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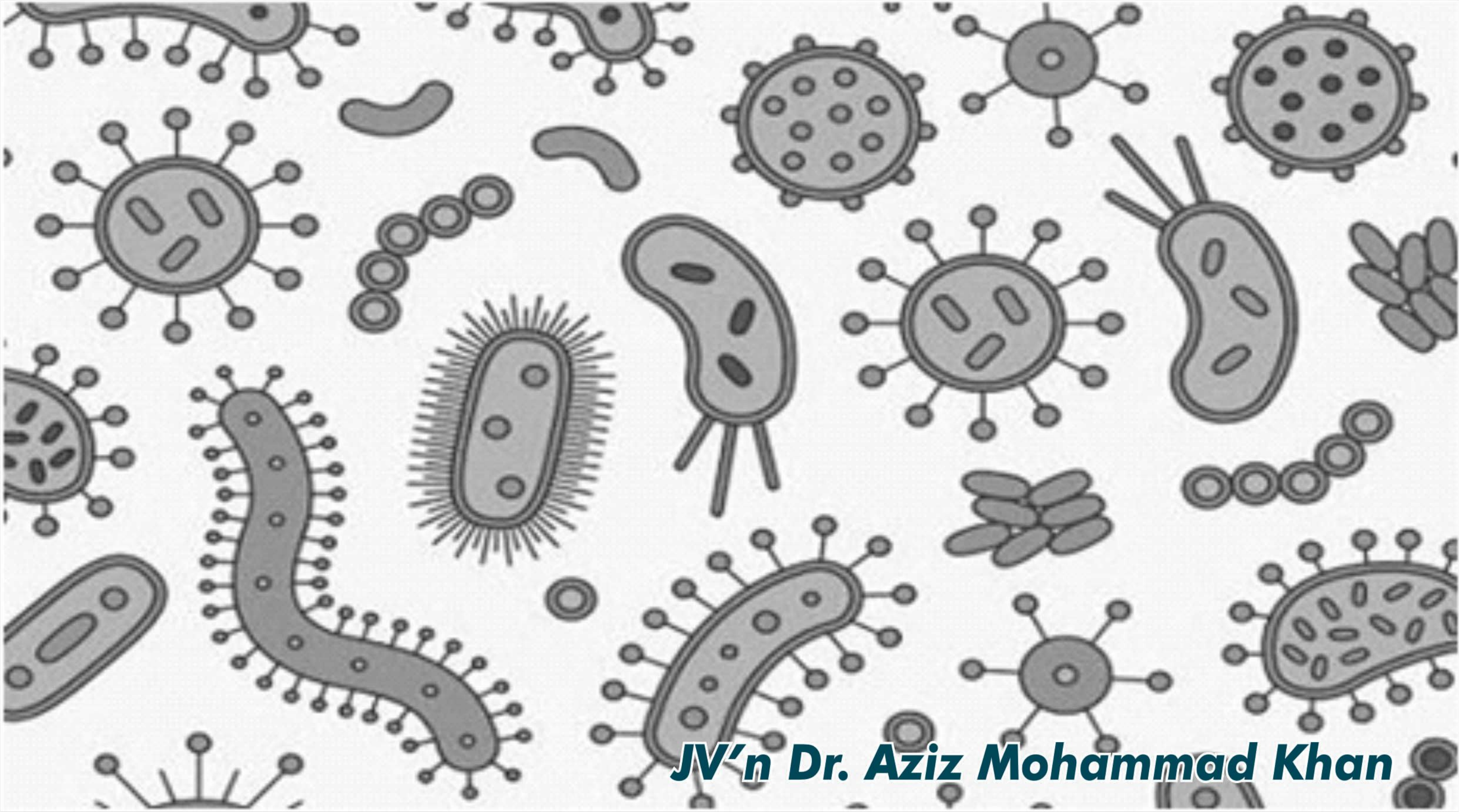
# A BOOK
# ON
# FOOD BIOTECHNOLOGY
## VOL-I



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# INDEX

# Chapter -1 Historical background of Food biotech

## FOOD BIOTECHNOLOGY

Biotechnology has a long tradition of use in food production and manufacturing. For ten thousand years fermentation, a form of biotechnology, has been used to manufacture wine, beer and bread. For decades, selective breeding of animals like horses and dogs has been going on. Compared to their wild ancestors, selective breeding of important foods such as corn, maize and wheat has produced thousands of local varieties with increased yield.

The use of technologies for manipulating the genes of our food supplies is food biotechnology. Animals, herbs, and microorganisms are our sources of food. With food biotechnology, we develop new animal and plant organisms, precisely the animals and plants we consume, for example. Nutritional, development, and marketing properties have been desired by these new animals.With food biotechnology, we use what we know about science and genetics to improve the food we eat. We also use it to improve how we produce food.

By change, we mean either making the food cheaper, longer-lasting, more resistant to disease, or more nutritional to make.

The International Food Intelligence Council Foundation writes about the use of biotechnology to help generate the food we need:

"Food biotechnology tools include both traditional breeding techniques, such as cross-breeding, and more advanced techniques, which involve using what we know about genes or specific trait instructions to improve the quantity and quality of plant species."

We may transfer beneficial characteristics from one plant or animal to another with science techniques.

## *The origins of food fermentation*

Pasteur took the more realistic view of an applied scientist, unlike Darwin and Mendel, who is identified as pure scientists. One of his main interests was the manufacture of vinegar, a method that had previously had mixed results due to infection by inappropriate bacteria. The first to recognize the type of bacteria necessary and isolate them in a pure form was Pasteur. From then on, under regulated conditions, vinegar could be produced in a reliable manner, facilitating large-scale, economical production of consistently high quality vinegar.

Now, commercial fermentation of micro-organisms produces multiple food products. In a procedure which is more cost-effective and convenient than the use of lemons, citric acid is extracted from the fungus, Aspergillus niger. The flavor enhancer, monosodium glutamate, is extracted from Corynebacterium glutamicum, a bacterium that produced more than 300,000 tons of this compound worldwide in 1993. Extracts of yeast for use as fruit flavouringYeast extracts for use as food flavourings are produced by fermentation; lactic acid is also made using this method.

## *The birth of gene technology*

The food industry has benefited from the pharmaceutical sector's investment in biotechnology this century, as fermentation methods have been developed to manufacture antibiotics and the knowledge of genetics by scientists has improved. James Watson and Francis Crick lay the basis of genetic modification with the discovery in the 1950s of the replication mechanism of DNA (deoxyribonucleic acid). During the 1970s, developments led to processes becoming more predictable and consistent than ever before, due to increasing molecular level regulation.

