MICROBIAL BIOTECHNOLOGY

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Lecture-16
Lec 16: Control methods: Physical methods

Hello everyone, welcome back to my course on microbial biotechnology. We are in Module 5, where we are discussing the control of microorganisms. In the last lecture, we discussed bacterial growth, culture media, and different cultural conditions and requirements. In this lecture, we are going to discuss the control methods. We will start with the physical methods of control.

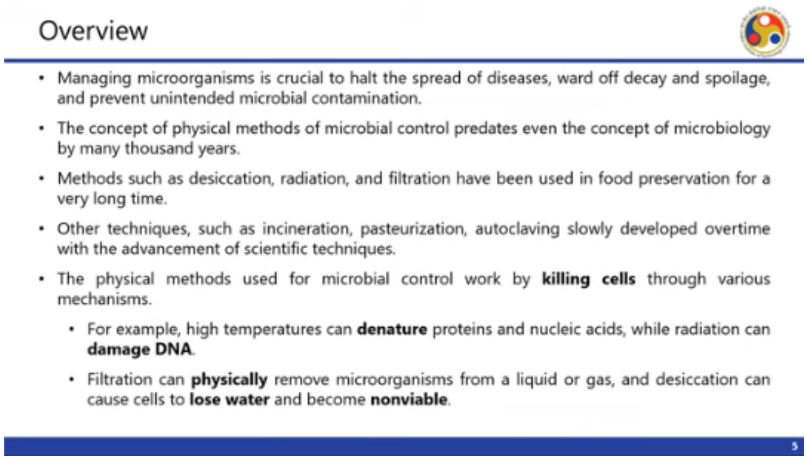
What are the methods for controlling microorganisms, and why do we need to control microbial growth? We know that there are beneficial microbes, but there are also harmful ones that can cause disease, damage materials, and compromise hygiene and safety standards. The primary goal of controlling microorganisms is to mitigate the risks they pose, such as the ones we just discussed.

This includes preventing the spread of infectious diseases, ensuring the safety and shelf life of food products, maintaining sterile environments in medical and laboratory settings, and managing waste to protect public health and the environment. Briefly, effective control of microorganisms is crucial for healthcare—preventing infections and ensuring sterile conditions for surgeries and medical procedures—and for food safety, inhibiting the growth of pathogens and spoilage organisms in food products, as well as environmental protection, safeguarding water, air, and soil quality by controlling microbial contamination. It is also important in laboratory research for maintaining contaminant-free conditions to ensure accurate experimental results. In any case, if you consider all these different aspects of controlling microorganisms, it is ultimately for improving our health and well-being. In this lecture, we will briefly discuss some of the important terms related to microbial control and then the various methods we use—particularly physical methods like temperature, pressure, desiccation, radiation, sonication, and filtration.

So, let us start with the physical methods of microbial control one by one. We start with an overview. So, we already know that microorganisms management will help us in halting the spread of diseases, world of decay and spoilers and prevent unintended microbial contamination. In this context, the concept of physical methods of microbial control predates even the concept of microbiology by many thousands of years, although people didn't knew that microbiology There are microbes which are existing in this world.

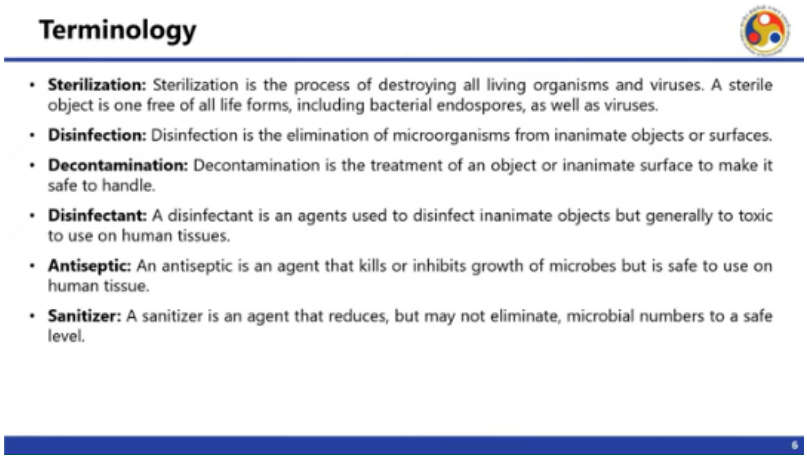
They had some kind of control mechanisms unknowingly. For example, people adopted for desiccation and even radiation by drying the food materials under the sunshine and also filtration in food preservation for a very long time. Other techniques such as incineration, burning, pasteurization, autoclaving slowly developed over time with the advancement of scientific techniques. The physical methods used for microbial control work by killing cells through various mechanisms. For example, high temperature can damage proteins, nucleic acids, while radiation can damage DNA.

Filtration can physically remove microorganisms from a liquid or gas and desiccation can cause cells to lose water and become non-viable. So some of the important terminologies useful from the control of microorganisms point of view is sterilization. Sterilization is a process of destroying all living organisms and viruses. A sterile object is one free of all life forms including bacterial endospores as well as viruses. Then there is a term called disinfection, which is the elimination of microorganisms from inanimate objects or surfaces.



And then we have decontamination, which is the treatment of an object or inanimate surface to make it safe to handle. Then we have disinfectants, which are agents used to disinfect inanimate objects, but generally too toxic if we use them on human tissues. This is basically chemical in nature. Then we have antiseptics, which are again chemical agents.

We will be discussing disinfectants and antiseptics in the next lecture, as well as sanitizers, which are agents that reduce but may not eliminate microbial numbers to a safe level. In the next lecture, we will discuss both sanitizers and static agents. So briefly, if we look into the overall methods employed for microbial control, they are physical, chemical, and biological. Today we are discussing the physical methods of microbial control here, which include heat and cold. Then pressure, and again, the heat may be dry or moist. Then we have desiccation, radiation, sonication, and filtration.



So let us start with temperature as a control method. Microbial growth is influenced by temperature. Each organism has a minimum, optimum, and maximum temperature for growth. So, here in this graph, you can see the distribution of microorganisms according to

the temperature they prefer for growth. For example, you have psychrophiles, which grow from sub-zero temperatures to around 20 degrees.

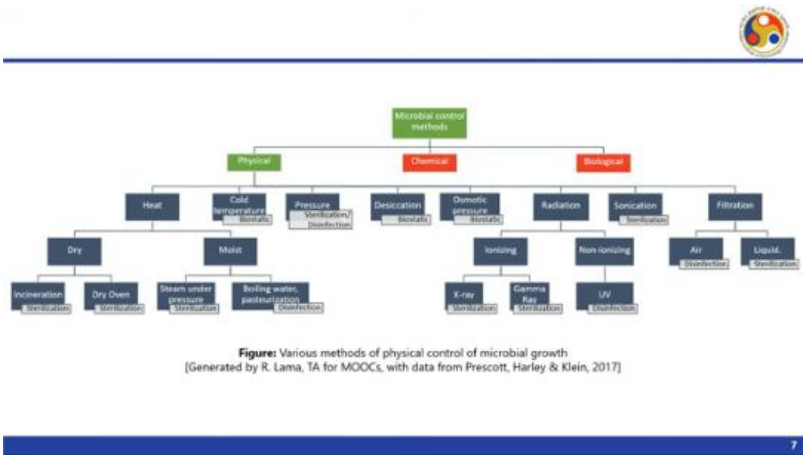


Figure: Various methods of physical control of microbial growth
(Generated by R. Lama, TA for MOOCs, with data from Prescott, Harley & Klein, 2017)

As you can see, then you have the mesophiles, which grow from around 15 to 45 degrees centigrade. Then you have the thermophiles, which can grow above 40 degrees and up to 80 degrees, and then you have the hyperthermophiles, which grow somewhere from around 65 to over 100 degrees centigrade. So, temperatures below the minimum preferred range slow metabolism without necessarily killing the microbes, while temperatures above the maximum cause enzyme denaturation, leading to microbial death. For example, in the case of mesophiles, if we lower the temperature below 10 degrees, their metabolism will slow down. But the same is not going to happen in the case of psychrophiles.

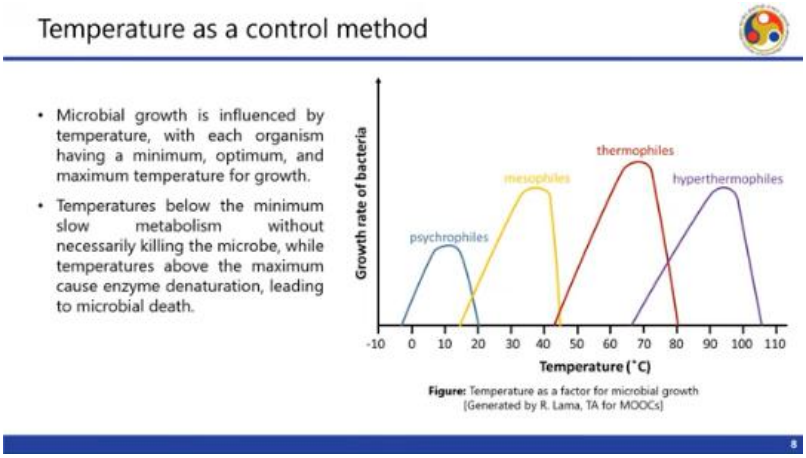


Figure: Temperature as a factor for microbial growth
(Generated by R. Lama, TA for MOOCs)

They will infect grow very happily because 10 degrees is their optimum temperature. Again, if we increase the temperature above 45 degrees centigrade, many of the mesophiles will die. Most of them will die. But for the thermophiles, that will be a very favorable temperature to start their growth.

