II YEAR – III SEMESTER
COURSE CODE: 7BZOA3

ALLIED COURSE - III – FOOD MICROBIOLOGY

Unit - I

Introduction and History of Microbiology – The theory of spontaneous generation, gene theory of disease, Louis pasteur's experiment. Different terminology of Nutrition Heterotrophic nutrition, autotrophic nutrition, saprophytic, holozoic, host, culture, parasite.

Bacteria – Morphology, reproduction, growth curve, nomenclature, genera of bacteria importance in food microbiology. Observation of motility of bacteria in bottle milk.

Mold – Morphology, reproduction, physiology and nutrition. Demonstration of mold growth in bread.

Unit - II

Yeast – classification Morphology, physiology, nutrition and reproduction process of hybridization, importance of yeast in food. Observation of yeast cells

Virus – Occurrence, morphology, reproduction, human viral disease caused by virus.

Algae – Occurrence, morphology, reproduction, importance of algae.

General principles underlying spoilage – fitness and unfitness of food for consumption, causes for spoilage, factors affecting the growth of micro organism in food.

Unit - III

Contamination and kinds of micro organisms causing spoilage of cereal products – grains, flour, baked products and cake. Contamination and kinds of micro organisms causing spoilage of fruits and vegetables and their products – fruit juice, pickles. Contamination and kinds of micro organisms causing spoilage of fleshy foods–meats, oultry and fish. Observation of milk spoilage.

Unit - IV

Contamination and kinds of micro organisms causing spoilage of eggs, milk and milk products – cream and butter.

Contamination and kinds of micro organisms and spoilage of fats and oils, bottled beverages, spices and condiments.

Food poisoning, food infection and food borne diseases.

Micro organism in air, air borne diseases.

Unit - V

Micro-organisms in Water – sources, bacteriological examinations, total count, test of *E.coli*, purification of water, water borne diseases.

Micro organisms in sewage and sewage disposal.

Destruction of bacteria – sterilization, physical agents, light, desiccators, electricity, heat and chemical agents.

Visit to microbiology lab to learn most probable number.

Books for Reference:

1. Frazier WC, Food Microbiology Mc Green Hill Book, 1985
2. Sullia SB and S Shantharam – "General Microbiology" Oxford and IBH Publishing Ltd., 1998.
3. Michael J.Pelczar, E.C.S.Cahn & Noel R.Kruef – Microbiology, Tata McGraw-Hill Edition – 1993

4. Nicklin J. Graeme – Cook K, Page& Killington R – “Notes in Microbiology” Bros Scientific Publishers – Preprinted 2001, 2002.
5. Eugene Rosenlarg & Irun R. Cohea – Microbial Biology – Holt-Saunders International Editions 1983
6. James M.Jay ‘Modern Food Microbiology International Thomson Publishing – Fifth Edn. – 1996

♣♣♣♣♣♣♣♣♣♣

UNIT I

INTRODUCTION

The word **MICROBIOLOGY** describes exactly what the discipline is: the study of small living things. MICRO = small, BIO = living, and LOGY = to study. **Microbiology** (or specifically, bacteriology) is still a very young science and not yet completely understood.

Micro-organisms are living entities of microscopic size and include bacteria, viruses, yeasts and molds (together designated as fungi), algae, and protozoa. While bacteria are classified as prokaryotes (cells without definite nuclei), the fungi, algae, and protozoa are eukaryotes (cells with nuclei); viruses do not have regular cell structures and are classified separately. Micro-organisms are present everywhere on earth, which includes humans, animals, plants and other living creatures, soil, water, and atmosphere, and they can multiply everywhere except in the atmosphere. Together, their numbers far exceed all other living cells on this planet. They were the first living cells to inhabit the earth over 3 billion years ago; and since then they have played important roles, many of which are beneficial to the other living systems.

THE SCIENCE OF MICROBIOLOGY Microbiology is the branch of the biological sciences that deals with microorganisms, i.e. bacteria, fungi, some algae, protozoa, viruses, viroids and prions.

Most micro-organisms have the following characteristics:-

1) They are generally too small to be seen with the unaided human eye, and some form of microscopy is required for the study of their structure.

2) Cells or other structures are relatively simple and less specialized than those of higher plants and animals.

3) They are handled and cultured in the laboratory in ways that are generally quite similar. Microbiology has developed into a science that can be studied from a number of perspectives.

A specialist study can be made of each of the individual groups giving rise to the following disciplines: Bacteriology - the study of bacteria;

- Mycology - the study of fungi;
- Protozoology - the study of protozoa;
- Phycology (algology) - the study of algae;

- Virology - the study of viruses.

• Micro-organisms can also be studied from the applied viewpoint, i.e. the relationship between micro-organisms, the environment and human activity. This again gives rise to a number of areas of specialist study: Medical microbiology includes some aspects of pathology (the study of• diseases), immunology (how the immune system operates to prevent invasion by micro-organisms) and epidemiology (how diseases are distributed and spread). Agricultural microbiology: The study of micro-organisms for crop/• plant health and related areas. Industrial microbiology / biotechnology: The study of the use of Microorganisms in large scale industrial processes.

Who is known as father of microbiology

Antonie van Leeuwenhoek

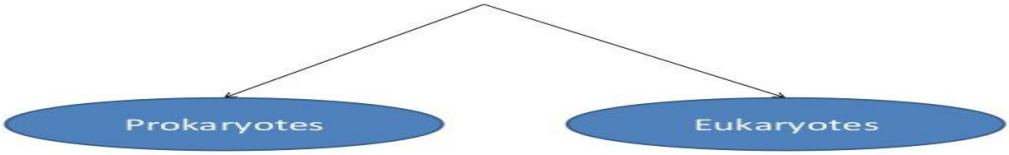
Antonie van Leeuwenhoek is considered a father of microbiology as he observed and experimented with microscopic organisms in the 1670s, using simple microscopes of his own design. Scientific microbiology developed in the 19th century through the work of **Louis Pasteur** and in medical microbiology Robert Koch.

Who is the mother of microbiology

Fanny Hesse

Fanny Hesse, acknowledged as the **mother of microbiology**, whose birthday would have been today, is best known for her work developing agar for cell culture.Jun 21, 2016

Classification of microorganisms

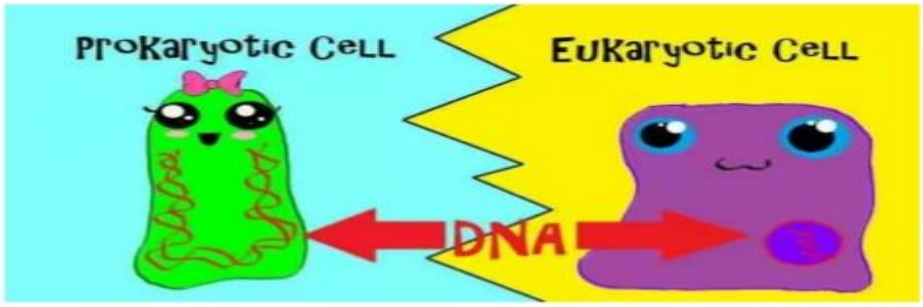


Prokaryotic cells

- Genetic material is not enclosed by the nuclear membrane.
- Absence of nuclear membrane

Eukaryotic cells

- Genetic material is enclosed by the nuclear membrane.
- Presence of nuclear membrane.