Plant breeding has since been changed by genetic modification techniques. Plant breeding is historically aimed at matching the optimal traits of two types of plants. Tomato variety X, for instance, can yield high yields but is vulnerable to diseases, while variety Y is disease-resistant but produces low yields. It may take several years for high yield to be combined with disease resistance in a new variety. Gene technology now has the ability to recognize and spread the disease-resistance gene in variety Y directly to variety X, without the need for time-consuming breeding programs.

Gene technology will also allow genetic material to be mixed in a way that could not exist in nature, in addition to speeding up breeding programs and improving their chances of success. For starters, it is possible to inject copies of animal genes into plants and to incorporate copies of plant genes into bacteria. It is this capacity that increases the diversity of ethical and safety issues currently being addressed across Europe, a dialogue to which the food industry wishes to make a complete and transparent commitment.

# Chapter -2 History of Food Microbiology

Food Microbiology does not have a precise start as a discipline. In the end, occurrences over many decades lead to the understanding of the importance and role of microorganisms in foods. From the dawn of our race, food-borne illness and food spoilage have become part of the human condition. While for thousands of years the true cause of these issues will remain a mystery, many early cultures found and applied successful methods to conserve and safeguard their food:

7000 BC- Evidence that beer was made by the Babylonians (fermentation). About 3500 BC, wine emerged. Alcoholic drinks such as beer and wine were far better to drink in early cultures (and still still in underdeveloped countries where modern hygiene is lacking) than the local water source, since the water was sometimes polluted with intestinal microorganisms that triggered cholera, dysentery and other severe illnesses.6000 BC – The first apparent reference to food spoilage in recorded history.

3000 BC-Cheese (fermentation) and butter produced by Egypt (fermentation, low aw). Again, fermented foods such as cheese and sour milk (yogurt) were safer to consume than their raw agricultural counterparts, and avoided spoilage better. In order to protect meat and other foods at this time, many cultures have learnt to use salt (low aw).

1000 BC-Romans used snow to sustain shrimp (low temp), as well as accounts of smoked and fermented meats.

Although early human civilizations found efficient ways to conserve food (fermentation, salt, ice, drying, and smoking), they did not understand how food spoilage or foodborne illness was inhibited by these activities. Their confusion was exacerbated by the assumption that living life arose from non-living matter naturally. (Theory of Spontaneous Generation).

> 1665-Francesco Redi's Italian physician proved that maggots on putrefying meat did not emerge naturally, but instead were the larval stages of flies (put meat in a jar filled with fine gauze such that flies could not have access to eggs). This was the first step away from the random generation doctrine.
>
> 1683-Bacteria through a microscope were studied and identified by Anton van Leeuwenhoek from the Netherlands. At around the same time, in order to interact and publish experimental work, the Royal Society was founded in England and they invited Leeuwenhoek to share his findings. Before his death in 1723, he did so for almost 50 years.As a result, Leeuwenhoek's

reports were widely disseminated and he is justifiably regarded as the person who discovered the microbial world.

1765-The Italian Spallanzani sought to disprove the hypothesis of spontaneous life generation by showing that boiled and then sealed beef broth remained sterile. The theory's proponents dismissed his work because they believed that his treatment omitted O2, which they believed was necessary for spontaneous generation.

1795- 12,000 francs were given by the French government to anybody who could create a realistic method of storing food. The patent was given to a French confectioner named Nicholas Appert after proving that meat could be stored when put in glass bottles and cooked. This was the beginning of the conservation of food by canning.

1837-Schwann reveals that in the presence of air (which he passed in by heated coils), healed infusions remain sterile again to disprove spontaneous generation. It is important to note that while Spallanzani and Schwann both used heat to preserve food, the importance of making these findings into a commercial tool for food preservation was obviously not understood by either individual. (Critics say that heating somehow changed the influence of air as spontaneous generation required it.)

Louis Pasteur was the first human to fully appreciate and understand the causal relationship between microorganisms in infusions and the chemical changes that resulted in those infusions. Via his experiments, Pasteur persuaded the science community that microorganisms were responsible for all fermentative processes and that particular forms of fermentation (e.g. alcoholic, lactic or butyric) were the product of specific microorganism types.

Pasteur showed that souring milk was caused by microbes in 1857, and he showed that heat in wine and beer killed undesirable microbes in 1860. For a variety of foods, the latter method is still used and is called pasteurization. Pasteur is regarded as the father of food microbiology and microbiological research because of the relevance of his work. Using his famed swan-necked flasks that finally disproved spontaneous generation, he showed that air doesn't have to be heated to stay sterile.Some of Pasteur’s most notable achievements include:

- Fermentation has been found to be a result of microbial action and different forms of fermentation (i.e. lactic, butyric, etc.) have been induced by various types of microorganisms. The knowledge that fermentation and putrefaction were the responsibility of microbes led Pasteur to argue that microbes were also causative agents of disease. Eventually, these claims hit Joseph Lister, an English surgeon who used them to establish the first aseptic surgical procedures.

- Developed a vaccine to protect sheep from anthrax by isolating Bacillus anthracis, the attenuated (virulent) form of the causative bacterium. By developing them at higher temperatures, Pasteur isolated the attenuated organisms (42oC). Sheep is resistant to virulent strains exposed to the attenuated bacterium. While the basis for attenuation was not known by Pasteur, we now know that in this bacterium, virulence relies on the existence of a plasmid that does not reproduce at 42oC.

-A method to make chickens resistant to cholera caused by Pasteurella septica was also developed by Pasteur, again using an attenuated bacterium which he had isolated in his laboratory.

Microbiological discoveries and inventions started to progress more quickly from the time of Pasteur. In many pathogens, bacteria were active, heat-resistant spores were detected, toxins were detected, and by the late 1800s, legislatures started enacting laws to protect food safety.

Many food industries in the U.S. refused to follow broad microbiological standards in the sector until they were economically challenged by the ads surrounding foodborne disease outbreaks. In the early 1920s, many unpleasant outbreaks of botulism gradually forced the U.S. canning industry to introduce a rather restrictive heat treatment, known as the 12D system, which decreases the likelihood of the most heat tolerant C. survival. Up to one in a billion botulinum spores (10-12). This tradition continues today, and since 1925, with just 5-6 documented cases of botulism, the canning field has created more than a trillion containers. Faulty containers were involved in most of these cases, not under packaging.

