

## Control (Management) of Microbial Populations

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### Abstract

Basic significance of knowing the fundamental properties of the marine framework, as Dr. Morel examined. Albeit the vast majority of our point here is microbial defilement-that is, things that we have been adding to the marine conditions and issues with those options-it will be significant that we see how that marine framework functions before we could see how we could stop or take care of a portion of the sullyng issues. We quickly talk about that subject, yet generally we cover the subject of microbial tainting of marine conditions. We endeavor to characterize it and quickly portray what we are doing now and what we can do later on to enhance it. When we utilize the term microbial defilement, we mean an arrival of microorganisms into nature, for the most part from discharged waste items. Individuals utilize amazing measures of water. Its greater part experiences siphoned frameworks. It ends up blended with human waste and a wide range of other waste and is dumped once again into the water cycle and out into the sea. An essential worry that the vast majority have is human security identified with sickness; obviously, a significant number of us are additionally extremely worried about corrupting the territories in common frameworks.

Microorganisms (MO) are abundant in the environment (on or in bodies, vegetation, surfaces, air, water, soils)

Grossly MO can be grouped into two categories with their relationship to the host, namely:

- Normal flora (majority)
- Pathogenic/conditionally pathogenic MO (fewer, but can be fatal)

Pathogenic MO are found either in/on diseased bodies of animals and plants or in their surrounding environment (air, water, soil).

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### Introduction

Microorganisms that reason pollution incorporate microbes (moderately little [~1 mm direct dimension] cell prokaryotes) and infections that are non-cell and little (~30 to 200 nm). The infections don't utilize without their hosts, which is vital in light of the fact that it means that they are altogether different from microscopic organisms, which duplicate and get things done individually. Notwithstanding microorganisms and infections, protists can now and again be sickness life forms, as Dr. Burkholder will talk about.

We don't know particularly about debasement of the living

spaces of characteristic frameworks from organisms that we are discharging out there, contrasted and arrival of, for instance, supplements or synthetic substances. There may be some intense issues in that absence of information. Generally what we think about is the point at which we put a microorganism in nature and it returns to us as a conceivable infection operator, and that is the essential subject of our discussion.

The one wellspring of pollution that quickly rings a bell, sewage treatment plant emanating, is quite very much directed. Individuals know all in all what is turning out from sewage treatment plants, and on the West Coast it will in general be discharged from some profound pipe seaward (pardon our West

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Coast incline). In the marine condition on the East Coast, there are commonly estuaries, and a significant part of the treated sewage may continue through an estuary out to ocean. In this manner, in spite of the fact that there is a lot of near shore presentation, generally few individuals go swimming in a large number of the real estuaries close where the sewage turns out [1-5].

### Why control MOs?

1. Situations where the control of MO is necessary include:
  - When need of specific or pure cultures of MOs (e.g. in research, probiotics and vaccine/immune serum preparation)
  - To keep pathogenic MOs away (e.g. in sterilization, disinfection, antiseptics or treatment)
  - Minimize total numbers of MOs to prevent or delay spoilage of foodstuffs (e.g. pasteurization, tyndalization and irradiation).

### Agents of microbial control

1. Agents of microbial control (antimicrobials) are diverse and can either be target specific or non-specific. They include: heat, irradiation, disinfectants, antiseptics, antibiotics or chemotherapeutics, bacteriophages (Figures 1-5).

### Microbial resistance or susceptibility to control mechanisms

1. MOs can resist control (destruction or death) by developing

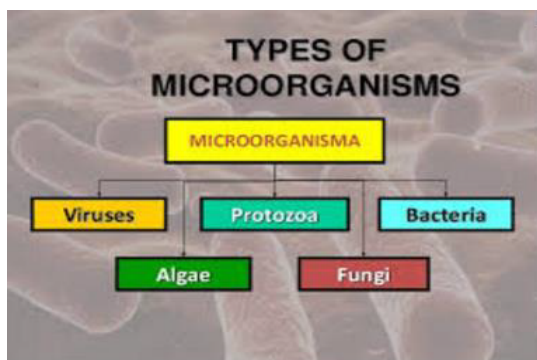


Figure 1 Types of microorganisms.

resistance mechanisms against the control agents or can succumb) to the control agents.