Scope and Importance of microbiology

1. Production of antibiotic Eg: penicillin from penicillium.
2. Production of enzymes , vaccines, biosurfactants,alcoholic and other pharmaceutical product.
3. Diagnosis of disease and treatment Eg: ELISA, Widal test.
4. Treatment of industrial waste and material
5. Plant growth promotion
6. Sterile product preparation
7. Sterilization (process of killing microorganisms). Eg: moist heat sterilization, dry heat sterilization, and membrane filtration.
8. Steroid biotransformation. Eg: progesterone, testosterone.
9. Identification of microorganisms. Eg: morphological, cultural or microscopic study.
10. Testing of Pharmaceuticals products and raw materials.

History of microbiology

- **Anton van Leeuwenhoek (1632–1723):** was the first microbiologist and the first person to observe bacteria using a single-lens microscope of his own design.

- **Louis Pasteur (1822–1895):** Pasteur developed a process (today known as pasteurization) to kill microbes. pasteurization is accomplished by heating liquids to 63 to 65 C for 30 minutes or to 73 to 75 C for 15 seconds.

- **Robert Koch (1843–1910):** was a pioneer in medical microbiology and worked in cholera, anthrax and tuberculosis. He was awarded a Nobel prize in 1905 (Koch's postulates) he set out criteria to test.

- **Alexander Fleming (1929):** Discovered penicillin.

History of Microbiology

The Germ Theory of Disease

1835: Agostino Bassi showed a silkworm disease was caused by a fungus.

1865: Pasteur believed that another silkworm disease was caused by a protozoan.

1840s: Ignaz Semmelweis advocated handwashing to prevent transmission of puerperal fever from one OB patient to another.

AUTOTROPHIC NUTRITION

Autotrophic nutrition is a process where an organism prepares its own food from a simple inorganic material like water, mineral salts and carbon dioxide in the presence of sunlight."

The term "autotrophic" is formed by the combination of two terms, "**auto"** meaning **self,** and **"trophic"** meaning **nutrition**. The literal meaning of this term is self-nutrition.

The autotrophic organisms contain a green coloured pigment called chlorophyll which helps in trapping energy from the sun. All green plants possess an autotrophic mode of nutrition. They prepare their own food by utilizing solar energy, water, and carbon dioxide by the process of photosynthesis. This results in the formation of glucose.

Plants like blue-green algae and bacteria such as cyanobacteria are considered to be examples of autotrophs.

Heterotrophic Nutrition

"Heterotroph is an organism that is unable to synthesize its own food, and therefore, has to rely on other sources, specifically plant and animal matter."

All animals and non-photosynthetic plants are classified as heterotrophs since they are unable to prepare food. So these organisms resort to other various forms of nutrition. Hence, from an ecological perspective, heterotrophs are always secondary or tertiary consumers in a food chain.

Humans and other vertebrates rely on converting organic, solid or liquid food into energy. Other organisms such as fungi rely on converting dead organic matter into nutrients. In essence, heterotrophs break down complex food into its readily usable constituents.

Types of Heterotrophic Nutrition

In nature, organisms exhibit various types of heterotrophic nutrition. They are as follows:

- Holozoic Nutrition
- Saprophytic Nutrition
- Parasitic Nutrition

Holozoic Nutrition

Holozoic nutrition involves the ingestion and internal processing of solid and liquid food in an organism. This involves the steps of ingestion, digestion, absorption, assimilation and excretion.

Ingestion is the intake of food, which is broken down into simpler organic matters by a process called digestion. After extraction of useful components, the unwanted and undigested particles are excreted out.

Examples of animals that exhibit holozoic nutrition include all vertebrates. Even some unicellular organisms such as amoeba also exhibit holozoic nutrition

Holozoic Nutrition in Amoeba

Amoeba exhibits holozoic nutrition. The process takes place in the following steps:

- The amoeba projects its pseudopodia and encircles the food. It then engulfs the food by the process of phagocytosis.
- The food vacuoles of amoeba are rich in digestive enzymes, which help break the food into simpler substances. This process is known as digestion.
- The digested food is absorbed into the cytoplasm leaving behind the undigested materials. This absorbed food is utilised to produce energy for the growth and development of the cell.
- The undigested food material is ejected out by the rupturing of the cell membrane.

Types of Holozoic Organisms

Holozoic organisms can be divided into three types:

- **Herbivores-** These organisms depend upon plants for their food. Cows, buffaloes, deer, elephants are herbivores.
- **Carnivores-** These animals feed on other animals for their food. Lions, tigers and leopards are carnivores.
- **Omnivores-** These animals can survive on either plants or animals for their food. Cockroach, pig, chimpanzees, raccoons and ant are some examples of omnivores.

Saprophytic Nutrition

Saprophytes (animals which follow saprophytic nutrition) feed on dead and decayed organisms for energy. They are an important part of the ecosystem as they help to keep **our environment** clean and recycle nutrient back into the ecosystem.

Some examples of saprophytes are fungi and certain types of bacteria. These are also responsible for the staling of bread and other similar food products.

Saprophytes release certain enzymes to act on the complex organic matter. It works by breaking it down into its constituents, which can be easily consumed by them.

Parasitic Nutrition

Organisms that live in or on other organisms and acquire food at the expense of its host are called parasites. Most parasites are harmful to the hosts' health; sometimes, they even kill the host. Both animals and plants may serve as a host. Unlike commensalism, the parasite causes some harm to its host. A few examples of parasites are louse on a human head, Cuscuta plant and tapeworms.

***Cymothoa exigua* is an unusual parasite. It is also known as the tongue-eating louse and is aptly named so as it is found in the mouth of the marine fish Lithognathus. It essentially severs the fish's tongue, cutting off the blood supply and causing the tongue to fall off. The**

louse then attaches itself to the remains of the tongue and essentially acts as the fish's new tongue.

Difference Between Parasitic And Saprotrophic Nutrition?

Parasite and saprotrophic nutrition belongs to the heterotrophic mode of nutrition.

- A mode of nutrition in which organisms obtain nutrition from dead and decaying matters is called the Saprotrophic Nutrition.
- Parasitic Nutrition refers to heterotrophic nutrition, in which parasites depend on other living organisms for their food.

The differences between Parasitic and Saprotrophic Nutrition are tabulated below:

Difference Between Parasitic and Saprotrophic Nutrition

Parasitic Nutrition	**Saprotrophic Nutrition**
It has an intracellular digestion.	It shows extracellular digestion.
Feed on the living organism by causing harm to the living organism.	Feeds on dead organic matter.
Example:-Bacteria, Plasmodium, etc.	Example: -Fungi, etc.

MOLD

What Is Mold?

Mold is a fungus. A fungus is an organism that lives by decomposing and absorbing the organic matter on which it grows. Molds, mushrooms, yeasts, smuts, rusts, and mildew are all examples of fungi. There are thousands of varieties of molds. The term mildew is sometimes used interchangeably with mold. Mildew is often used to describe a specific mold fungus that grows on plants and is characterized by a downy, whitish or silvery appearance. Mildew is also sometimes used to describe mold growing on textiles, leather, or building exteriors.