At the the same time, because of several infamous outbreaks of milk-borne typhoid fever, diphtheria, tuberculosis and brucellosis, the dairy industry was forced to introduce microbiological regulation on milk. Requirements covering animal welfare, hygiene, pasteurization (which had an immediate and very successful impact on the problems) and refrigeration were developed by the public health authorities, both of which were strengthened by bacterial requirements. As a consequence, by the mid-1900s, pasteurized milk was among our safest foods.

"The New York state government institutionalized a woman who came to be known as "Typhoid Mary" in one of the most peculiar episodes of early food microbiology. Mary was an asymptomatic typhoid carrier who served at the turn of the century as a cook for many families. Seven typhoid infections have been specifically attributed to her for more than ten years, and reports indicate that she could have been responsible for 51 cases of typhoid fever. New York police arrested her and threatened to remove her gall bladder, but finally released her after she decided that she would never function again as a chef. A few years after, after another epidemic was linked to her, she was imprisoned as a public safety threat and institutionalized until her death in 1938.

We establish an environment free of competition when we extract microbes from food, which could encourage other microorganisms to develop and cause disease. For this cause, there is great interest in finding healthy bacteria (e.g. lactic acid bacteria) that would prevent the growth of pathogens when purposely applied to food but would not easily ruin the substance itself (though some lost shelf life seems inevitable).

# Chapter -3 -Types of Microorganisms in food

## Microorganisms

Microorganisms in the food industry play a significant part. Microorganisms are used in the manufacturing of various agricultural products and are also responsible for the spoilage of food, causing poisoning and disease.

Microbial infection of food products typically happens on the way to the processing plant from the farm, or during processing, packaging, transportation and delivery, or prior to use. Bacteria, molds and yeasts are predominantly the microorganisms that cause food spoilage and also find optimum exploitation in the processing of food and food products.

## Bacteria

The largest community of unicellular microorganisms is bacteria. In-cocci or circular cells; bacilli or cylindrical or rod-shaped cells; and spiral or curved forms are known as shapes of medically important bacteria. Pathogenic or disease-causing bacteria are normally gram-negative, although it is recognized that three gram-positive rods cause food poisoning: Clostridium botulinum,C. Bacillus cereus, perfringens, and

Acinetobacter, Aeromonas, Escherichia, Proteus, Alcaligenes, Flavobacterium, Pseudomonas, Arcobacter, Salmonella, Lactococcus, Serratia, Campylobacter, Shigella, Citrobacter, Listeria, Staphylococcus, Micrococcus, Corynebacterium, Vibrio Enterobacter, Paenibacillus, Weissella, Enterococcus, Yersinia are some of the other most common bacteria causing food spoilage, infection and disease.

In the processing of various food and dairy products, separate types of bacteria are often used. Streptococcus strains, Bifidobacterium Lactobacillus, Erwiniaetc. They are used in the manufacturing of fermented food and milk products. The processing of yogurt is carried out by Streptococcus thermophilus and Lactobacillusbulgaricus.

## Molds:

Molds are multicellular filamentous fungi and are typically easily identified by their fuzzy or cottony appearance for food growth. They are largely responsible for the spoilage of food at room temperature of 25-30oC and low pH, and have minimal requirements for moisture. When these goods are processed under wet conditions, moulds can grow rapidly on grains and maize. For growth, molds need free oxygen and thus grow on the surface of polluted food.

Molds are also found to be used in the manufacture of various foods and dairy items. They are used to ripen different kinds of food items, such as cheese (e.g. Roquefort,Camembert). Molds are also cultivated as feed and food and are used in soft drinks to produce ingredients such as enzymes such as amylase used in the manufacturing of bread or citric acid. Molds play a significant role in the ripening of many Oriental foods. The *Bothrytiscinerea* is used in the rotting of grapes for wine processing. The product of lactic fermentation using molds is a distinctive Finnish.

**Yeasts**:

Yeasts are capable of fermenting ethanol and carbon dioxide sugars, and are thus commonly used in the food industry. The yeast most widely used, the baker's yeast, is produced industrially. Most commonly, Saccharomyces carlsbergensis is used in the fermentation of most beers. *Brettanomyces, Schizosaccharomyce,, Candida, Cryptococcus, Debaryomyces, Zygosaccharomyces, Hanseniaspora, Saccharomyces* are the other yeast strains of significance.

**Points to remember**

- The most significant microorganisms that cause food spoilage and also find optimum exploitation in the processing of food and food products are bacteria, molds and yeast.
- For the fermentation of dairy products, different types of bacteria and fungi are used for the processing of a wide range of cultured milk products. Both bacteria and fungi are used in these cheese processing processes.
- For milk coagulation, lactic acid bacteria are used and can be processed to produce a wide range of cheeses, including soft unripened, soft ripened, semisoft, strong, and very hard forms.
- As in the food and health sector, microorganisms such as Lactobacillus and Bifidobacterium are included.
- For the development of various wine varieties, molds are used to rot grapes.
- Spirulina, a cyanobacterium, is a common source of food sold in specialty stores as well.
- Mushrooms are one of the most crucial fungi used as a food source (Agaricusbisporus).
- One of the most important fungi used as a food source is mushrooms (Agaricusbisporus).
- Ferme manufactures soft beverages such as beer. For the processing of various types of wines, moulds are used for the rotting of grapes.

- By fermenting cereals and grains using various strains of yeasts, alcoholic drinks are produced as beer.

## Pathogenic micro-organisms

Pathogenic micro-organisms, including microbes, viruses, fungi and moulds, cause food-borne illnesses or intoxication. It is important to remember that pathogenic bacteria and viruses typically do not cause food spoilage, and it is difficult to see or taste their infection.

The major contributing factors to the outbreak of foodborne diseases are:

1. Usage of raw produce and products coming from unhealthy sources
2. Insufficient cooking or heat processing
3. Improper cooling and drying, such as holding cooking food for extended periods of time at room temperature or storing food in large containers in the refrigerator.
4. Allowing many hours to pass between food preparation and feeding
5. Inadequate reheating inadequate
6. Improper warm keeping, which means below 65 ° C
7. Handling of food by sick people or carriers of infection
8. From raw to prepared food, cross contamination. For example, you cut vegetables for salad on a cutting board where you cut raw meat until you cut it.
9. Improper washing of equipment and utensils

## Bacteria

- Campylobacter jejuni: is a frequent cause of human diarrhea as well as of some species of animals. Transmission may occur by direct human interaction with infected animals or their waste. More frequently, it is transmitted by the ingestion of infected food or drink, passing from person to person. Symptoms vary from moderate diarrhea to extreme invasive illness, including stomach pain, fever, and stool blood and mucus.