2. The behavior of MOs to control agents (such as heat, dryness, high pressure, light intensity or antibiotics etc.. is expressed in three general properties thus: MOs can be:
  - Susceptible (i.e. easily destroyed) to the control agent
  - Low/moderate resistant
  - Highly resistant.

### General statement on susceptibility/resistance of MOs to control agents

1. Under normal environmental conditions, vegetative forms of MOs are susceptible or moderately resistant to control agents, while sporulated forms are relatively more resistant.
2. E.g. Vegetative forms of most MOs are normally destroyed by heat (60°C for 30 min)
3. Spores of most MOs may resist heat (100°C) at normal atmospheric pressure for many hours.
4. Some spores are exceptionally resistant to destruction (e.g. *Bacillus anthracis* spores can survive in the environment for over 30 y).

### Definition of terms in microbial control

1. Sterilization: Is the total (absolute) destruction of all living cells by physical means. Sterilization is not selective. It kills pathogenic and non-pathogenic MOs as well;
2. Disinfection: Is selective destruction of undesired MOs on unviable surfaces. Generally, disinfectants are chemicals that are toxic to living animal cells;

Therefore, disinfectants are used in low concentrations and with precaution!!!

3. Antiseptics: Prevention of infection by inhibiting the growth of MOs on a surface of a living body ie. animal or plant body. Antiseptics are generally less toxic than disinfectants.

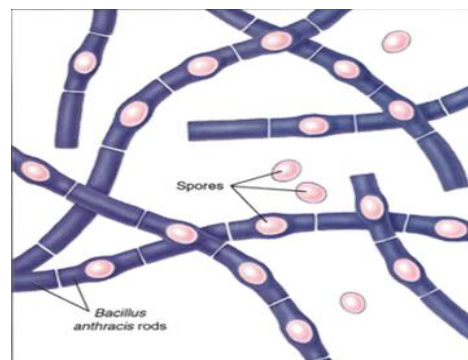
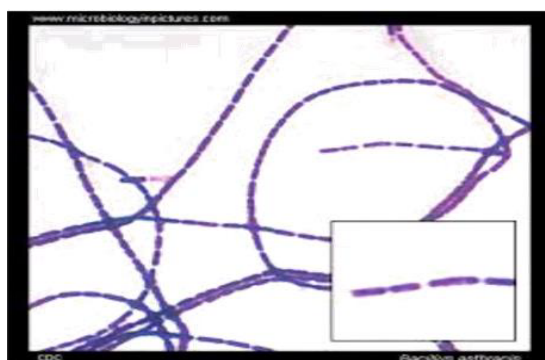


Figure 2 *Bacillus anthracis*: Vegetative (L) Sporulated (R).

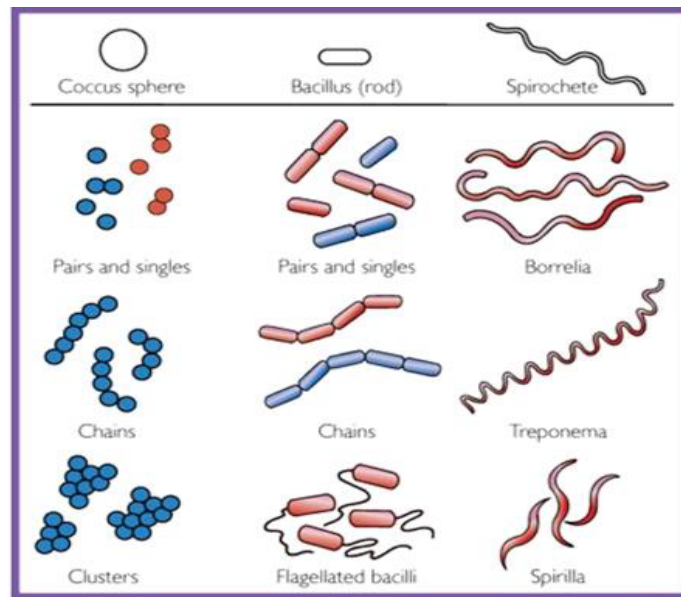


Figure 3 Shapes of bacteria.