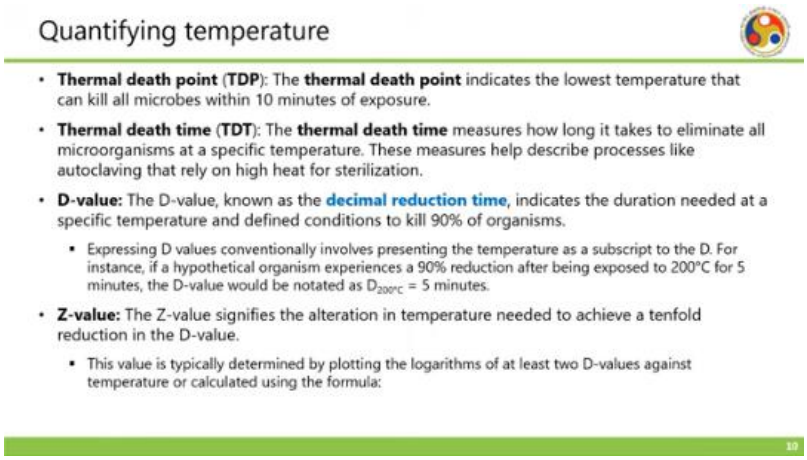
So, this temperature versus growth rate of bacteria curve is very useful when we are going to use temperature to control microbial growth. So, a high temperature in microbial growth—before you define that—that high temperature will actually vary for each and every type of these bacteria. For psychrophiles, 25 degrees is actually a very high temperature. While for hyperthermophiles, that may go as high as 110 degrees centigrade. So, heating is one of the oldest and most common methods for controlling microbes, which has been used in practices like cooking and canning.

Heat kills microbes by altering their membranes and modifying proteins. Microbes vary in heat resistance, as we have seen from that growth versus temperature curve, with some like C. botulinum, which forms endospores, being particularly heat-resistant. Heating methods for sterilization are classified into dry heat and moist heat techniques. Dry heat methods, such as incineration or direct high heat, are often used in labs for sterilizing tools like inoculating loops. So there, we burn the loops directly in the flame.

Dry heat sterilizers, such as ovens, can reach up to 170 degrees centigrade for extended periods, but moist heat sterilization is generally more effective due to better cell penetration. Quantifying temperature. Thermal death point indicates the lowest temperature that can kill all microbes within 10 minutes of exposure. TDT, or thermal death time, measures how long it takes to eliminate all microorganisms at a specific temperature.

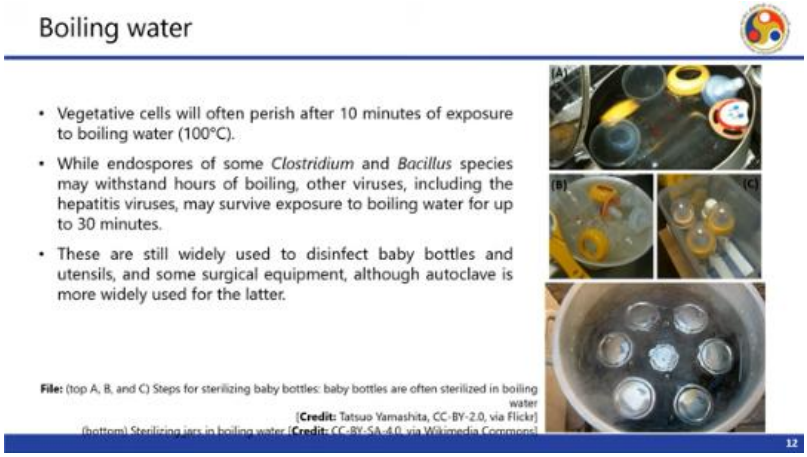
These measures help describe processes like autoclaving, which rely on high heat for sterilization. Then there is a concept called D-value, which is known as decimal reduction time. This indicates the duration needed at a specific temperature and defined condition to kill 90% of the organisms. Expressing D-values conventionally involves presenting the temperature as a subscript to the D. For instance, if a hypothetical organism experiences a 90% reduction after being exposed to 200 degrees centigrade for 5 minutes,

The D value would be notated as D 200 degrees centigrade is equal to 5 minus. Then we have a Z value, which signifies the alteration in temperature needed to achieve a tenfold reduction in the D value. This value is typically determined by plotting the logarithmic of at least two D values against temperature, calculated using the formula Z is equal to T1 minus T2, where T refers to the temperature or two different temperatures, and this is divided by the difference of log D1 minus log D2, which stands for the D value. Moist heat control.



Moist heat, when used for controlling microbes, involves the application of water-based methods like boiling or using steam to kill microbes. These methods are effective at eliminating many types of microorganisms. This is due to the ability of water to transfer heat more efficiently into cells compared to dry heat. Some widely used techniques using moist heat are simple boiling, autoclaving where we use an autoclave, or pasteurization.

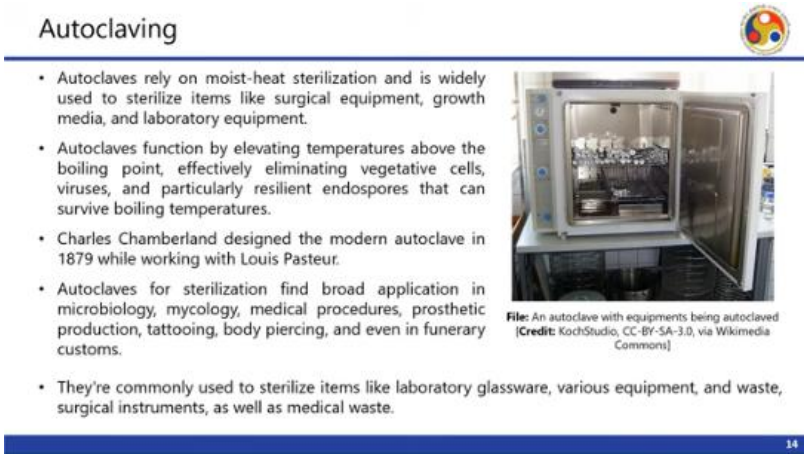
Let us start with a simple technique like boiling, where vegetative cells will often perish after 10 minutes of exposure to boiling water, roughly around 100 degrees centigrade. While endospores of some Clostridium and Bacillus species may withstand hours of boiling, other viruses, including the Hepatitis virus, may survive exposure to boiling water for up to 30 minutes. So in this figure, in A, B, and C, we see steps for sterilizing baby bottles or milk bottles by simply boiling them in water, thereby ensuring that the baby is safe when milk is loaded into this bottle for feeding. This is a simple technique and is still a widely used method, not only for disinfecting baby feeding bottles and utensils but also for disinfecting surgical equipment, although autoclaving is most widely used in a clinical context.



Tyndallization is a method that was devised by John Tyndall in the 19th century and is used to sterilize food. Tyndallization primarily targets heat-resistant endospores and has now been replaced by more rapid methods such as autoclaving. The process involves repeatedly heating the substance to near boiling for 15 minutes over three consecutive days. Between each heating, a resting period allows any surviving spores to germinate into bacterial cells, which are then eliminated during the subsequent heating. The resting period maintains a moist environment at room temperature, encouraging spore germination.

This environment doesn't favor spore formation from cells but instead supports the growth of bacteria from germinated spores. Autoclaving is a much more popular method. It relies on moist heat sterilization and is widely used to sterilize items like surgical equipment, growth media, and laboratory equipment. In this picture, you can see an autoclave with equipment loaded for sterilization. Autoclaves function by elevating temperatures above the boiling point, effectively eliminating vegetative cells, viruses, and particularly resistant endospores that can survive boiling temperatures.

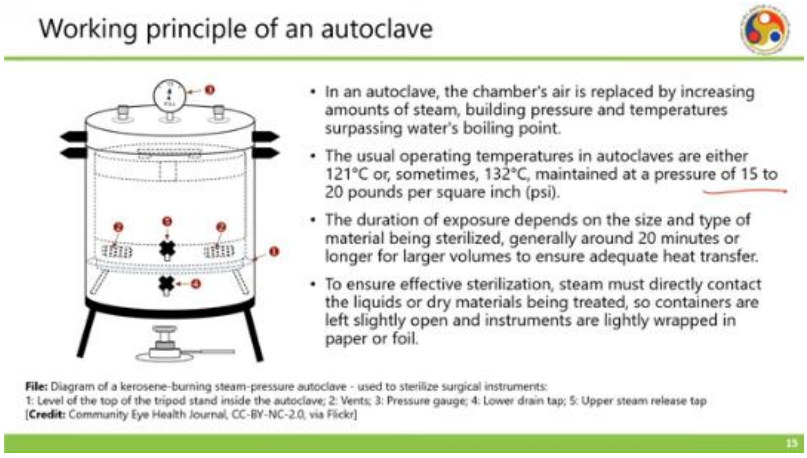
Charles Chamberlain designed a modern autoclave in 1879 while working with Louis Pasteur. Autoclaves for sterilization find broad applications in microbiology, mycology, medical procedures, prosthetic production, tattooing, body piercing, and even in funerary customs. They are commonly used to sterilize items like laboratory glassware, various equipment, surgical instruments, and medical waste. However, we cannot use autoclaves for sterilizing certain chemicals like vitamins and growth media.



growth factors particularly we can sterilize growth media but the growth factors if we autoglyph its potency will be destroyed. So what is the working principle of an autoglyph? In a way it is actually a pressure cooker. The chambers of these cooker are tightly packed with a lid.

The air is replaced inside this chamber by increasing amounts of steam. Building pressure and temperatures will surpass water's boiling point. The usual operating temperature in autoclaves are either 121 degree centigrade or sometimes it can go up to 132 degree centigrade. Now, if we remember the temperature versus growth of various bacteria, the maximum temperature was for the hyperthermophiles, which can survive somewhere up to around 110 degrees centigrade. So in these autoclaving, we cross that limit of 110 degrees.