- Non-typhi salmonellosis: Salmonella spp has more than 2000 serotypes, of which only a handful cause human Salmonella gasteroenteritis. Acute watery diarrhea followed by nausea, cramps and fever are the symptoms. Blood can be found in the stools. Animals are the primary reservoir, and infection happens by absorption of infected materials. Poultry, fruit, eggs and milk are foods which are mainly at risk.

- Salmonella typhi and paratyphi, respectively, cause typhoid fever and paratyphoid fever. Transmission happens primarily by person-to-person contact or food contamination by food handlers, as the source for all of these bacteria is generally human.

- **Staphylococcus aureus**: Humans are the cause of this infection. In the nose and on the skin of genetically healthy individuals, bacteria are also present in smaller concentrations. In skin lesions, such as contaminated eczema, psoriasis or some other pus drainage lesion, higher levels can be detected. Thus, these persons should not be handling fruit. Food poisoning caused by this bacteria is caused by staphylotoxin, which is immune to heat, leading to diarrhea, vomiting, cramps and fever. The symptoms unexpectedly start and usually go away within 24 hours.

- **Escherichia coli**: There are many serotypes, several of which can cause gastroenteritis, while others are harmless to humans. The most frequent cause of traveller's diarrhea is Enterotoxigenic E.coli. Humans are the root, and transmission typically takes place by polluted food and water.

- **Listeria monocytogenes**: This bacterium is strongly correlated with food kept in the refrigerator for long periods of time because it is omnipresent and is capable of developing slowly, even at low temperatures. In immunocompromised cases, where it can cause septicemia and meningitis, it can be fatal.

- **Shigella**: Humans and primates are the cause. Since it has a low contagious dose, contact from person to person is the main mode of transmission. It can also be spread by food and drink that is contaminated. Fever and watery diarrhea are the symptoms of shigellosis. The infection may also present itself as a dysenteric condition involving fever, stomach cramps and tenesmus, as well as regular, limited amount, bloody stools.

- **Vibrio Cholerae 01**:People are the cause of this infection. In overcrowded, unhygienic conditions, the primary mode of transmission is by polluted water and food, or person-to-person diffusion. Extreme watery diarrhea, which can reach up to 20 liters a day, is induced.

- **Clostridium Botulinum**: The digestive tract of fish, birds, and mammals is its source. It is also spread extensively in nature. The bacterium is an anaerobic spore with a very strong heat-labile toxin that affects the nervous system.

**Viruses**

Viruses do not replicate in foods, unlike bacteria. Therefore, the predominant mode of transmission by food handlers and the use of filthy utensils that spread the virus to food is eaten by humans.

- The main causes of gastroenteritis are Rotaviruses and Norwalk viruses.
- Viral hepatitis A outbreaks are caused mostly by asymptomatic food handling carriers.

**Parasites**

Many parasites, such as helminths, have more than one host involved in a complicated lifecycle. For these parasites, the primary path of transmission to humans is the food route. The pattern tends to be the eating of undercooked pork or beef, or the consumption of raw salads washed in polluted water.

**Solium of Taenia and T. Saginat**a: also called tapeworms for pigs and beef. Their cysts are swallowed, present in the muscle of the species, and the adult worm grows in the gut. The ova will grow into larvae and, as a result, can enter other tissues, such as the brain, forming cysticercosis and significant neurological disorders.

**Trichinella spiralis:** It is present in pork that is undercooked. Tissues may be attacked by the larvae to develop a febrile disease.

**Giardia lambila:** This infection may be transmitted through food, water or transferred by interpersonal communication. It induces acute or subacute diarrhea, with malabsorption, stomach pain and bloating, and oily stools.

**Entamoeba histolytica:** Transmission is primarily transmitted through food or drink. Because they are extremely immune to chemical disinfectants, including chlorination, the cysts pose a serious issue. Typically, the virus is asymptomatic, but may occur either as a moderate chronic diarrhea or as a fulminant dysentery.

## Food Spoilage

It is the alteration in food's texture, consistency, taste and scent, and is caused by bacteria, moulds andyeasts.

**Bacteria**: Examples of action of bacteria involved in food spoilage:

1. Lactic acid formation: Lactobacillus, Leuconostoc
2. Lipolysis: Pseudomonas, Alcaligenes, Serratia, Micrococcus
3. Pigment formation: Flavobacterium, Serratia, Micrococcus
4. Gas formation: Leuconostoc, Lactobacillus, Proteus
5. Slime or rope formation: Enterobacter, Streptococcus

**Moulds**: Some strains produce mycotoxins under certain conditions

1. Aspergillus produces aflatoxin, ochrtoxin, citrinin and patulin
2. Fusarium
3. Cladosporium
4. Alternaria

Mycotoxins can penetrate into the parts of food that are not visibly mouldy as well. It is therefore necessary to throw away all of the food if any part of it is mouldy. They are also notoriously difficult to destroy as they are stable to both heat and chemicals.

- Hepatotoxins: aflatoxins, sporidesmins, luteoskyrin
- Nephrotoxins: ochratoxin, citrinin
- GIT toxins: trichocetens
- Neuro- and myotoxins: tremorgens, citreoviridin
- Dermatotoxins: verukarins, psoralen, sporidesmins, trichocetes
- Respiratory tract toxins: patulin

Foods most at risk for moulds:

1. Grains and grain products - many mycotoxin types
2. Peanuts, nuts and pulses - aflatoxin
3. Fruits and vegetables (raw and preserved) - patulin
4. Milk and milk products - aflatoxin

It is important to note that if any contaminated fodder is fed to animals, this is metabolized and the toxic derivatives can be found in animal products consumed by humans, e.g. milk and meat.

# Chapter -4 Role of Microorganisms in Food Industry

## *In household Food processing*

The members of the family produce household food for their own consumption. Some microorganisms, such as bacteria and fungi, play a variety of roles in household food production.

Lactobacillus, for example, the bacteria involved in the formation of milk and yogurt curds, is produced by Lactobacillus bulgaricus.

Saccharomyces cerevisiae is a type of yeast used in the household and food processing industries to produce bread.

In order to prepare certain popular beverages like Toddy, microorganisms are often used.

Besides these, some bacteria prepare the most popular foods such as dosa and idly from fermented rice.