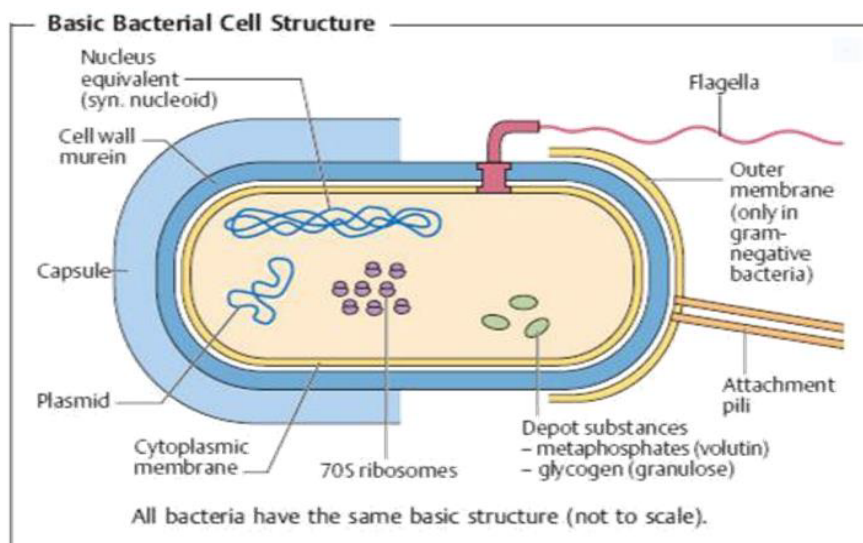


Figure 4 Basic bacterial cell structure.

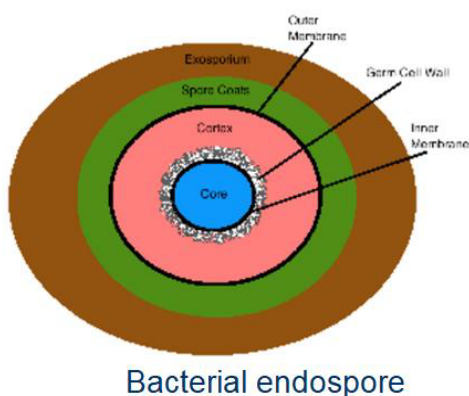


Figure 5 Bacterial endospore.

**More definitions**

- Bacteriostatic agent: Physical or chemical agent that will INHIBIT bacterial growth (certain antibiotics, e.g. Penicillin are bacteriostatic).
- Bactericidal agent: Physical or chemical agent that will KILL bacteria.
- Most disinfectants and physical agents (e.g. heat, irradiation with ultraviolet rays are bactericidal).
- Certain classes of antibiotics are also bactericidal (e.g. tetracyclines).

**Methods of microbial control**

1. **Physical methods:** These can be grossly grouped into three classes:

- Heat (high temperature)
- Irradiation
- Mechanical

a) Heat (high temperature) will kill a living cell of a particular organism at a certain heat intensity, so called the thermal death point (TDP) of that cell.

TDP will depend on:

- whether the heat is dry or moist (e.g. boiling/steaming)
  - the duration and surface exposed
  - atmospheric pressure
  - pH/ionic strength of the medium the cell is found
  - stage of growth (i.e maturity level) of the microbial cell
  - Heat kills MOs by causing denaturation of proteins and nucleic acids of the microbial cells.
  - Sterilization by dry heat can be achieved in a hot oven (160°C, 2 h) or on an open (direct) flame (i.e. flaming the loop, incineration of carcasses, contaminated clothing's or wooded materials)
  - Sterilization by moist heat can be achieved in the form of: autoclaving, boiling, steaming
  - Tyndalization and pasteurization are selective MO control methods using moist heat.
1. **Autoclaving:** Sterilizing by pressurized steam (T=121°C, 1.5 atm). This method is commonly used as safest and most effective means of achieving sterilization. It kills all vegetative bacteria and SPORES within 20-30 min.
  2. **Boiling (100°C, at 1 atm):** Can kill most vegetative forms in 30 min BUT NOT 100% EFFECTIVE vs. SPORES
  3. **Steaming:** Used for materials (e.g. sugars) that will be destroyed if boiling were to be used.
  4. **Tyndalization (syn. fractional sterilization):** Method used to sterilize materials e.g. seeds, mushrooms or solutions, which are easily degradable (e.g. sugars) to kill vegetative cells and spores gradually:

### How is it done?

By steaming (at 100°C, 15 mn) and cooling (at 37°C) repeatedly (3-4 cycles) to allow germination of the spores into vegetative forms.