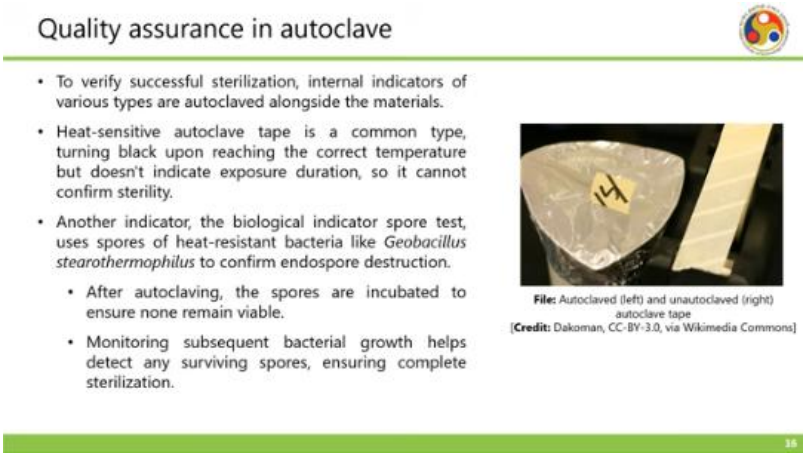
10 degree centigrade and achieve a temperature of 121 degree centigrade and it can go up to 132 degree centigrade and this ensures that all bacteria microbes are eliminated by this process of heating. And maintaining a pressure of around 15 to 20 psi, pounds per square inch is also very, very critical. So the duration of exposure depends on the size and type of material being sterilized. Generally around 15 minutes to 20 minutes or longer for larger volumes to ensure adequate heat transfer is necessary. To ensure effective sterilization, steam must directly contact the liquids or dry material being treated so containers are left slightly open and instruments are lightly wrapped in paper or foil.



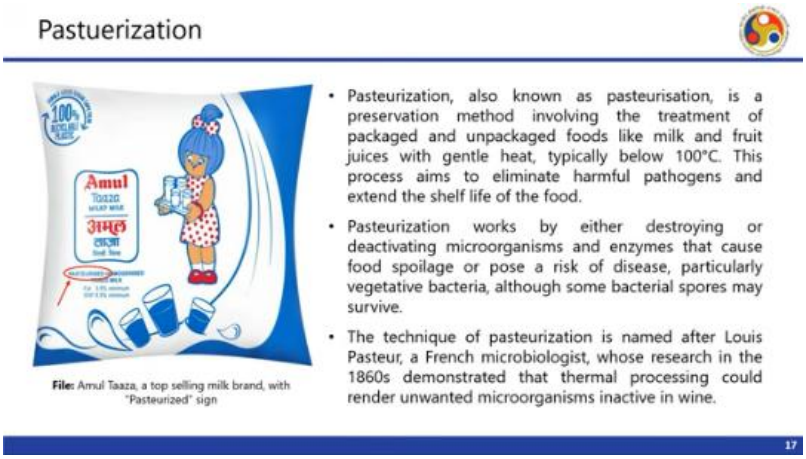
Quality assurance in autoclaving. To verify successful sterilization, internal indicators of various types are autoclaved alongside the material. Heat-sensitive autoclave tape is a common type, turning black upon reaching the correct temperature, but it does not indicate exposure duration, so it cannot confirm sterility. Anandaya indicator, the biological indicator spore test, uses spores of heat-resistant bacteria like Geobacillus stearothermophilus to confirm endospore destruction. After autoclaving, the spores are incubated to ensure none remain viable.

Monitoring subsequent bacterial growth helps detect any surviving spores, ensuring complete sterilization. Let us now discuss pasteurization, which is very common in the

dairy industry. So, whenever you pick up a milk pouch, you will see this particular labeling: pasteurized. Pasteurization is a preservation method involving the treatment of packaged and unpackaged foods like milk and fruit juices with gentle heat, typically below 100 degrees Celsius. But even at this temperature, if you go back to that graph, a large number of bacteria will be eliminated except some of the hyperthermophiles.



This process aims to eliminate harmful pathogens and extend the shelf life of the food. Pasteurization works by either destroying or deactivating microorganisms and enzymes that cause food spoilage or pose a risk of disease, particularly vegetative bacteria, although some bacterial spores may survive. The technique of pasteurization is named after Louis Pasteur, the famous French microbiologist whose research in the 1860s demonstrated that thermal processing could render unwanted microorganisms inactive in wine. What is the principle of pasteurization? So, in this figure, you can see the pasteurization process.

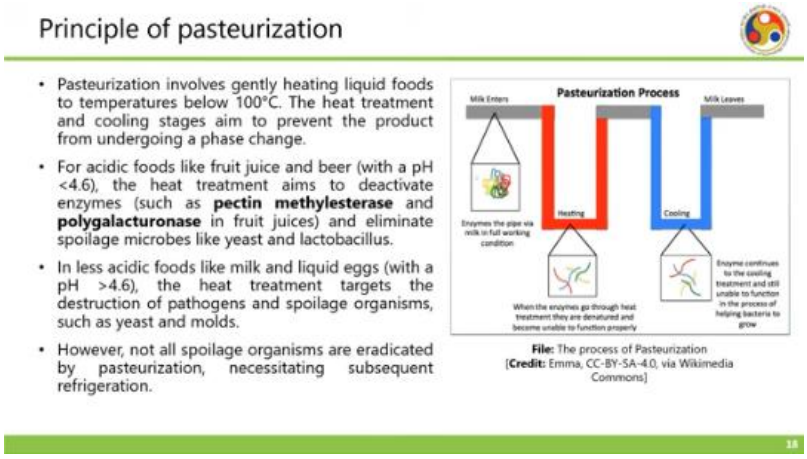


So, here the milk is put in. Or you can see the enzymes, the pipes via milk in full working condition. Then we go for heating first. When the enzymes go through heat treatment, they

denature and become unable to functional property. And then this heating is followed by cooling.

The enzyme continues to the cooling treatment and still unable to function in the process of helping bacteria to grow. And then after this cooling process, the milk exits or the milk leaves. So, this is in brief entry of the milk, then heating, followed by cooling and then exit of the milk is the simple method of pasteurization. The heat treatment and cooling stages aim to prevent the product from undergoing a phase change. For acidic foods like fruit juice and beer with a pH of around 4.6, the heat treatment aims to deactivate enzymes such as pectin, methyl esterase and polygalactouranase in fruit juices and eliminate spoilage microbes like yeast and some of the lactobacillus.

In less acidic foods like milk and liquid eggs with a pH a little bit higher than that of 4.6, the heat treatment targets the destruction of pathogens and spoilage organisms such as yeasts and moles. However, not all spoilage organisms are eradicated by pasteurization, necessitating subsequent refrigeration. Now there are other methods like AST, ST and UHT which is high temperature short time. These are used for pasteurization which involves heating liquids to high temperature for short time periods. This method is useful in places where access to refrigeration is

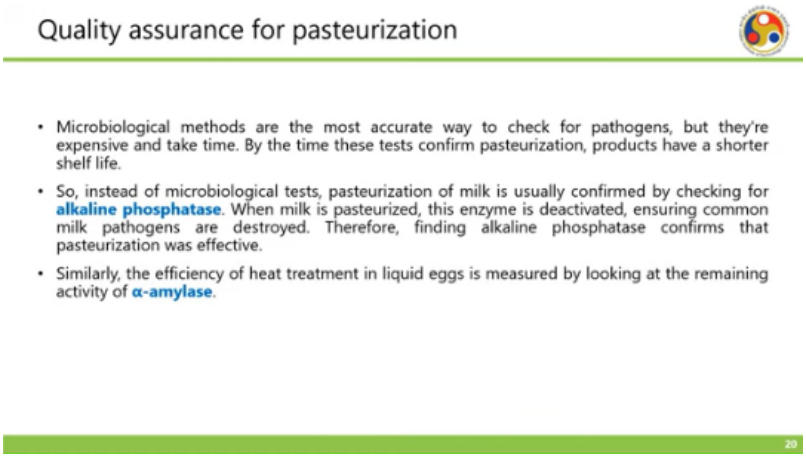


File: The process of Pasteurization
[**Credit:** Emma, CC-BY-SA-4.0, via Wikimedia Commons]

Limited high-temperature short-term pasteurization, like that used for milk, which is heated to 71.5 degrees centigrade for 15 seconds, ensures milk safety and provides a refrigerated shelf life of approximately two weeks. Then we have ultra-high-temperature pasteurization. This involves refrigeration. Heating milk to 135 degrees centigrade for 1 to 2 seconds. This method guarantees the same safety level while, when combined with suitable packaging, extends the refrigerated shelf life to 3 months.

However, the super-high temperatures change the proteins in the milk, making slight changes in the taste and also the aroma or smell. So, quality assurance for pasteurization is also very, very important. Here, microbial methods are the most accurate way to check for pathogens, but they're expensive and time-consuming. By the time these tests confirm pasteurization, the products have a shorter shelf life. So, instead of microbiological tests, pasteurization of milk is usually confirmed by checking for alkaline phosphatase.

When milk is pasteurized, this enzyme is deactivated, ensuring common milk pathogens are destroyed in the process. Therefore, the absence of alkaline phosphatase confirms that pasteurization was effective. Similarly, the efficiency of heat treatment in liquid eggs is measured by assessing the remaining activity of alpha-amylase. Let us now discuss dry heat control. Dry-heat-based methods of microbial control employ techniques



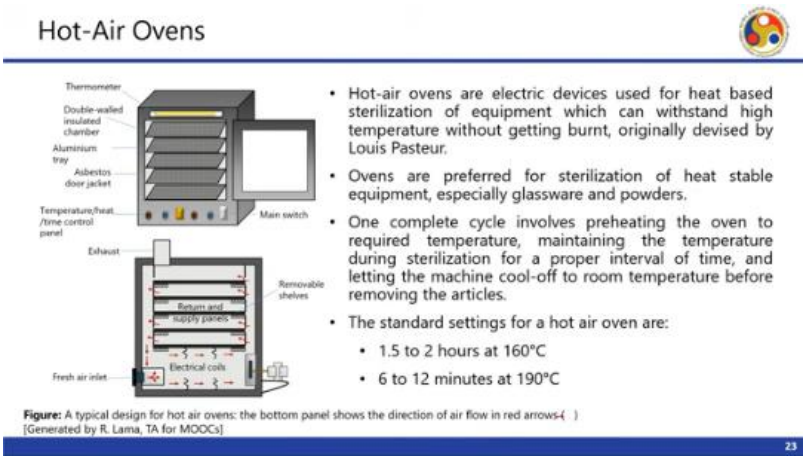
of directly applying heat to the objects in question instead of using water or steam. These techniques rely on the principle of protein oxidation instead of coagulation seen in the case of moist heat methods. Some widely used methods in dry heating include incineration or burning, hot air ovens. In incineration, we generally burn the objects. For example, here we see large-scale incineration of waste in the first picture.