## Industrial Production

Food engineering is one of the most sophisticated ways of using microorganisms to improve the consistency and quantities of food. The method of planning and upgrading the production process of food products includes food engineering. New foods and high quality biological products can be prepared using microorganisms by food engineering. Even, microorganisms are used in industries to sustain food and its consistency.

Microorganisms play a vital role in the processing of a variety of foodstuffs in commercial food production.

1. Antibiotics against pathogens and diseases are essential components of human welfare. These are produced in factories that use bacteria. Penicillin, for instance, is one of the essential antibiotics and is produced by the bacteria Penicillium notatum.

2. Saccharomyces cerevisiae carries out the processing and storage of drinks such as bourbon, brandy, cider, and rum.
3. In the industrial development of enzymes, microorganisms are also involved. Example: Pro
4. One of the essential commercial chemicals that Saccharomyces cerevisiae produces is ethanol.
5. From the fungus, Trichoderma, immunosuppressive agents like Cyclosporin are prepared.

6. Any of the microorganisms in food processing technology are also used for the preservation of packed food.

Significant Microorganisms in Food Production

Microorganisms such as molds, yeasts, and bacteria may develop in food and cause spoilage. Bacteria can cause foodborne illnesses as well. Viruses and parasites can cause foodborne illness, such as tapeworms, roundworms, and protozoa, but they are not capable of developing in food and do not cause spoilage.

A list of diseases and infectious agents of importance to public health is as follows. This list is not complete, but includes most foodborne pathogens that impact beef, poultry, and egg products that have been processed.

- Bacteria
- *Bacillus cereus* (*B. cereus*)
- *Brucella* species (*Brucella* spp)
- *Campylobacter* spp
- *Clostridium botulinum* (*C. botulinum*)
- *Clostridium perfringens* (*C. perfringens*)
- *Escherichia coli*
- *Listeria monocytogenes* (*L. monocytogenes*)
- *Salmonella* spp
- *Shigella* spp
- *Staphylococcus aureus* (*S. aureus*)
- *Yersinia enterocolitica* (*Y. enterocolitica*)

**Viruses**

- Hepatitis A and D
- Norovirus
- Rotaviruses

# Chapter -5 Microorganisms in food production

Yeasts, bacteria, moulds, or a mixture of these are the most widely used microorganisms. The fermentation process, resulting in the production of organic acids, alcohols, and esters, is a clear example of the use of microorganisms in food production. They help in:

1. Preserve the food
2. generate distinctive new food products

## Yeast in food production

Leavened bread and bakery products: Saccharomyces cervisiae ferments CO2-producing sugars, the gas that gives bakery products their porous shape. By forming alchols, aldehydes, esters etc., it also adds to the flavor.

- Beer
- Wine
- Vinegar
- Pickles

## Bacteria in food production

- Fermented milk products: *Lactobacillus, Lactococcus, Bifidobacterium*
- A variety of foods, including Indian dosa, rabri: Leuconostoc mesenteroides fermentation, S. Fecalis
- Probiotics: live dietary additives found in yoghurt and other products of fermented milk. Lactobacillus acidophillus and Bifidobacterium bifidum are included. To have some meaningful impact, a minimum of 108 bacteria per 1 ml must get to the colon alive. The microbial spectrum in the gut is strengthened by these bacteria and thus leads to the following effects:
- 1. Influences immunity and thereby eliminates or mildens diarrheal diseases

- 2. Lowering the risk of bowel cancer
- 3. Diminish the synthesis of cholesterol
- 4. It creates acids that reduce the pH of the intestine, thus increasing the absorption of minerals such as calcium and phosphorus.

**Mould in food production**

- Cheese: Penicillium roqueforti and Penicillium camemberti (note that at 25 ° C this produces mycotoxin, so the processing of cheese must take place at 15 ° C)
- Dry salami: making use of moulds of Penicillium and Scopulariopis.
- Soy sauce: Aspergillus spp, especially A. Oryzae are interested in this manufacturing. A subsequent lactic fermentation is often carried out in which lactic bacteria produce lactic acid.
- Sake: developed using a mixture of yeast and Aspergillus oryzae mould.

# Chapter-6 Factors Affecting Growth of Microorganisms

The food processor eliminates microorganisms' possible issues in many ways:

Remove or kill them by trimming, cleaning, boiling, choosing, applying additives, or promoting competition from species that form acid or alcohol.

Minimizing pollution from buildings, persons, the environment, and unprocessed food.

Minimizing microbial growth on facilities, by washing and sanitizing, and by changing storage temperature, pH, and other environmental variables in the substance itself.

While the presentchapter discusses each factor influencing development independently, these factors exist concurrently in nature. Their inhibitory effects are cumulative where more than one situation is very detrimental to microbial development.

## Temperature

The most powerful method of regulating microbial growth is temperature. Microorganisms are loosely categorized as follows, based on their tolerance to wide temperature ranges:

1. Psychrophies only develop at the temperature of refrigeration.

2. At refrigeration temperatures, psychrotrophs grow well, but best at room temperature.

3. At or above human body temperature, mesophiles grow best, but grow well at room temperature.

4. Thermophiles only thrive at temperatures that are almost as hot as the human hand can tolerate, and normally not at or below body temperature at all.

To be more detailed on these limits of growth temperature is to step into the controversy that has continued from the beginning of microbiology, and in temperature ranges there are many species that overlap them. However, for food microbiology, these assumptions are relevant: to be more precise about these growth temperature limits is to step into the debate that has persisted since the beginning of microbiology, because there are several organisms that overlap these temperature ranges. However, these conclusions are relevant for food microbiology:

1. In foods below freezing, some psychrotrophic microorganisms develop very slowly, but typically not below 19 ° F. There are a few growth reports, typically of molds, at 14°F, but there are no credible growth reports below that temperature. This suggests that microbial growth is not allowed by the normal storage temperature for frozen foods, O°F.

Few microorganisms withstand freezing, however (Michener and Elliott, 1964).Most psychrotrophs have difficulty growing above 90°F.

2. It is difficult for most psychrotrophs to grow above 90°F.

3. Many species with foodborne pathogens are mesophiles. In the awareness that foods kept above or below the limits in Figure 1 and properly rotated will stay healthy, the food processor will feel comfortable. Storing perishable foods below 40°F or over 140°F is a safe rule of thumb.

4. The psychrotrophs develop more quickly in the temperature range where both mesophilic and psychrotrophic species live (from 41 ° F. to around 90 ° F), causing spoilage and often frequently interfering with the development of foodborne disease species (Elliott and Michener, 1965).

