GURUNANAK INSTITUTE OF TECHNOLOGY

157/F, Nilgunj Road, Panihati

Kolkata -700114

Website: www.gnit.ac.in

Email: info.gnit@jisgroup.org

Approved by A.I.C.T.E., New Delhi

Affiliated to MAKAUT, West Bengal

ONLINE COURSE WARE

SUBJECT: Food Microbiology

SUBJECT CODE: FT 302

CREDIT: 3

NAME OF THE COORDINATOR: **Dr. Souptik Bhattacharya (GNIT)**

NAME OF THE TEAM MEMBER: **Dr. Dolanchapa Sikdar (GNIT)**

Module I

Introduction

Microorganisms are living entities of microscopic size and include bacteria, viruses, yeasts and molds (together designated as fungi), algae, and protozoa. For a long time, bacteria have been classified as procaryotes (cells without definite nuclei), and the fungi, algae, and protozoa as eucaryotes (cells with nuclei); viruses do not have regular cell structures and are classified separately.

Among the microorganisms, some molds, yeasts, bacteria, and viruses have both desirable and undesirable roles in our food. Except for a few sterile foods, all foods harbor one or more types of microorganisms. Some of them have desirable roles in food, such as in the production of naturally fermented food, whereas others cause food spoilage and foodborne diseases. To study the role of microorganisms in food and to control them when necessary, it is important to isolate them in pure culture and study their morphological, physiological, biochemical, and genetic characteristics.

Microscopes are optical instruments having a magnifying lens or combination of lenses for inspecting objects too small to be seen distinctly and in detail by the unaided eye.

Microscopes

Some of the most important types of microscopes that used in biology are as follows:

1. Simple microscope

2. Compound microscope

3. Electron microscopes

4. Phase-Contrast microscope

5. Interference microscope.

The simple dissection microscope to advanced electron microscopes finds application in studies of living organisms.

Microscope as the name suggests are instruments that help to enlarge minute (micro = very small) organisms or their parts. A microscope not only presents a magnified view of the object but also 'resolves' it better.

Resolution is the feature which makes it possible to differentiate between two points present close together in the objects being viewed. The first microscope was constructed by Anton Van Leeuwenhoek (1632-1723). This, microscope consisted of a single biconvex lens fitted in a small window of a "board" and the object was viewed through it. This was a simple microscope.

After this compound microscope, were developed using combinations of two lenses. Improvements continued, newer and newer' microscopes were designed and are still being improved.

Different types of microscopes being used in biological studies are the following:
Resolving Power: It is the ability of a microscope to show two closely lying points as two distinct points.

Magnification:

In simple microscope, convex lens of short focal length is used to see magnified image of a small object. The object is placed between the optical centre and the focus of a convex lens, its image is virtual, erect and magnified and on the same side as the object. The position of the object is so adjusted that the image is formed at the least distance of distinct vision (D).

Magnifying power (M) of a simple microscope is the ratio of the angle subtended by the image at the eye to the angle subtended by the object seen directly, when both lie at the least distance of distinct vision or the near point.

$M = 1 + D/ f$

Where D is the least distance of distinct vision and f is the focal length of the lens.

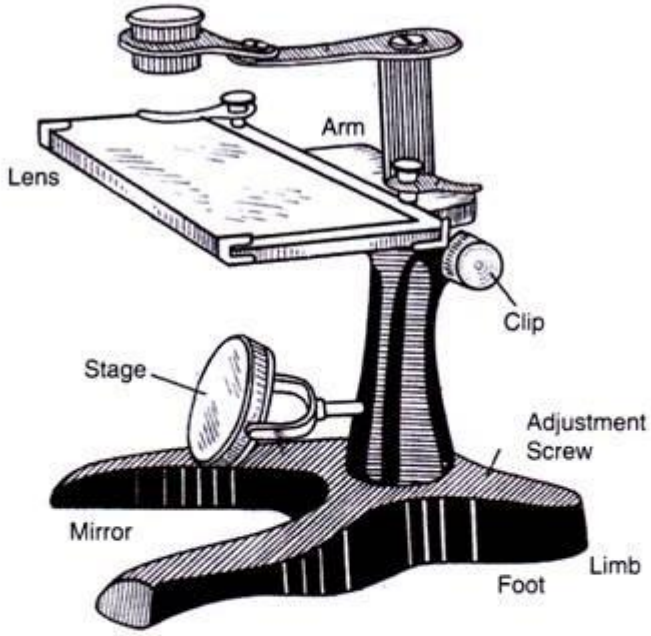


Fig. 6.1 Simple/dissecting microscope

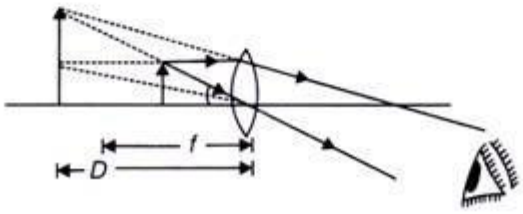


Fig. 6.1

2. A

Compound Microscope:

A compound microscope consists of two set of convex lenses. A lens of short aperture and short focal length facing the object is called objective. Another set of lens of relatively moderate focal length and large aperture facing the eye is called the eye piece. The objective and the eye piece are placed coaxially at the two end of a tube (Fig. 6.2).

The object is placed between the centre of curvature and focus of the objective – it forms real, inverted and magnified image on the other side of the objective. This image acts as an

object for the eye piece which then acts as a simple microscope to produce virtual, erect and magnified image.

Magnifying power (M) of a compound microscope will be

$M = L/ f_0 (1+D/f_e)$

Where $f_0$and f_e are focal length of objective and eye piece respectively, L is the length a the microscope tube and D is the least distance of distinct vision.

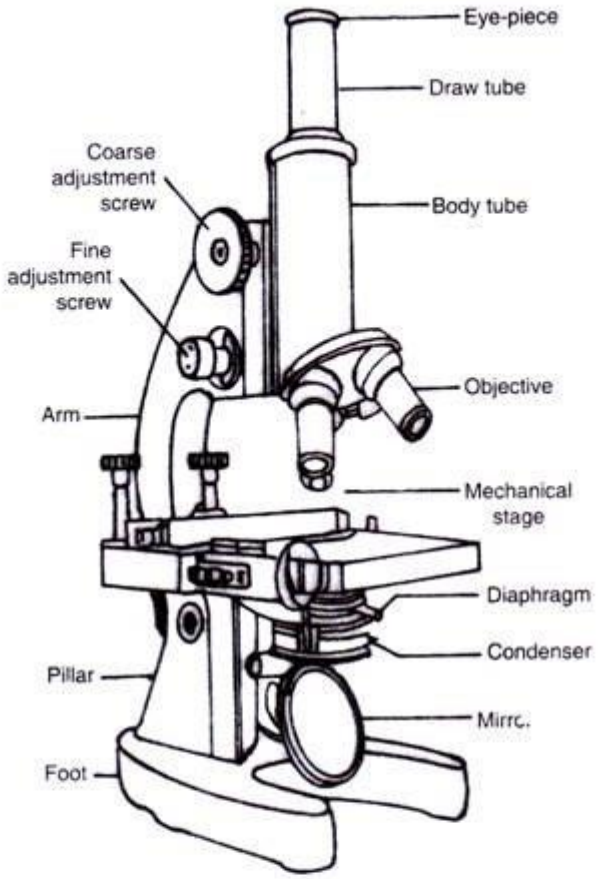


Fig. 6.2 A compound microscope

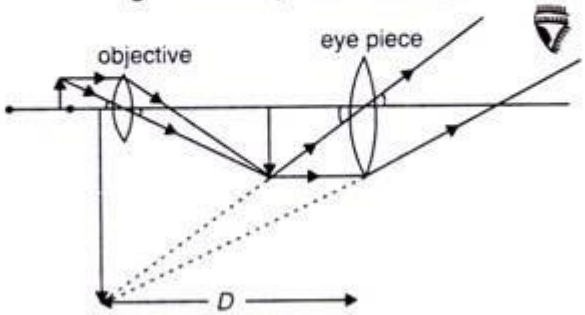


Fig. 6.2

3. The Electron Microscope:

The organelles of the cell became known after the electron microscope was invented. The electron microscope was developed in 1932 by M. Knoll and Ruska in Germany. It consist of

a source of supplying, a beam of electron of uniform velocity, a condenser lens for concentrating the electron on the specimen, a specimen stage for displacing the specimen which transmits the electron beam, an objective lens, a projector lens and a fluorescent screen on which final image is observed (fig. 6.3).

For permanent record of the image, the fluorescent screen is replaced by photographic film. This microscope utilizes a stream of high speed electrons which are deflected by an electromagnetic field in the same way as a beam of light is reflected when it crosses a glass lens. There are two types of electron microscope.

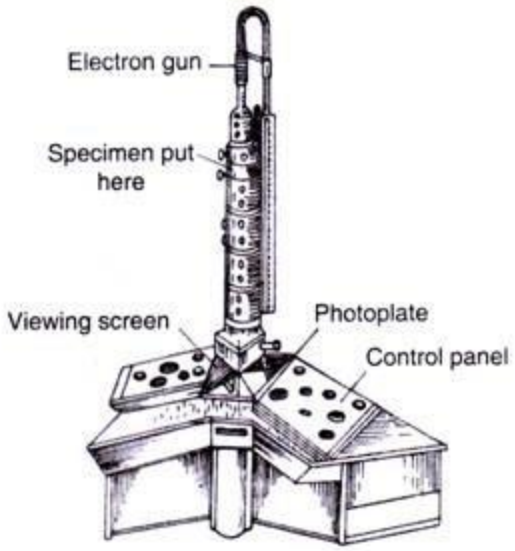


Fig. 6.3 An electron microscope

(a) Transmission electron microscope (TEM):

This is used to observe fine structure of cells. Ultra thin sections of the object are prepared and they are stained with a heavy metal (gold or palladium) to make certain part dense, and inserted in the vacuum chamber of the microscope. A 100, 00 volt electron beam is focused on the section and manipulated prepared from the image may be enlarged with enough resolution to achieve a total magnification of over 20 million times.

(b) Scanning electron microscope (SEM):

It is used to study the surfaces of the cell and organisms. In this microscope, the image is formed by electrons reflected back from the object. The image formed by this microscope has a remarkable three dimensional appearance. Typically magnification of scanning electron microscope is around 20,000 times.

4. Phase-Contrast microscope:

This is used to study the behavior of living cells, observe the nuclear and cytoplasmic changes taking place during mitosis and the effect of different chemicals inside the living cells. By using the phase-contrast microscope, an image of strong contrast of the object is obtained (fig. 6.4).

It is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens, such as living cells (usually in culture), microorganisms, thin tissue slices, fibers, glass fragments, and sub-cellular particles (including nuclei and other organelles). In effect, the phase contrast technique employs an optical mechanism to translate minute variations in phase into corresponding changes in amplitude, which can be visualized as differences in image contrast.

One of the major advantages of phase contrast microscopy is that living cells can be examined in their natural state without previously being killed, fixed, and stained. As a result, the dynamics of ongoing biological processes can be observed and recorded in high contrast with sharp clarity of minute specimen detail.

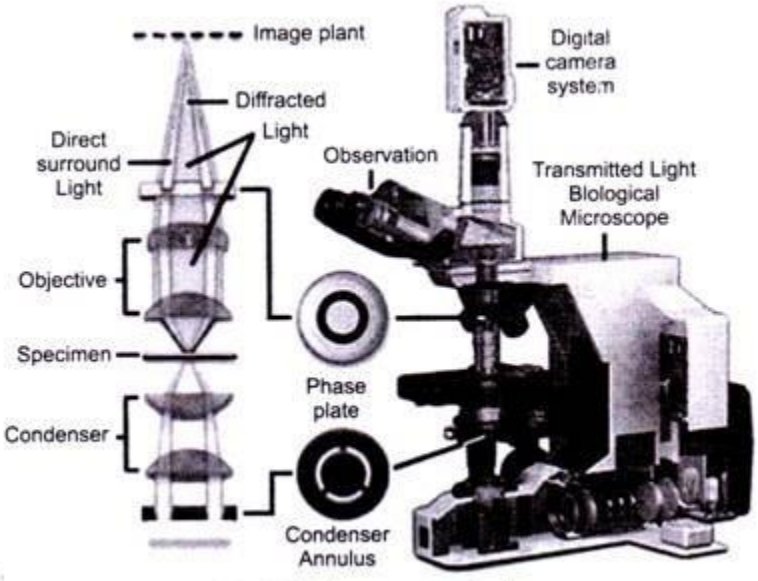


Fig. 6.4 Phase-contrast microscope

5.

Interference microscope:

Interference microscope is used for quantitative studies of macromolecules of the cell components, for example it is used for determination of lipid, nucleic acids and protein contents of the cell. Interferometry is a traditional technique in which a pattern of bright and

dark lines (fringes) result from an optical path difference between a reference and a sample beam.

The incoming light is split inside an interferometer, one beam going to an internal reference surface and the other to the sample. After reflection, the beams recombine inside the interferometer, undergoing constructive and destructive interference and producing the light and dark fringe pattern.

A precision translation stage and a CCD camera together generate a 3D interferogram of the object that is stored in the computer memory. This 3D interferogram of the object is then transformed by frequency domain analysis into a quantitative 3D image providing surface structure analysis (fig. 6.5).

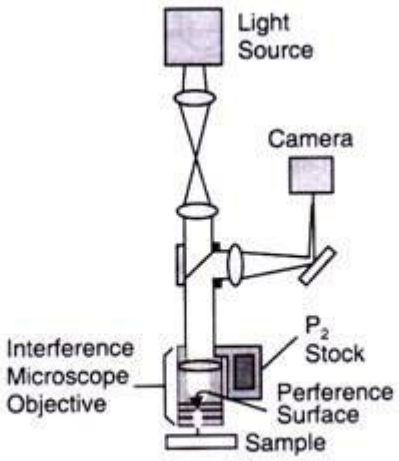
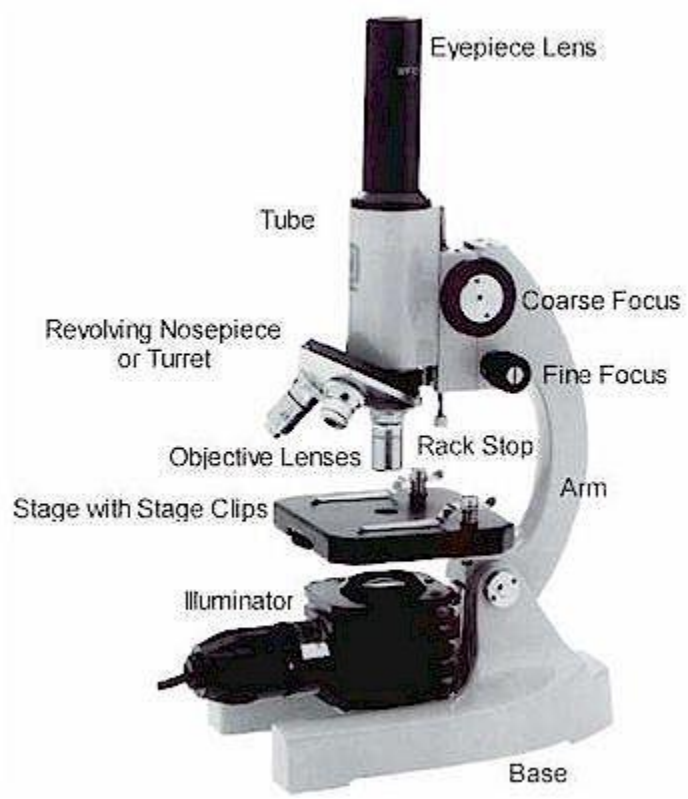


Fig. 6.5 Interference microscope

Compound Microscope and its different parts



Historians credit the invention of the compound microscope to the Dutch spectacle maker, Zacharias Janssen, around the year 1590. The compound microscope uses lenses and light to enlarge the image and is also called an optical or light microscope (vs./ an electron microscope)**.** The simplest optical microscope is the magnifying glass and is good to about ten times (10X) magnification. The **compound** micro scope has two systems of lenses for greater magnification, 1) the ocular, or eyepiece lens that one looks into and 2) the objective lens, or the lens closest to the object. Before purchasing or using a microscope, it is important to know the functions of each part.

Eyepiece Lens: the lens at the top that you look through. They are usually 10X or 15X power.

Tube: Connects the eyepiece to the objective lenses

Arm: Supports the tube and connects it to the base

Base: The bottom of the microscope, used for support

Illuminator: A steady light source (110 volts) used in place of a mirror. If your microscope has a mirror, it is used to reflect light from an external light source up through the bottom of the stage.

Stage: The flat platform where you place your slides. Stage clips hold the slides in place. If your microscope has a mechanical stage, you will be able to move the slide around by turning two knobs. One moves it left and right, the other moves it up and down.

Revolving Nosepiece or Turret: This is the part that holds two or more objective lenses and can be rotated to easily change power.

Objective Lenses: Usually you will find 3 or 4 objective lenses on a microscope. They almost always consist of 4X, 10X, 40X and 100X powers. When coupled with a 10X (most common) eyepiece lens, we get total magnifications of 40X (4X times 10X), 100X , 400X and 1000X. To have good resolution at 1000X, you will need a relatively sophisticated microscope with an Abbe condenser. The shortest lens is the lowest power, the longest one is the lens with the greatest power. Lenses are color coded and if built to DIN standards are interchangeable between microscopes. The high power objective lenses are retractable (i.e. 40XR). This means that if they hit a slide, the end of the lens will push in (spring loaded) thereby protecting the lens and the slide. All quality microscopes have achromatic, parcentered, parfocal lenses.

Rack Stop: This is an adjustment that determines how close the objective lens can get to the slide. It is set at the factory and keeps students from cranking the high power objective lens down into the slide and breaking things. You would only need to adjust this if you were using very thin slides and you weren't able to focus on the specimen at high power. (Tip: If you are using thin slides and can't focus, rather than adjust the rack stop, place a clear glass slide under the original slide to raise it a bit higher)

Condenser Lens: The purpose of the condenser lens is to focus the light onto the specimen. Condenser lenses are most useful at the highest powers (400X and above). Microscopes with in stage condenser lenses render a sharper image than those with no lens (at 400X). If your microscope has a maximum power of 400X, you will get the maximum benefit by using a condenser lenses rated at 0.65 NA or greater. 0.65 NA condenser lenses may be mounted in the stage and work quite well. A big advantage to a stage mounted lens is that there is one less focusing item to deal with. If you go to 1000X then you should have a focusable condenser lens with an N.A. of 1.25 or greater. Most 1000X microscopes use 1.25 Abbe condenser lens systems. The Abbe condenser lens can be moved up and down. It is set very close to the slide at 1000X and moved further away at the lower powers.