And then in the second picture, we see the incineration of a nichrome loop by the application of direct flame in a microbiological laboratory. So, here we are directly burning the objects to control microbial growth. This is often used for the treatment of clinical waste where high temperatures not only destroy the pathogens present but also traces of toxins that might be left behind. Heat-labile equipment is often reduced to ash post-incineration, while heat-stable equipment could be thoroughly sterilized through these methods. On a smaller scale, incineration could also refer to sterilization by the application of direct flame, a technique often used to sterilize inoculating loops during streaking experiments.

Let us now discuss the hot air ovens. So here you can see a diagram of a hot air oven which has a double-walled insulated chamber, and then you may have some metallic trays, often aluminum trays, but we can also have stainless steel. Then we have an asbestos door jacket. Then we have a temperature, heat, and time control panel and a main switch. And then we also have a thermometer through which we can know how much temperature has been achieved.

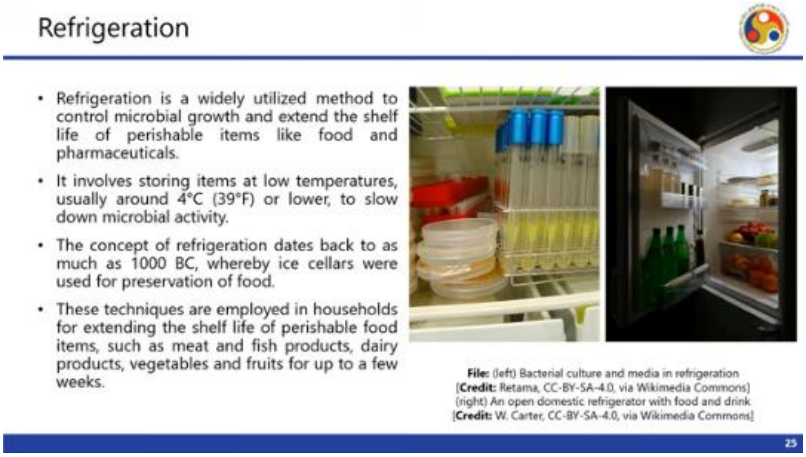
So, these hot air ovens are operated with the help of electricity. So, they are electric devices used for heat-based sterilization of equipment which can withstand high temperatures without getting burned. Originally devised by Louis Pasteur, these ovens are preferred for sterilization of heat-stable equipment, especially glassware and certain powders. One complete cycle involves preheating the oven to the required temperature,

maintaining the temperature during sterilization for the proper interval of time, and letting the machine cool off to room temperature before removing the articles. The standard setting for a hot air oven is 1.5 to 2 hours at 160 degrees centigrade or 6 to 12 minutes at 190 degrees centigrade. This is preceded by preheating. We can also use lower temperatures for controlling microbial growth. So lower temperatures work by decelerating microbial metabolism, which impedes microbial growth.



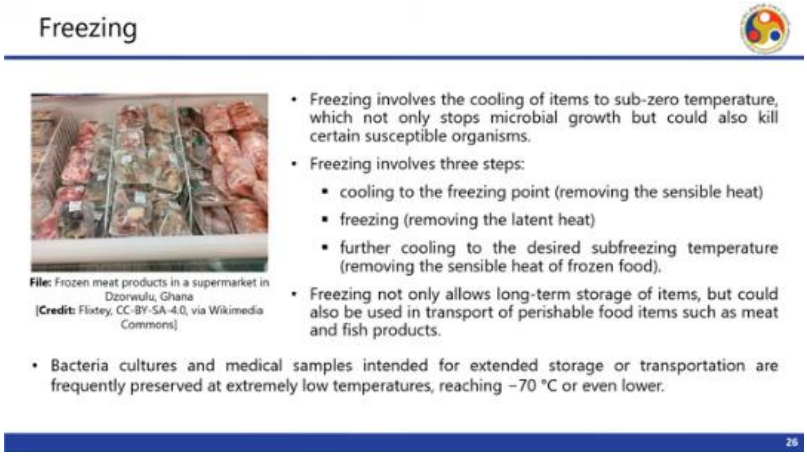
These techniques are often used to store food items, ensuring freshness for several months by hindering microbial growth, although they typically don't eliminate microorganisms. The best example is the household refrigerator many of us have in our homes or those used by stores for preserving food and beverages. So, methods using lower temperatures include refrigeration and freezing, Refrigeration is a widely utilized method to control microbial growth and extend the shelf life of perishable items like food and pharmaceuticals. So, here you can see bacterial culture and media, which we are keeping in refrigeration in the first picture.

Then we see this domestic refrigerator with food and drinks. Refrigeration involves storing items at low temperatures, usually around 4 degrees Celsius or lower, which may be as low as minus 20 degrees Celsius, to slow down microbial activity. The concept of refrigeration dates back to as early as 1000 BC, when ice cellars were used for food preservation. These techniques are employed in households to extend the shelf life of perishable food items such as meat and fish products, dairy products, and vegetables and fruits for up to a few weeks. Freezing is another low-temperature method.



This involves cooling items to sub-zero temperatures, which not only stops microbial growth but could also kill certain susceptible organisms. Here, you can see frozen meat products kept in a freezer or deep freezer. Freezing involves three steps: cooling to the freezing point, removing the sensible heat; freezing, removing the latent heat; then further cooling to the desired sub-freezing temperature, removing the sensible heat of frozen food. Freezing not only allows long-term storage of items but could also be used to transport perishable food items such as meat and fish products over long distances.

Bacterial cultures and medical samples intended for extended storage or transportation are frequently preserved at extremely low temperatures, which may be as low as minus 70 degrees or even lower. Next, let us discuss the use of atmospheric pressure in microbial control. Pascalization, also known as bridgmanization or high-pressure processing, is a method used to preserve and sterilize food by subjecting it to extremely high pressure. In this picture, you can see a researcher loading bottles into a 2-liter high-pressure processing unit in a laboratory in Australia.



So this technique involves applying more than 50,000 pounds per square inch for about 15 minutes, effectively deactivating yeast, mold, vegetative bacteria, and some viruses and parasites. High-pressure processing can achieve pasteurization-equivalent log reduction or go further to achieve sterilization, including the elimination of endospores, based on the temperatures and pressure settings. Pasteurization-equivalent HPP can be carried out in cold temperatures, while sterilization techniques require at least 90 degrees centigrade under pressure. So, what is the principle of pascalization?

High pressure in microbial control

- Pascalization, also known as **bridgmanization** or **high pressure processing** (HPP), is a method used to preserve and sterilize food by subjecting it to extremely high pressure.
- This technique involves applying more than 50,000 pounds per square inch for about fifteen minutes, effectively deactivating yeast, mold, vegetative bacteria, and some viruses and parasites.
- HPP can achieve pasteurization-equivalent log reduction or go further to achieve sterilization, including the elimination of endospores, based on the temperature and pressure settings.
- Pasteurization-equivalent HPP can be carried out in cold temperatures, while sterilization requires at least 90 °C under pressure.

File: A scientist loading bottles into a two-litre high-pressure processing unit, CSIRO, Australia
[**Credit:** CSIRO, CC-BY-3.0, via Wikimedia Commons]

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So, in this figure, you can see a pressure vessel here. Now, this pressure vessel has two enclosures. So, within this pressure vessel, we have a carrier basket in which we load the food or pack the food. So, during pascalization, selective disruptions occur in the hydrogen bonds of food, disrupting key elements of the bacterial cell and the integrity of many key enzymes. Pressures of around 400 to 1000 MPa could cause the removal of pathogenic microorganisms such as Listeria, E. coli, Salmonella, and Vibrio.

Since this method does not rely on heat, it leaves covalent bonds intact, preserving the taste and the nutritional value. Some components within foods can undergo changes during this process, such as carbohydrates getting gelatinized and an increase in lipid oxidation rates. Let us now discuss hyperbaric oxygen therapy, or briefly called HBOT, which involves the administration of oxygen at increased atmospheric pressure within a specialized chamber. So, in this picture, you can see a hyperbaric chamber here, inside which you can see a patient sleeping.