The rate of growth grows exponentially within the growth spectrum as the temperature is increased. Conversely, as the temperature is reduced, microbial growth rates decline quickly and, thus, food spoilage happens even more slowly. Near the freezing point, this impact is extremely marked. Note that a decline would more than double the s from about 41 ° F to about 32 ° F(time before spoilage).

## Water Activity

Water activity (aw) is a concept that describes microorganisms' supply of water. It is only roughly related to the moisture percentage. Pure water has 1.00 aw, and a 100 percent equilibrium relative humidity (ERH) would provide the atmosphere above the water in a closed bottle. If we apply an ounce of rock in such a bottle to a quart of water, the ERH and aw will not alter. But if we apply an ounce of salt, it's going to reduce the ERH to around 98% and the aw to 0.98. Rocks do not dissolve in water, but salt does, decreasing the percentage of water that will penetrate the environment. Similarly, there is a decrease in the amount of water accessible to microorganisms found in the solution. Yet in the container with rocks, the percent moisture is the same as in the container with salt, namely, 98 percent.

Water behavior is defined by the GMP regulations for low-acid canned foods as the vapor pressure of the food component separated by the vapor pressure of pure water under equal pressure and temperature conditions. The regulations define low-acid foods as foods, other than beverages, with a finished equilibrium pH value greater than 4.6 and a water activity greater than 0.85.

Table 1. The water activity ($a_w$) limits for growth of principal foodborne disease organisms.*

| Microorganism | Minimal $a_w$ for growth | Reference |
|---|---|---|
| *Salmonella* | 0.945 | Christian & Scott, 1953 |
| *Clostridium botulinum* | 0.95 | Scott, 1957 |
| *Clostridium perfringens* | 0.93 | Kang, et al., 1969 |
| *Staphylococcus aureus* | 0.86** | Scott, 1962 |
| *Vibrio parahaemolyticus* | 0.94 | Beuchat, 1974 |

* These limits are the lowest stated, with optimal conditions for all other growth. The minimum aw would be higher if other parameters are less than ideal.

** Troller and Stinson (1975) have shown that the minimum aw in their experiments for toxin output is greater than 0.93 for growth.

In a food or other medium where the aw is less than 0.94, most bacteria struggle to expand. Bacteria need a higher aw than yeasts, requiring a higher aw than molds in exchange. Thus, bacteria, then yeasts, and finally molds are inhibited by any condition that reduces the aw first (Elliott and Michener, 1965). But there are limitations for each species that are interrelated with other growth factors. The aw limits for the development of key foodborne disease species kept under otherwise ideal conditions are presented in Table 2.

On fish immersed in saturated salt solution where the aw is around 0.75, some molds and bacteria can emerge. Any molds with AW 0.62-0.655 can develop in foods (Elliott and Michener, 1965). Development is very sluggish at these lower limits. The aw is around 0.10 for entirely dried foods, such as crackers or sugar, and these items are microbiologically stable solely because of this element. Combinations of variables such as low aw, low pH, pasteurization, organic contaminants, and impervious packaging rely on the consistency of intermediate moisture foods (aw 0.75-0.90), such as dried fruits, preserves, and soft moist pet foods.

## pH

pH is a term used to describe the acidity or alkalinity of a solution. At pH 7, there is an equal amount of acid (hydrogen ion: H +) and alkali (hydroxyl ion: OH-), so the solution is “neutral”. pH values below 7 are acidic, while those above 7 are alkaline. pH expresses the H +

concentration logarithmically, that is, in multiples of 10. For example, at pH 5 there are 10 times as many H **+** as at pH 6; at pH 3 there are 100 times as many H **+** as at pH 5, and so on.

pH has a profound effect on the growth of microorganisms. Most bacteria grow best at about pH 7 and grow poorly or not at all below pH 4. Yeasts and molds, therefore, predominate in low pH foods where bacteria cannot compete. The lactic acid bacteria are exceptions; they can grow in high acid foods and actually produce acid to give us sour milk, pickles, fermented meats, and similar products. Some strains, called Leuconostoc contribute off-flavors to orange juice. The pH values of certain foods are given in Table 2.

Table 2. Mean pH Values of Selected Foods (Lopez, 1987)

| **pH Value** | **Selected Foods** |
|---|---|
| 2.3 | Lemon juice (2.3), Cranberry sauce (2.3) |
| 3.0 | Rhubarb (3.1)<br>Applesauce (3.4), Cherries, RSP (3.4)<br>Berries (3.0 – 3.9), Sauerkraut (3.5)Peaches (3.7), Orange juice (3.7)<br>Apricots (3.8) |
| 4.0 | Cabbage, red (4.2), Pears (4.2)<br>Tomatoes (4.3) |
| 4.6 | Ravioli (4.6)<br>Pimientos (4.7) |
| 5.0 | Spaghetti in tomato sauce (4.9)<br>Figs (5.0)Onions (5.2)<br>Carroes (5.2)<br>Green Beans (5.3), Beans with pork (5.3)Asparagus (5.5), Potatoes (5.5) |
| 6.0 | Lima beans (5.9), Tuna (5.9), Tamales (5.9)<br>Codfish (6.0), Sardines (6.0), Beef (6.0)<br>Pork (6.1), Evaporated milk (6.1)<br>Frankfurters (6.2), Chicken (6.2)<br>Corn (6.3)<br>Salmon (6.4) |
| 7.0 | Crabmeat (6.8), Milk (6.8)<br>Ripe olives (6.9)<br>Hominy (7.0) |

The lowest pH limits for growth of foodborne disease organisms are shown in Table 3. Many of the investigators who reported these values also determined that adverse factors, such as low temperature or low water activity, increased the minimal pH for growth. But the processor can be sure that these minimal values will prevent growth of these pathogens under any and all circumstances.

Table 3. The minimal pH minimal for growth of principal foodborne disease organisms*

| Microorganism | Growth reported at but not below | Reference |
|---|---|---|
| Staphylococcus aureus | pH 4.5 | |
| Salmonella | 4.0 | Chung and Goepfer, 1970 |
| Clostridium botulinum | | |
| Types A and B | 4.8 | National Canners Assn., 1971a |
| Type E | 5.0 | National Canners Assn., 1971a |
| Clostridium perfringens | 5.0 | |
| Vibrio parahaemolyticus | 4.8 | Beuchat, 1973 |
| Bacillus cereus | 4.9 | Kim and Goepfer, 1971 |

*Note: These limits are the lowest recorded, with all other growth conditions optimal. If other conditions are less than optimal, the pH limit will be higher.