Diaphragm or Iris: Many microscopes have a rotating disk under the stage. This diaphragm has different sized holes and is used to vary the intensity and size of the cone of light that is projected upward into the slide. There is no set rule regarding which setting to use for a particular power. Rather, the setting is a function of the transparency of the specimen, the degree of contrast you desire and the particular objective lens in use.

How to Focus Your Microscope: The proper way to focus a microscope is to start with the lowest power objective lens first and while looking from the side, crank the lens down as close to the specimen as possible without touching it. Now, look through the eyepiece lens and **focus upward only** until the image is sharp. If you can't get it in focus, repeat the process again. Once the image is sharp with the low power lens, you should be able to simply click in the next power lens and do minor adjustments with the focus knob. If your microscope has a fine focus adjustment, turning it a bit should be all that's necessary. Continue with subsequent objective lenses and fine focus each time.

Biological Classification of Microorganisms:

Microorganisms may be defined as living creatures that are microscopic in size and relatively small, unicellular in structure. The diameter of the smallest body that can be resolved and seen clearly with the naked eye is about 100 µ (1 µ, or micron = 0.001 millimeter). All microorganisms are smaller than 100 µ.

Therefore, a microscope is necessary for the observation. Light microscope can resolve down to 0.2 n in diameter and thus includes all microbes except viruses which can be seen only under electron microscope whose limit of resolution is 0.0005 µ (i.e., 0.5 mµ = millimicron = 5 Angstrom Units).

One Angstrom Unit = 1/10th of 1 mµ. When microorganisms grow on solid or semi-solid media, their progenies accumulate locally to form masses or colonies which are visible to the naked eye. Microorganisms constitute a very antique group of living organisms which appeared on the Earth's surface almost 1.5 billion years ago. Some scientists believe that the microbes were the first living microorganisms Of the Earth. Staining is an important technique to observe the shape, size, and characteristics of an organism.

Staining is an auxiliary technique used in microscopic techniques used to enhance the clarity of the microscopic image.Stains and dyes are widely used in the scientific field to highlight the structure of the biological specimens, cells, tissues etc.

The most widely used staining procedure in microbiology is the Gram stain, discovered by the Danish scientist and physician **Hans Christian Joachim Gram** in 1884. Gram staining is a differential staining technique that differentiates bacteria into two groups: gram-positives and gram-negatives. The procedure is based on the ability of microorganisms to retain color of the stains used during the gram stain reaction. Gram-negative bacteria are decolorized by the alcohol, losing the color of the primary stain, purple. Gram-positive bacteria are not decolorized by alcohol and will remain as purple. After decolorization step, a counterstain is used to impart a pink color to the decolorized gram-negative organisms.

The Gram stain is a very important preliminary step in the initial characterization and classification of bacteria. It is also a key procedure in the identification of bacteria based on staining characteristics, enabling the bacteria to be examined using a light microscope. The bacteria present in an unstained smear are invisible when viewed using a light microscope. Once stained, the morphology and arrangement of the bacteria may be observed as well. Furthermore, it is also an important step in the screening of infectious agents in clinical specimens such as direct smears from a patient.

The Gram stain procedure enables bacteria to retain color of the stains, based on the differences in the chemical and physical properties of the cell wall.

1**. Gram positive bacteria**: Stain dark purple due to retaining the primary dye called Crystal Violet in the cell wall.

Example: *Staphylococcus aureus*

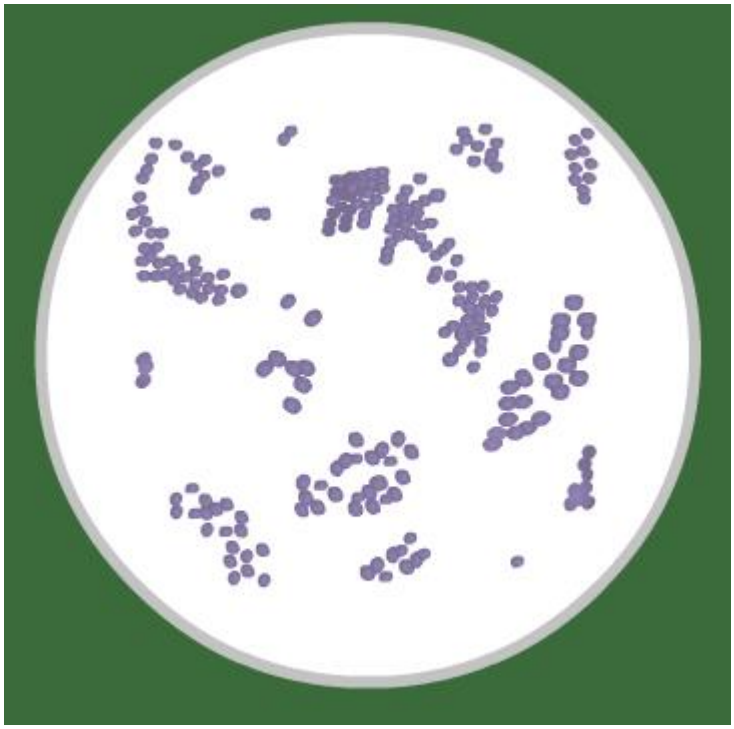


Fig: Gram positive bacteria

2. **Gram negative bacteria**: Stain red or pink due to retaining the counter staining dye called Safranin.

Example: *Escherichia coli*

Fig: Gram negative bacteria

Bacterial Morphology:

Bacteria are very small unicellular microorganisms ubiquitous in nature. They are micrometers (1µm = 10^{-6} m) in size. They have cell walls composed of peptidoglycan and reproduce by binary fission. Bacteria vary in their morphological features.

The most common morphologies are:

Coccus (pleural: Cocci):

Spherical bacteria; may occur in pairs (diplococci), in groups of four (tetracocci), in grape-like clusters (*Staphylococci)*, in chains (*Streptococci*) or in cubical arrangements of eight or more (sarcinae).
For example: *Staphylococcus aureus, Streptococcus pyogenes*

Bacillus (pleural: Bacilli):

Rod-shaped bacteria; generally occur singly, but may occasionally be found in pairs (diplo-bacilli) or chains (streptobacilli).
For example: *Bacillus cereus, Clostridium tetani*

Spirillum (pleural: Spirilla):

Spiral-shaped bacteria

For example: *Spirillum, Vibrio, Spirochete* species.

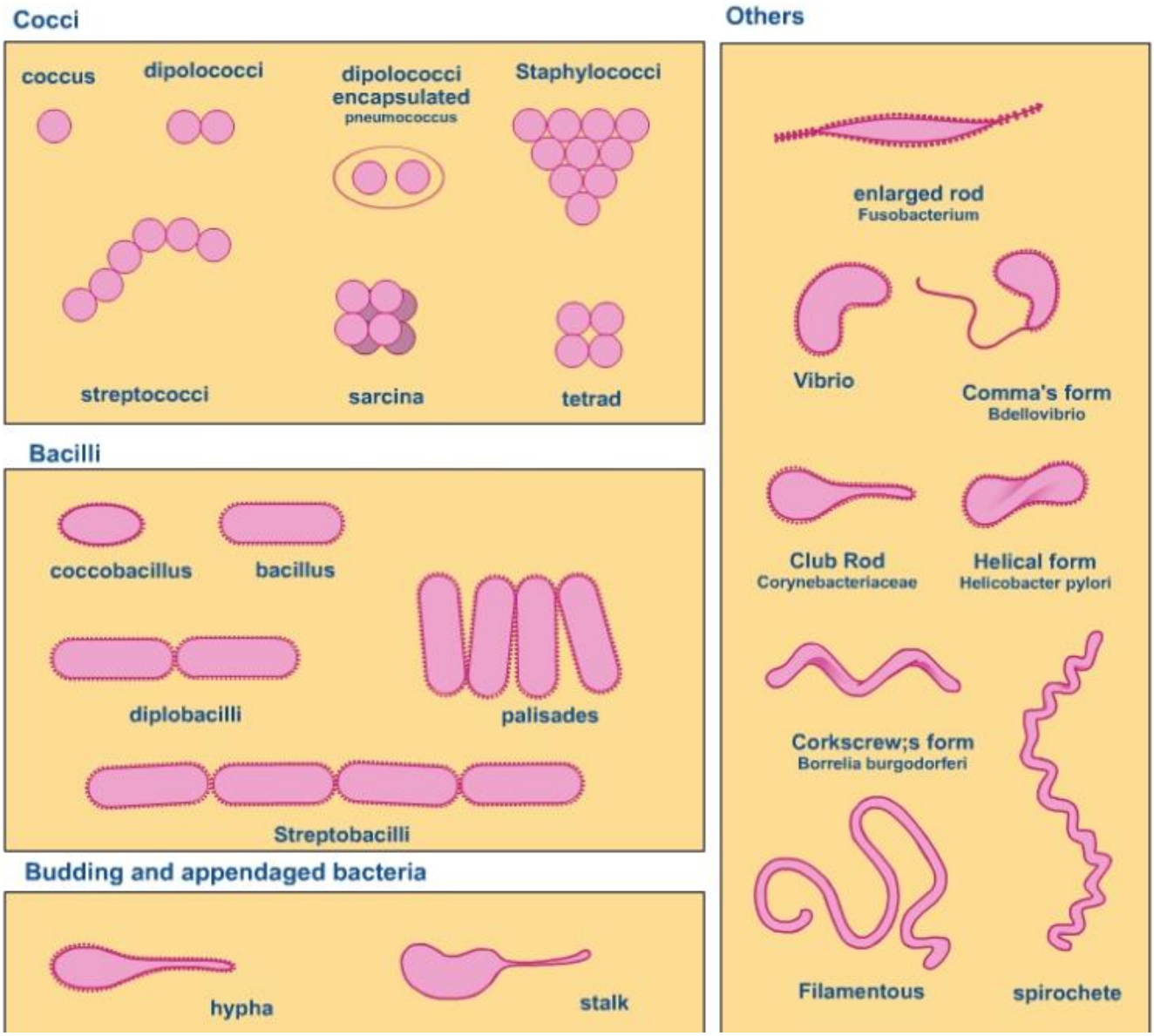
Some bacteria have other shapes such as:

Coccobacilli: Elongated spherical or ovoid form.

Filamentous: Bacilli that occur in long chains or threads.

Fusiform: Bacilli with tapered ends.

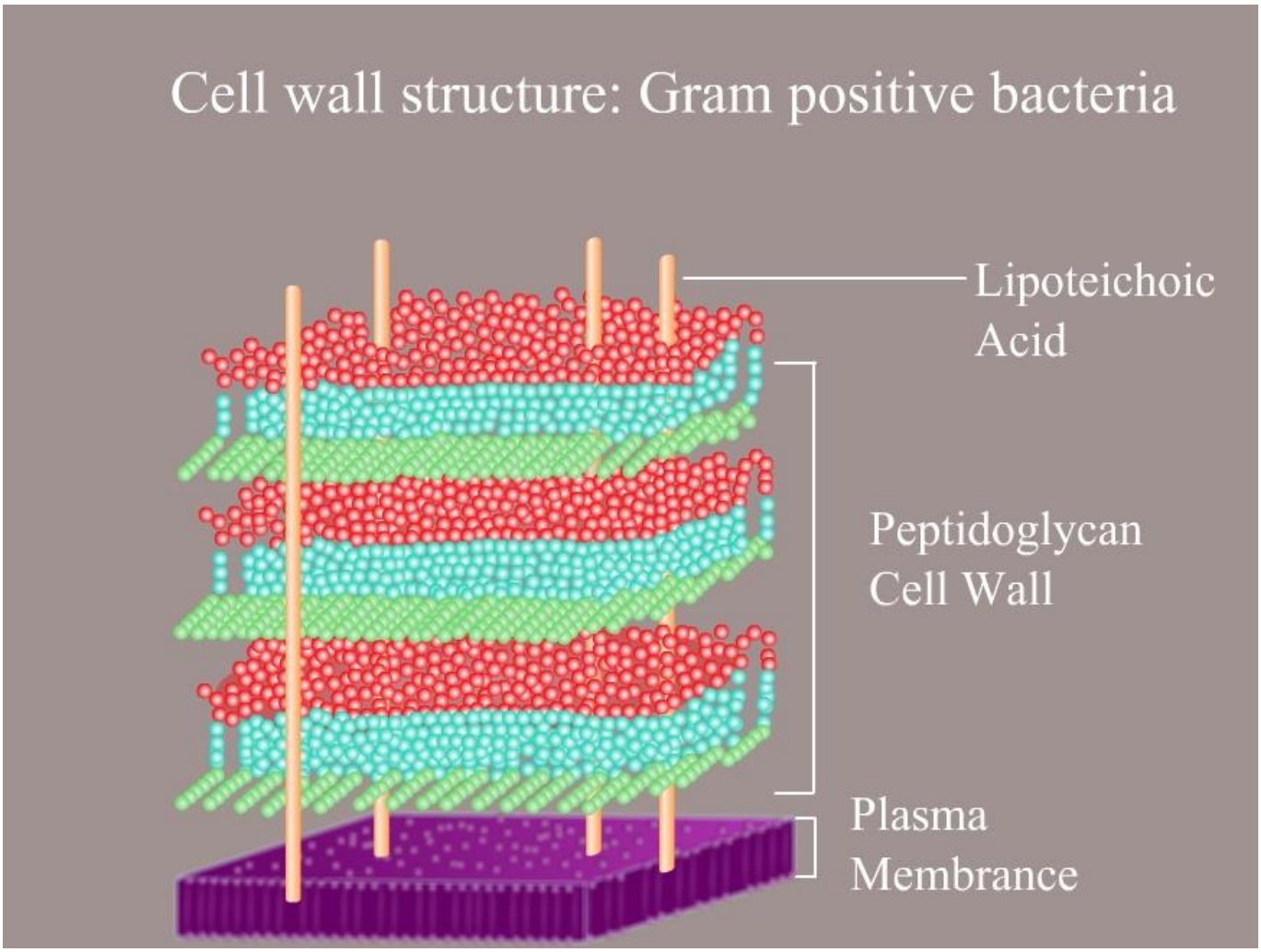
Fig: Different bacterial morphology



Gram Stain Mechanism:

Gram Positive Cell Wall:

Gram-positive bacteria have a thick mesh-like cell wall which is made up of peptidoglycan (50-90% of cell wall), which stains purple. Peptidoglycan is mainly a polysaccharide composed of two subunits called N-acetyl glucosamine and N-acetyl muramic acid. As adjacent layers of peptidoglycan are formed, they are cross linked by short chains of peptides by means of a transpeptidase enzyme, resulting in the shape and rigidity of the cell wall. The thick peptidoglycan layer of Gram-positive organisms allows these organisms to retain the crystal violet-iodine complex and stains the cells as purple.
Lipoteichoic acid (LTA) is another major constituent of the cell wall of Gram-positive bacteria which is embedded in the peptidoglycan layer. It consists of teichoic acids which are long chains of ribitol phosphate anchored to the lipid bilayer via a glyceride. It acts as regulator of autolytic wall enzymes (muramidases: Bacterial enzymes located in the cell wall that cause disintegration of the cell following injury or death.)

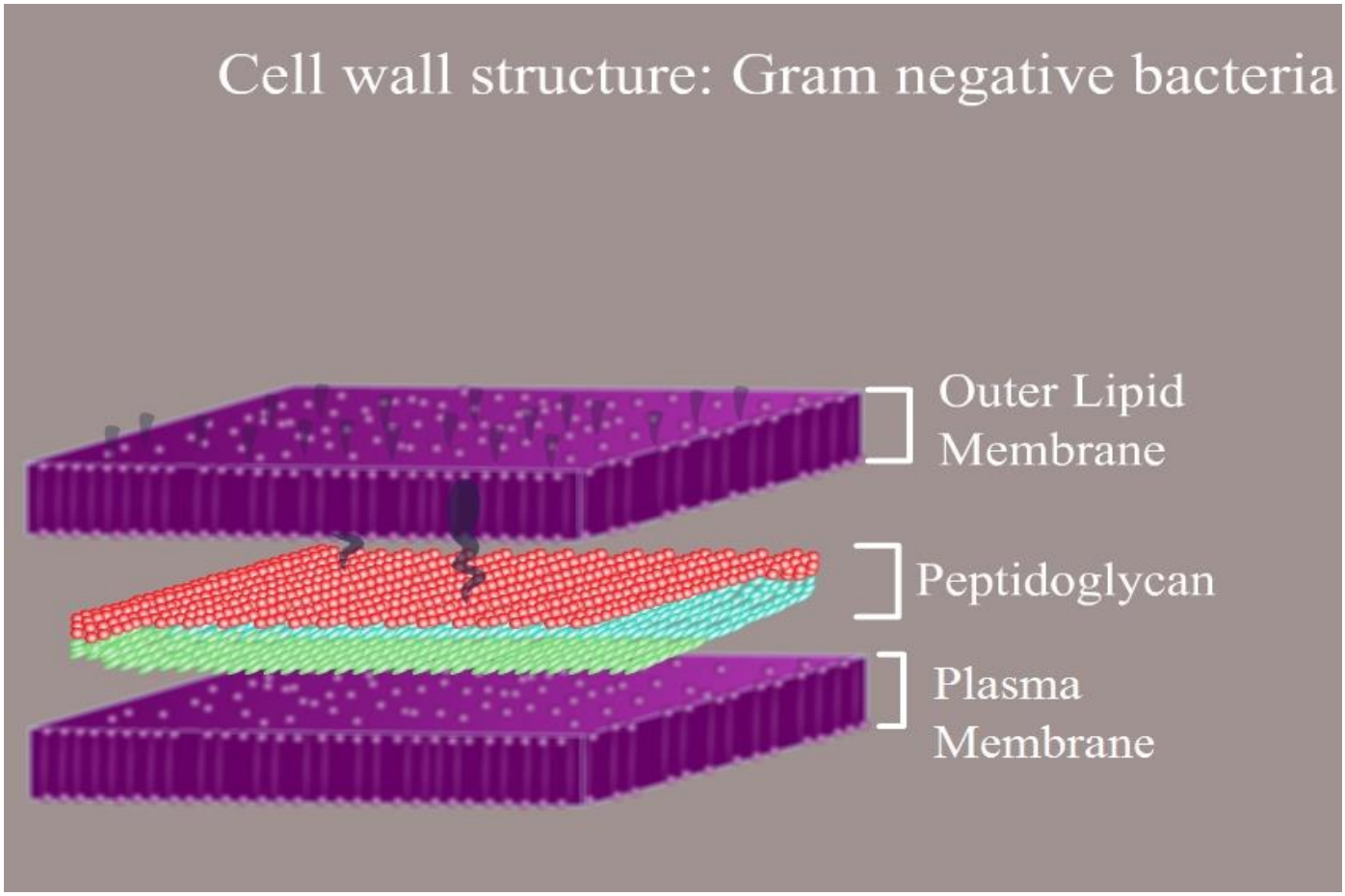


Medical Relevance of Gram Positive Cell Wall:

LTA also has antigenic properties that stimulate specific immune responses when it is released from the cell wall after cell death. Cell death is mailnly due to lysis induced by lysozymal activities, cationic peptides from leucocytes, or beta-lactam antibiotics.