Mold is natural and it is everywhere. Tiny particles of molds exist in both the indoor and outdoor environment. Molds are saprotropic, meaning that they gather their food from dead, moist organic matter. Molds play a major role in the ecosystem as they digest organic matter, such as dead leaves, and prevent accumulation of nature's debris. It would be impossible to eliminate molds from our environment. Molds grow or spread by extending hyphae, which are tiny root hairs or filament chains of cells. These hyphae extend and intertwine to form a mass, which is called the mycelium. The hyphea can grow through or into a material as well as on the surface, and often much of the mold growth is not visible on the surface of a material. Molds reproduce by spores. The spores, which are microscopic cells, are released into the air. Acting much like seeds, the spores spread the mold colonies. Mold spores can remain dormant for long periods of time, until the right growing conditions are available. Fragments of broken hyphae can also be transplanted to start growing new mold colonies. Some molds produce mycotoxins. A mycotoxin is a toxic substance or poison produced by a fungus. Molds that produce mycotoxins may only produce them under certain conditions, and some experts think the mycotoxins may be a defense mechanism. The mycotoxins are found in the spores.

DEMONSTRATION OF MOLD GROWTH IN BREAD

Have you seen mold growth on bread? It is outright gross, right?
You must be wondering why we are talking about germs and mold today.
Well, here are some cool science experiments you can try out by using bread.

Mold is a microscopic, living organism in the Fungi kingdom, related to yeast and mushrooms. Although it can be harmful if handled incorrectly, mold is not a bacteria or virus. In the first stage of its life cycle, mold lays dormant as a spore, a reproductive structure that is similar to the seeds of plants. When these spores find a warm, moist, nutrient-rich environment they set up a colony, mature and produce more spores. That's the growth that you see on your food trillions of mold spores.
These spores are a constant part of our environment, but they rarely cause health problems. People with sensitive respiratory or immune systems may have an allergic reaction to these background levels of spores.

Guidelines:

• Before you engage in any of these experiments seek the permission of an adult in charge.

• Inform everyone in the house to not go near the experiments or consume them.

• Keep it away from any house pets.

• Keep it away from other consumable items.

• After every experiment, you will need to throw the slice of bread and the plastic bags and containers used, even if you do not see any mold. Remember mold is not always visible to the eyes.

1. Things You'll Need for the experiment:

Bread, water, plastic zipper bag, tape, marker, book, pen and a phone with a camera.

Experiment:

• Sprinkle water on a slice of bread.

• Put the bread in the plastic bag and seal it with tape.

• Label the bag with the start date.

• Place this in your balcony or some place outside of the house where it will be undisturbed for 7 days.

• Track the growth of your mold by checking on it daily.

• Take a photograph on each day and make a note about the size and color of the mold growth.

• Once you've completed the experiment, throw away the sealed bag containing the moldy bread. You do not want to be around when the bag opens. Inhaling mold spores is harmful.

Make a note of your observations over the 7-day period. What changes did you observe each day? What is the end result? What is your conclusion?

Conclusion Hint: Mold loves to grow in warm, moist places and will eat just about anything organic.

2. Things You'll Need for the experiment:

3 slices of bread, water, 3 plastic zipper bags, disposable tray, tape, marker, book, pen and a phone with a camera.

Experiment:

• Label the plastic bags 'Dry,' 'Moist' and 'Wet' to write the start date on the bags.

• Place a slice of bread into the plastic bag marked 'Dry' without wetting it.

• On the second slice of bread, sprinkle it with water and put the bread in the plastic bag labelled 'Moist'.

• Submerge the third slice of bread in a bowl of water and place the slice into the bag labelled 'Wet'.

• Close and seal all three plastic bags with tape.

• Place the bags on a disposable tray.

• For 10 days you will need to store these three bags outside the house.

• Take pictures daily and make notes about the size and color of the mold on each slice of bread.

• Throw it away once the experiment is complete.

Conclusion Hint: So what did you find? does the moisture affect mold growth? Among the slices you experimented with, which ones grew mold fastest and which least?

3. Things You'll Need for the experiment:

3 slices of bread, water, 3 plastic zipper bags, glass jar, tape, marker, book, pen and a phone with a camera.

Experiment:

• Label the bags 'Cold' and 'Room temperature'. Use a double bag for the bag labelled 'Cold'.

• Label the glass jar 'Refrigerated'.

• Don't forget to write the start date on the bags and jar.

• Sprinkle an equal amount of water on each slice of bread.

• Place a slice of bread inside each plastic bag and one in the glass jar.

• Close the bags and tape shut. Seal the jar and tape around the lid to prevent leakage.

• Place the bag labelled 'Cold' in the freezer, the bag labelled 'Room Temperature' in a safe place outside the house and the jar in the refrigerator.

• Take photographs each day over the course of 10 days. Make notes about the size and color of the mold growth.

MORPHOLOGY OF BACTERIA

CONTENTS

INTRODUCTION

- Bacteria is unicellular, free-living, microscopic microorganisms capable of performing all the essential functions of life.
- They possess both deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA).
- Bacteria are prokaryotic microorganisms that do not contain chlorophyll.
- They occur in water, soil, air, food, and all natural environment.
- They can survive extremes of temperature, pH, oxygen, and atmospheric pressure.

SIZE OF BACTERIA

- Bacteria are very small microorganisms which are visible under the microscope.
- They are having the size range in microns.
- Bacteria are stained by staining reagents and then visualised under high power of magnification (1000X) of compound microscope.
- An electron microscope is used for clear visualization of internal structure of bacteria.

SIZE OF BACTERIA

- Bacteria are very small microorganisms which are visible under the microscope.
- They are having the size range in microns.
- Bacteria are stained by staining reagents and then visualised under high power of magnification (1000X) of compound microscope.
- An electron microscope is used for clear visualization of internal structure of bacteria.

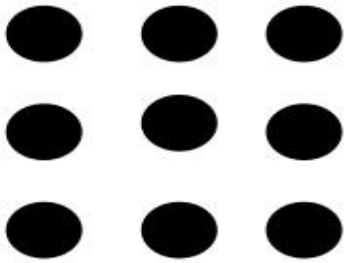
SHAPE OF BACTERIA

On the basis of shape bacteria are classified as

1. Cocci
2. Bacilli
3. Vibrios
4. Spirilla
5. Spirochetes
6. Actinomycetes
7. Mycoplasma

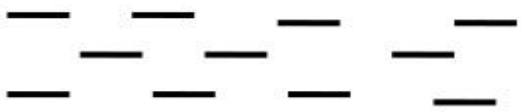
1. Cocci

- Cocci are small, spherical or oval cells. In greek 'Kokkos' means berry. Eg: micrococcus



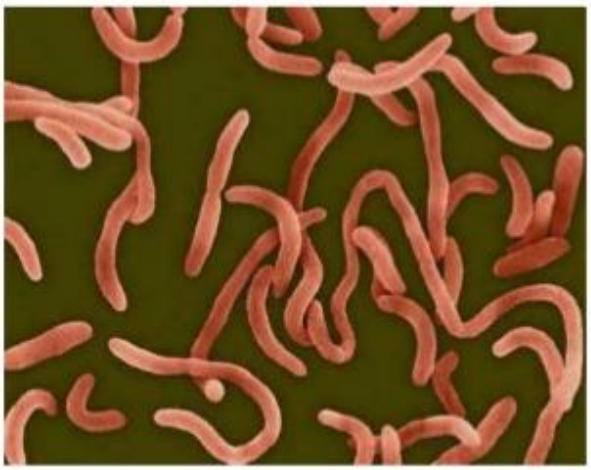
2. Bacilli

- They are rod shaped cells. Eg: Bacillus anthracis.
- It is derived from greek word " Bacillus" meaning stick.
- In some of the bacilli the length of cell may be equal to width. Such bacillary forms are known as coccobacilli. Eg: Bracella.