*Condition:* the medium containing the materials must be able to support germination of the spores at 37°C

5. **Pasteurization:** Use of temperatures safe enough to kill most vegetative forms of MOs BUT without denaturation of proteins or caramization of sugars

(e.g. Pasteurization of milk: Best apply 62°C for 30 min - UHT- or 72°C for 15 s. (also called flash pasteurization)

6. Pasteurization is effective vs. vegetative forms of bacteria

in milk, honey, juices (e.g. Brucella spp. Salmonella spp, Escherichia coli, staphylococci, streptococci and mycobacteria).

7. *Condition:* The appearance /natural taste of pasteurized foods must not be significantly altered in the process.
  8. Pasteurization is practical but requires special conditions for rapid heating and cooling.
2. **Irradiation:** Is a physical method of sterilization which use of non ionizing rays (e.g. UV) and ionizing rays (e.g. X or  $\gamma$  rays) to damage MOs.

### Non ionizing irradiation (e.g. UV rays) can be from the sun or from an UV generator (at 200-300 nm)

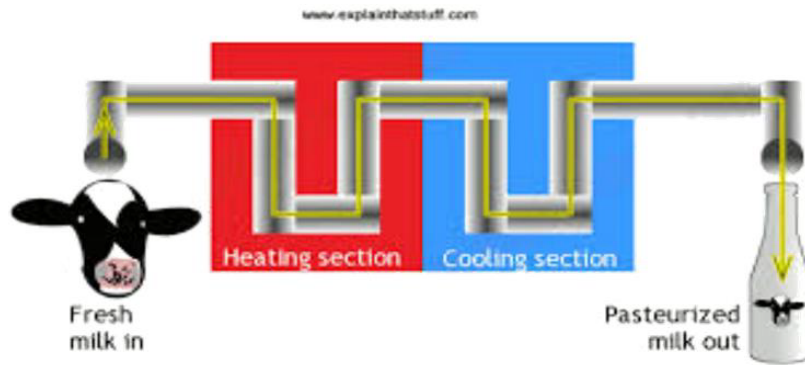
- UV is a low penetrant irradiation. It is, therefore, mostly used to sterilize clean surfaces (e.g. tops of sterile benches (biosafety cabinets) but also glassware and occasionally surgical gowns
- Destruction of MOs by irradiation occurs by rupture of cell membranes and denaturation of cytoplasmic materials and nucleic acids (**Figures 5-10**).



Figure 6 Sterilization by dry heat-Bunsen burner; incineration.



Figure 7 Autoclaves.



Typical pasteurization cycle

**Figure 8** Typical pasteurization cycle.



**Figure 9** Industrial pasteurization line.



**Figure 10** The appearance/natural taste of pasteurized foods (eg. milk, honey, juices.....) must not be significantly altered in the process.

**Irradiation**

Ionizing irradiation employs highly penetrant rays and is more damaging on DNA than UV rays.

- Used to sterilize plastics and some foodstuffs and canned foods (milk, fruit, meat..)
- Irradiation thus kills vegetative MOs

- It also destroys the chemical bonds in foods (fruits, meat...) thus delays decay and consequently increases product shelf life.

Mechanical methods of sterilization: There are variable examples:

- Violent shaking with glass beads or grinding in a mortar can destroy cells but not totally, therefore it is not very effective.

- Ultrasonic waves (i.e sound at high frequency 20,000 cycles/sec will disrupt most vegetative bacterial cells through vacuolization)
- High and rapid pressure changes (500-600 atm.) are variably disruptive to cells
- Filtration/microfiltration: Is a mechanical method of sterilization by size exclusion of bacterial and fungal spores. It is a useful method for sterilizing contaminated materials that denture easily, like sera, sugar solutions, enzymes, vitamins.

For exclusion of bacteria, filters of the pore size of 0.2  $\mu$  or less are used. Viruses are filterable i.e., they can pass through such pore sizes.

- Bacterial filters are commonly made up of cellulose acetate fiber, asbestos pads, sintered glass or earthenware materials and are used to handle small volumes of fluids in ordinary laboratories.