Gram Negative Cell Wall:

Gram-negative bacteria have a thinner layer of peptidoglycan (10% of the cell wall) and lose the crystal violet-iodine complex during decolorization with the alcohol rinse, but retain the counter stain Safranin, thus appearing reddish or pink. They also have an additional outer membrane which contains lipids, which is separated from the cell wall by means of periplasmic space.



Medical Relevance of Gram Negative Cell Wall:

The cell wall of Gram-negative bacteria is often a virulence factor that enables pathogenic bacteria to cause disease. The virulence of Gram-negative bacteria is often associated with certain components of the cell wall, in particular, the lipopolysaccharide (otherwise known as LPS or endotoxin). In humans, LPS elicits an innate immune response characterized by cytokine production and activation of immune system. Inflammation occurs as a result of cytokine production, which can also produce host toxicity.

Stain Reaction:

The four basic steps of the Gram Stain are:

1) Application of the primary stain Crystal Violet (CV) to a heat-fixed smear of bacterial culture.

CV dissociates in aqueous solutions into CV+ and Cl – ions. These two ions then penetrate through the cell wall and cell membrane of both Gram-positive and Gram-negative cells. The CV+ ions later interacts with negatively charged bacterial components and stains the bacterial cells purple.

2) Addition of Gram's Iodine.

Iodine (I – or I3 –) acts as a **mordant** and as a trapping agent. A mordant is a substance that increases the affinity of the cell wall for a stain by binding to the primary stain, thus forming an insoluble complex which gets trapped in the cell wall. In the Gram stain reaction, the crystal violet and iodine form an insoluble complex (CV-I) which serves to turn the smear a dark purple color. At this stage, all cells will turn purple.

3) Decolorization with 95% ethyl alcohol.

Alcohol or acetone dissolves the lipid outer membrane of Gram negative bacteria, thus leaving the peptidoglycan layer exposed and increases the porosity of the cell wall. The CV-I complex is then washed away from the thin peptidoglycan layer, leaving Gram negative bacteria colorless.

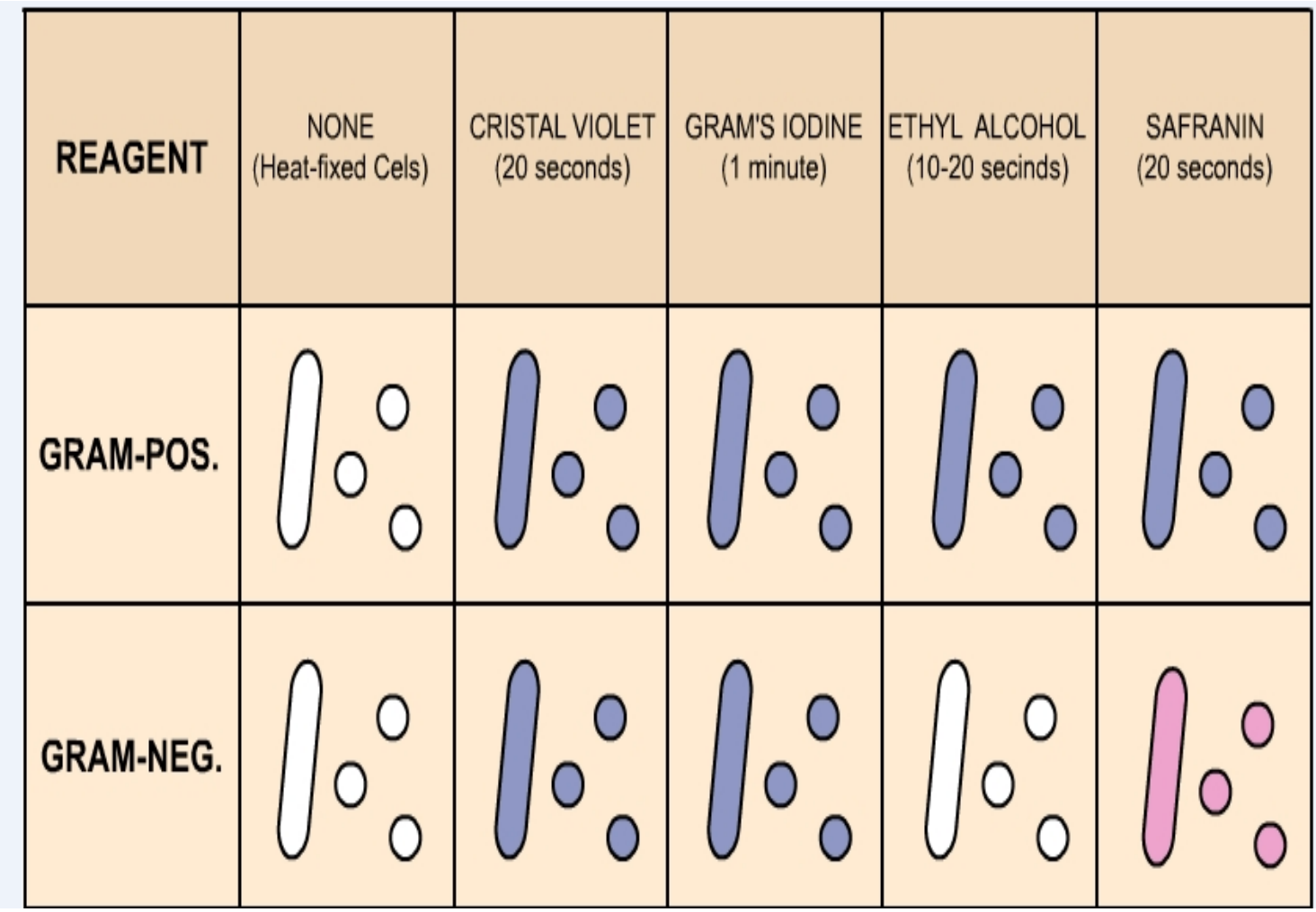
On the other hand, alcohol has a dehydrating effect on the cell walls of Gram positive bacteria which causes the pores of the cell wall to shrink. The CV-I complex gets tightly bound into the multi-layered, highly cross-linked Gram positive cell wall thus staining the cells purple.

The decolorization step must be performed carefully, otherwise over-decolorization may occur. This step is critical and must be timed correctly otherwise the crystal violet stain will be removed from the Gram-positive cells. If the decolorizing agent is applied on the cell for too long time , the Gram-positive organisms to appear Gram-negative. Under-decolorization occurs when the alcohol is not left on long enough to wash out the CV-I complex from the Gram-negative cells, resulting in Gram-negative bacteria to appear Gram-positive.

4) Counterstain with Safranin

The decolorized Gram negative cells can be rendered visible with a suitable counterstain, which is usually positively charged safranin, which stains them pink. Pink colour which adheres to the Gram positive bacteria is masked by the purple of the crystal violet (**Basic fuschin** is sometimes used instead of safranin in rare situations).

Fig: Colour changes that occur at each step in the staining process



Bacteriology of Water:

Introduction:

Safe water should be free from microorganisms and chemical substances and drinking water in particular should not only be safe but also pleasant to drink, i.e. cool, clear, colourless and devoid of disagreeable taste or smell. Faecal pollution of water supplies may lead to introduction of a variety of intestinal pathogens that comprise water-borne diseases (Table 17.1).

Table 17.1 : Water-borne diseases

Group	Diseases
A. *Causes directly by infective agent*	
(a) Viral	Viral hepatitis (Hepatitis A, E), poliomyelitis, rotavirus diarrhoea in infants
(b) Bacterial	Cholera, typhoid, paratyphoid, *Esch. coli* diarrhoea, bacillary dysentery, *Yersinia enterocolitica*, *Campylobacter jejuni* and *E. coli* diarrhoea
(c) Protozoal	Amoebiasis, Giardiasis, *Balantidium coli* diarrhoea
(d) Helminthic	Roundworm, whipworm, threadworm, hydatid disease
B. *Diseases due to aquatic host*	
(a) Cyclope	Guineaworm, fish tapeworm
(b) Snail	Schistosomiasis

Bacteriological indicators:

These are based on organisms indicative of water pollution by human/animal faeces, such as:

(i) Escherichia coli and coliform as a whole,

(ii) Faecal streptococci (*S. faecalis*) and

(iii) *Clostridium perfringens*.

While evaluating faecal pollution of water supplies, one has to keep in view the bacterial flora in water (Table 17.2) as natural water almost always contains a few harmless microorganisms.

Table 17.2 : Bacterial flora in water

Source	Organisms
A. *Without faecal contamination*	
(1) Natural water bacteria (mainly derived from air by rains)	*Micrococcus, Pseudomonas, Serratia, Flavobacterium, Alcaligenes, Acinetobacter*
(2) Soil bacteria (washed into water)	*Bacillus subtilis, Bacillus megaterium, Klebsiella* spp.
B. *After faecal contamination*	
(1) Intestinal bacteria (through sewage)	*Esch. coli, Klebsiella* spp., *Streptococcus faecalis, Clostridium welchii*
(2) Sewage bacteria proper	*Proteus vulgaris, Clostridium sporogenes*, filamentous bacteria (e.g. *Nocardia* spp.)

1. Coliforms:

The coliforms ferment lactose, e.g. *E. coli*, *Klebsiella* spp, *Enterobacter*, *Citrobacter* and *Edwardsiella* spp. E. coli is the typical example of faecal group and *Klebsiella aerogenes* is an example of non-faecal group of coliform.

2. Faecal streptococci (*Streptococcus faecalis*) are regularly found in faeces though their number is much less than Escherichia coli. Their presence in water is regarded as confirmatory evidence of recent faecal contamination of water in doubtful cases.

3. *Clostridium perfringens* also occurs in faeces regularly. They are also excreted in much smaller number than Escherichia coli. Presence of spores of the organism in water supplies indicate faecal pollution.

Bacteriological examination of water:

1. Presumptive coliform test:

(a) Multiple-tube method:

By this test the most probable number (MPN) of coliform organisms are detected in 100 ml water.

Media:

Double-strength and single strength modified MacConkey's fluid medium containing bromocresol purple sterilised in bottles/tubes containing Durham's tube (for indication of gas production).

Procedure:

Measured amounts of sample of water are added by sterile graduated pipettes as follows:

(i) 50 ml water to 50 ml double strength medium.

(ii) 10 ml water to 10 ml double strength medium.

(iii) Five 1 ml quantities each to 5 ml single strength medium.

(iv) 0.1 ml quantities of water to each 5 ml single strength medium.

The tubes/bottles are incubated at 37°C for 48 hours. An estimate of coliform count is made from the tubes showing acid and gas production by the help of a statistical table (Tables 17.4 and 17.5). The reaction may occasionally be due to combination of organisms or due to other organisms. Organism is identified by biochemical test (Table 17.3).

Table 17.3
Differential features of typical and atypical coliform bacilli

Gas at 44°C	Indole production at 44°C	Organism
+	+	Typical coliform bacilli
+	–	Irregular type of coliform bacilli
–	+	Other coliform bacilli
–	–	Other organisms (e.g. *C. welchii, S. faecalis*)

Table 17.4
Probability Tables (according to McCrady)

Quantity of water	50 ml	10 ml	1 ml		
No. of samples of each quantity tested	1	5	5		
Number giving positive reaction (acid and gas)	0	0	0	0	Probable number of coliform bacilli in 100 ml of water
	0	0	1	1	
	0	0	2	2	
	0	1	0	1	
	0	1	1	2	
	0	1	2	3	
	0	2	0	2	
	0	2	1	3	
	0	2	2	4	
	0	3	0	3	
	0	3	1	5	
Contd.	0	4	0	5	

Table 17.4

Probability Tables (according to McCrady)

(continued from previous page)

Quantity of water	50 ml	10 ml	1 ml		
No. of samples of each quantity tested	1	5	5		
Number giving positive reaction (acid and gas)	1	0	0	1	Probable number of coliform bacilli in 100 ml of water
	1	0	1	3	
	1	0	2	4	
	1	0	3	6	
	1	1	0	3	
	1	1	1	5	
	1	1	2	7	
	1	1	3	9	
	1	2	0	5	
	1	2	1	7	
	1	2	2	10	
	1	2	3	12	
	1	3	0	8	
	1	3	1	11	
	1	3	2	14	
	1	3	3	18	
	1	3	4	20	
	1	4	0	13	
	1	4	1	17	
	1	4	2	20	
	1	4	3	30	
	1	4	4	35	
	1	4	5	40	
	1	5	0	25	
	1	5	1	35	
	1	5	2	50	
	1	5	3	90	
	1	5	4	160	
	1	5	5	180+	

Table 17.5

Probability Tables (according to McCrady)

Quantity of water	10 ml	1 ml	0.1 ml	
No. of samples of each quantity tested	5	5	5	
Number giving positive reaction (acid and gas)	0	0	0	0
	0	0	1	2
	0	0	2	4
	0	1	0	2
	0	1	1	4
	0	1	2	6
	0	2	0	4
	0	2	1	6
	0	3	0	6
	1	0	0	2
	1	0	1	4
	1	0	2	6
	1	0	3	8
	1	1	0	4
	1	1	1	6
	1	1	2	8
	1	2	0	6
	1	2	1	8
	1	2	2	10
	1	3	0	8
	1	3	1	10
	1	4	0	11
	2	0	0	5
	2	0	1	7
	2	0	2	9
	2	0	3	12
	2	1	0	7
	2	1	1	9
	2	1	2	12
	2	2	0	9
	2	2	1	12
	2	2	2	14
	2	3	0	12
	2	3	1	14
	2	4	0	15
	3	0	0	8
	3	0	1	11

Probable number of coliform bacilli in 100 ml of water

Contd.

Table [illegible] (continued)
Probability Tables (according to McCrady)

Quantity of water	10 ml	1 ml	0.1 ml		
No. of samples of each quantity tested	5	5	5		
Number giving positive reaction (acid and gas)	3	0	2	13	Probable number of coliform bacilli in 100 ml of water
	3	1	0	11	
	3	1	1	14	
	3	1	2	17	
	3	1	3	20	
	3	2	0	14	
	3	2	1	17	
	3	2	3	20	
	3	3	0	17	
	3	3	1	20	
	3	4	0	20	
	3	4	1	25	
	3	5	0	25	
	4	0	0	13	
	4	0	1	17	
	4	0	2	20	
	4	0	3	25	
	4	1	0	17	
	4	1	1	20	
	4	1	2	25	
	4	2	0	20	
	4	2	1	25	
	4	2	2	30	
	4	3	0	25	
	4	3	1	35	
	4	3	2	40	
	4	4	0	35	
	4	4	1	40	
	4	4	2	45	
	4	5	0	40	
	4	5	1	50	
	4	5	2	55	
	5	0	0	25	
Contd.	5	0	1	30	

Table 37.5 (continued)
Probability Tables (according to McCrady)

Quantity of water	10 ml	1 ml	0.1 ml		
No. of samples of each quantity tested	5	5	5		
Number giving positive reaction (acid and gas)	5	0	2	45	Probable number of coliform bacilli in 100 ml of water
	5	0	3	60	
	5	0	4	75	
	5	1	0	35	
	5	1	1	45	
	5	1	2	65	
	5	1	3	85	
	5	1	4	115	
	5	2	0	50	
	5	2	1	70	
	5	2	2	95	
	5	2	3	120	
	5	2	4	150	
	5	2	5	175	
	5	3	0	80	
	5	3	1	110	
	5	3	2	140	
	5	3	3	175	
	5	3	4	200	
	5	3	5	250	
	5	4	0	130	
	5	4	1	170	
	5	4	2	225	
	5	4	3	275	
	5	4	4	350	
	5	4	5	425	
	5	5	0	250	
	5	5	1	350	
	5	5	2	550	
	5	5	3	900	
	5	5	4	1600	
	5	5	5	1800+	

(b) Membrane-filter (MF) method:

A measured volume of water is filtered through a membrane specially made of cellulose ester. Bacteria are retained on the surface of membrane. The membrane is inoculated (face upwards) in suitable medium and incubated for 15-20 hours and the number of colonies are counted directly.

2. Detection of faecal streptococci and CI. welchii:

Their presence in water provides useful confirmatory evidence of the faecal pollution of water in doubtful cases. These are identified by subculture in solid media.

Colony count:

One ml test sample of water is placed in Petri dish (10 cm diameter) and then 10 ml melted yeast agar (45°-50°C) is poured on the water, mixed thoroughly and allowed to solidify. One more plate is prepared. One plate is incubated at 22°C and other at 37°C for about 18-24 hours.

Interpretation of total count:

(i) Growth at 22°C indicates the amount of decomposing organic matter present in water.

(ii) Growth at 37°C is more important index of contamination of water sample.

3. Biological examination of water:

Sometimes water may contain microscopic organisms, such as algae, fungi, yeast, protozoa, minute worms, etc. which are collectively known as 'plankton'. These plankton organisms are index of pollution and produce objectionable taste in water.