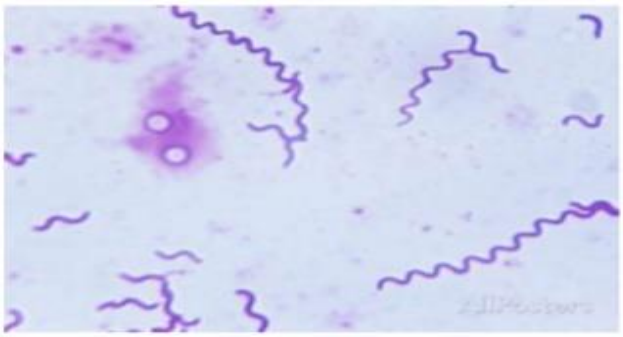
3. Vibrios

- They are comma shaped curved rods. Eg: Vibrio comma.



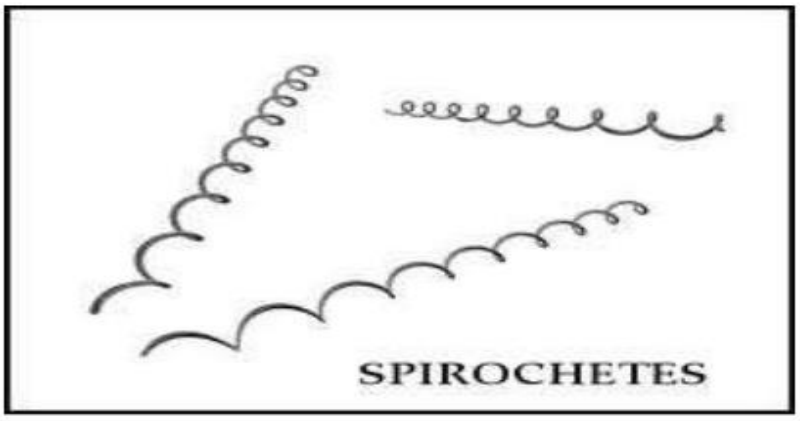
4. Spirilla

- They are longer rigid rods with several curves or coils.
- They have a helical shape and rigid body.
- Eg: Spirillum ruprem.



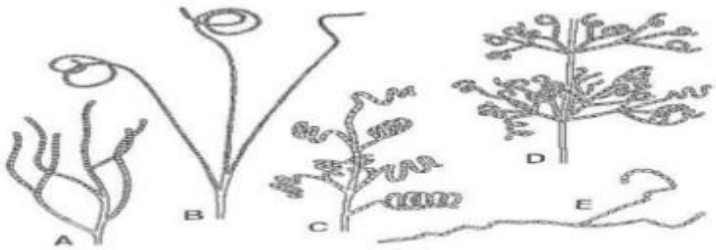
5. Spirochetes

- They are slender and flexuous spiral forms.



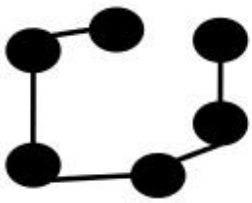
6. Actinomycetes

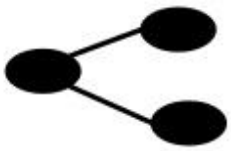
- The characteristic shape is due to the presence of rigid cell wall. Eg: Streptomyces.
- They are branching filamentous bacteria.
- Eg: Streptomyces species.



7. Mycoplasma

- They are cell wall deficient bacteria and hence do not possess stable morphology. They occur as round or oval bodies with interlacing filaments.





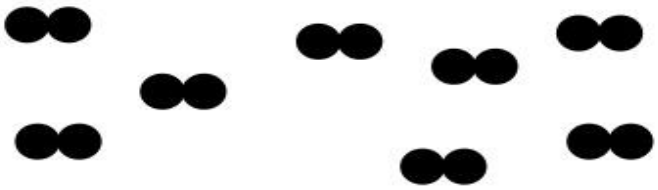
ARRANGEMENT OF BACTERIAL CELLS

Cocci appears as several characteristics arrangement or grouping.

1. Diplococci
2. Streptococci
3. Tetracocci
4. Staphylococci
5. Sarcinae

1. Diplococci

- They split in one plane and remains in pair. Eg: diplococcus pneumoniae.

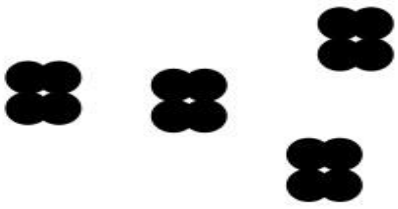


2. Streptococci

These cells divide in one planes and remain attached , to form chains. Eg: streptococcus lactis.

3. Tetracocci

- They divide in two planes and live in groups of four. Eg: Gaffyka tetragena.

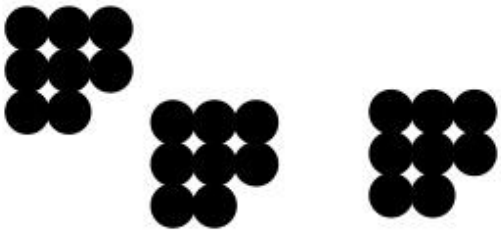


4. Staphylococci

- Cocci cells divide in three planes in an irregular pattern. These cells produce bunches of cocci as in grapes. Eg: staphylococcus aureus, staphylococcus albus.

5. Sarcinae

- Sarcinae cells divide in three planes in a regular pattern.
- These cells produces a cuboidal arrangement of group of a eight cells.
- Eg: Micrococcus tetragena.



Arrangement of grouping formed by bacilli species

1. Diplobacilli
2. Streptobacilli
3. Trichomes

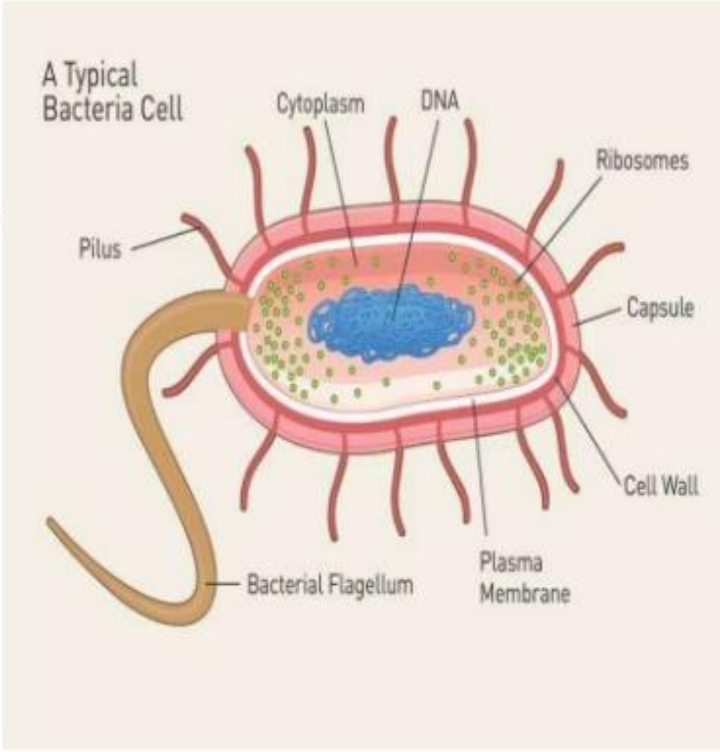
Diplobacilli

Trichomes

streptobacilli

Bacterial Structures

- Flagella
- Pili
- Capsule
- Plasma Membrane
- Cytoplasm
- Cell Wall
- Ribosomes
- mesosomes
- Inclusions
- Spores