## Population

A high initial bacterial load increases the likelihood that spoilage will occur under marginal circumstances (Chung and Goepfert, 1970) (see Figures 4 and 5) (see Figures 4 and 5). This fact is of major importance to the processor of refrigerated foods, the shelf-life of which is enhanced by good sanitation. A high level of spores also increases the possibility that a few will survive to spoil heat processed products.

## Oxygen

Oxygen is essential for growth of some microorganisms; these are called aerobes. Others cannot grow in its presence and are called anaerobes. Still others can grow either with or without oxygen and are called microaerophilic. Strict aerobes grow only on food surfaces and cannot grow in foods stored in cans or in other evacuated, hermetically sealed containers. Anaerobes grow only beneath the surface of foods or inside containers. Aerobic growth is faster than anaerobic. Therefore, in products where both conditions exist, such as in fresh meat, the surface growth is promptly evident, whereas subsurface growth is not.

# Chapter 7 The “Indicator” Organisms

The “indicator” organisms are so called because their presence in large numbers in food signifies one of three contamination possibilities: disease bacteria or filth; spoilage or low quality; or preparation under insanitary conditions.

### Aerobic Plate Count

The aerobic plate count (APC) measures only that fraction of the bacterial flora that is able to grow to visible colonies under the arbitrary test conditions provided in the time period allowed. It does not measure the total bacterial population in a food sample, but is the best estimate. Altering conditions, such as composition of the agar medium or temperature of incubation, changes the spectrum of organisms that will grow. It is necessary to adhere rigidly to the standardized test conditions that have encouraged some to call the APC a “standard plate count.”

Depending on the circumstances, a high APC may indicate that a food has been grossly mishandled or that it contains a poor quality ingredient. Interpretation depends on knowing what the normal APC is for this food. An abnormal APC indicates that something is out of control. The microbiologist can frequently suggest that cause, thereby aiding the sanitarian. Some of the problems that investigation of a high APC might reveal include:

- Failure of sorting, trimming, washing, and destroying operations to remove or destroy bacteria from raw ingredients adequately.
- Inadequate heat processing.
- Insanitary equipment, particularly near the end of the process.
- The food has reached or is approaching the end of its refrigerated shelf-life.
- The food has been stored at or above room temperature for too long.
- The food is at least partly decomposed.

### Coliform Bacteria

The coliform bacteria in human and animal waste are non-spore-forming rods that exist in vast quantities. They are commonly found in raw animal products, such as beef, milk, and eggs, and are often found naturally in soil, water, and plant surfaces. They are heat sensitive and, during blanching or pasteurizing, die easily. Significant numbers of coliforms indicate an inappropriate degree of post-heating contamination during a heat phase or indicate adequate time-temperature abuse of the food to permit development. In order to identify the cause of infection or temperature mishandling, elevated coliform quantities require investigations.

The appearance in the diet of Escherichia colia, a member of the coliform community, typically suggests overt or indirect fecal infection of humans or livestock. While this may be valid in a general context, a quantitative relationship between the E numbers must not be believed. Coli and the degree of stool infection. E. Coli grows well outside the body of the animal and thrives in Uncle

### *Food Poisoning*

Human infections induced by foodborne microorganisms are commonly referred to as food poisoning. The widespread use of a single grouping is largely due to the resemblance of symptoms of multiple food-related diseases (see Table 5). Foodborne disease may be classified into two main groups, food infection and food overdose, aside from illness related to food allergy or food reaction. When foods infected by pathogenic, invasive, food poisoning bacteria are ingested, food contamination occurs. In the human body, these bacteria then proliferate and ultimately cause disease. The intake of preformed toxic compounds that develop during the development of certain bacterial forms in foods is accompanied by food intoxication.

The incubation period is called the period of time between the ingestion of infected foods and the occurrence of illness. Depending on the causative species or the harmful substance, the incubation time can be anything from less than one hour to more than three days.

Table 5. Characteristics of the important bacterial food intoxications and foodborne infections. (NAS-NRC, 1975)*

| Disease | Etiologic Agent | Incubation Period | Symptons |
|---|---|---|---|
| Botulism | *Clostridium botulinum*<br>A.B.E.F toxin | Usually 1 to 2 days; range 12 | Difficulty in swalling, double vision, difficulty in speech. |

| | | | |
|---|---|---|---|
| | | hours to more than 1 week | Occasionally nausea, vomiting, and diarrhea in early stages. Constipation and subnormal temperature. Respiration becomes difficult, often followed by death from paralysis of muscles of respiration. |
| Staphylococcal food poisoning | Staphyloccal enterotoxin | 1 to 6 hours; average 3 hours | Nausea, vomiting, abdominal cramps, diarrhea, and acute prostration. Temperature subnormal during acute attack, may be elevated later. Rapid recovery-usually within 1 day. |
| Salmonellosis | Specific infection by *Salmonella* spp. | Average about 18 hours; range 7 to 72 hours | Abdominal pains, diarrhea, chills, fever, frequent vomiting, prostration. Duration of illness: 1 day to 1 week. |
| Shigellosis (bacillary dysentery) | Shigella sonnei, s. flexneri, s. dysenteriae, s. boydii | Usually 24 to 48 hours; range 7 to 48 hours | Abdominal cramps, fever, chills, diarrhea, watery stool (frequently containing blood, mucus, or pus), spasm, headache, nausea, dehydration, prostration. Duration: a few days. |
| Enteropathogenic *Escherichia coli* infection | *Escherichia coli* serotypes associated with infant and adult infections | Usually 10 to 12 hours; range 5 to 48 hours | Headache, malaise, fever, chills, diarrhea, vomiting, abdominal pain. Duration: a few days. |
| *Clostridium perfringens* food poisoning | *Clostridium perfringens* | Usually 10 to 12 hours; range 8 to 22 hours | Abdominal cramps and diarrhea, nausea, and malaise, vomiting very rare. Meat and poultry products usually involved. Rapid Recovery. |
| *Bacillus cereus* food poisoning | *Bacillus cereus* | Usually about 12 hours; range about 8 to 16 hours | Similar to *Clostridium perfringens* poisoning |

| | | | |
|---|---|---|---|
| *Vibrio Parahaemolyticus* food poisoning | *Vibrio Parahaemolyticus* | Usually 12 to 14 hours; range 2 to 48 hours | Abdominal pain, server watery diarrhea, usually nausea and vomiting, mild fever, chills and headache. Duration: 2 to 5 days. |

# Chapter 8 Microbial Nutrition and Growth

## Growth Requirements

The term growth is used by microbiologists to denote a rise in a microbe population rather than an increase in size. Microbial development is based on nutrient metabolism and results in the creation of a separate colony, an assembly of cells from a single parent cell. Any chemical needed for microbial communities to expand is a nutrient. The most significant of these are carbon, oxygen, nilTngen, and/or hydrogen containing compounds.