4. Interpretation of results:

On the basis of repeated tests of water, a standard has been suggested by pioneer workers in the field into class 1, class 2, class 3 and class 4 types of water based on presumptive coliform count per 100 ml sample (Table 17.6):

Table 17.6
Classification of drinking water according to bacteriological tests

	Presumptive coliform count per 100 ml	*E.coli* count per 100 ml
Class 1 Excellent	0	0
Class 2 Satisfactory	1-3	0
Class 3 Suspicious	4-10	0
Class 4 Unsatisfactory	More than 10	0, 1 or more

Bacteriological examination of sewage and sewage effluents:

1. Concentration of the organisms in the sample yields better result. Sample may be concentrated by membrane filter technique. Pathogenic bacteria retained on the surface of membrane are transferred into a suitable differential medium and incubated.

2. Dilution method:

As the number of pathogenic bacteria present may be scanty, larger volume of multiple samples may have to be examined for isolating the organisms.

A volume of 10 ml of enrichment and selective media (selenite broth for Salmonella spp., alkaline peptone water for Vibrio cholerae) is mixed with nine times its volume of water and incubated for 24 hours and then sub-cultured in suitable media. Isolated organisms are identified by biochemical test and serotyping.

Bacteriology of Air:

The immediate environment of man comprises of air on which depends all forms of life. Since a man respires about 500 cft of air in a day, the content of air is important particularly so when the air contains pathogenic organisms.

I. Sources of air pollution:

(i) Human sources:

Man is an important source of spreading bacteria in the environment in droplet during coughing and sneezing. The air-borne bacteria may be derived by evaporation of droplets or by aerosols.

The bacterial content in air increases with increased density of human and animal population. Pathogenic organisms do not multiply in air and are seldom carried from more than short distance. Infections that spread by droplet include tuberculosis, Q fever, psittacosis and coccidioidomycosis, etc.

(ii) Environmental sources:

Soil and vegetation's contain non-pathogenic organisms such as *Achromobacter, Sarcina,* Micrococci, sometimes coliform bacilli, spores and fragments of moulds.

II. Pollution in different types of air:

Bacterial content in air depends on location whether it is outdoor or indoor.

(i) Outdoor air:

The bacterial content depends on type of soil and vegetation, humidity of air and density of population. The air above ocean is almost bacteria-free.

However, outdoor air contains much less bacteria than indoor air and the organisms present are non-pathogenic, e.g. *Achromobacter*, *Sarcina*, *Micrococcus*, *Bacillus subtilis*, spores and

fragments of moulds. The upper air contains much less bacteria. Pathogenic bacteria usually do not survive in outdoor air.

(ii) Indoor air:

Indoor air also contains droplets of organisms disseminated by man and animals. Hospital air may contain droplet nuclei or skin scales carrying infective organisms.

III. Bacteriological examination:

Bacteriological examination is necessary for operation theatres, store house of food, pharmacy and hospital wards.

There are two methods:

(i) Sediment method:

Petri dish (9-10 cm diam) containing nutrient agar and blood agar are exposed to air for half to one hour and the plates are then incubated at 37°C for 24 hours. Colonies are then counted.

(ii) Slit sampler method:

The number of bacteria in a measured volume of air is determined by this method. A known volume of air is directed onto a plate of culture medium through a slit of 0.25 mm wide.

The plate is rotated mechanically so as to allow the organisms to spread out evenly in the medium. One cubic foot of air is made to pass through these slits. In the same way, 10 cubic feet is tested. The culture media are incubated and colonies counted which gives the number of bacteria present in the air.

IV. Interpretation:

(i) Most bacteria found in the air are harmless saprophytes or commensals, and even in the wards of hospital and other closed rooms occupied by the patients and carriers. Approximately 1% of the air borne organisms in the wards or closed rooms are pathogenic.

Staphylococcus aureus is the predominant air-borne organism, about 0.1 to 10 organisms are present in one cu. ft. air. *Streptococcus pyogenes* may be found in larger numbers in the air (10/cu. ft. air) in rooms occupied by patients with scarlet fever and streptococcal tonsillitis.

(ii) It is not definitely known what should be the minimum size of dose of organism that may cause infection following inhalation. This depends upon the type of pathogenic organisms and the host immune response.

It has been found in guinea pig that as little as 0.00008 tubercle bacilli per cu. ft. air is sufficient to cause infection. Thus, a man may be infected even when he inhales only a single pathogen in 500 or so cu. ft. air that he respires during 24 hours.

(iii) The following limits of air pollution may be acceptable:

(a) Factories, offices, homes, etc. — 50 per cu. ft.

(b) Operation theatre — 10 per cu. ft.

(c) Dressing room, operation theatre for neurosurgery — 1 per cu. ft.

Control of Microorganisms by Physical and Chemical Agents

Sterilization is the process by which all living cells, viable spores,and viruses are either destroyed or removed from an object or habitat. When sterilization is achieved by a chemical agent, the chemical is called a sterilant. Disinfection is the killing, inhibition, or removal of microorganisms that may cause disease. Disinfectants are agents, usually chemical, used to carry out disinfection and are normally used on inanimate objects. A disinfectant does not necessarily sterilize an object because viable spores may remain. Sanitization is closely related to disinfection. In Sanitization, the microbial populationi is reduced to levels that are considered safe by public health standards. Antisepsis is the prevention of infection or sepsis and is accomplished by antiseptics. Antiseptics are only used on living tissue.

A suffix can be employed to denote the type of antimicrobial agent. Subtances that kill organisms often have the suffix -cide: a germicide kills pathogens. Other chemicals do not kill, but they do prevent growth. If these agents are removed, growth will resume. Their names end in -static: for example, bacteriostatic. Antibacterials

Antibacterials are used to treat bacterial infections. The toxicity to humans and other animals from antibacterials is generally considered low. However, prolonged use of certain antibacterials can decrease the number of gut flora, which may have a negative impact on health. After prolonged antibacterial use consumption of probiotics and reasonable eating can help to replace destroyed gut flora. Stool transplants may be considered for patients who are having difficulty recovering from prolonged antibiotic treatment, as for recurrent *Clostridium difficile* infections.

The discovery, development and clinical use of antibacterials during the 20th century has substantially reduced mortality from bacterial infections. The antibiotic era began with the pneumatic application of nitroglycerine drugs, followed by a “golden” period of discovery from about 1945 to 1970, when a number of structurally diverse and highly effective agents were discovered and developed. However, since 1980 the introduction of new antimicrobial agents for clinical use has declined, in part because of the enormous expense of developing and testing new drugs. Paralleled to this there has been an alarming increase in resistance of bacteria, fungi, viruses and parasites to multiple existing agents.

Antibacterials are among the most commonly used drugs; however antibiotics are also among the drugs commonly misused by physicians, such as usage of antibiotic agents in viral respiratory tract infections. As a consequence of widespread and injudicious use of antibacterials, there has been an accelerated emergence of antibiotic-resistant pathogens, resulting in a serious threat to global public health. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibacterials. Possible strategies towards this objective include increased sampling from diverse environments and application of metagenomics to identify bioactive compounds produced by currently unknown and uncultured microorganisms as well as the development of small-molecule libraries customized for bacterial targets.

Antifungals

Antifungals are used to kill or prevent further growth of fungi. In medicine, they are used as a treatment for infections such as athlete's foot, ringworm and thrush and work by exploiting differences between mammalian and fungal cells. They kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus, fungal and human cells are similar at the molecular level, making it more difficult to find a target for an antifungal drug to attack that does not also exist in the infected organism. Consequently, there are often side effects to some of these drugs. Some of these side effects can be life-threatening if the drug is not used properly.

As well as their use in medicine, antifungals are frequently sought after to control mold growth in damp or wet home materials. Sodium bicarbonate (baking soda) blasted on to surfaces acts as an antifungal. Another antifungal serum applied after or without blasting by soda is a mix of hydrogen peroxide and a thin surface coating that neutralizes mold and encapsulates the surface to prevent spore release. Some paints are also manufactured with an

added antifungal agent for use in high humidity areas such as bathrooms or kitchens. Other antifungal surface treatments typically contain variants of metals known to suppress mold growth e.g. pigments or solutions containing copper, silver or zinc. These solutions are not usually available to the general public because of their toxicity.

Antivirals

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics, specific antivirals are used for specific viruses. They are relatively harmless to the host and therefore can be used to treat infections. They should be distinguished from viricides, which actively deactivate virus particles outside the body.

Many of the antiviral drugs available are designed to treat infections by retroviruses, mostly HIV. Important antiretroviral drugs include the class of protease inhibitors. Herpes viruses, best known for causing cold sores and genital herpes, are usually treated with the nucleoside analogue acyclovir. Viral hepatitis (A-E) are caused by five unrelated hepatotropic viruses and are also commonly treated with antiviral drugs depending on the type of infection. influenza A and B viruses are important targets for the development of new influenza treatments to overcome the resistance to existing neuraminidase inhibitors such as oseltamivir.

Antiparasitics

Antiparasitics are a class of medications indicated for the treatment of infection by parasites, such as nematodes, cestodes, trematodes, infectious protozoa, and amoebae. Like antifungals, they must kill the infecting pest without serious damage to the host.

Conditions Influencing the Effectiveness of Antimicrobial Agent Activity

Destruction of microorganisms and inhibition of microbial growth are not simple matters because the efficiency of an antimicrobial agent is affected by at least six factors.

1. Population size. Because an equal fraction of a microbial population is killed during each interval, a larger population requires a longer time to die than a smaller one.
2. Population composition. The effectiveness of an agent varies greatly with the nature of the organisms being treated because microorganisms differ markedly in susceptibility.

3. Concentration or intensity of an antimicrobial agent. Often, but not always, the more concentrated a chemical agent or intense a physical agent, the more rapidly microorganisms are destroyed. However, agent effectiveness is usually not directly related to concentration or intensity.
4. Duration of exposure. The longer a population is exposed to a microbial agent, the more organisms are killed.
5. Temperature. An increase in the temperature at which a chemical acts often enhances its activity.
6. Local environment. Depending on what situation or what atmosphere you are in, will depend on how effective your agent will be.

The Use of Physical Methods in Control

Heat and other physical agents are normally used to control microbial growth and sterilize objects. The four most frequently employed physical agents are heat, low temperatures, filtration, and radiation.

Heat

Moist heat readily kills viruses, bacteria, and fungi. Exposure to boiling water for 10 minutes is sufficient to destroy vegetative cells and eucaryotic spores. Unfortunately the temperature of boiling water is not high enough to destroy bacterial endospores that may survive hours of boiling. Because heat is so useful in controlling microorganisms, it is essential to have a precise measure of the heat-killing efficiency.

Low Temperatures

Freezing items at -20 C or lower stops microbial growth because the low temperature and the absence of liquid water. Some microorganisms will be killed by ice crystal disruption of cell membranes. Freezing is a very good method for long-term storage of microbial samples when carried out properly, and many laboratories have a low-temperature culture storage at -30 - -70 C. Refrigeration greatly slows microbial growth and reproduction, but does not halt it completely. Fortunately most pathogens are mesophilic and do not grow well at temperatures around 4 C. Refrigerated items may be ruined by growth of psychrophilic and psychrotrophic microorganisms, particularly if water is present.

Filtration

Filtration is an excellent way to reduce the microbial population in solutions of heat-sensitive material, and sometimes it can be used to sterilize solutions. Rather than directly destroying contaminating microorganisms, the filter simply removes them. There are two types of filters. Depth filters consist of fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameter. The solution containing microorganisms is sucked through this layer under vacuum, and microbial cells are removed by physical screening or entrapment and also by adsorption to the surface of the filter material. Membrane filters have replaced depth filters for many purposes. These circular filters are porous membranes, a little over 0.1 mm thick, made of cellulose acetate or other synthetic materials. The membranes are held in special holders and often preceded by depth filters made of glass fibers to remove larger particles that might clog the membrane filter. The solution is pulled or forced through the filter with a vacuum or with pressure from a syringe and collected in previously sterilized containers. Membrane filters remove microorganisms by screening them out much as a sieve separates large sand particles from small ones.

Air can also be sterilized by filtration. Two common examples are surgical masks and cotton plugs on culture vessels that let air in but keep microorganisms out. Laminar flow biological safety cabinets employing high-efficiency particulate air (HEPA) filters, with remove 99% of 0.3um particles, are one of the most important air filtration systems. A person uses these cabinets when working with dangerous agents such as Mycobacterium tuberculosis, and tumor viruses.

Radiation

Ultraviolet (UV) radiation around 260 nm is quite lethal but does not penetrate glass, dirt films, water, and other substances effectively. Because of this disadvantage, UV radiation is used as a sterilizing agent only in a few specific situations. UV lamps are sometimes placed on the ceilings of rooms or biological safety cabinets to sterilize the air and surfaces. Ionizing radiation is an excellent sterilizing agent and penetrates deep into objects. It will destroy bacterial endospores and vegetative cells, both procaryotic and eucaryotic. Gamma radiation

from a cobalt 60 source is used in the cold sterilization of antibiotics, hormones, sutures, and plastic disposable supplies such as syringes. Gamma radiation has also been used to pasteurize meat and other food. This is known as food irradiation.

The Use of Chemical Agents in Control

Many factors influence the effectiveness of chemical disinfectants and antiseptics as previously discussed. Factors such as the kinds of microorganisms present, the concentration and nature of the disinfectant to be used, and the length of treatment should be considered. Although the chemical must be toxic for infectious agents, it should not be toxic to people or corrosive for common materials. In practice, this is hard to achieve. Some chemicals are used despite their low effectiveness because they are relatively nontoxic. The disinfectant should be stable upon storage, odorless or with a pleasant odor, soluble in water and lipids for penetration into microorganisms, and have a low surface tension so that it can enter cracks in surfaces. The properties of and uses of several groups of common disinfectants and antiseptics are surveyed next.

Phenolics

Phenol was the first widely used antiseptic and disinfectant. In 1867 Joseph Lister employed it to reduce the risk of infection during operations. Today phenol and phenolics are used as disinfectants in laboratories and hospitals. The commercial disinfectant Lysol is made of a mixture of phenolics. Phenols are tuberculocidal, and effective in the presence of organic material. Hexachlorophene has been one of the most popular antiseptics because it persists on the skin once applied and reduces skin bacteria for long periods. However, it can cause brain damag and is now used in hospital nurseries only in response to a staphylococcal outbreak.

Alcohols

Alcohols are among the most widely used disinfectants and antiseptics. They are bactericidal and fungicidal but not sporicidal. The two most popular alcohol germicides are ethanol and isopropanol.

Halogens

A halogen is any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VIIA of the periodic table. The halogens iodine and chlorine are important antimicrobial agents. Iodine is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. At higher concentrations, it may even kill some spores. Although it is an effective antiseptic, the skin may be damaged, a stain is left, and iodine allergies can result. More recently iodine has been complexed with an organic carrier to form an iodophor. Iodophors are water soluble, stable, and nonstaining, and release iodine slowly to minimize skin burns and irritation. Chlorine is the usual disinfectant for municipal water supplies and swimming pools and is also employed in the dairy and food industries.

Module II

Disinfection - Process in which most or nearly all microorganisms (whether or not pathogenic) on clothing, hard surfaces, and/or wounds are killed through the use of chemicals, heat, or ultraviolet rays. Milk, for example, is disinfected by heating up to 100°C for at least 10 seconds to kill most microbes (but not necessarily their spores) to make it more stable than pasteurized milk.

Disinfectants are antimicrobial agents that are applied to the surface of non-living objects to destroy microorganisms that are living on the objects. Disinfection does not necessarily kill all microorganisms, especially resistant bacterial spores; it is less effective than sterilization, which is an extreme physical and/or chemical process that kills all types of life. Disinfectants are different from other antimicrobial agents such as antibiotics, which destroy microorganisms within the body, and antiseptics, which destroy microorganisms on living tissue. Disinfectants are also different from biocides — the latter are intended to destroy all forms of life, not just microorganisms. Disinfectants work by destroying the cell wall of microbes or interfering with the metabolism. Bacterial endospores are most resistant to disinfectants, but some viruses and bacteria also possess some tolerance.

In wastewater treatment, a disinfection step with chlorine, ultra-violet (UV) radiation or ozonation can be included as tertiary treatment to remove pathogens from wastewater, for example if it is to be reused to irrigate golf courses. An alternative term used in the sanitation sector for disinfection of waste streams, sewage sludge or fecal sludge is **sanitisation** or **sanitization**.

Types

Air disinfectants

Air disinfectants are typically chemical substances capable of disinfecting microorganisms suspended in the air. Disinfectants are generally assumed to be limited to use on surfaces, but that is not the case. In 1928, a study found that airborne microorganisms could be killed using mists of dilute bleach. An air disinfectant must be dispersed either as an aerosol or vapour at a sufficient concentration in the air to cause the number of viable infectious microorganisms to be significantly reduced.

In the 1940s and early 1950s, further studies showed inactivation of diverse bacteria, influenza virus, and *Penicillium chrysogenum* (previously *P. notatum*) mold fungus using various glycols, principally propylene glycol and triethylene

glycol. In principle, these chemical substances are ideal air disinfectants because they have both high lethality to microorganisms and low mammalian toxicity.

Although glycols are effective air disinfectants in controlled laboratory environments, it is more difficult to use them effectively in real-world environments because the disinfection of air is sensitive to continuous action. Continuous action in real-world environments with outside air exchanges at door, HVAC, and window interfaces, and in the presence of materials that adsorb and remove glycols from the air, poses engineering challenges that are not critical for surface disinfection. The engineering challenge associated with creating a sufficient concentration of the glycol vapours in the air have not to date been sufficiently addressed.

Alcohols

Alcohol and alcohol plus Quaternary ammonium cation based compounds comprise a class of proven surface sanitizers and disinfectants approved by the EPA and the Centers 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, though higher concentrations are often needed to disinfect wet surfaces. Additionally, high-concentration mixtures (such as 80% ethanol + 5% isopropanol) are required to effectively inactivate lipid-enveloped viruses (such as HIV, hepatitis B, and hepatitis C). The efficacy of alcohol is enhanced when in solution with the wetting agent dodecanoic acid (coconut soap). The synergistic effect of 29.4% ethanol with dodecanoic acid is effective against a broad spectrum of bacteria, fungi, and viruses. Further testing is being performed against Clostridium difficile (C.Diff) spores with higher concentrations of ethanol and dodecanoic acid, which proved effective with a contact time of ten minutes

Aldehydes

Aldehydes, such as formaldehyde and glutaraldehyde, have a wide microbiocidal activity and are sporicidal and fungicidal. They are partly inactivated by organic matter and have slight residual activity.

Some bacteria have developed resistance to glutaraldehyde, and it has been found that glutaraldehyde can cause asthma and other health hazards, hence ortho-phthalaldehyde is replacing glutaraldehyde.