Pascalization principle

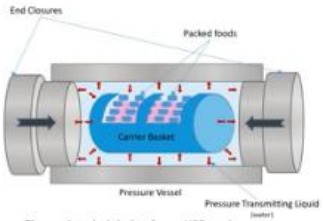

Figure: A typical design for an HPP equipment
[Generated by R. Lama, TA for MOOCs, reference: Farid *et al.*, 2021]

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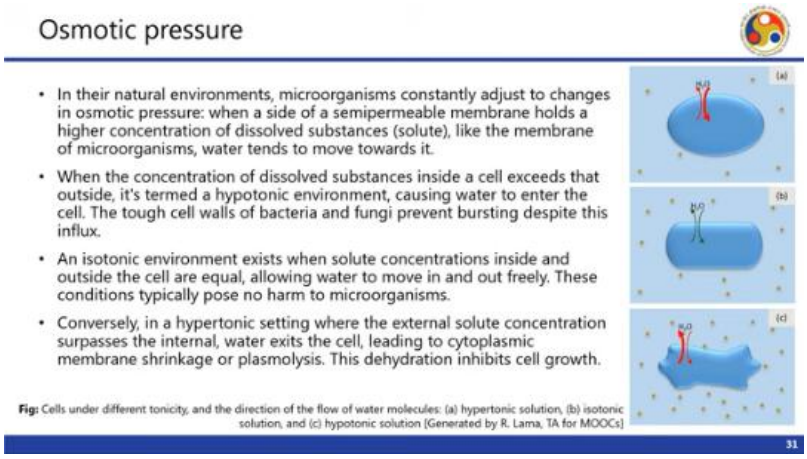
This is a picture taken at the Moose Jaw Union Hospital in Saskatoon, Canada. During this treatment method, patients are exposed to pure oxygen at pressures above normal atmospheric levels, typically ranging between 1 to 3 atmospheres. This is accomplished by placing the individual in a hyperbaric chamber or administering pressurized oxygen through a specialized breathing tube. While primarily used for various medical conditions like decompression sickness, carbon monoxide poisoning, and chronic wounds, HBOT has also shown some effectiveness in controlling certain microorganisms. Let us discuss the principle and applications of HBOT.

Hyperbaric oxygen therapy functions by elevating oxygen levels in hypoxic tissues resulting from infections and inflammations. These heightened oxygen concentrations amplify the body's immune response by increasing the activities of infection-fighting white blood cells such as neutrophils and macrophages. Additionally, the augmented oxygen levels contribute to the creation of harmful free radicals, which impede the growth of oxygen-sensitive anaerobic bacteria such as Clostridium perfringens, a common agent responsible for gas gangrene. In such cases of infections, this therapy can also diminish the production of bacterial toxins that cause tissue damage.

Another method used for controlling microbial growth is osmotic pressure. In their natural environments, microorganisms constantly adjust to changes in osmotic pressure. When one side of a semi-permeable membrane holds a higher concentration of dissolved substrates, like the membrane of microorganisms, water tends to move toward it. So, in this picture, you can see the effect of osmotic pressure. When the concentration of dissolved substances inside a cell exceeds that of the outside, it's termed a hypotonic environment, causing water to enter the cell.

The tough cell walls of bacteria and fungi prevent bursting despite this influx because, due to the entry of water, there will always be pressure exerted on the wall. An isotonic environment exists when solute concentrations both inside and outside the cell are equal, allowing water to move in and out freely. These conditions typically pose no harm to the microorganisms. Conversely, in a hypertonic setting where the external solute concentration surpasses the internal, water exits the cell, leading to cytoplasmic membrane shrinkage or plasmolysis. This dehydration inhibits cell growth.



Let us now discuss another very old method of microbial control: salting. This is a traditional method used for preserving food, which involves applying salt to food to inhibit the growth of microorganisms, thereby extending its shelf life. Salt draws out moisture from food, through osmosis, reducing water availability for microbes and slowing their ability to reproduce and spoil the food. The high concentration of salt creates an inhospitable environment for many bacteria, fungi, and other spoilage microorganisms,

effectively preserving the food. Here, you can see different kinds of salted food. For example, women preparing salt-cured fish in this first picture. In the second picture, you have salted fish at a traditional market in Indonesia. Then, in this last picture, you have the salting of meat.

Salting

- Salting is a traditional method used for preserving food, which involves applying salt to food items to inhibit the growth of microorganisms, thereby extending the shelf life.
- Salt draws out moisture from food through osmosis, reducing water availability for microbes, slowing down their ability to reproduce and spoil the food.
- The high concentration of salt creates an inhospitable environment for many bacteria, fungi, and other spoilage microorganisms, effectively preserving the food.

File: (L-R) (a) Women preparing salt-cured fish in St. Louis, Senegal [**Credit:** T.K. Naliaka, CC-BY-SA-4.0, via Wikimedia Commons]
(b) Salted fish at traditional market in North Jakartz, Indonesia [**Credit:** Sakurai Midori, CC-BY-SA-2.1-JP, via Wikimedia Commons]
(c) Salting of meat while preparing prosciutto [**Credit:** Deathworm, Public domain, via Wikimedia Commons]

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Similar to salting, the use of sugar can also—high concentration of sugar can also control microbial growth. For example, we see in jams and jellies; fruits are generally preserved by boiling with sugar and sometimes pectin to make these jams and jellies. So, the canning of jam or preserves with a high sugar concentration inhibits bacterial growth through hypertonicity. The boiling process kills most bacteria and molds, and the sugar content inhibits the growth of microorganisms by drawing out water through osmosis. So, we see a variety of jams and jellies over here made from different fruits, and here the process of making jam by boiling in a pot.

So, this boiling will take care of most of the bacteria and the molds. Additionally, the natural acidity of the fruits inherent in them also contributes to their preservation by creating an environment where bacteria find it hard to survive. However, molds might better tolerate hypertonic conditions, and if not sealed to prevent oxygen, germs may develop molds over time. The next method we are going to discuss is desiccation. This is basically drying or dehydration.

Jams and jellies

- Fruits are generally preserved by boiling with sugar and sometimes pectin to make jams and jellies.
- The canning of jams or preserves with a high sugar concentration inhibits bacterial growth through hypertonicity.
- The boiling process kills most bacteria and molds, and the sugar content inhibits the growth of microorganisms by drawing out water through osmosis.
- Additionally, the natural acidity of fruits also contributes to their preservation by creating an environment where bacteria find it hard to survive.
- However, molds might better tolerate hypertonic conditions and if not sealed to prevent oxygen, jams may develop mold.

File: (top) A variety of jams made from different fruits [**Credit:** eGuide Travel, CC-BY-2.0, via Wikimedia Commons]
(bottom) Jam being made in a pot [**Credit:** Baryonic Being, CC-BY-SA-3.0, via Wikimedia Commons]

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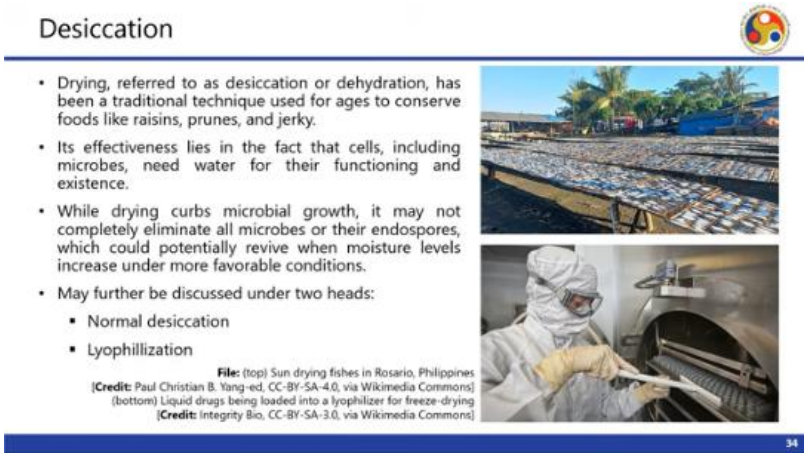
It has been used traditionally for a long time to preserve foods like raisins, prunes, and jerky, and you can see here fish getting dried. And in this picture below, you can see liquid drugs being loaded into a lyophilizer for freeze-drying. So, this is altogether a different method. This is not sun drying, unlike in the first picture. But both these processes help us in removing the water content—that is, dehydrating the samples.

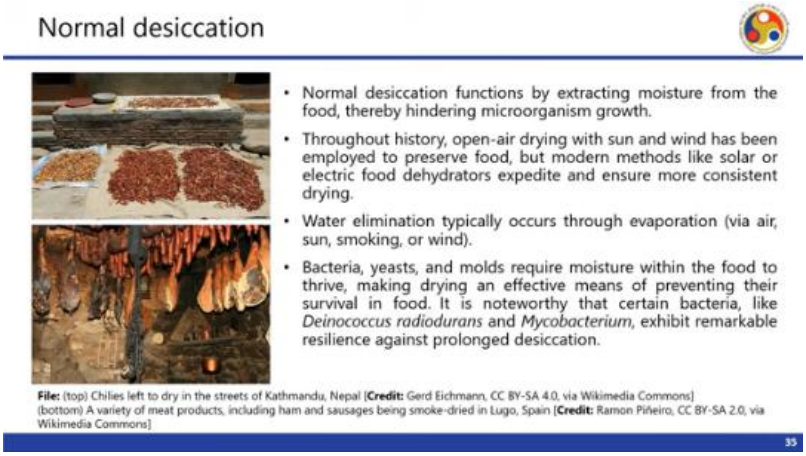
The effectiveness of desiccation lies in the fact that cells, including microbes, need water for their functioning and existence. While drying curbs microbial growth, it may not completely annihilate all microbes or their endospores, which could potentially revive when moisture levels increase under more favorable conditions. There are two types of desiccation. One is normal desiccation, and the other is lyophilization. In normal desiccation, the removal of moisture from food takes place, thereby hindering microorganism growth.



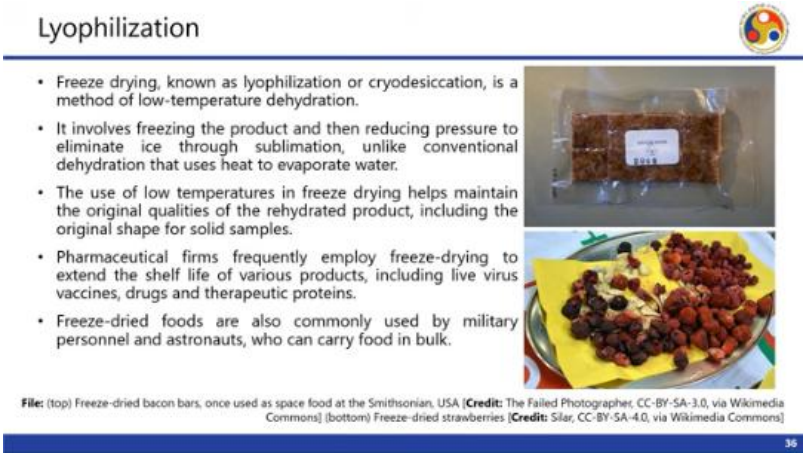
In this picture, you can see chilies left to dry on the roadside using freely available sunlight. Then you have a variety of meat products, including ham and sausages, being smoke-dried in the lower picture. These are what we call normal desiccation. It has been known to human civilization for quite a long time. This is basically an open-air drying method with the help of sun and wind.