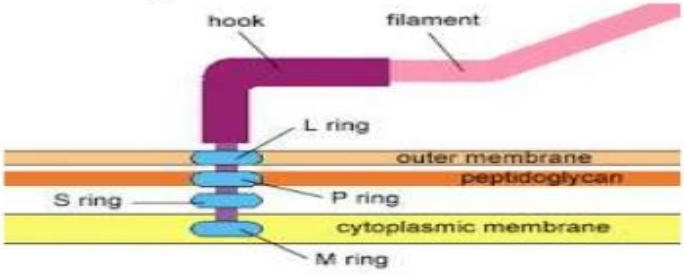


Flagella

- Flagella are long, slender, thin hair-like cytoplasmic appendages, which are responsible for the motility of bacteria.
- These are the organs of locomotion.
- They are 0.01 to 0.02 µm in diameter, 3 to 20 µm in length.
- Flagella are made up of a protein- flagellin.
- The flagellum has three basic parts ,

1. Filament
2. Hook
3. Basal body

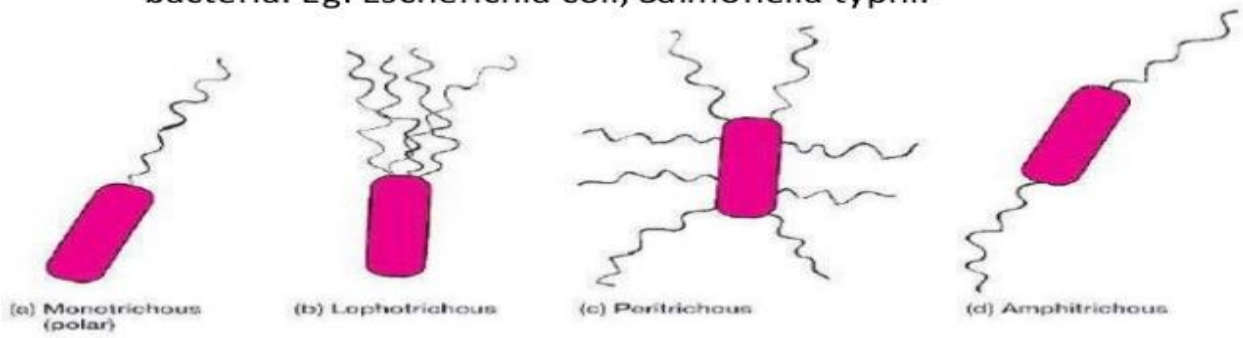
- Filament is the thin, cylindrical, long outermost region with a constant diameter.
- The filament is attached to a slightly wider hook.
- The basal body is composed of a small central rod inserted into a series of rings.



- Gram negative bacteria contain four rings as L-ring, P-ring, S-ring, M-ring whereas gram positive bacteria have only S and M rings in basal body.

Flagella may be seen on bacterial body in following manner.

1. **Monotrichous:** These bacteria have single polar flagellum. Eg: vibrio cholera
2. **Lophotrichous:** These bacteria have two or more flagella only at one end of the cell. Eg: pseudomonas fluorescence.
3. **Amphitrichous:** These bacteria have single polar flagella or tuft of flagella at both poles. Eg :Aquaspirillum serpens.
4. **Peritrichous:** Several flagella present all over the surface of bacteria. Eg: Escherichia coli, Salmonella typhi.



Pili or fimbriae

- Pili are hair-like microfibrils, 0.5 to 2 µm in length and 5 to 7 nm in diameter.
- They are thinner, shorter and more numerous than flagella.
- They are present only on gram negative cells.
- They are composed of protein known as pillin.
- They are unrelated to motility and are found on motile and non-motile cells.
- Fimbriae and pili, these two terms are used interchangeably but they can be distinguished.
- Fimbriae can be evenly distributed over the entire surface of the cell or they occurs at the poles of the bacterial cell. Each bacteria possess 100 to 200 fimbriae.
- Pili are usually longer than fimbriae and number only one or two per cell.

Function:

- Pili play an important role in attachment to surfaces. Hence pili is also called organ of adhesion.

GROWTH CURVE, GROWTH MEASUREMENT

Introduction

Growth is an orderly increase in the quantity of cellular constituents. It depends upon the ability of the cell to form new protoplasm from nutrients available in the environment. In most bacteria, growth involves the following steps.

1. Increase in cell mass and number of ribosomes
2. Duplication of the bacterial chromosome
3. Synthesis of new cell wall and plasma membrane
4. Partitioning of the two chromosomes
5. Septum formation
6. Cell division.

This asexual process of reproduction is called binary fission . For unicellular organisms such as the bacteria, growth can be measured in terms of two different parameters: changes in cell mass and changes in cell numbers .

Growth Curve

Batch experiment

It has been observed that if one of the essential requirements for growth is present in only limited amounts, the limiting factor affects on the rate of growth.

A batch culture system is one containing a limited amount of nutrient, which is inoculated with the microorganism. Cells grow until some component is exhausted or until the environment changes so as to inhibit growth. During batch fermentations the population of microorganisms goes through several distinct growth phases:

1. Lag phase
2. Accelerating growth phase
3. Log (exponential growth) phase
4. Declining growth phase
5. Stationary phase
6. Death or declining death phase

Log death phase

The Bacterial Growth Curve

The salient points of the growth phases are as under

1. Lag Phase

a. **Adaptation (acclimation) period**

The lag phase is the initial phase which represents the period (time) required for bacteria to adapt to their new environment.

b. **Constant number of cells**

During this phase, the individual bacterial cells increase in size, but the number of cells remains unchanged.

c. **Physiologically active**

They are very active physiologically and are synthesizing new enzymes and activating factors.

2. Accelerating Growth Phase

Transition period from the lag phase to the log phase. .Cell is beginning to grow (increase in numbers) noticeably as enzyme systems are gearing up.

3. Log (Exponential Growth) Phase

a. Exponential growth during this phase, the bacterial cells divide regularly at a constant rate.

b. Straight line on semilog scale - The logarithms of the number of cells plotted against time results in a straight line.

c. Maximum Rate of Substrate utilization. A maximum growth rate occurs under optimal conditions, and substrate is removed from the medium at the maximum rate. The growth rate is limited only by the bacteria's ability to process the substrate. Food is in excess (not limiting) so that the rate of growth is only limited by the ability to process the food. Sometimes called "0-order growth" and growth rate is constant and maximum.

4. Declining Growth Phase

a. Transition period from the log phase to the stationary phase.

b. Decreasing growth rate

c. Exhaustion of essential nutrients

d. Accumulation of toxic metabolic products - the growth rate can be limited either by the exhaustion of essential nutrients or by the accumulation of toxic metabolic products. - food becomes limiting factor and therefore growth rate and mass of bacteria are dependent on the amount of food present.

5. Stationary Phase

a. The number of cells remains constant perhaps as a results of complete cessation of division or the balancing of reproduction rate by an equivalent death rate. Growth of new cells is balanced by the death of old cells. No increase in cell mass - population is "stable". net growth rate = 0

6. Death or declining Phase

a. The number of viable cells decreases slowly while the total mass may remain constant due to the fact that the death rate exceeds the production rate of new cells.

b. Depletion of essential nutrients

c. Accumulation of inhibitory products. - Death occurs primarily as a result of depletion of essential nutrients and/or the accumulation of inhibitory products.

7. Log Death Phase

a. Exponential death - "wholesale die-off" - system is dead - even if you add food, you will get no growth.