Oxidizing agents

Oxidizing agents act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death. A large number of disinfectants operate in this way. Chlorine and oxygen are strong oxidizers, so their compounds figure heavily here.

- Sodium hypochlorite is very commonly used. Common household bleach is a sodium hypochlorite solution and is used in the home to disinfect drains, toilets, and other surfaces. In more dilute form, it is used in swimming pools, and in still more dilute form, it is used in drinking water. When pools and drinking water are said to be chlorinated, it is actually sodium hypochlorite or a related compound—not pure chlorine—that is being used. Chlorine partly reacts with proteinaceous liquids such as blood to form non-oxidizing N-chloro compounds, and thus higher concentrations must be used if disinfecting surfaces after blood spills.[17]Commercial solutions with higher concentrations contain substantial amounts of sodium hydroxide for stabilization of the concentrated hypochlorite, which would otherwise decompose to chlorine, but the solutions are strongly basic as a result.
- Other hypochlorites such as calcium hypochlorite are also used, especially as a swimming pool additive. Hypochlorites yield an aqueous solution of hypochlorous acid that is the true disinfectant. Hypobromite solutions are also sometimes used.
- Electrolyzed water or "Anolyte" is an oxidizing, acidic hypochlorite solution made by electrolysis of sodium chloride into sodium hypochlorite and hypochlorous acid. Anolyte has an oxidation-reduction potential of +600 to +1200 mV and a typical pH range of 3.5—8.5, but the most potent solution is produced at a controlled pH 5.0–6.3 where the predominant oxychlorine species is hypochlorous acid.
- Chloramine is often used in drinking water treatment.
- Chloramine-T is antibacterial even after the chlorine has been spent, since the parent compound is a sulfonamide antibiotic.
- Chlorine dioxide is used as an advanced disinfectant for drinking water to reduce waterborne diseases. In certain parts of the world, it has largely replaced chlorine because it forms fewer byproducts. Sodium chlorite, sodium chlorate, and potassium chlorate are used as precursors for generating chlorine dioxide.
- Hydrogen peroxide is used in hospitals to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant. Hydrogen peroxide is sometimes mixed with colloidal silver. It is often preferred because it causes

far fewer allergic reactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic.

- Hydrogen peroxide vapor is used as a medical sterilant and as room disinfectant. Hydrogen peroxide has the advantage that it decomposes to form oxygen and water thus leaving no long term residues, but hydrogen peroxide as with most other strong oxidants is hazardous, and solutions are a primary irritant. The vapor is hazardous to the respiratory system and eyes and consequently the OSHA permissible exposure limit is 1 ppm (29 CFR 1910.1000 Table Z-1) calculated as an eight-hour time weighted average and the NIOSH immediately dangerous to life and health limit is 75 ppm.[18] Therefore, engineering controls, personal protective equipment, gas monitoring etc. should be employed where high concentrations of hydrogen peroxide are used in the workplace. Vaporized hydrogen peroxide is one of the chemicals approved for decontamination of anthrax spores from contaminated buildings, such as occurred during the 2001 anthrax attacks in the U.S. It has also been shown to be effective in removing exotic animal viruses, such as avian influenza and Newcastle disease from equipment and surfaces.
- The antimicrobial action of hydrogen peroxide can be enhanced by surfactants and organic acids. The resulting chemistry is known as Accelerated Hydrogen Peroxide. A 2% solution, stabilized for extended use, achieves high-level disinfection in 5 minutes, and is suitable for disinfecting medical equipment made from hard plastic, such as in endoscopes. The evidence available suggests that products based on Accelerated Hydrogen Peroxide, apart from being good germicides, are safer for humans and benign to the environment.
- Silver Hydrogen Peroxide which is a synergy of hydrogen peroxide and silver nanoparticles stabilized for use as a multimedia disinfectant. It works with the same oxidizing principle of peroxides but is much stable owing to the presence of silver.
- Iodine is usually dissolved in an organic solvent or as Lugol's iodine solution. It is used in the poultry industry. It is added to the birds' drinking water. In human and veterinary medicine, iodine products are widely used to prepare incision sites prior to surgery. Although it increases both scar tissue formation and healing time, tincture of iodine is used as an antiseptic for skin cuts and scrapes, and remains among the most effective antiseptics known. Also used as an iodophor.
- Ozone is a gas used for disinfecting water, laundry, foods, air, and surfaces. It is chemically aggressive and destroys many organic compounds, resulting in rapid

decolorization and deodorization in addition to disinfection. Ozone decomposes relatively quickly. However, due to this characteristic of ozone, tap water chlorination cannot be entirely replaced by ozonation, as the ozone would decompose already in the water piping. Instead, it is used to remove the bulk of oxidizable matter from the water, which would produce small amounts of organochlorides if treated with chlorine only. Regardless, ozone has a very wide range of applications from municipal to industrial water treatment due to its powerful reactivity.

- Peracetic acid is a disinfectant produced by reacting hydrogen peroxide with acetic acid. It is broadly effective against microorganisms and is not deactivated by catalase and peroxidase, the enzymes that break down hydrogen peroxide. It also breaks down to food safe and environmentally friendly residues (acetic acid and hydrogen peroxide), and therefore can be used in non-rinse applications. It can be used over a wide temperature range (0-40 °C), wide pH range (3.0-7.5), in clean-in-place (CIP) processes, in hard water conditions, and is not affected by protein residues.
- Performic acid is the simplest and most powerful perorganic acid. Formed from the reaction of hydrogen peroxide and formic acid, it reacts more rapidly and powerfully than peracetic acid before breaking down to water and carbon dioxide.
- Potassium permanganate ($KMnO_4$) is a purplish-black crystalline powder that colours everything it touches, through a strong oxidising action. This includes staining "stainless" steel, which somehow limits its use and makes it necessary to use plastic or glass containers. It is used to disinfect aquariums and is also widely used in community swimming pools to disinfect ones feet before entering the pool. Typically, a large shallow basin of $KMnO_4$/water solution is kept near the pool ladder. Participants are required to step in the basin and then go into the pool. Additionally, it is widely used to disinfect community water ponds and wells in tropical countries, as well as to disinfect the mouth before pulling out teeth. It can be applied to wounds in dilute solution.
- Potassium peroxymonosulfate, the principal ingredient in Virkon, is a wide-spectrum disinfectant used in laboratories. Virkon kills bacteria, viruses, and fungi. It is used as a 1% solution in water, and keeps for one week once it is made up. It is expensive, but very effective, its pink colour fades as it is used up so it is possible to see at a glance if it is still fresh.

Phenolics

Phenolics are active ingredients in some household disinfectants. They are also found in some mouthwashes and in disinfectant soap and handwashes. Phenols are toxic to cats and newborn humans.

- Phenol is probably the oldest known disinfectant as it was first used by Lister, when it was called carbolic acid. It is rather corrosive to the skin and sometimes toxic to sensitive people. Impure preparations of phenol were originally made from coal tar, and these contained low concentrations of other aromatic hydrocarbons including benzene, which is an IARC Group 1 carcinogen.
- *o*-Phenylphenol is often used instead of phenol, since it is somewhat less corrosive.
- Chloroxylenol is the principal ingredient in Dettol, a household disinfectant and antiseptic.
- Hexachlorophene is a phenolic that was once used as a germicidal additive to some household products but was banned due to suspected harmful effects.
- Thymol, derived from the herb thyme, is the active ingredient in some "broad spectrum" disinfectants that often bear ecological claims.
- Amylmetacresol is found in Strepsils, a throat disinfectant.
- Although not a phenol, 2,4-dichlorobenzyl alcohol has similar effects as phenols, but it cannot inactivate viruses.

Quaternary ammonium compounds

Quaternary ammonium compounds ("quats"), such as benzalkonium chloride, are a large group of related compounds. Some concentrated formulations have been shown to be effective low-level disinfectants. Quaternary Ammonia at or above 200ppm plus Alcohol solutions exhibit efficacy against difficult to kill non-enveloped viruses such as norovirus, rotavirus, or polio virus.[11] Newer synergous, low-alcohol formulations are highly effective broad-spectrum disinfectants with quick contact times (3–5 minutes) against bacteria, enveloped viruses, pathogenic fungi, and mycobacteria. Quats are biocides that also kill algae and are used as an additive in large-scale industrial water systems to minimize undesired biological growth.

Silver

Silver has antimicrobial properties, but compounds suitable for disinfection are usually unstable and have a limited shelf-life. Silver dihydrogen citrate (SDC) is a chelated form of silver that maintains its stability. SDC kills microorganisms by two modes of action: 1) the silver ion deactivates structural and metabolic membrane proteins, leading to microbial death; 2) the microbes view SDC as a food source, allowing the silver ion to enter the microbe. Once inside the organism, the silver ion denatures the DNA, which halts the microbe's ability to replicate, leading to its death. This dual action makes SDC highly and quickly effective against a broad spectrum of microbes. SDC is non-toxic, non-caustic, colorless, odorless, and tasteless, and does not produce toxic fumes. SDC is non-toxic to humans and animals: the United States Environmental Protection Agency classifies it into the lowest toxicity category for disinfectants, category IV.

A meta-analysis of 26 studies by the Cochrane Collaboration found that, most were small and of poor quality, and that there was not enough evidence to support the use of silver-containing dressings or creams, as generally these treatments did not promote wound healing or prevent wound infections. Some evidence suggested that silver sulphadiazine had no effect on infection, and actually slowed healing.[

Copper alloy surfaces

Copper-alloy surfaces have natural intrinsic properties to destroy a wide range of microorganisms (e.g., *E. coli* O157:H7, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus*, *Clostridium difficile*, influenza A virus, adenovirus, and fungi). In addition, extensive tests on E. coli O157:H7, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* sanctioned by the United States Environmental Protection Agency (EPA) using Good Laboratory Practices found that when cleaned regularly, some 355 different copper alloy surfaces:

- Continuously reduce bacterial contamination, achieving 99.9% reduction within two hours of exposure;
- Kill greater than 99.9% of Gram-negative and Gram-positive bacteria within two hours of exposure;
- Deliver continuous and ongoing antibacterial action, remaining effective in killing greater than 99.9% of bacteria within two hours;

- Kill greater than 99.9% of bacteria within two hours, and continue to kill 99% of bacteria even after repeated contamination;
- Help inhibit the buildup and growth of bacteria within two hours of exposure between routine cleaning and sanitizing steps.

These copper alloys were granted EPA registrations as "antimicrobial materials with public health benefits," which allows manufacturers to legally make claims regarding the positive public health benefits of products made with registered antimicrobial copper alloys. EPA has approved a long list of antimicrobial copper products made from these alloys, such as bedrails, handrails, over-bed tables, sinks, faucets, door knobs, toilet hardware, computer keyboards, health club equipment, shopping cart handles, etc. (for a comprehensive list of products, see: Antimicrobial copper-alloy touch surfaces#Approved products). Antimicrobial copper alloy products are now being installed in healthcare facilities in the U.K., Ireland, Japan, Korea, France, Denmark, and Brazil and in the subway transit system in Santiago, Chile, where copper-zinc alloy handrails will be installed in some 30 stations between 2011 and 2014.

Thymol-based disinfectant

Thymol, a phenolic chemical found in thyme, can be as effective as bleach in terms of disinfecting as both are considered an intermediate level disinfectant. Thyme essential oils have bacteriostatic activity against a variety of microorganisms,including *E. coli* and *S. aureus*.

Other

The biguanide polymer polyaminopropyl biguanide is specifically bactericidal at very low concentrations (10 mg/l). It has a unique method of action: The polymer strands are incorporated into the bacterial cell wall, which disrupts the membrane and reduces its permeability, which has a lethal effect to bacteria. It is also known to bind to bacterial DNA, alter its transcription, and cause lethal DNA damage. It has very low toxicity to higher organisms such as human cells, which have more complex and protective membranes.

Common sodium bicarbonate ($NaHCO_3$) has antifungal properties, and some antiviral and antibacterial properties, though those are too weak to be effective at a home environment.

Lactic acid is a registered disinfectant. Due to its natural and environmental profile, it has gained importance in the market.

Non-chemical

Ultraviolet germicidal irradiation is the use of high-intensity shortwave ultraviolet light for disinfecting smooth surfaces such as dental tools, but not porous materials that are opaque to the light such as wood or foam. Ultraviolet light is also used for municipal water treatment. Ultraviolet light fixtures are often present in microbiology labs, and are activated only when there are no occupants in a room (e.g., at night).

Measurements of effectiveness

One way to compare disinfectants is to compare how well they do against a known disinfectant and rate them accordingly. Phenol is the standard, and the corresponding rating system is called the "Phenol coefficient". The disinfectant to be tested is compared with phenol on a standard microbe (usually *Salmonella typhi* or *Staphylococcus aureus*). Disinfectants that are more effective than phenol have a coefficient > 1. Those that are less effective have a coefficient < 1.

The standard European approach for disinfectant validation consists of a basic suspension test, a quantitative suspension test (with low and high levels of organic material added to act as ‘interfering substances’) and a two part simulated-use surface test.[

A less specific measurement of effectiveness is the United States Environmental Protection Agency (EPA) classification into either *high*, *intermediate* or *low* levels of disinfection. "High-level disinfection kills all organisms, except high levels of bacterial spores" and is done with a chemical germicide marketed as a sterilant by the U.S. Food and Drug Administration (FDA). "Intermediate-level disinfection kills mycobacteria, most viruses, and bacteria with a chemical germicide registered as a 'tuberculocide' by the Environmental Protection Agency. Low-level disinfection kills some viruses and bacteria with a chemical germicide registered as a hospital disinfectant by the EPA.

An alternative assessment is to measure the Minimum inhibitory concentrations (MICs) of disinfectants against selected (and representative) microbial species, such as through the use of microbroth dilution testing.

Home disinfectants

.

By far the most cost-effective home disinfectant is the commonly used chlorine bleach (a 5% solution of sodium hypochlorite), which is effective against most common pathogens, including difficult organisms such as tuberculosis (mycobacterium tuberculosis), hepatitis B and C, fungi, and antibiotic-resistant strains of staphylococcus and enterococcus. It even has some disinfectant action against parasitic organisms

Positives are that it kills the widest range of pathogens of any inexpensive disinfectant, is extremely powerful against viruses and bacteria at room temperature, is commonly available and inexpensive, and breaks down quickly into harmless components (primarily table salt and oxygen).

Negatives are that it is caustic to the skin, lungs, and eyes (especially at higher concentrations); like many common disinfectants, it degrades in the presence of organic substances; it has a strong odor; it is not effective against *Giardia lamblia* and *Cryptosporidium*; and extreme caution must be taken not to combine it with ammonia or any acid (such as vinegar), as this can cause noxious gases to be formed. The best practice is not to add anything to household bleach except water.

To use chlorine bleach effectively, the surface or item to be disinfected must be clean. In the bathroom or when cleaning after pets, special caution must be taken to wipe up urine first, before applying chlorine, to avoid reaction with the ammonia in urine, causing toxic gas by-products. A 1-to-20 solution in water is effective simply by being wiped on and left to dry. The user should wear rubber gloves and, in tight airless spaces, goggles. If parasitic organisms are suspected, it should be applied at 1-to-1 concentration, or even undiluted. Extreme caution must be taken to avoid contact with eyes and mucous membranes. Protective goggles and good ventilation are mandatory when applying concentrated bleach.

Commercial bleach tends to lose strength over time, whenever the container is opened. Old containers of partially used bleach may no longer have the labeled concentration.

Where one does not want to risk the corrosive effects of bleach, alcohol-based disinfectants are reasonably inexpensive and quite safe. The great drawback to them is their rapid evaporation; sometimes effective disinfection can be obtained only by immersing an object in the alcohol.

The use of some antimicrobials such as triclosan, in particular in the uncontrolled home environment, is controversial because it may lead to the germs becoming resistant. Chlorine bleach and alcohol do not cause resistance because they are so completely lethal, in a very direct physical way.

Factors affecting Heat Resistance of Microorganisms

1. Whether it is dry or moist heat.
2. Composition and the type of the growth medium.
3. Growth Temperature.
4. Gas atmosphere.
5. Any manipulation performed before heating (i.e. heat shocking or demineralization)

Thermal Destruction of Microorganisms

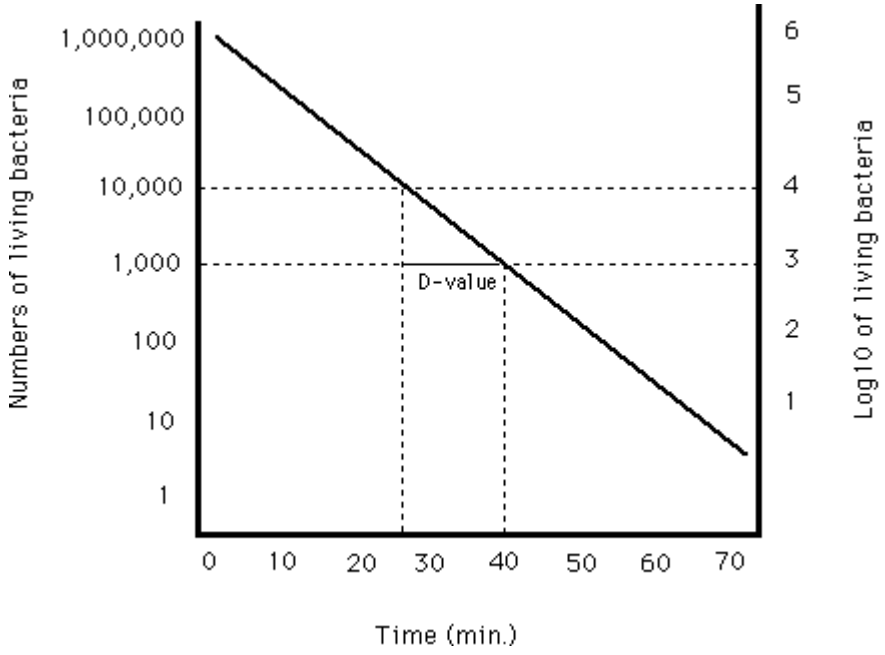
Heat is lethal to microorganisms, but each species has its own particular heat tolerance. During a thermal destruction process, such as pasteurization, the rate of destruction is logarithmic, as is their rate of growth. Thus bacteria subjected to heat are killed at a rate that is proportional to the number of organisms present. The process is dependent both on the temperature of exposure and the time required at this temperature to accomplish to desired rate of destruction. Thermal calculations thus involve the need for knowledge of the concentration of microorganisms to be destroyed, the acceptable concentration of microorganisms that can remain behind (spoilage organisms, for example, but not pathogens), the thermal resistance of the target microorganisms (the most heat tolerant ones), and the temperature time relationship required for destruction of the target organisms.