This has been very effective in preserving food for thousands of years. I mean, as a practice—a cultural practice. But today, we have modern methods like solar or electric food dehydrators, which expedite and ensure more consistent drying. The elimination of water typically occurs through evaporation via air, sun, smoking, or wind. Bacteria, yeast, and molds require moisture within the food to thrive.

Making drying an effective means of preventing their survival in food. It is noteworthy that certain bacteria like Deinococcus radiodurans and Mycobacterium exhibit remarkable resilience against prolonged desiccation. Another type of drying or dehydration is lyophilization. This is basically freeze-drying or cryo-desiccation. This is a method of low-temperature dehydration.

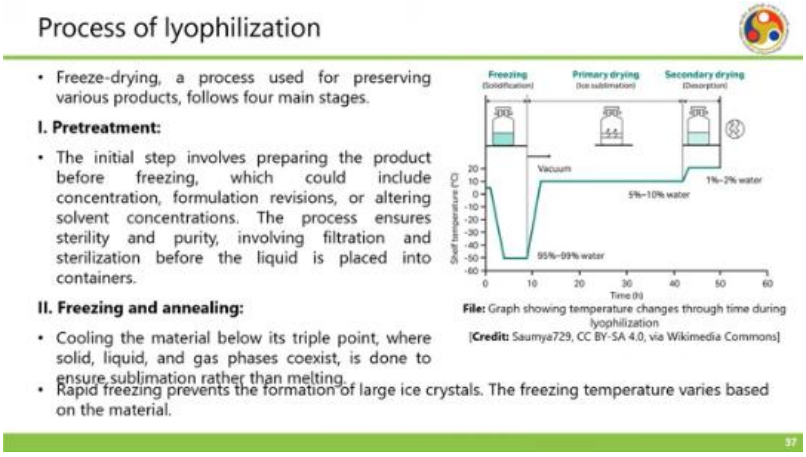


It involves freezing the product and then reducing pressure to eliminate ice through sublimation, unlike conventional dehydration that uses heat to evaporate water. The use of low temperature in freeze-drying helps maintain the original qualities of the rehydrated product, including the original shape of our solid samples. Pharmaceutical firms frequently employ freeze-drying to extend the shelf life of various products, including live viruses, vaccines, drugs, and therapeutic proteins. Freeze-dried foods are also commonly used by military personnel and astronauts who can carry food in bulk because the weight is reduced after removing the water content. You can see here some bacon bars, which are freeze-dried and were used as space food at the exhibition in the Smithsonian in the USA. Below, you have freeze-dried strawberries and



Let us discuss the process of lyophilization. This process, used for preserving various products, follows four main stages. Number one is the pre-treatment stage. The initial step involves preparing the product before freezing, which could include concentration, formulation revisions, or altering solvent concentrations.

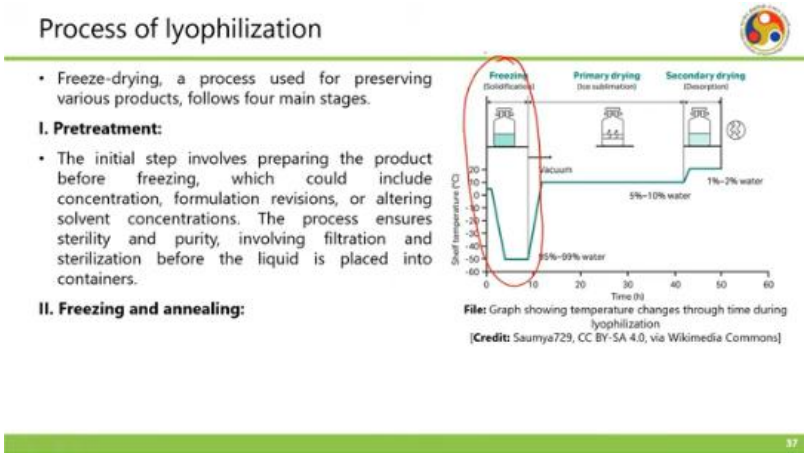
The process ensures sterility and purity, involving filtration and sterilization before the liquid is placed into containers. Then, the second stage is the freezing and annealing stage, where the material is cooled below its triple point, where solid, liquid, and gas phases coexist, to ensure sublimation rather than melting. Then, rapid freezing prevents the formation of large ice crystals. The freezing temperature varies based on the material. We have the third stage, which is primary drying.



This involves lowering pressure and applying controlled heat to the frozen material, promoting sublimation of about 95% of the water content. The pressure reduction accelerates sublimation, which is a slow process to prevent structural damage due to excessive heat. The vacuum facilitates the water vapor's re-liquification on cold condenser surfaces. And then we proceed to the secondary drying. This stage focuses on removing unfrozen water molecules left after primary drying.

This phase requires raising the temperature above freezing to break physicochemical interactions between water molecules and the material. Additionally, pressure is further lowered to increase desorption. At the end of this stage, the product attains an extremely low residual water content, typically ranging from 1% to 4%. Now, let us see this overall graph, which shows the temperature change over time during lyophilization. So, in the beginning, there is solidification.

So, the temperature can go as low as minus 50 degrees. Then we have the primary drying, where the ice sublimation takes place, so the temperature is raised, and here the water content will get reduced to as low as 5% to 10%. from as high as 95% to 99%. Then we have the secondary drying or the desorption, where only 1% to 2% of the water remains. So, there are various kinds of freeze dryers.



Some of them are for domestic use, and there are also industrial-scale freeze dryers, which are very, very large. As you can see over here, Basically, these freeze dryers consist of a chamber for material placement, a vacuum system for low pressure, a condenser to collect the water vapor, and a heating system for controlled heat application. The process begins by freezing the material in the chamber, reducing pressure to increase sublimation, and applying gentle heat to facilitate the transition of ice to vapor. And this process you can understand from the temperature ranges in the graph, showing the pretreatment, the primary drying, and the secondary drying.

The vaporized water is collected in the condenser, leaving behind a freeze-dried product with minimal moisture content. Another physical agent that is used for controlling microbial growth is radiation. Electromagnetic radiation encompasses electromagnetic field waves that travel through space, bearing both momentum and radiant energy, and you can see here the electromagnetic spectrum showing the frequencies and wavelengths of various types of radiation. So, you have radio frequency at one end, which is very large, and then you have gamma rays, which are very, very short.

Freeze dryers

- Freeze dryers consist of a chamber for material placement, a vacuum system for low pressure, a condenser to collect water vapor, and a heating system for controlled heat application.
- The process begins by freezing the material in the chamber, reducing pressure to encourage sublimation, and applying gentle heat to facilitate the transition of ice to vapor.
- Vaporized water is collected in the condenser, leaving behind a freeze-dried product with minimal moisture content.

File: (L-R) (a) Freeze-dryer for domestic use [**Credit:** JK Nair, CC-BY-SA-4.0, via Wikimedia Commons] (b) Industrial scale freeze-dryer [**Credit:** Millrock, Public Domian, via Wikimedia Commons] (c) A bench-top freeze-dryer [**Credit:** Matylda Sęk, CC BY-SA 3.0, via Wikimedia Commons]

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And the penetration of these is also very, very deep. So, these radiations have found wide applications in the control of microorganisms, especially for controlling microorganisms in equipment that is heat-labile. We cannot heat them, but we can use radiation to make them free from microbes. So, the types of radiation that can be used for sterilization can be classified into two groups. Ionizing radiation includes X-rays and gamma rays.

Radiation

- Electromagnetic radiation (EMR) encompasses electromagnetic field waves that travel through space, bearing both momentum and radiant energy.
- These radiations have found wide application in control of microorganisms, especially for control of microorganisms in equipment which are heat-liable.
- The types of radiation used can widely be classified into two groups:

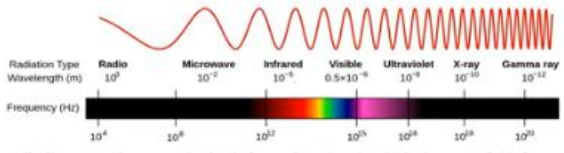

Fig: Electromagnetic spectrum showing the frequencies and wavelength of various types of radiations [**File:** Inductiveload, NASA, CC-BY-SA-3.0, via Wikimedia Commons]

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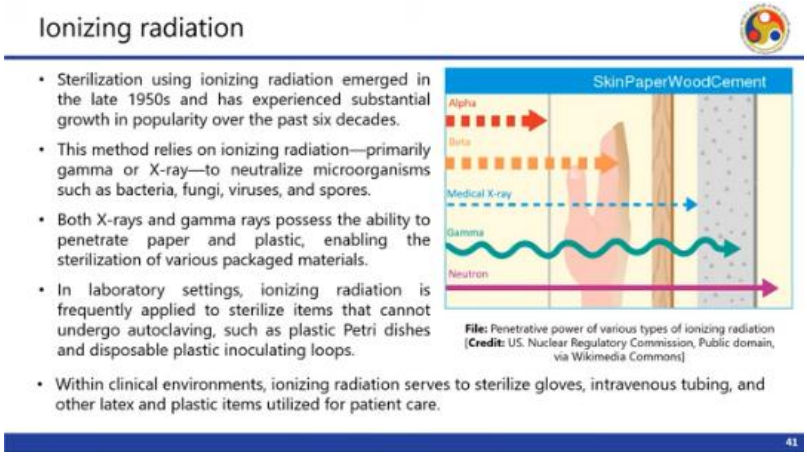
Then we have the non-ionizing ultraviolet rays. Let us first discuss ionizing radiation. So, this picture shows the penetrative power of various types of ionizing radiation. For example, we have here the alpha beam, which can penetrate up to a particular depth, but here you can see there is skin, paper, wood, and cement. So, medical X-rays, for example, can cross our body, and gamma rays can actually cross

physical structures like wood and cement over here and up to that point, and neutrons can actually penetrate much deeper. So, the beta particles, of course, have better penetration than the alpha particles. So, this is very, very important: alpha followed by beta. Then X-rays, gamma rays, and neutrons are the sequence in which the penetration of these

radiations can be understood. Sterilization using ionizing radiation emerged in the 1950s and has experienced substantial growth in popularity over the past six decades.