In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc. or 2^0, 2^1, 2^2, 2^3.....2^n **exponential growth**. In reality, exponential growth is only part of the bacterial life cycle, and not representative of the normal pattern of growth of bacteria in nature. (where n = the number of generations). This is called

When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of time, plotting the data will yield a **typical bacterial growth curve**

When bacteria are grown in a closed system (also called a batch culture), like a test tube, the population of cells almost always exhibits these growth dynamics: cells initially adjust to the new medium (lag phase) until they can start dividing regularly

by the process of binary fission (exponential phase). When their growth becomes limited, the cells stop dividing (stationary phase), until eventually they show loss of viability (death phase). Note the parameters of the x and y axes. Growth is expressed as change in the number viable cells vs time. Generation times are calculated during the exponential phase of growth. Time measurements are in hours for bacteria with short generation times.

3.4.1 Four phases of the growth cycle

3.4.1.1 Lag phase

Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity.

The length of the lag phase is apparently dependent on a wide variety of factors including the size of the inoculum; time necessary to recover from physical damage or shock in the transfer; time required for synthesis of essential coenzymes or division factors; and time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium.

3.4.1.2 Exponential (log) phase

The exponential phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. The rate of exponential growth of a bacterial culture is expressed as **generation time**, also the **doubling time** of the bacterial population. Generation time (G) is defined as the time (t) per generation (n = number of generations). Hence, G=t/n is the equation from which calculations of generation time derive.

3.4.1.3 Stationary phase

Exponential growth cannot be continued forever in a **batch culture** (e.g. a closed system such as a test tube or flask). Population growth is limited by one of the three factors *viz.*, 1. exhaustion of available nutrients; 2. accumulation of inhibitory metabolites or end products; 3. exhaustion of space, in this case called a lack of "biological space".

During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase,

like the lag phase, is not necessarily a period of quiescence. Bacteria that produce **secondary metabolites**, such as antibiotics, do so during the stationary phase of the growth cycle (Secondary metabolites are defined as metabolites produced after the active stage of growth). It is during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process.

3.4.1.4 Death phase

If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines. However, if counting is done by turbidimetric measurements or microscopic counts, the death phase cannot be observed. During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

3.5 Growth Rate and Generation Time

As mentioned above, bacterial growth rates during the phase of exponential growth, under standard nutritional conditions (culture medium, temperature, pH, etc.), define the bacterium's generation time. Generation times for bacteria vary from about 12 minutes to 24 hours or more. The generation time for *E. coli* in the laboratory is 15-20 minutes, but in the intestinal tract, the coliform's generation time is estimated to be 12-24 hours. For most known bacteria that can be cultured, generation times range from about 15 minutes to 1 hour. Symbionts such as *Rhizobium* tend to have longer generation times. Many lithotrophs, such as the nitrifying bacteria, also have long generation times. Some bacteria that are pathogens, such as *Mycobacterium tuberculosis* and *Treponema pallidum*, have especially long generation times, and this is thought to be an advantage in their virulence. Generation times for a few bacteria

Thermal death time (TDT)

It is the time taken to kill a given number of microorganisms or spores at a certain temperature under specified conditions.

12.4.2 Thermal death point

It is the temperature necessary to kill all the organisms in ten minutes.

Heat resistance of different microorganisms is different. Microorganisms are more heat resistant than their spores. Heat resistance of vegetative yeast is 50-58°C in 10-15

min and the ascospores is 60°C for 10-15 min. However, yeast and spores are killed by pasteurization.

12.5 Heat Resistance of Microorganisms

Heat resistance of mold is 60°C in 5 to 10 min and asexual spores are more heat resistance than the ordinary mycelium and require a temperature 5-10°C higher for their destruction. *Aspergillus, Muco, ,Penicillium* are more resistant than yeast. Heat resistance of bacteria and bacterial spores is different. Cells high in lipid content and capsule containing bacteria are harder to kill. Higher the optimal and maximal temperature for growth , the greater the resistance to killing.

12.6 Heat Resistance of Enzymes

Most of the food and microbial enzymes are destroyed at 79.4°C . Some hydrolases will retain a substantial levels of activity after an ultra high temperature treatment. Bovine phosphatase, if present, in processed milk indicates that the milk was not properly pasteurized.

12.7 D Value

It is the decimal reduction time, or the time required to destroy 90% of the organisms. Mathmatically, it is equal to reciprocal of the slop of the survivor curve and is a measure of the death rate of a microorganisms. When D is determined at 250°F, it is expressed as D_r.

Fig. 12.2 D value of organisms

12.8 Z Value

It refers to the degree F required to reduce TDT ten fold. Mathematically, this value is equal to the reciprocal of the slope of the TDT curve.

Fig. 12.3 z value of organisms

12.9 F Value

This value is the equivalent time, in min at 250°F, of all heat considered, with respect to its capacity to destroy spores or vegetative cells of a particular organisms or F is the time in minute required to destroy the microorganisms in a specified medium at 250°F.

12.10 Thermal Death Time Curve

Mean viable counts determined at intervals of 5 minute are as follows-

Time Mean viable count 5 3120.0 10 65.015 19.0

Time of heating in min is plotted on semi-log paper along the linear axis and the number of survivors is plotted along the log scale to produce the TDT curve .

UNIT II

YEAST

INTRODUCTION

Yeasts are single-celled microorganisms that predate humans by...hundreds of millions of years. There are more than 1,500 species of yeast, but the species we're concerned with today is *saccharomyces cerevisiae*, derived from Latinized Greek meaning "sugar-fungus." This group of yeasts includes strains of baker's yeast and brewer's yeast, responsible for producing our favorite carb-heavy treats: bread and alcohol. They work by feeding on sugars and converting that food into carbon dioxide (and alcohol, given enough time), giving your baked goods that soft, airy structure you love and your beer that bubbly nature.

Yeasts are actually microbial eukaryotes which belong to ascomycetes that are good source of vitamin B nd protein. Yeasts are plant-like unicellular fungi thriving on every living organism. Being living organism fungi require warmth, water, albumen or nitrogenous material and sugars to remain alive (The Artisan, The Yeast Treatise, 2002).

DEFINITION:

A MICROSCOPIC FUNGI CONSISTING OF SINGLE OVAL CELLS THAT REPRODUCE BY BUDDING AND ARE CAPABLE OF CONVERTING SUGAR INTO ALCOHOL AND CARBOHYDRATE YEAST FORMENTATIONS ARE INVOLVED IN THE MANIFACTURE OF FOODS SUCH AS BREAD,BEAR,WINES,VINEGAR & SURFACE RIPENED CHEESE & YEASTS ARE GROWN FOR ENZYMES & FOR FOOD.

MORPHOLOGY OF YEAST

Vacuole is single,large and centrally located

 The protoplasm is surrounded by cell membrane which contain all the usual cell organelles like ribosomes,mitochondria,ER,nucleus etc.