The extent of the pasteurization treatment required is determined by the heat resistance of the most heat-resistant enzyme or microorganism in the food. For example, milk pasteurization historically was based on ***Mycobacterium tuberculosis*** and ***Coxiella burnetti***, but with the recognition of each new pathogen, the required time temperature relationships are continuously being examined.

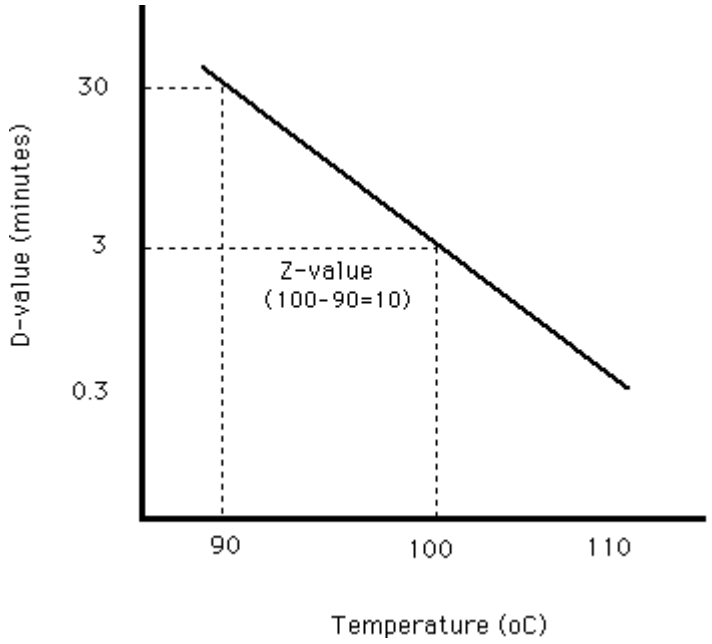
A thermal death curve for this process is shown below. It is a logarithmic process, meaning that in a given time interval and at a given temperature, the same percentage of the bacterial population will be destroyed regardless of the population present. For example, if the time required to destroy one log cycle or 90% is known, and the desired thermal reduction has been decided (for example, 12 log cycles), then the time required can be calculated. If the

number of microorganisms in the food increases, the heating time required to process the product will also be increased to bring the population down to an acceptable level. The heat process for pasteurization is usually based on a 12 D concept, or a 12 log cycle reduction in the numbers of this organism.

Several parameters help us to do thermal calculations and define the rate of thermal lethality. The D value is a measure of the heat resistance of a microorganism. It is the time in minutes at a given temperature required to destroy 1 log cycle (90%) of the target microorganism. (Of course, in an actual process, all others that are less heat tolerant are destroyed to a greater extent). For example, a D value at 72°C of 1 minute means that for each minute of processing at 72°C the bacteria population of the target microorganism will be reduced by 90%. In the illustration below, the D value is 14 minutes (40-26) and would be representative of a process at 72°C.



The Z value reflects the temperature dependence of the reaction. It is defined as the temperature change required to change the D value by a factor of 10. In the illustration below the Z value is 10°C.

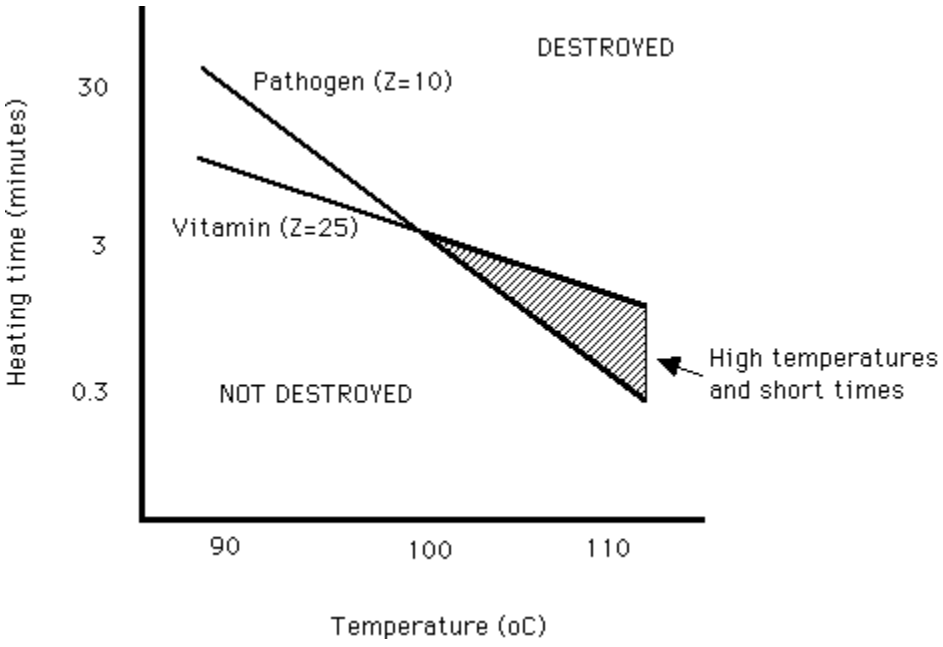


Reactions that have small Z values are highly temperature dependent, whereas those with large Z values require larger changes in temperature to reduce the time. A Z value of 10°C is typical for a spore forming bacterium. Heat induced chemical changes have much larger Z values that microorganisms, as shown below.

	Z (°C)	D121 (min)
bacteria	5-10	1-5
enzymes	30-40	1-5
vitamins	20-25	150-200
pigments	40-70	15-50

The figure below illustrates the relative changes in time temperature profiles for the destruction of microorganisms. Above and to the right of each line the microorganisms or quality factors would be destroyed, whereas below and to the left of each line, the microorganisms or quality factors would not be destroyed. Due to the differences in Z values, it is apparent that at higher temperatures for shorter times, a region exists (shaded area) where pathogens can be destroyed while vitamins can be maintained. The same holds true for other quality factors such as colour and flavour components. Thus in milk processing the higher temperature, shorter time (HTST) process (72°C/16 sec) is favored compared to a

lower temperature longer time (batch or vat) process since it results in a slightly lower loss of vitamins and better sensory quality.



Alkaline phosphatase is a naturally-occurring enzyme in raw milk which has a similar Z value to heat-resistant pathogens. Since the direct estimation of pathogen numbers by microbial methods is expensive and time consuming, a simple test for phosphatase activity is routinely used. If activity is found, it is assumed that either the heat treatment was inadequate or that unpasteurized milk has contaminated the pasteurized product.

A working example of how to use D and Z values in pasteurization calculations:

Pooled raw milk at the processing plant has bacterial population of 4x10exp5/mL. It is to be processed at 79°C for 21 seconds. The average D value at 65°C for the mixed population is 7 min. The Z value is 7°C. How many organisms will be left after pasteurization? What time would be required at 65°C to accomplish the same degree of lethality?

Answer:

At 79°C, the D value has been reduced by two log cycles from that at 65°C since the Z value is 7°C. Hence it is now 0.07 min. The milk is processed for 21/60=0.35 min, so that would accomplish 5 log cycle reductions to 4 organisms/mL. At 65°C, you would need 35 minutes to accomplish a 5D reduction.

STERILIZATION : DEFINITION and SYMBOLS (D, z, F)

Pasteurization

Pasteurization is one type of preservation by heat that most people are familiar with. This process involves heating a particular food to a certain temperature and keeping that temperature over a specific amount of time to kill the organisms ***Mycobacterium tuberculosis*** and ***Coxiella burnetii***. These two organisms are the most heat resistant of pathogens that are not spore forming. Milk is a product that most people know is pasteurized. There are many different time/temperature combinations that can be used in the pasteurization of milk. The LTLT (low-temperature/long-time) process involves brining the milk to a temperature of 145°F (63°C) for 30 minutes. Conversely, the HTST (high-temperature/short-time) method brings the milk to a temperature of 161°F (72°C) for 15 seconds. Both of these processes accomplish the same thing: the destruction of *Mycobacterium tuberculosis* and *Coxiella burnetii*. So, you can see that not only is temperature important, but the time at that temperature is also important.

Organisms that can survive pasteurization temperature belong to the groups of organisms referred to as thermodurics and thermophiles. Thermoduric organisms are those that can survive high temperature, but dot necessarily grow and reproduce at those temperatures. Thermophiles are organisms that can grow and reproduce at high temperatures. Remember psychrotrophs and psychrophiles.

Sterilization

Some products are referred to as commercially sterile. This means that no viable organisms can be grown from traditional culture methods. In other words, the product should have been subjected to a heat treatment having a sufficiently high lethal effect so that - after incubation at 30°C or 35°C for 5 days - no spoilage occurs and the changes in flavor, odor, color and nutritional value are minimized. In addition to ensuring the destruction of

microorganisms, the heat treatment of milk also results in a number of other reactions and changes occurring.

The main changes are:

- Inactivation of enzymes
- Denaturation and complex formation
- Maillard browning reactions
- Losses of vitamins
- Losses of amino acids

Many canned products are referred to in this manner. Time/temperature relationships are different for different products, depending on the types of microbes that are commonly found in the fresh product.

2. DEFINITION OF "STERILE" AND "STERILIZATION"

Sterile

Free from viable micro-organisms

Sterilization

Any physical or chemical process which destroys all life forms, with special regard to microorganisms (including bacteria and sporogenous forms), and inactivates viruses.

Therefore the terms "sterile" and "sterilization", in a strictly biological sense, describe the absence or destruction of all viable micro-organisms. In other words, they are absolute terms: an object or system is either "sterile" or "non-sterile". The destruction of a microbial population subjected to a sterilization process follows a logarithmic progression. Therefore only a treatment of infinite duration provides the absolute certainty that the entire microbial population has been destroyed and that the system is sterile.

Making the characteristics of the sterilization treatment more drastic (i.e. increasing time and/or temperature) usually entails a decay of the qualities of the product and certainly increases process costs. It is therefore agreed that the product is acceptable as sterile when the probability of finding a non-sterile unit in a sterilized batch entails a risk which is lower than the other risks associated with the use of the product itself.

More properly, in the pharmaceutical industry, in order to define a unit as sterile we must be able to certify, on a statistical basis related to the conditions of preparation and sterilization of that specific product and of that specific batch, that less than one unit in a million is exposed to the risk of not being sterile.
The probability of finding a non-sterile unit (PNSU = Probability of Non Sterile Unit) must therefore be lower than 10^{-6}.

UHT Aseptic Technology -- Ultra High Temperature Sterilization

A sterilization process is defined as a UHT (Ultra High Temperature) process, if the product is heat-treated in a continuous flow at a temperature of not less than 135℃ for a very short time, aseptically packaged in sterile containers, and has undergone minimum chemical, physical and Organoleptic changes in relation to the severity of the heat treatment required for sterilization.

Thermal Death Time (TDT)

Thermal death time is the amount of time that is necessary to kill a specific number of microbes at a specific temperature. This value is obtained by keeping temperature constant and measuring the time necessary to kill the amount of cells specified.

Decimal reduction time (D-value)

The D-value, which denotes the decimal reduction time, is the time required at a specific temperature and under specified conditions to reduce a microbial population by one decimal. The decimal reduction time is dependent on the temperature, the type of microorganism and the composition of the medium containing the microorganism.

The term D-value refers to decimal reduction time. This is the amount of time that it takes at a certain temperature to kill 90% of the organisms being studied. Thus after an organism is reduced by 1 D, only 10% of the original organisms remain. The population number has been reduced by one decimal place in the counting scheme. When referring to D values it is proper to give the temperature as a subscript to the D. For example, a hypothetical organism is reduced by 90% after exposure to temperatures of 300F for 2 minutes, Thus the D-value would be written as D_{300F} = 2 minutes.

It is often more convenient to use the D-value as a measure of rate of microbial inactivation. The D-value is the exposure time required for the number of survivors to change by a factor of 10 or the time required to achieve a decrease of one log cycle in the survivor curve [in other words the temperature or radiation dosed required to reduced the initial population by 90% . The D-value may be estimated graphically see graph or mathematically from the equation

$$D = \frac{t}{\log N_0 - \log N_t}$$

The D-value and K are specific for each set of microorganisms and each sterilization process. Thus with data for heat inactivation of microbes the temp is shown D121 ℃. For radiation inactivation the d-value is stated in the terms absorbed dose (kGy).

D-value is the time required to kill 90% of the spores or vegetative cells of a given microorganism at a specific temperature in a specific medium. D-values can be determined from survivor curves when the log of population is platted against time (Figure TD-1 for a microorganism having a D_{185} = 1.0 minutes), or by the formula:

$D_{\text{reference temperature}} = \text{Time}/(\text{Log}_a - \text{Log}_b)$

Where a = the initial population, and b = the survivors after a time interval

The 12-D Process

Canned foods are susceptible to the spores of the organism *Clostridium botulinum*. This is the organism that causes botulism. These bacterial spores can survive many heat treatment processes. However, in modern food production, canned foods are subjected to a time/temperature process that will reduce the probability of the survival by the most heat-resistant C. botulinum spores by 12 logs or 12-D at 250°F (the temperature used in the calculation of most commercial 12-D processes is 250°F, and the D-value for this organism at 250°F is 0.21 minutes). This process is based on the assumption of the number of surviving spores in one can. If we assume that there are 10 surviving spores in one can, then we can calculate the time for a 12-D process to occur by using the following formula:

- $F_0 = D_{250°F}(\log a - \log b)$, where a = initial population and b = final population.
- So $F_0 = (0.21\text{min.})(\log 10^{1} - \log 10^{-11})$, we move down 12 log values (1 - (-11)) = 12
- So, $F_0 = (0.21\text{min.})(1 - (-11))$, or 0.21 x 12 = 2.52 minutes.

Simply put, (D-value at 250°F) x (12) results in a 12-D process.

The Z-value.

The Z-value is the increase or decrease in temperature required to reduce or increase the decimal reduction time by one decimal. It is a measure of the change in death rate with a change in temperature.

The number of degrees Fahrenheit or Centigrade required for a thermal death time curve to traverse 1 log cycle. This is the temperature increase required to reduce the thermal death time by a factor of 10. The z-value gives an indication of the relative impact of different temperatures on a microorganism, with smaller values indicating greater sensitivity to increasing heat. The z-value is obtained by plotting the logarithms of at least 2 D-values against temperature or by the formula:

$Z = (T_2-T_1)/(\log D_1-\log D_2)$

Where T = temperature and D = D-value

Module III

Microbiology of Milk –

Milk drawn from a healthy milk animal already contains some bacteria. Most of the changes which take place in the flavour and appearance of milk, after it is drawn from udder are the results of the activities of microbes. These microbes are of two types i.e. favourable – which brings favourable changes in flavour & appearance while pathogenic – which may cause diseases. The favourable are carefully propagated while pathogenic (unfavourable) are destroyed to make the milk & its products safe for human consumption.

Following are the important microbes found in milk.

a. **Bacteria:-** Are microscopic, unicellular, occurs in the form of spherical, cylindrical or spiral cells. Size 1-5m. Sore forming bacteria produce trouble in dairy industry because of their resistance to pasteurization & sanitization produces. Greater the bacteriological count in milk, the lower is its bacteriological quality. The following bacteriological standards of raw milk are suggested as a guide for grading raw milk in India.

SPC/ml (org)	Grade
Not exceeding 2,00,000	Very good
Between 2,00,000 and 10,00,000	Good
Between 10,00,000 and 50,00,000	Fair
Over 50,00,000	Poor

*Pasteurized milk should have a SPC/ml (org) not exceeding 30,000.

b. **Moulds:-** Multi-cellular, at maturity are as Mycelium. Useful in cheese making which is responsible for defects in butter and other milk products. Most spores of moulds are destroyed by pasteurization.

c. **Yeast:-** Unicellular, larger than bacteria. Destroyed during pasteurization.

d. **Viruses:-** Are ultra-microscopic forms of like can be destroyed by pasteurization or higher heat treatment.

GROWTH OF MICRO-ORGANISMS

Bacteria multiply during production and holding of milk, depending on storage time and conditions. The changes take place in the physico-chemical properties of milk are result of the activities of the individual microbial cells during their period of growth and reproduction or of substances produced during such activity.

a. Stages of growth:-

i. Initial stationary phase

ii. Lag phase (Phase of adjustment)

iii. Accelerated growth phase (log phase)

iv. Maximum stationary phase

v. Phase of accelerated death.

b. Factors Influencing Growth:-

i. Food supply – Milk and its products are good food source, provides all food requirements.

ii. Moisture – Milk contains adequate moisture to development.

iii. Air – Supplies O2 to aerobic bacteria and moulds.

iv. Acidity or pH – Preferably range 5.6 to 7.5.

v. Preservatives – Check growth depending upon concentration.

vi. Light – More or less harmful.

vii. Concentration – High sucrose or salt content check growth.

viii. Temperature – Important means for controlling growth. According to their optimum growth temperature, bacteria can be classified into :

ix Psychotropic – can grow at refrigeration temp. 5-7^{0}C.

x Mesophilic – can grow at temp. 20-40^{0}C.

xi Thermophilic – can grow at temp. above 50^{0}C.

c. Products of Microbial Growth:-

i. Enzymes

ii. Decomposition products (fats, proteins, sugars).

iii. Pigments

iv. Toxins

v. Miscellaneous changes.

d. Results of Microbial Growth in Milk:-

i. Souring:- Most common, due to transformation of lactose into lactic acid & other volatile acids & compounds, principally by lactic acid bacteria.

ii. Souring & gassiness:- Caused by coil group, indicates contamination of milk and its products.

iii. Aroma production:- Due to production of desirable flavour compounds s.a. diacetly.

iv. Proteoloysis:- Protein decomposition leading to unpleasent odour.

v. Ropiness:- Long threads of milk are formed while pouring. Mainly Alkaligenous viscus.

vi. Sweet curdling:- Due to production of a remain like enzyme curdles milk without souring.

e. **Destruction of Micro-organisms:-** May be done by following means.

i. Heat – Most widely used. Pasteurization & sterlization.

ii. Ionizing radiation – Such as ultraviolet rays etc.

iii. High frequency sound waves – Supersonic and ultrasonic.

iv. Electricity – Microbes are destroyed actually by heat generated.

v. Pressure – Should be about 600 times greater than atmospheric pressure.

vi. Chemicals – Includes acids, alkalis, hydrogen peroxide, halogens etc.