This method relies on ionizing radiation, primarily gamma, or X-rays to neutralize microorganisms such as bacteria, fungi, viruses, and spores. Both X-rays and gamma rays possess the ability to penetrate paper and plastic, enabling the sterilization of various packaged materials. In laboratory settings, ionizing radiation is frequently applied to sterilize items that cannot undergo autoclaving, such as plastic petri dishes and disposable plastic inoculating loops. Within clinical environments, ionizing radiation serves to sterilize gloves, intravenous tubing, and other latex and plastic items utilized for patient care.



Let us now try to understand the effect of ionizing radiation. During the sterilization process, high-energy electromagnetic radiation bombards the targeted sample, producing highly unstable free radicals, molecular ions, and secondary electrons. These radiation byproducts then interact with nearby molecules, causing disruptions and changes in chemical bonds. DNA, particularly sensitive to radiation, can fracture, depolymerize, mutate, and undergo structural alterations when exposed to ionizing radiation. Inadequate repair of DNA damage ultimately results in genetic information loss and cell death.

Hence, radiation serves as an effective method to eliminate harmful microorganisms and achieve sterilization. So, here we can see the ionizing radiation causing membrane damage. So, it can directly break down the DNA. Or it generates reactive oxygen species, which will indirectly damage the DNA. And then these DNA damages may result in cell death.

Effects of ionizing radiation

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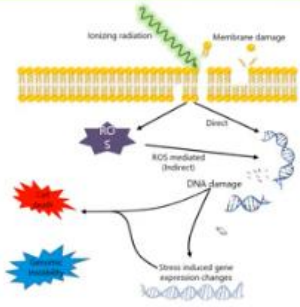

Figure: Effects of ionizing radiation on cells [Generated by R. Lama, TA for MOOCs, reference: Jeong & Jeong, 2017]

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Or sometimes the cell will survive. This will lead to stress-induced gene expression changes, and then it results in genomic instability and can cause cell death. D-value, the susceptibility of a specific organism to radiation is measured by its decimal reduction dose or D10 value, denoting the radiation dose causing a tenfold reduction in the microorganism population. To ensure efficacy, sterilization treatments must consider the D10 values of the microbes present, the initial bioburden level, and the diversity of microbes present in the sample.

Additionally, the ideal radiation dose depends on the desired sterility assurance level, indicating the likelihood of microorganisms surviving the sterilization process. A sterility assurance level of 10 to the power of minus 6 or higher is typically the ideal target. So, you have here various microorganisms like Salmonella, Vibrio, and HIV, and the D10 value for all of them will vary. Let us now discuss gamma rays.

D-value

- The susceptibility of a specific organism to radiation is measured by its **decimal reduction dose** (D_{10} value), denoting the radiation dose causing a tenfold reduction in the microorganism population.
- To ensure efficacy, sterilization treatment must consider the D_{10} values of the microbes present, the initial bioburden level, and the diversity of microbes in the sample.
- Additionally, the ideal radiation dose depends on the desired sterility assurance level (SAL), indicating the likelihood of microorganisms surviving the sterilization process: a SAL of 10^{-6} or better is typically targeted.

Organism	D_{10} value
Salmonella typhimurium	0.3 kGy
Vibrio cholera	0.48 kGy
Human Immunodeficiency Virus	4.0 - 8.4 kGy

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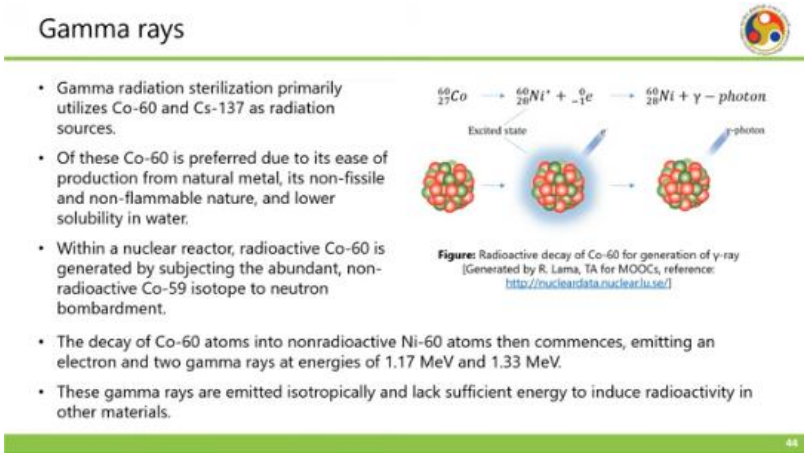
Gamma-ray radiation sterilization primarily utilizes Cobalt-60 and cesium-137 radiation sources. Here, Cobalt-60 is preferred due to its ease of production from natural metal, its

non-fissile and non-flammable nature, and lower solubility in water. Within a nuclear reactor, radioactive cobalt-60 is generated by subjecting the abundant non-radioactive cobalt-59 isotope to neutron bombardment. The decay of cobalt-60 atoms into non-radioactive nickel-60 atoms then commences, emitting an electron and two gamma rays at energies of around 1.17 MeV and 1.33 MeV. These gamma rays are emitted isotropically and lack sufficient energy to induce radioactivity in other metals.



So here in this picture, we can see the radioactive decay of cobalt-60 for the generation of gamma rays. Now let us discuss X-rays as microbial control agents. X-rays are generated by high-energy electrons interacting with nuclei possessing high atomic numbers, such as tungsten or tantalum. The passing of these electrons near the nucleus results in the emission of X-rays. Commercially, electron energies within the range of 5 to 7 MeV are employed, generating X-rays that span a spectrum from zero to the energy of the electron beam.

X-rays propagate in the same direction as the incident electrons, because of which a focused beam of X-rays tends to be directed toward the article for sterilization. As such, X-rays are more penetrating in sterilization than gamma rays. So, here we can see the generation of X-rays within a Crookes tube in this picture. Then we have the non-ionizing radiations, which are used for sterilization.

X-rays

- X-rays are generated by high-energy electrons interacting with nuclei possessing high atomic numbers, such as tungsten or tantalum.
- Passing of these electrons near the nucleus results in the emission of X-rays.
- Commercially, electron energies within the range of 5-7 MeV are employed, generating X-rays that span a spectrum from zero to the energy of the electron beam.
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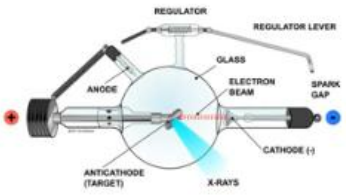

Fig: Generation of X-ray within a Crookes tube
[**File:** JhelebrantCC0, via Wikimedia Commons]

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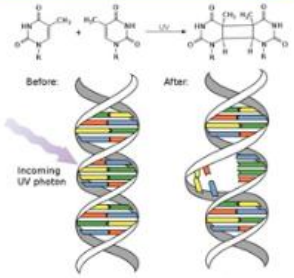
The ability of ultraviolet light, with wavelengths between 100 nanometers and 400 nanometers, to eliminate microbes depends on the duration of exposure. The longer the exposure, the stronger its ability to eliminate them. The most effective germicidal wavelengths fall within the range of 260 nanometers to 270 nanometers. UV light is absorbed by microbial DNA, prompting adjacent thymine bases to form thymine-thymine dimers through covalent bonding. This prevents nucleotides from properly pairing with these dimers during replication.

So, in this picture, we can see the timing dimerization by UV. So this is before the dimerization process. And once the UV comes in, we can see here the dimerization effect in this DNA structure. The microbial cell will attempt to repair the DNA damaged by this dimerization process through a mechanism called SOS repair which may lead to mutations leading to errors in DNA replication and faulty protein synthesis ultimately blocking bacterial metabolism and causing cell death. So, here we see a UV ray hitting the DNA backbone.

Non-ionizing radiation

- The ability of ultraviolet light (wavelength between 100 nm and 400 nm) to eliminate microbes depends on:
  - the exposure duration: the longer the exposure, the stronger its ability to eliminate them.
  - the specific wavelength: the most effective germicidal wavelengths fallis within the range of 260 nm to 270 nm.
- UV light is absorped by microbial DNA, prompting adjacent thymine bases to form **thymine-thymine dimers** through covalent bonding: this prevents nucleotides from properly pairing with these dimers during replication.
- Attempts to repair the DNA through SOS repair may cause mutations, leading to errors in DNA replication and faulty protein synthesis, ultimately blocking bacterial metabolism and causing cell death.



File: Thymine dimerization by UV:
(top) effects of UV on adjacent thymidine units [**Credit:** Steffen 962, Public domain, via Wikimedia Commons]
(bottom) Overall effect of UV on a DNA molecule
[**Credit:** NASA, Public domain, via Wikimedia Commons]

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So, here two thymine are there and then these thymine dimer has been formed over here. Now, these thymine dimerization can be reversed through a process known as photoreactivation, which involves the enzyme photolize. So, here this is the enzyme photolize and due to the activity of these enzyme, we can see that these thymine dimer is removed and normalcy restored. So, this enzyme is activated by visible light, specifically in the blue or near UV spectrum and the activated fertilizer binds to the damaged DNA. It then uses the energy from light to break the covalent bonds between the thymine, thymine dimers, effectively repairing the DNA structure.

