlled areas (e.g. biosafety cabinets and operating rooms) and for sewage water treatment where is no packaging because of its low penetration power so it can't penetrate even plastic.

C. Filtration

In some preparations, we can't use neither heat nor radiation because that might damage the materials we are dealing with, such as insulin and growth factors preparation. (Hence: Heat denatures proteins). Also, we can't expose

them for chemical solutions like formaldehydes because they are water soluble and will remain in the materials therefore they can't be used by patients. So, we want a method that doesn't destruct microorganisms but removes it mechanically which is **FILTRATION**.

- Filtration is complete **destruction*** or **removal** of all types of microorganisms and it's used only with fluids (liquid & gas). NOT used with powder or solid. It is the last resort of sterilization, which means last method you think to use when there is thermolabile fluids.
  *the doctor said it in the lecture although the slide said that it is just "removal"
    - Liquid filtration: HOW?
      0.2 – 0.45 µm **membrane filters** (defined pore size which is smaller than all known bacteria and other microorganisms) are usually used for the sterilization of heat-sensitive liquids. We pour the solution into the filer which will pass through pores but the microorganisms will retention on the filter which is removed after that.
      --> here there is a problem! Viruses is much smaller than bacteria (20-300 nm) therefore most of them will pass with solution! So, we want filters with smaller pores, actually there is filters with pore size small than that but they don't practically used. So, it must be highly controlled. **DISADVANTAGE**

    - Air filtration:
      Sometimes we have to sterilize air like in aseptic manufacturing sites, hospital isolation units, operating theaters which are controlled rooms that want a large continuous supply with sterilized air, but HOW? Filtration is the key, but with special filters “HEPA filters”, they are microfibers which don’t have a defined pore size. Also, they are much thicker than membranes and don't depends on sieving but on the adsorption and entrapment of particular matter.

Depth filters also named as a HEPA filters when it can remove more than 99.97% of particles greater than 0.3 µm in diameter. They have better handling capacity. isn't closed easily because they don't have defined pore size.

Note: Depth filter for gas, Membrane filter for liquid.

HEPA filters are also used for: Decontamination of air in mechanical ventilators – Clarification and sterilization of medical gases.

☺ SORRY FOR MISTAKES ☺