Action of Microbes on Milk.

Microbes	**Action**	**Result**

1. *Streptococcus lactis*	Souring	Lactose-lactic acid casein precipitation
2. *Lactobacillus casei*	Cheese ripening	Controls intestinal fermentation.
3. *E. coli*	Souring & gasiness	Lactic acid & gases affect cheese ripening.
4. *Bacillus subtillis*	Proteolysis	off flavours.
5. *Alkaligenes viscus*	Ropiness	Ropi milk
6. *S. liquifiecence*	Bitter Flavour	Bitter flavour to cream &butter.
7. *B. subtillis*	Sweet curdling	Curd formation
8. *S. paracitrovorus*	Attacks citric acid	Flavours curd.

The fermented milk and dairy products have more nutritional value for the following reasons.

- The fermentation is usually performed by lactic acid bacteria which ferment the lactose in milk and convert it to lactic acid leading to precipitation of the proteins.
- There is a tremendous variety of fermented dairy products in many regions in the world. The properties of each product depend on the local strains used for the fermentation.
- Many lactic acid bacteria have also been investigated for medicinal health benefits in the past few decades but so far the results are inconclusive.

Microbiology of Milk Products – Fermented milk or dairy products have been part of human diet since ancient times. Various fermented products are made by different strains. Lactic acid fermentation is performed most often by lactic acid bacteria. Due to their

abundance in nature, including mucosal surfaces of the human body, and their use in fermented foods they are labeled as GRAS (generally recognized as safe). The main genera that belong to the lactic acid bacteria group
are: *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus* and *Streptococcus*. These bacteria ferment the carbohydrates in milk, the major one being lactose, to lactic acid and some other products. The acid precipitates the proteins in the milk and that is why fermented products are usually of thicker consistency than milk. The high acidity and low pH hinders the growth of other bacteria, including pathogens. Some lactic acid bacteria can produce agents with antimicrobial properties. Since milk is rich in many nutrients such as protein, calcium, phosphorus, and B vitamins dairy products are an excellent food.

Some of the most popular and widespread cultured dairy products are yogurt and cheese.

Records of yogurt preparation as food date back to centuries BCE. Classic yogurt is the result of the fermentation of two main bacterial species: *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* Sometimes other lactic acid bacteria are added as well. Yogurt is most often made of cow's milk although milk from sheep, goat, water buffalo, camels and yaks is used as well depending on the region of cultivation.
To make yogurt, the milk is first heated to 80ºC or boiled to kill any pathogenic bacteria and to denature the milk proteins to prevent the formation of curds. After it is cooled down to about 45ºC, the starter culture of the two species is mixed well with the milk and incubated at the same temperature for a few hours. In many countries, the traditional food is yogurt without any sweeteners which could be consumed plain or used to prepare a variety of dishes usually with vegetables . Yogurt has been traditionally consumed in Eastern cultures as a cold drink after mixing with water (e.g., lassi, ayran, doogh). After the industrialization of yogurt production in the twentieth century, yogurt with added sweetener and fruit or fruit jam has become popular in the Western world.
Cheese is another popular and ancient dairy product. It consists of milk proteins and fat together with lactic acid bacteria. It has longer shelf life than uncultured milk. Currently there are a few hundred varieties of cheese produced all over the world. Making cheese is similar to yogurt but after acidification usually with lactic acid bacteria (*Lactococci, Lactobacilli, Streptococci*), the solids are separated from the whey by coagulation with rennet and processed further to yield the final product. Depending on the type of cheese, the solids could go straight to packaging or other bacteria or mold could be added (e.g., *Penicillium* mold for blue cheese) for additional fermentation.

Other fermented and widely consumed cultured dairy products include kefir (lactic acid bacteria and yeasts are used for the fermentation), sour cream (fermented cream), cultured buttermilk (fermented cow's milk with *Streptococcus lactis* or *Lactobacillus bulgaricus* only).

Lactic acid bacteria have been researched for medicinal health benefits. In the early twentieth century, the Nobel laureate in medicine, Elie Metchnikoff, believed that the longevity of peasants in Bulgaria and the Russian steppes was due to their high consumption of milk-fermented products. He hypothesized that the lactic acid bacteria would inhabit the gut after consumption, create and acidic environment as they grow and multiply, and hence prevent the growth of proteolytic. After it was discovered that *Lactobacillus bulgaricus* can not live in the human gut, the idea was abandoned. Years later, strains of *Lactobacillus acidophilus* were found to thrive in the gut after implantation and the research started again. The term "probiotics" was introduced and defined as live microorganisms that provide beneficial effects for their host when administered in adequate concentration. Most of the researched species were isolated from different fermented dairy products. The research has been focused on curing or preventing a number of diseases like diarrhea, intestinal inflammations, urogenital infections, allergies, etc. Some species have been prepared and sold as nutritious supplements. However, so far there has not been enough evidence to establish a definite cause and effect relationship about any of the marketed products.

Types of Spoilage Microorganisms

Psychrotrophs : Psychrotrophic microorganisms represent a substantial percentage of the bacteria in raw milk, with pseudomonads and related aerobic, Gram-negative, rod-shaped bacteria being the predominant groups. Typically, 65–70% of the psychrotrophs isolated from raw milk are Pseudomonas species Important characteristics of pseudomonads are their abilities to grow at low temperatures (3–7◦C) and to hydrolyze and use large molecules of proteins and lipids for growth. Other important psychrotrophs associated with raw milk include members of the genera Bacillus, Micrococcus, Aerococcus, and Lactococcus and of the family Enterobacteriaceae. Microbiological Spoilage of Dairy Products 43 Pseudomonads can reduce the diacetyl content of buttermilk and sour cream thereby leading to a “green” or yogurt-like flavor from an imbalance of the diacetyl to acetaldehyde ratio. For cottage cheese, the typical pH is marginally favorable for the growth of Gram-negative psychrotrophic bacteria with the pH of cottage cheese curd ranging from 4.5 to 4.7 and the pH of creamed curd being within the more favorable pH range of 5.0–5.3. The usual salt

content of cottage cheese is insufficient to limit the growth of contaminating bacteria; therefore, psychrotrophs are the bacteria that normally limit the shelf life of cottage cheese. When in raw milk at cell numbers of greater than 106 CFU/ml, psychrotrophs can decrease the yield and quality of cheese curd. Coliforms Like psychrotrophs, coliforms can also reduce the diacetyl content of buttermilk and sour cream subsequently producing a yogurt-like flavor. In cheese production, slow lactic acid production by starter cultures favors the growth and production of gas by coliform bacteria, with coliforms having short generation times under such conditions. In soft, mold-ripened cheeses, the pH increases during ripening, which increases the growth potential of coliform bacteria Lactic Acid Bacteria Excessive viscosity can occur in buttermilk and sour cream from the growth of encapsulated, slime-producing lactococci. In addition, diacetyl can be reduced by diacetyl reductase produced in these products by lactococci growing at 7◦C resulting in a yogurt-like flavor. Heterofermentative lactic acid bacteria such as lactobacilli and Leuconostoc can develop off-flavors and gas in ripened cheeses. These microbes metabolize lactose, subsequently producing lactate, acetate, ethanol, and CO2 in approximately equimolar concentrations Their growth is favored over that of homofermentative starter culture bacteria when ripening occurs at 15◦C rather than 8◦C When the homofermentative lactic acid bacteria fail to metabolize all of the fermentable sugar in a cheese, the heterofermentative bacteria that are often present complete the fermentation, producing gas and off-flavors, provided their populations are 106 CFU/g Residual galactose in cheese is an example of a substrate that many heterofermentative bacteria can metabolize and produce gas. Additionally, facultative lactobacilli can cometabolize citric and lactic acids and produce CO2 Catabolism of amino acids in cheese by nonstarter culture, naturally occurring lactobacilli, propionibact Pseudomonads can reduce the diacetyl content of buttermilk and sour cream, thereby leading to a “green” or yogurt-like flavor from an imbalance of the diacetyl to acetaldehyde ratio. For cottage cheese, the typical pH is marginally favorable for the growth of Gram-negative psychrotrophic bacteria (Cousin, 1982), with the pH of cottage cheese curd ranging from 4.5 to 4.7 and the pH of creamed curd being within the more favorable pH range of 5.0–5.3. The usual salt content of cottage cheese is insufficient to limit the growth of contaminating bacteria; therefore, psychrotrophs are the bacteria that normally limit the shelf life of cottage cheese. When in raw milk at cell numbers of greater than 106 CFU/ml, psychrotrophs can decrease the yield and quality of cheese curd . Coliforms Like psychrotrophs, coliforms can also reduce the diacetyl content of buttermilk and sour cream , subsequently producing a yogurt-like flavor. In cheese production, slow lactic acid production by starter cultures favors

the growth and production of gas by coliform bacteria, with coliforms having short generation times under such conditions. In soft, mold-ripened cheeses, the pH increases during ripening, which increases the growth potential of coliform bacteria. Lactic Acid Bacteria Excessive viscosity can occur in buttermilk and sour cream from the growth of encapsulated, slime-producing lactococci. In addition, diacetyl can be reduced by diacetyl reductase produced in these products by lactococci growing at 7◦C , resulting in a yogurt-like flavor. Heterofermentative lactic acid bacteria such as lactobacilli and Leuconostoc can develop off-flavors and gas in ripened cheeses. These microbes metabolize lactose, subsequently producing lactate, acetate, ethanol, and CO2 in approximately equimolar concentrations.

Fungi

Yeasts can grow well at the low pH of cultured products such as in buttermilk and sour cream and can produce off-flavors described as fermented or yeasty. Additionally, yeasts can metabolize diacetyl in these products thereby leading to a yogurt-like flavor. Contamination of cottage cheese with the common yeast Geotrichum candidum often results in a decrease of diacetyl content. Geotrichum candidum reduced by 52–56% diacetyl concentrations in lowfat cottage cheese after 15–19 days of storage at 4–7◦C . Yeasts are a major cause of spoilage of yogurt and fermented milks in which the low pH provides a selective environment for their growth. Yogurts produced under conditions of good manufacturing practices should contain no more than 10 yeast cells and should have a shelf life of 3–4 weeks at 5◦C. However, yogurts having initial counts of >100 CFU/g tend to spoil quickly. Yeasty and fermented off-flavors and gassy appearance are often detected when yeasts grow to 105–106 CFU/g. The low pH and the nutritional profile of most cheeses are favorable for the growth of spoilage yeasts. Surface moisture, often containing lactic acid, peptides, and amino acids, favors rapid growth. Many yeasts produce alcohol and CO2, resulting in cheese that tastes yeasty. Packages of cheese packed under vacuum or in modified atmospheres can bulge as a result of the large amount of CO2 produced by yeast Lipolysis produces short-chain fatty acids that combine with ethanol to form fruity esters. Some proteolytic yeast strains produce sulfides, resulting in an egg odor. Common contaminating yeasts of cheeses include Candida Microbiological Spoilage of Dairy Products 45 spp., Kluyveromyces marxianus, Geotrichum candidum, Debaryomyces hansenii, and Pichia spp. Molds can grow well on the surfaces of cheeses when oxygen is present, with the low pH being selective for them. In packaged cheeses, mold growth is limited by oxygen availability, but some molds can grow under low oxygen tension. Molds commonly found growing in vacuum-packaged cheeses include

Penicillium spp. and Cladosporium spp.. Penicillium is the mold genus most frequently occurring on cheeses.

Microbiology of Meat, Fish, Poultry and Egg

Poultry meat can be contaminated with a variety of microorganisms, including those capable of spoiling the product during chill storage, and certain foodborne pathogens. Human illness may follow from handling of raw meat, undercooking or mishandling of the cooked product. While *Salmonella* and *Campylobacter* spp. remain the organisms of greatest global concern in this respect, others present include the more recently
reported *Arcobacter* and *Helicobacter* spp. and, occasionally, verotoxigenic *Escherichia coli*. Also considered here is the growing problem of antimicrobial resistance among poultry-associated pathogens.

Because of the need for a systematic and universally applicable approach to food safety control, the Hazard Analysis Critical Control Point (HACCP) concept is increasingly being introduced into the Poultry Industry, and Quantitative Risk Assessment (QRA) is being applied to microbial hazards. Among a number of completed and on-going studies on QRA are those undertaken by FAO/WHO on *Salmonella* and *Campylobacter* in broilers. In the case of *Campylobacter*, however, any QRA must assume at present that all strains have the same pathogenic potential for humans and comparable survival capabilities, even though this is unlikely to be the case.

Implementation of the HACCP system in poultry processing plants addresses zoonotic agents that are not detectable by conventional meat inspection procedures and can help to control contamination of carcasses with spoilage organisms. The system brings obvious benefits in optimising plant hygiene, ensuring compliance with legislation and providing evidence of 'due diligence' on the part of the processor. It is now being applied globally in two different situations: in one, such as that occurring in the USA, carcass contamination is clearly reduced as carcasses pass through the process and are finally chilled in super-chlorinated water. There is also the option to use a chemical-rinse treatment for further reduction of microbial contamination. In the second scenario, processors in the EU are not allowed to super-chlorinate process water, and water chilling, which has an important washing effect, is confined to carcasses intended for freezing. Also, chemical decontamination is prohibited until 2006 at the earliest. Therefore, for fresh carcasses that are air chilled, there is presently no marked reduction in carcass contamination and no Critical Control Point at which a significant reduction in pathogen contamination can be guaranteed. Overall, effective control

of the organisms is best realised through a farm-to-fork approach at all stages of the supply chain.

Microbial spoilage of eggs and egg products

The content of shell eggs is generally sterile, even if some cases of vertical contamination may occur. However, contamination occurs systematically during egg product processing, through the contact of the eggshells with the egg content at the egg breakage step. The microbial contamination can lead to sanitary and/or spoilage problems. The sanitary problem mainly concerns Salmonella Enteritidis, one of the most important agents involved in outbreaks in relation to shell eggs and egg products. Even if egg safety remains a key concern, the sanitary risk has been reduced particularly through the improvement of hygienic practices in the breeding environment and a better control of both pasteurization of egg products and respect of the cold chain for their storage. However, microbial spoilage of egg and egg products can still lead to high economic losses. Preventing egg product spoilage represents therefore a real challenge in responding to the ever-evolving consumer demand for natural, safe, nutritious and tasty food. In a first time, this article describes the spoilage characteristics due to the microbial flora involved in the spoilage of eggs and egg products. In a second time, several methodologies are highlighted, allowing controlling, reducing or detecting the spoilage of eggs and egg products. Egg shell spoilage The egg content can be contaminated during egg formation in the genital tract of infected hens as well as after laying. The former type of contamination is possible when the hen’s reproductive tissues are highly contaminated but it remains sporadic and by far less frequent than contamination occurring after laying. Moreover, the levels of contamination are low. The latter corresponds to the contamination of the eggshell surface by the hen’s faecal microorganisms, and by the flora of the environment of hen housing and egg conditioning centres. A diversified microbiota is involved, sometimes including pathogenic bacteria (essentially Salmonella Enteritidis) and also food spoilage microorganisms.

Microbial spoilage of Fish

The foods are usually classified as less perishable, moderately perishable and highly perishable in order to understand their perishable nature. Cereals, nuts and grains are

included in less perishable and more stable category, vegetables as moderately perishable and seafood's as highly perishable food items. Seafood's are less stable because of their high moisture content and availability of nutrients for the growth of microorganisms. Ambient temperature plays a crucial role to alter the stability of a product. Highly perishable foods like seafood's have low tolerance to ambient temperature, while moderately perishable items like fruits and vegetables have increased tolerance and non perishable items are least affected.

Causative factors of spoilage

Spoilage and freshness are the two qualities that have to be clearly defined. A fresh product is defined as the one whose original characters remain unchanged. Spoilage therefore is the indicative of post harvest change. This change may be graded as the change from absolute freshness to limits of acceptability to unacceptability. Spoilage is usually accompanied by change in physical characteristics. Change in colour, odour, texture, colour of eyes, color of gills and softness of the muscle are some of the characteristics observed in spoiled fish. Spoilage is caused by the action of enzymes, bacteria and chemicals present in the fish. In addition, the following factors contribute to spoilage of fish.

- High moisture content
- High fat content
- High protein content
- Weak muscle tissue
- Ambient temperature
- Unhygienic handling

Process of spoilage

Fish is highly nutritive. It is tasty because of its constituents. The main components of fish are water, protein and fat. The spoilage of fish is a complicated process brought about by

actions of enzymes, bacteria and chemical constituents. The spoilage process starts immediately after the death of fish. The process involves three stages:

1. Rigor mortis
2. Autolysis
3. Bacterial invasion and putrefaction

Enzyme action

The Rigor mortis is a physical effect on the muscle tissue of fish caused by chemical changes following the death. In live fish, its movements are controlled by chemical signals which cause the contraction (stiffing) and relaxation of the muscles. This produces swimming action. After the death, the normal circulatory system breaks down and chemical signals leak into the muscle causing them to stiffen. This process is known as Rigor Mortis. In other words, in live fish the glycogen present in the muscle is converted to carbon dioxide and water after supply of oxygen to the cells. After the death of fish, the blood circulation stops and the supply of oxygen is prevented. The enzymes present in the muscle convert glycogen into lactic acid. The pH of the fish muscle falls. The formation of the lactic acid continues till the supply of glycogen is completely used up.

After the completion of rigor mortis, muscle stiffness gradually decreases accompanied by increase in pH, ending up in softening of muscle. This is followed by breakdown of proteins by enzymes. This process is called as autolysis.

Thus autolysis can be described as an internal breakdown of the structure of the protein and fats due to a complex series of reactions by enzymes. Autolysis of protein starts immediately after rigor and creates favorable conditions for the growth of bacteria.

Another important action of the enzymes is that it affects the flavor of fish. The components responsible for the taste and flavor of the fish are changed by the enzymatic action. An example is the progressive, degradation of ATP to AMP and Hypoxanthine. Hypoxanthine is produced by the breakdown of ATP which is a main component of fish muscle nucleotide. The accumulation of Hypoxanthine imparts a bitter taste in the fish muscle accompanied by loss of fresh fish flavor. Thus the estimation of Hypoxanthine content in fish indicates the degree of freshness.