This process allows the DNA to resume its normal function by restoring the proper base pairing between timing bases. So what are the different applications of UV light? It is an efficient tool for consumers and laboratory staff in managing microbial proliferation and is frequently integrated into household water purification systems for domestic uses. Moreover, campers often utilize compact portable UV light to sterilize water from natural sources before consumption.

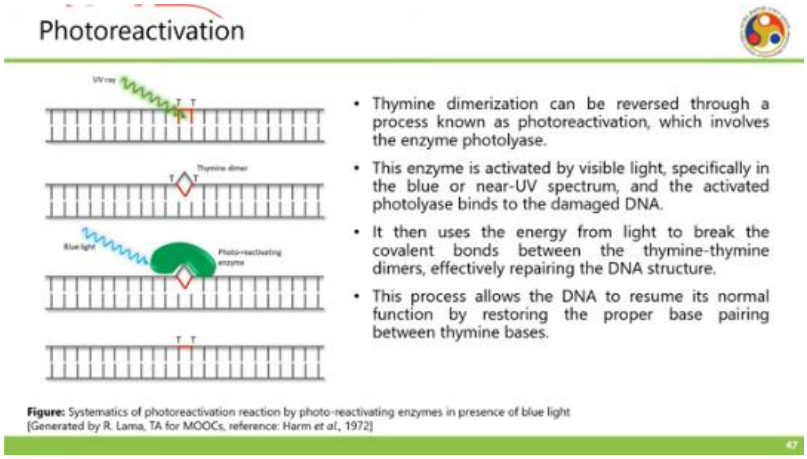
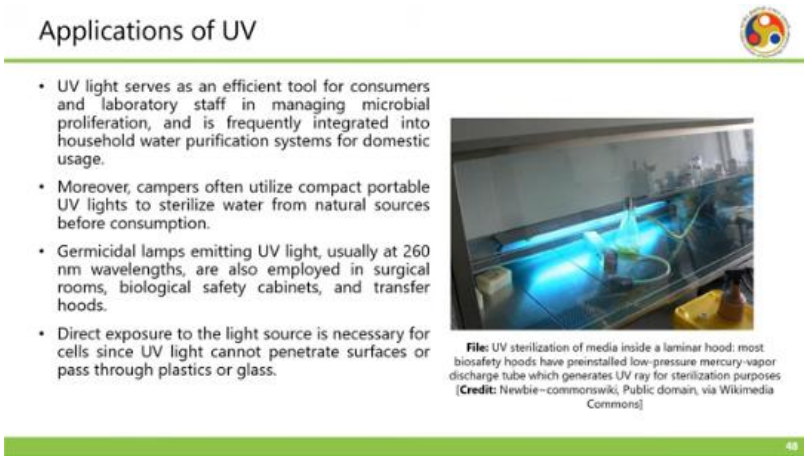


Figure: Systematics of photoreactivation reaction by photo-reactivating enzymes in presence of blue light [Generated by R. Lama, TA for MOOCs, reference: Harm *et al.*, 1972]

Germicidal lamps emitting UV light, usually at 260-nanometer wavelengths, are also employed in surgical rooms, biological safety cabinets, and transfer hoods. Direct exposure to the light source is necessary for cells since UV light cannot penetrate surfaces or pass through plastics or glass. So here, you can see UV sterilization of media inside a laminar hood in a laboratory setting. Another method used for the removal or killing of microbes is sound or sonication, which employs high-frequency ultrasound waves. This is a method used to disturb cell structures.



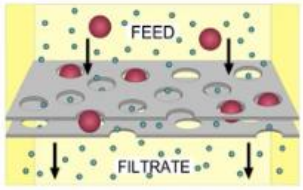
The application of these high-frequency ultrasound waves induces swift changes in pressure within the fluid inside cells, resulting in a phenomenon known as cavitation. This creates bubbles within the cells, which can interfere with their structures and ultimately lead to cell breakdown or collapse. In research laboratory settings, sonication efficiently breaks down cells to extract their contents for subsequent research purposes. Beyond the lab, sonication finds application in cleaning surgical tools, lenses, and various items like coins, tools, and musical instruments. One of the oldest and simplest methods is filtration, but now with sophisticated techniques like membrane filtration, it has also become a very high-end technique.

This is commonly used for sterilizing heat-sensitive liquids and gases, such as antibiotics, hazardous chemicals, radioisotopes, vaccines, and carbohydrates. But then we have, for example, sand filters through which water can be purified, and these can take care of a large percentage of the microbes present in the water sample. The process involves passing the liquid or gas through a filter featuring pores that are too small for microorganisms to pass through while enabling the passage of the liquid or gas. Rather than destroying the contaminants, the present filtration only removes them by retaining them in the filter. So, it doesn't kill but physically retains the contaminants in the filter media itself.

The widely employed filter, the nitrocellulose filter, has a pore size of around 0.22 microns. It can enter bacteria, which typically range in size from 0.3 to 0.5 micrometers, whereas viruses with sizes between 20 nanometers to 0.36 micrometers may pass through these pores. This is the reason why viruses in the beginning were also named as filterable agents. Let us now have a brief discussion about HEPA filters, which actually stands for high-efficiency particulate air filters that are frequently used to filter the air. So here, you can see the diagram.

Filtration

- Filtration is a commonly used technique for sterilizing heat-sensitive liquids and gases, such as antibiotics, hazardous chemicals, radioisotopes, vaccines, and carbohydrates.
- The process involves passing the liquid or gas through a filter featuring pores which are too small for microorganisms to pass through, while enabling the passage of the liquid or gas.
- Rather than destroying the contaminants present, **filtration only removes them by retaining them in the filter.**
- The widely employed filter **nitrocellulose filter**, which has a pore size of 0.22µm, for example, can entrap bacteria which typically range in size from 0.3 to 0.5 µm, whereas viruses, with size between 20 nm to 0.36 µm, may pass through the pores.



File: Schematics of a simple filtration system (**Credit:** Wikiwayman, CC-BY-SA-3.0, via Wikimedia Commons)

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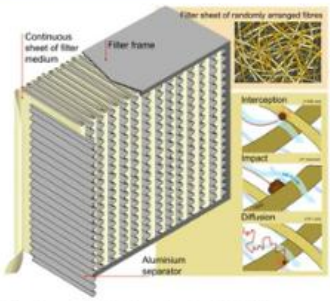
In the main parts of a HEPA filter, on this side, you can see the continuous sheet of the filter medium, and then these are stacked one after the other. These are basically sheets of randomly arranged fibers. Any particulate material that comes over and tries to cross this filter will end up hitting some of these fibers and thereby will be intercepted. Then, while the incoming air flows through these fibers, there may also be some kind of diffusion effect. So, basically, these filters have effective pore sizes of around 0.3 microns and are small enough to trap bacterial cells, endospores, and many viruses. As air flows through HEPA filters, the air on the other side of the filter is nearly sterilized.

HEPA filters are frequently used in hospital settings, vehicles, aircraft, and even homes. They can be found, for instance, in air purifiers, heating and cooling systems, Hoover cleaners, and, as I already told you, in laminar hoods, which we use in the microbiological laboratory. So another kind of filter is the membrane filter. This membrane filtration can be used to filter out germs from liquid samples. So, here is a Curtis membrane filter used in filtering wine in large quantities, shown here on the left side.

HEPA filters



File: Diagram showing the main parts of a HEPA filter (**Credit:** LadyofHats, Public domain, via Wikimedia Commons)

- High-efficiency particulate air (HEPA) filters are frequently used to filter air.
- These filters have effective pore sizes of 0.3 microns, and are thus small enough to trap bacterial cells, endospores, and many viruses.
- As air flows through HEPA filters, the air on the other side of the filter is nearly sterilized.
- HEPA filters are frequently used in hospital settings, vehicles, aircraft, and even homes. They can be found, for instance, in air purifiers, heating and cooling systems, and hoover cleaners.

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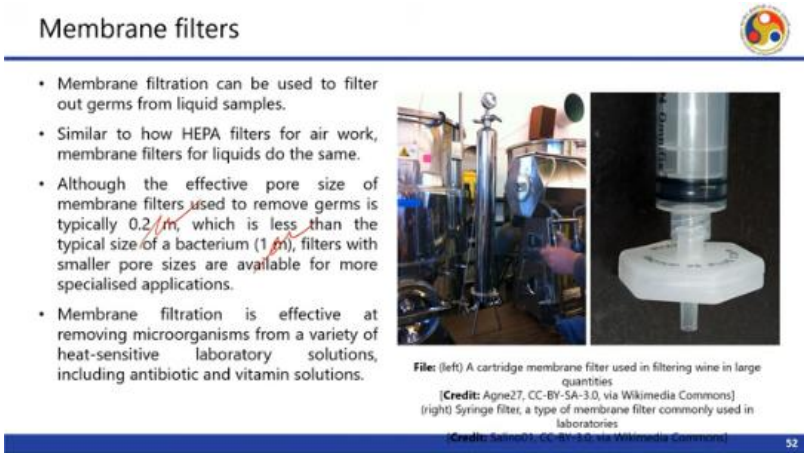
And then in the right side is a syringe filter which is a type of a membrane filter which is commonly used in laboratories for example in sterilizing labile, heat labile substances which can be dissolved in liquids. Similar to how HEPA filters work for air purification, membrane filters work for liquid purification. Although the effective pore size of membrane filters used to remove germs is typically 0.2 micron, which is less than the typical size of a bacteria, 1 micron. Filters with smaller pore sizes are available for more specialized applications. Memon filtration is effective at removing microorganisms from a variety of heat-sensitive laboratory solutions, as I have already told you, including antibiotic and vitamin solutions.



So, with this, we come to end of this lecture. Thank you for your kind attention. Thank you.