### Properties of ideal disinfectants

An ideal disinfectant must have:

- High bactericidal effect
- High stability in the environment where applied
- High solubility in water (or fat), depending on the substrate it is being applied to
- Low corrosive property to the surface applied
- High penetrative property
- Relatively low toxicity to cells/tissues of the individual who is applying it.

### Targets of disinfectants

Target microbial cellular components for the disinfectants include:

1. Cell wall; 2. Cytoplasm; 3. Microbial enzymes.
- These components are commonly destroyed through irreversible oxido-reductive processes (e.g. Potassium permanganate is a potent oxidative disinfectant)
  - Some disinfectants are based on heavy metals (e.g. phenols and cresols) which will coagulate cellular proteins.
  - The relative potency of a disinfectant is determined by comparing its killing power to that of pure phenol under defined conditions, and is expressed as a ratio known as the phenol coefficient (PC) of that disinfectant
1. The effectiveness of a disinfectant is dependent on:
    - Contact with the target MO in terms of surface exposed and exposure time
    - Presence or absence of organic matter shielding the target MO
    - Concentration of the disinfectant

- Nature/state of the MO exposed to it (e.g. spores or vegetative)

P.S. some vegetative MOs (e.g. *Pseudomonas* spp. are resistant to disinfectants.

### Examples of common disinfectants

1. Acids and alkalines (NaOH, citric acid)
2. Phenolic derivatives (e.g. dettol, hexa-chlorophene- for surgical objects)
3. Ammonium compounds (e.g. citrimade), which are used for hand wash
4. Halogen compounds (e.g. Na-hypochlorite, iodine....)
5. Oxidising agents e.g. eusol ( $H_2O_2$ ), Potassium, permanganate
6. Anilic dyes (e.g. Gentian violet)
7. Gaseous disinfectants are used with excellent efficiency in fumigation of chambers/rooms. Most commonly used are:
  - Alkylating agents containing  $-NH_2$ ; OH groups
  - $CO_2H$  and  $-SH$  carrying compounds e.g. Formalin, ethylene dioxide, methyl bromide or sulphur dioxide. Most of the gaseous disinfectants are also sporocidal (**Figure 11**).

### Antiseptics

An ideal antiseptic must be: mild, with pH around neutral and non corrosive common antiseptics include

- i) Soaps containing unsaturated fatty acids
  - ii) Alcohols (e.g. methylated spirit 70%),
  - iii) Dilute phenolic compounds (e.g. dettol)
  - iii) Oxidizing agents (e.g. iodine, Na-permanganate,  $H_2O_2$ -eusol)
- The control of MOs inside the body (ie. organs and tissues, blood, lymph, cerebrospinal fluid) is essentially the responsibility of the body's own defence mechanisms (non-specific as well as specific)
  - Specific body defence vs. MO is also referred to as immunity.



**Figure 11** International logo for irradiated foodstuffs.

- Augmentation of body's defence mechanism is achieved through antibiotics, chemotherapeutics and immunotherapeutics [5-10].

## Conclusion

This entire system may be enhanced by the improvement of fast strategies to think huge volumes of water and keep up high affectability. Location might be accelerated with some conceivable methodologies, for example, tests, called sub-atomic reference points. These tests are inside to the PCR item and have a quencher. When they are bound to their objective, they unquench, getting to be fluorescent when the objective is available. Such tests can be utilized in the PCR test and identified while the test is being done, taking into consideration a brisk quantitative answer. Taqman is another comparative test, and it depends on enzymatic arrival of a fluorophore from a marked oligonucleotide test.

Looking more distant into the future, a moment test would be a colossal help. The majority of you presumably have seen that a doctor can rub a throat swab on a little plate and test for strep throat in almost no time. It used to take multi day, with test transport to a remote lab and work serious tests. Envision if a comparable test enables lifeguards to roll an instrument around on the shoreline, testing for microbes and possibly for infections at various areas. They may test a couple of liters, place it in a machine, and find quantitative solutions in minutes. It may be conceivable to create tests like that for seawater, with adequate assets contributed. A similar machine could test drinking water, stores, waterways, among different conceivable outcomes, and could likewise be utilized to follow wellsprings of tainting. It isn't difficult to envision it being savvy, however the underlying interest being developed is the troublesome part.

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