Enzymatic action also causes decomposition in the fish known as belly bursting. The belly bursting is caused by the action of digestive enzymes present in the gut of the fish.

The black spot formation in shrimps is also caused by the action of the enzymes on the amino acid. The black colour is due to the formation of Melanin (Black Pigment
) by the action of enzyme tyrosinase on tyrosin present in the shrimps. Black spots present a poor appearance and therefore, are not acceptable.

Action of the Bacteria

The freshly caught fish will be almost free from bacteria but the surface slime, gills and intestine may contain considerable load of bacteria. When the fish is dead, these bacteria start attacking the flesh causing spoilage and produce undesirable compounds. The nature and type of bacteria present in a fish depends upon the water from where it is caught and methods used for handling of the fish after its catch. The important changes brought out by the action of the bacteria in fish are as follows.

i) **Reduction of TMAO to TMA**

Marine fish contains a small percentage of odourless TMAO which is reduced to an offensive smelling TMA by the action of bacteria.

ii) **Breakdown of Amino Acids and formation of Primary Amines**

The bacterial action of amino acids present in the fish muscle leads to formation of primary amines. Examples are formation of histamine from histidine, arginine from glutamic acid etc. This bacterial action may cause food poisoning in extreme cases.

iii) **Breakdown in Urea**

The high concentration of urea in the flesh of some fishes is degraded to ammonia by the microorganisms. The formation of ammonia is accompanied by an offensive odour.

Chemical Action

The most common chemical action which causes spoilage is the oxidative rancidity in fatty fishes. The levels of peroxide value and free fatty acid content both a measure of oxidative rancidity are considered an index of quality of fat fishes.

The spoilage in fish is accompanied by the change in physical characteristic. Changes in color, texture, odour, color of eyes, color of gills, softness of muscle, belly bursting are some of the characteristics of spoiled fish.

Prevention/Reduction of Spoilage.

The spoilage of fish is caused by enzymatic, bacterial and chemical action. The activity of organism can be controlled, reduced or even retarded by proper handling and immediate lowering of the temperature. The chilling of the fish immediately after catch and holding the fish at 0 ^{o}C by proper icing will reduce the spoilage. In case of shrimps, removing head immediately after catch will reduce the rate of spoilage. In the case of big fishes, beheading and eviscerating will reduce the enzymatic actions which cause spoilage.

The spoilage is reduced or prevented in a number of ways like drying, salting, chilling, canning and freezing. Chilling is a means of short term preservation of seafoods achieved by the reduction in temperature using ice. Freezing is the most satisfactory method currently available for a long term preservation of seafood. It is, in fact by far the best way of preventing fish from spoilage, since fish continues to remain in almost the same natural conditions even after freezing. It is effective for retaining flavour, colour and nutritive value of seafoods. Freezing is a process by which the water in the fish muscle is crystallized into ice. The crystallization will be complete at -40 ^{o}C.

After freezing, the fish must be stored at a temperature maintained constantly at -18 ^{o}C or below. Fluctuation in this temperature will cause spoilage of products. If there is a wide variation in the temperature recrystallisation takes place. Dehydration is another important reaction of a physical nature caused by the evaporation of ice due to differences in vapour pressure over the product surface and in the air of the store room. Loss of the moisture by evaporation of ice causes the product surface to dry resulting in dull appearance and even discolouration in some cases. The evaporated water eventually condenses and freezes on the

cooling surfaces of the store room and the transfer of moisture from the product will be continuous. Proper glazing and packaging eliminates this evaporation.

Microbial spoilage of Meat

The organisms spoiling meat may infect the animal either while still alive ("endogenous disease") or may contaminate the meat after its slaughter ("exogenous disease"). There are numerous diseases that humans may contract from endogenously infected meat, such as anthrax, bovine tuberculosis, brucellosis, salmonellosis, listeriosis, trichinosis or taeniasis.

Infected meat, however, should be eliminated through systematic meat inspection in production, and consequently, consumers will more often encounter meat exogenously spoiled by bacteria or fungi after the death of the animal.[3] One source of infectious organisms is bacteraemia, the presence of bacteria in the blood of slaughtered animals. The large intestine of animals contains some 3.3×10^{13} viable bacteria,[3] which may infect the flesh after death if the carcass is improperly dressed. Contamination can also occur at the slaughterhouse through the use of improperly cleaned slaughter or dressing implements, such as powered knives, on which bacteria persist. A captive bolt pistol's bolt alone may carry about 400,000 bacteria per square centimeter. After slaughter, care must be taken not to infect the meat through contact with any of the various sources of infection in the abattoir, notably the hides and soil adhering to them, water used for washing and cleaning, the dressing implements and the slaughterhouse personnel.

Bacterial genera commonly infecting meat while it is being processed, cut, packaged, transported, sold and handled include Salmonella spp., Shigella spp., E. coli, B. proteus, S. epidermidis and Staph. aureus, Cl. welchii, B. cereus and faecal streptococci. These bacteria are all commonly carried by humans; infectious bacteria from the soil include Cl. botulinum.Among the molds commonly infecting meat are Penicillium, Mucor, Cladosporium, Alternaria, Sporotrichium and Thamnidium.

As these microorganisms colonize a piece of meat, they begin to break it down, leaving behind toxins that can cause enteritis or food poisoning, potentially lethal in the rare case of botulism. The microorganisms do not survive a thorough cooking of the meat, but several of their toxins and microbial spores do. The microbes may also infect the person eating the meat, although against this the microflora of the human gut is normally an effective barrier.

Module IV

Microbiological Spoilage of Fruits and Vegetables

About 20% of vegetables and fruits harvested for human consumption in the world is lost due to microbial spoilage. Fermented vegetables and fruits can be contaminated with different microorganisms during processing, which can cause spoilage. Spoilage is any change occurring in fruits and vegetables, making them inedible for human. Fresh vegetables and fruits contain natural microflora coming from soil, water, air, and other sources. The presence of air, high humidity, and high temperature as extrinsic factors during storage of vegetables and fruits increases the chances of microbial growth and spoilage. The common fruit fly can contaminate plant foods with microorganisms such as Rhizopus spp. Microbial spoilage in fruits represents significant economic loss throughout the fruit distribution chain. Refrigeration, vacuum packaging (VP), modified atmosphere packaging, washing, freezing, drying, heat treatment, and chemical preservatives are used to reduce microbial spoilage of vegetables and fruits. Deterioration of flesh vegetable and fruits may result from mechanical damage, action of food enzyme , microbial action or by combination of this factor . Mechanical damage caused by insect , bird , animal etc . increase susceptibility to deterioration by their enzyme and microbial spoilage .Most commonly occurring microbial spoilage of fresh vegetable and fruits are:

- **Bacterial soft rot :**Caused by *Erwinia carotovora* and related pectin fermenting bacteria . Rot appears water soaked , soft often with bad odour.
- **Gray mold rot :** It is caused by Botrytis species . This spoilage is enhanced by high humidity and warm temperature.
- **Rhopus soft rot :** Caused by species of *Rhizopus*. Rot is often soft and watery the cottony growth of the mold with small black dots of sporangia cover the food.
- **Alterriae rot :** Caused by *Alternaria tenuis* and other species . Spoiled area becomes greenish brown early in growth of mold and later turned in to brown or black dots.
- **Anthrecnose :** Caused by *Collectotrictum lindemuthioum* and other species . It is characterized by spotting of leaves and fruits.
- **Blue mold rot :**Caused by *Penicillum digitatum* and other species. Blue colour of rot is due to blue colour of spores .
- **watery soft rot :** It is mainly caused by *Sclerotinia scterotiorum* . It is found mainly in vegetables.
- **Black mold rot :** Caused by *Aspergillus niger* . Rot appears black to black spores of the mold .

- **Black rot**: It is maianly caused by Alternaria and sometimes by Ceratostomella ,Physalospora and other
- **Pink mold rot** :Cause by Trichothecium roseum
- **Fusarium rot :** It is includes variety of root , it caused by Fusarium .
- **Greenytic mold rot :**It isusually caused by Cladosporium and somerimea by green coloured mold such as Trichoderma.
- **Brown rot :**Casued by Monilinia Fructicold.
- **Stem end rot :**Caused by several genera of the mold such as Diplodia. Phomopsis, Alternaria and Fusarium species
- **Sliminiess or souring :** Caused by saprophtic bacteri in piled , wet and heating vegetables.

Spoilage of vegetable and fruit juice

Acidity of fruit juice range from 2.4-4.2 depending on fruit juice , sugar concentration vary from 2-17 %. Although mold can grow on surface of fruit juice . High moisture concerned also favours yeast and some bacteria . Deficiency of vitamin B discoverage growth of some bacteria common type of spoilage of fruit juice are ;

- Alcoholic fermentation by yeast .
- Lactic acid fermentaion by heterofermentative lactic acid bacteria
- Fermentation of organic acid of ice e.g., fermentation of malic acid into lactic acid and succinic acid
- Slime production by leuconostoc species.

pH of vegetable juice is less acidic (5-5.8) and also contai plentiful supply of growth factors. Therefore , vegetable juice supports growth of fastidious lactic acid bacteria. Common spoilage of vegetable juice acid fermentation , smile production etc. Vegetable juice contains sugar are less acidic fruits juices having pH value in the range of 5-5.8 for the most of the part. Vegetable juices contain plentiful supplied of accessory factor for microorganism and hence support the growth of fastidious organism including lactic acid bacteria. Acid fermentation of raw juice by lactic acid bacteria may cause spoilage juice.

Microbiology of Cereals

Cereals and cereal products are significant and important human food resources and livestock feeds worldwide. Cereal grains and legumes are food staples in many, if not most, countries and cultures and are the raw materials of many of our foods and certain beverages. The main cereal grains used for foods include corn (maize), wheat, barley, rice, oats, rye, millet, and sorghum. Soybeans are not a cereal product, but rather, are legumes or a pulse, but are often considered with cereals because of their importance as a food source.

Examples of cereal products derived from cereal grains include wheat, rye, and oat flours and semolina, cornmeal, corn grits, doughs, breads, breakfast cereals, pasta, snack foods, dry mixes, cakes, pastries, and tortillas. In addition, cereal products are used as ingredients in numerous products, such as batters and coatings, thickeners and sweeteners, processed meats, infant foods, confectionary products, and beverages such as beer. Because of their extensive use as human foods and livestock feeds, the microbiology and safety of cereal grains and cereal products is a very important area. The sources of microbial contamination of cereals are many, but all are traceable to the environment in which grains are grown, handled, and processed. Microorganisms that contaminate cereal grains may come from air, dust, soil, water, insects, rodents, birds, animals, humans, storage and shipping containers, and handling and processing equipment. Many factors that are a part of the environment influence microbial contamination of cereals, including rainfall, drought, humidity, temperature, sunlight, frost, soil conditions, wind, insect, bird and rodent activity, harvesting equipment, use of chemicals in production versus organic production, storage and handling, and moisture control.

The microflora of cereals and cereal products is varied and includes molds, yeasts, bacteria (psychrotrophic, mesophilic, and thermophilic/thermoduric), lactic acid bacteria, rope-forming bacteria (*Bacillus spp.*), bacterial pathogens, coliforms, and Enterococci. Bacterial pathogens that contaminate cereal grains and cereal products and cause problems include *Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Escherichia coli, Salmonella,* and *Staphylococcus aureus*. Coliforms and enterococci also occur as indicators of unsanitary handling and processing conditions and possible fecal contamination.

Bacteria are frequent surface contaminants of cereal grains. For bacteria to grow in cereal grains, they require high moisture or water activity (a_w) in equilibrium, with high relative humidity. Generally, bacteria are not significantly involved in the spoilage of dry grain and become a spoilage factor only after extensive deterioration of the grain has occurred and high moisture conditions exist. However, bacterial pathogens and spoilage bacteria, such as spore-forming bacteria that cause ropiness in bread, may survive and carry through to processed products and become problems. Lactic acid bacteria may also be present in the raw grain and carry over into flour and cornmeal and spoil doughs prepared with them. Yeasts present on cereal grains may also carry through into processed products. The main spoilage organisms in cereal grains, however, are molds.

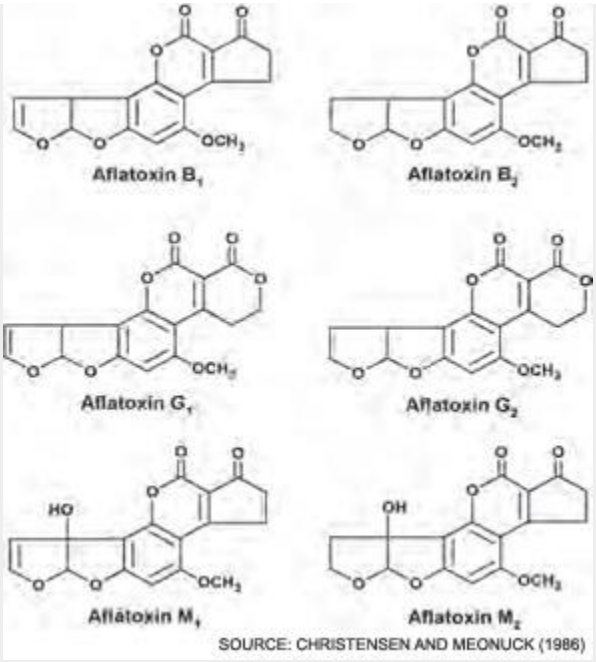


Figure 1. The chemical structure of aflatoxins.

There are more than 150 species of filamentous fungi and yeasts on cereal grains. But again, the most important of these are the filamentous fungi or molds. The filamentous fungi that occur on cereal grains are divided into two groups, depending on when they predominate in grain in relation to available moisture in the grain. These groups have been referred to as field fungi and storage fungi. Field fungi invade grain in the field when the grain is high in moisture (18 to 30%, i.e., at high a_w) and at high relative humidities (90 to 100%). Field fungi include species of *Alternaria*, *Cladosporium*, *Fusarium*, and *Helminthosporium*. Storage fungi invade grain in storage at lower moisture contents (14 to 16%), lower a_w and

lower relative humidities (65 to 90%). These main storage fungi are species of Eurotium, Aspergillus, and Penicillium. To prevent spoilage by storage fungi, the moisture content of starchy cereal grains should be below 14.0%, soybeans 12.0%, and other oilseeds, such as peanuts, and sunflower seeds, 8.5%. Certain molds, such as *Eurotium glaucus*, may initiate growth at low a_w and moisture contents (i.e., 15 to 16% moisture) and through their respiration increase a_w and raise the moisture content, facilitating molds to grow, thus ultimately leading to spoilage.

The major effects of fungal deterioration of grains include decreased germination, discoloration, development of visible mold growth, musty or sour odors, dry matter loss and nutritional heating, caking, and the potential for production of mycotoxins in the grain. Decreased germination of the grain occurs when storage fungi invade the germs or embryos of the grain kernel. The embryos are weakened and die as the storage fungi attack and parasitize the embryo to utilize its oils and other nutrients. Decreased germination caused by storage fungi usually precedes discoloration. However, discoloration can be caused by both field and storage fungi and can result in brown to black germs in wheat and corn and "blue eye" in corn, due to the presence of blue *Aspergillus* and *Penicillium* species. Musty odors may become apparent before mold growth becomes visible and is an early warning of mold activity, as is heating. Heating often starts in the fine materials or dust associated with the grain and is due to the growth of storage fungi. If sufficient heating occurs, the grain becomes dark and blackened. Further growth of storage fungi may result in surface growth and binding of the grain kernels together by mold hyphae, which is manifested as caking of the grain (i.e., large masses of the kernels bound together). By the time caking occurs, mold growth has become extensive and the grain is in advanced stages of decay. At this point the moisture content of the grain is increasing due to the respiration of the molds, and growth of yeasts and bacteria may also occur.

Mycotoxins

The word mycotoxin is derived from the Greek word mykes, meaning fungus or mold, and the Latin word toxicum, poison or toxin. Thus, mycotoxin is a general term meaning fungus poison or mold toxin. Mycotoxins are toxic secondary metabolites produced by filamentous microfungi or molds. These secondary metabolites are distinguished from primary

metabolites because they are not required for the growth of the fungus and have no apparent purpose in the metabolism of the organism. It has been speculated that mycotoxins are waste products or defense mechanisms. Mycotoxins are toxic and harmful in varying degrees to humans and animals, and may contaminate cereal grains in the field and in storage. Mycotoxins are stable compounds that resist destruction by food-processing methods and may carry through and contaminate finished processed foods.

There are numerous specific mycotoxins that may contaminate cereal grains, such as aflatoxins, ochratoxin, fumonisins, moniliformin, deoxynivalenol, T-2 toxin, and zearalenone. Mycotoxin research began in 1960 with the outbreak of Turkey "X" disease in England, where thousands of turkey poults and other young farm animals were lost due to poisoning by a fungal metabolite produced by *Aspergillus flavus* in peanut meal. The toxic substance was called aflatoxin (*A. flavus toxin*). Since 1960, many other toxic mold metabolites have been described. Those mycotoxins currently thought to be most important in cereal grains are listed in Table 2 (see p. 28) along with the molds that produce them.

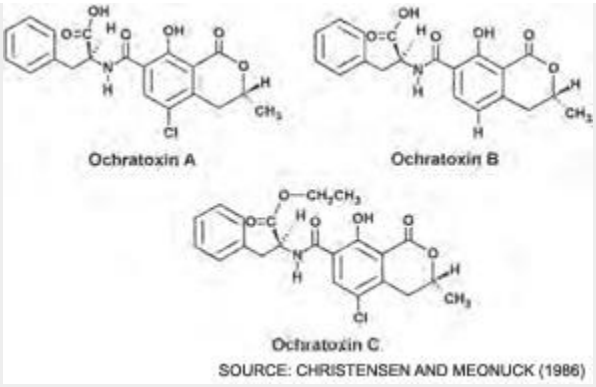


Figure 2. The chemical structure of ochratoxin.

Mycotoxins exhibit a range of toxicological properties, including acute toxicity or poisoning, which often results in death, and subacute or chronic toxicity, which may not result in death directly but which gradually weakens and lowers the general health of an animal or human due to effects on the immune system. Chronic toxicity may result in greater susceptibility to secondary bacterial infections. Some mycotoxins are carcinogenic and may cause cancers; some are mutagenic and are capable of causing mutations; they may also be teratogenic and embryo toxic, causing deformities and death in developing embryos