 Cell wall:composed of thin chitinous cell wall

 Yeast cell lacks flagella and other organ of locomotion

 Size and shape varies among species

 Size:generally larger than most bacteria(1 to 5 um)wide and (5 to 30 um)in length

 They are single celled fungi

REPRODUCTION IN YEAST CELLS

Yeast lack sex organs(anthridium

 Yeast generally reproduce by asexual method such as budding or fission

 & Sexual reproduction in yeast is highly variable

oogonium)

1. BUDDING: Septum formation and bud seperates into individual cell Enzymatic activities increases Parent nuclieus divides and moves towards daughter cell It occures during abundant supply of nutrition
2. SEXUAL REPRODUCTION Diplobiontic life cycle Haplobiontic life cycle Haplodiplobiontic life cycle Three different pattern of life cycle found in different genes Sexual reproduction is highly variable in yeast:
3. CULTURAL CHARACTERS YEAST Not Non – filamentous unicellular fungi ; cultures resemble bacteria when grown on the surface f lab media ,but are 5-10 times larger then bacteria ; spherical (or) cylindrical Yeast do not or do have aerial hyphae/supporting sporangia
4. Fission Budding
   One of two ways yeasts reproduce asexually , the parent cell elongates ,its nucleus divides,and it splits evenly into two daugter cells One of two ways yeast reproduce asexually an out growth from the parent cell pinches off ,producing a daugter cell 9. Houses ascospores ;when this structure ruptures , the ascospores are released and conjugate ,starting the sexual reproduction cycle again Four haploid nuclei (sexual spores);produced during yeast sexual reproduction Ascospore Ascus
5. 10. A carbohydrate that composes the cell wall of fungi The dye that permit to visualization of the fungi under optic microscope ,it adheres to the “chitin” of the fungi walls of hyphae Lactophenol cotton blue Chitin
6. 11. PHYSIOLOGICAL CHARACTERISTICS yeasts are commonly grown best with a plentiful supply of available moisture But some yeasts grow in the presence of greater concentration of solutes than most bacteria In general sugars are the best source of energy for yeasts,although oxidative yeasts Eg:the film yeasts,oxidise organic acids etc

TYPES OF YEAST

There are three main types of commercially produced baker's yeast: active dry, instant, and fresh. All of them will work to leaven doughs in any given yeasted baking recipe, but each has slightly different properties, and, for the more discerning palate, varying flavors.

Active dry yeast

Live yeast is partially dehydrated, rendered inert, and ground into granules. You'll often find this sold in ¼-oz. packets or a 4-oz. tinted

glass jar in the baking aisle. These dormant yeast cells can be stored at room temperature for several months until their expiration date but are heat-sensitive and potency can vary. Be sure to store it away from super warm areas up until the moment you're ready to bake—active dry yeast will begin to die once exposed to temperatures higher than 120°F. To extend shelf life, store sealed packets in an airtight bag in the freezer to keep the yeast in a more secure state of suspension.

Before being added to your recipe, active dry yeast should be dissolved in lukewarm (between 100°F and 110°F) water or milk, to ensure its activity level. If your yeast mixture does not increase in size and become foamy in 10 to 20 minutes, your packet was likely DOA and no longer viable. This likelihood is not unthinkable: The manufacturing process for active dry yeast can kill up to 25% of yeast cells.

Active dry yeast has a longer fermentation process when compared to other yeasts, meaning it is best suited for dough recipes that call for a double rise and a longer proofing time.

Instant yeast

Also known as "bread machine yeast" and "RapidRise," instant yeast is more shelf-stable and tends to be more reliably and consistently active than active dry yeast (talk about misleading names!). The most popular brand of instant yeast among pro-bakers is SAF-Instant, which is sold in a 16-oz. package. Instant yeast has finer, smaller grains than active dry yeast. This increased surface area allows for a faster rehydration process, and it can be used in recipes without blooming the yeast in a liquid beforehand. (Nonetheless, I still prefer to bloom instant yeast to ensure even distribution in the dough.)

When used in substitution, ¾ teaspoon of instant yeast is equivalent to 1 teaspoon of active dry due to its increased potency and shorter fermentation time. Instant yeast can be stored in the freezer in an airtight container for up to 2 years.

Fresh yeast

Also known as "cake yeast" and "compressed yeast," fresh yeast comes in big blocks or smaller, individually packaged cubes and can be found in

the refrigerated aisle near dairy and eggs. It is the only form of commercial yeast that isn't dehydrated: a solid but crumbly concoction of water and yeast, its rubbery texture is halfway between paste and modeling clay. Due to its high moisture content, this form of yeast is the least shelf-stable, requiring refrigeration and lasting only about two weeks under ideal conditions. If kept in too humid or unclean a container, white tufts of mold can develop within a week and the yeast is no longer safe to use. For longer storage, divide your fresh yeast into individual portions and wrap each piece tightly with plastic wrap before placing in your freezer for up to a year.

Some bakers prefer to use fresh yeast in sweeter recipes, such as donuts, for its more vibrantly yeasty perfume. When used in substitution, 1 oz. of fresh yeast is equivalent to 0.4 oz. of active dry yeast or 0.33 oz. of instant yeast. To substitute for a ¼-oz. packet of active dry yeast, use about ⅔ oz. of cake yeast. To substitute for a ¼-oz. packet of instant yeast, use about ¾ oz. of cake yeast.

NUTRITIONAL YEAST

Nutritional yeast is also derived from *saccharomyces cerevisiae*, but it undergoes a "deactivation" process during which the live yeast cultures are killed by heat. Because the yeast is essentially dead, it can no longer provide leavening magic to your baked goods. It is, however, a delicious seasoning that can provide a salty, cheesy, nutty kick to anything savory you're cooking up: popcorn, vegan mac and cheese, roasted veggies, fried rice—the list goes on!

REPRODUCTION OF YEAST

Yeasts, like all fungi, may have asexual and sexual reproductive cycles. The most common mode of vegetative growth in yeast is asexual reproduction by budding,[42] where a small bud (also known as a bleb or daughter cell) is formed on the parent cell. The nucleus of the parent cell splits into a daughter nucleus and migrates into the daughter cell. The bud then continues to grow until it separates from the parent cell, forming a new cell.[43] The daughter cell produced during the budding process is generally smaller than the mother cell. Some yeasts, including *Schizosaccharomyces pombe*, reproduce by fission instead of budding,[42] and thereby creating two identically sized daughter cells.

In general, under high-stress conditions such as nutrient starvation, haploid cells will die; under the same conditions, however, diploid cells can undergo sporulation, entering sexual reproduction (meiosis) and producing a variety of haploid spores, which can go on to mate (conjugate), reforming the diploid.[44]

VIRUS

INTRODUCTION

Viruses are typically described as **obligate intracellular parasites**, acellular infectious agents that require the presence of a host cell in order to multiply. Viruses that have been found to infect all types of cells – humans, animals, plants, bacteria, yeast, archaea, protozoa…some scientists even claim they have found a virus that infects other viruses! But that is not going to happen without some cellular help.

Virus Characteristics

Viruses can be extremely simple in design, consisting of nucleic acid surrounded by a protein coat known as a **capsid**. The capsid is composed of smaller protein components referred to as **capsomers**. The capsid+genome combination is called a **nucleocapsid**.

Viruses can also possess additional components, with the most common being an additional membranous layer that surrounds the nucleocapsid, called an **envelope**. The envelope is actually acquired from the nuclear or plasma membrane of the infected host cell, and then modified with viral proteins called **peplomers**. Some viruses contain viral enzymes that are necessary for infection of a host cell and coded for within the viral genome. A complete virus, with all the components needed for host cell infection, is referred to as a **virion**.

Virus Genome

While cells contain double-stranded DNA for their genome, viruses are not limited to this form. While there are **dsDNA** viruses, there are also viruses with single-stranded DNA (**ssDNA**), double-stranded RNA (**dsRNA**), and single-stranded RNA (**ssRNA**). In this last category, the ssRNA can either positive-sense (**+ssRNA**, meaning it can transcribe a message, like mRNA) or it can be negative-sense (**-ssRNA**, indicating that it is complementary to mRNA). Some viruses even start with one form of nucleic acid in the nucleocapsid and then convert it to a different form during replication.