## Nutrients: Chemical and Energy Requirements

All cells require three things to conduct metabolism: a carbon source, a source of energy, and a source of electrons or hydrogen atoms.

## Sources of Carbon, Energy, and Electrons

Organisms may be classified into one of four categories depending on their carbon source and their use as an energy source of either chemicals or light:

In order to make their own food, photoautotrophs use carbon dioxide as a carbon source and light energy from the atmosphere.

Chcmoautotrophs use carbon dioxide but catabolize organic molecules for energy as a source of carbon.

Photosynthetic species that obtain energy from light and acquire nutrients by organic compound catabolism are photoheterotrophs.

- For both energy and biomass, chemoheterotrophs use organic compounds.

In addition, **organotrophs** acquire electrons from organic sources, whereas **Iithotrophs**acquire electrons from inorganic sources.

## Oxygen Requirements

As the final electron acceptor of the electron transport chain, mandatory aerobes use oxygen, while mandatory anaerobes are unable to tolerate oxygen and use an electron acceptor other than oxygen. Toxic sources of oxygen induce a sequence of vigorous oxidation and are strongly reactive. Four oxygen forms are toxic:

Singlet oxygen (l02) is molecular oxygen with electrons that have been accelerated, usually during aerobic metabolism, to a higher energy state. Phototropic microorganisms also possess pigments called carotenoids, which, through eliminating the surplus energy of singlet oxygen, avoid toxicity.

- Supcroxide radicals (0:;.-) are formed by anaerobes in the presence of oxygen during the incomplete decrease in oxygen during electron transfer in aerobes and during metabolism. Superoxide dismutase detoxifies them.
- Peroxide anion (022-) is a hydrogen peroxide part formed during superoxide dismutase-catalyzed reactions. Peroxide anion is deroxified by the enzymes catalase and peroxidase.

The consequence of ionizing radiation and the incomplete removal of hydrogen peroxide is hydroxyl radicals (OH '). Hydroxyl radicals are the most reactive of the four toxic sources of oxygen, but the threat of hydroxyl radicals is practically reduced in aerobic cells because hydrogen peroxide does not accumulate in aerobic cells.

Neither strict aerobes nor anaerobes are not all organisms. Facultative anaerobes can sustain life by fermentation or anaerobic respiration, but, in the absence of oxygen, their metabolic efficiency is always decreased. Aerotolerant anaerobes prefer anaerobic environments, but since they have a sort of enzymes that detoxify the toxic sources of oxygen, they can withstand oxygen.

Low levels of oxygen are provided by microaerophiles. In addition to low levels of oxygen, capnophiles thrive better with elevated levels of carbon dioxide.

### Requirements for Nitrogen

Nitrogen for many microorganisms, which derive it from organic and inorganic nutrients, is a growth-limiting nutrient. While nitrogen comprises about 79 percent of the atmosphere, nitrogen gas can be used by comparatively few species. Via a mechanism called nitrogen fixation, which is important for life on Earth, a few bacteria convert nitrogen gas to ammonia.

### Other Chemical Requirements

In addition to the main elements found in microbes, very small amounts of **trace elements** such as selenium, zinc, etc., are required. Most microorganisms also require small amounts of certain organic chemicals that they cannot synthesize. These are called **growth factors**. For example, vitamins are growth factors for some microorganisms.

### Physical Requirements

In addition to chemical nutrients, organisms have physical requirements for growth, including specific conditions of temperature, pH, osmolarity, and pressure.

### Temperature

Since both proteins and lipids are temperature-sensitive, different temperatures have different effects on the survival and growth rates of microbes. Though microbes survive within the limits imposed by a minimum growth temperature and a maximum growth temperature, an organism's metabolic activities produce the highest growth rate at the **optimum growth temperature**.

Microbes are described in terms of their temperature requirements as (from coldest to warmest):

- **Psychrophiles** require temperatures below 20°C.
- **Mesophiles** grow best at temperatures ranging between about 20°C and 40°C.
- **Thermophiles** require temperatures above 45°C.
- **Hyperthermophiles** require temperatures above 80°C.

## pH

Organisms are sensitive to changes in acidity because hydrogen ions and hydroxyl ions interfere with hydrogen bonding within the molecules of proteins and nucleic acids; 54 Study Guide for Microbiology as a result, organisms have ranges of acidity that they prefer and can tolerate. Most bacteria and protozoa are called neutrophiles because they grow best in a narrow range around a neutral pH, between 6.5 and 7.5. By contrast, other bacteria and many fungi are acidophiles, and grow best in acidic environments where pH can range as low as 0.0. In contrast, alkalinophiles live in alkaline soils and water up to pH 11.5.

## Physical Effects of Water

In certain metabolic reactions, microorganisms require water to degrade enzymes and nutrients and to serve as a reactant. Cells are limited to some environments by osmotic pressure. Although some microbes' cell walls shield them from osmotic shock, osmosis can cause other cells to die from either swelling or bursting or shriveling (crenation). Obligatory halophiles, such as those present in salt water, need high osmotic pressure. Facultative halophiles do not need salty conditions, but can accommodate them.

In proportion to its depth, water exerts pressure and the pressure in deep ocean basins and trenches is immense. Organisms existing under intense pressure are referred to as barophiles. In order to preserve their three-dimensional functional forms, their membranes and enzymes rely on pressure, and they can usually not live at sea level.

## Ecological Associations

Relationships are called antagonistic in which one organism damages or even destroys another. Members of an association interact in synergistic relationships in such a manner that each gains advantages that outweigh those that would arise if each resided independently. Organisms exist in near dietary or physical association within symbiotic partnerships, being interdependent.

Biofilms are an example of dynamic interactions between multiple entities, often different species, that bind to surfaces together and exhibit metabolic and structural characteristics distinct from those displayed alone by each of the microorganisms. They also develop as a result of quorum sensing, a mechanism in which bacteria use signal and receptor molecules to respond to the density of surrounding bacteria.

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