Structure and Function

Viruses are small obligate intracellular parasites, which by definition contain either a RNA or DNA genome surrounded by a protective, virus-coded protein coat. Viruses may be viewed as mobile genetic elements, most probably of cellular origin and characterized by a long co-evolution of virus and host. For propagation viruses depend on specialized host cells supplying the complex metabolic and

biosynthetic machinery of eukaryotic or prokaryotic cells. A complete virus particle is called a virion. The main function of the virion is to deliver its DNA or RNA genome into the host cell so that the genome can be expressed (transcribed and translated) by the host cell. The viral genome, often with associated basic proteins, is packaged inside a symmetric protein capsid. The nucleic acid-associated protein, called nucleoprotein, together with the genome, forms the nucleocapsid. In enveloped viruses, the nucleocapsid is surrounded by a lipid bilayer derived from the modified host cell membrane and studded with an outer layer of virus envelope glycoproteins.

Classification of Viruses

Morphology: Viruses are grouped on the basis of size and shape, chemical composition and structure of the genome, and mode of replication. Helical morphology is seen in nucleocapsids of many filamentous and pleomorphic viruses. Helical nucleocapsids consist of a helical array of capsid proteins (protomers) wrapped around a helical filament of nucleic acid. Icosahedral morphology is characteristic of the nucleocapsids of many "spherical" viruses. The number and arrangement of the capsomeres (morphologic subunits of the icosahedron) are useful in identification and classification. Many viruses also have an outer envelope.

Chemical Composition and Mode of Replication: The genome of a virus may consist of DNA or RNA, which may be single stranded (ss) or double stranded (ds), linear or circular. The entire genome may occupy either one nucleic acid molecule (monopartite genome) or several nucleic acid segments (multipartite genome). The different types of genome necessitate different replication strategies.

Virus Structure

Viral nucleocapsids come in two basic shapes, although the overall appearance of a virus can be altered by the presence of an envelope, if present. **Helical viruses** have an elongated tube-like structure, with the capsomers arranged helically around the coiled genome. **Icosahedral viruses** have a spherical shape, with icosahedral symmetry consisting of 20 triangular faces. The simplest icosahedral capsid has 3 capsomers per triangular face, resulting in 60 capsomers for the entire virus. Some viruses do not neatly fit into either of the two previous categories because they are so unusual in design or components, so there is a third category known as **complex viruses**. Examples include the poxvirus with a brick-shaped exterior and a complicated

internal structure, as well as bacteriophage with tail fibers attached to an icosahedral head.

Virus Replication Cycle

While the replication cycle of viruses can vary from virus to virus, there is a general pattern that can be described, consisting of five steps:

1. **Attachment** – the virion attaches to the correct host cell.
2. **Penetration or Viral Entry** – the virus or viral nucleic acid gains entrance into the cell.
3. **Synthesis** – the viral proteins and nucleic acid copies are manufactured by the cells' machinery.
4. **Assembly** – viruses are produced from the viral components.
5. **Release** – newly formed virions are released from the cell.

Attachment

Outside of their host cell, viruses are inert or metabolically inactive. Therefore, the encounter of a virion to an appropriate host cell is a random event. The attachment itself is highly specific, between molecules on the outside of the virus and receptors on the host cell surface. This accounts for the specificity of viruses to only infect particular cell types or particular hosts.

Penetration or Viral Entry

Many unenveloped (or **naked**) viruses inject their nucleic acid into the host cell, leaving an empty capsid on the outside. This process is termed penetration and is common with bacteriophage, the viruses that infect bacteria. With the eukaryotic viruses, it is more likely for the entire capsid to gain entrance into the cell, with the capsid being removed in the cytoplasm. An unenveloped eukaryotic virus often gains entry via **endocytosis**, where the host cell is compelled to engulf the capsid resulting in an endocytic vesicle. An enveloped eukaryotic virus gains entrance for its nucleocapsid when the viral envelope fuses with the host cell membrane, pushing the nucleocapsid past the cell membrane. If the entire nucleocapsid is brought into the cell then there is an uncoating process to strip away the capsid and release the viral genome.

Synthesis

The synthesis stage is largely dictated by the type of viral genome, since genomes that differ from the cell's dsDNA genome can involve intricate viral strategies for genome replication and protein synthesis. Viral specific enzymes, such as RNA-dependent RNA polymerases, might be necessary for the replication process to proceed. Protein

production is tightly controlled, to insure that components are made at the right time in viral development.

Assembly

The complexity of viral assembly depends upon the virus being made. The simplest virus has a capsid composed of 3 different types of proteins, which self-assembles with little difficulty. The most complex virus is composed of over 60 different proteins, which must all come together in a specific order. These viruses often employ multiple assembly lines to create the different viral structures and then utilize scaffolding proteins to put all the viral components together in an organized fashion.

Release

The majority of viruses lyse their host cell at the end of replication, allowing all the newly formed virions to be released to the environment. Another possibility, common for enveloped viruses, is **budding**, where one virus is released from the cell at a time. The cell membrane is modified by the insertion of viral proteins, with the nucleocapsid pushing out through this modified portion of the membrane, allowing it to acquire an envelope.

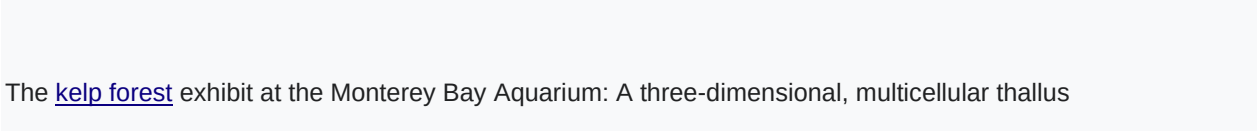
ALGEA

INTRODUCTION

Freshwater algae are globally ubiquitous and highly diverse, with tens or perhaps hundreds of thousands of species, in a myriad of forms and sizes (Andersen, 1992; Norton et al.; 2004; Mann and Vanormelingen, 2013; Guiry and Guiry, 2014). The algae represent between eight and 12 major evolutionary lineages (Graham et al., 2008; Cock et al., 2010), and all have representatives in inland waters. Current classifications consider most algae to be protists with chloroplasts, but there are also photosynthetic prokaryotes (the cyanobacteria) and a subset of the land plants, the Charales, which have been considered to be algae in previous texts (Patterson, 2014). With new molecular tools being applied to understanding algal taxonomy, systematics, and evolution, our understanding of this diversity is rapidly changing and expanding. Efforts to characterize this biological diversity

(such as the Tree of Life Project: Maddison et al., 2007) have contributed to a better understanding of many groups of freshwater algae

Morphology[



The kelp forest exhibit at the Monterey Bay Aquarium: A three-dimensional, multicellular thallus

A range of algal morphologies is exhibited, and convergence of features in unrelated groups is common. The only groups to exhibit three-dimensional multicellular thalli are the reds and browns, and some chlorophytes.[36] Apical growth is constrained to subsets of these groups: the florideophyte reds, various browns, and the charophytes.[36] The form of charophytes is quite different from those of reds and browns, because they have distinct nodes, separated by internode 'stems'; whorls of branches reminiscent of the horsetails occur at the nodes.[36] Conceptacles are another polyphyletic trait; they appear in the coralline algae and the Hildenbrandiales, as well as the browns.[36]

Most of the simpler algae are unicellular flagellates or amoeboids, but colonial and nonmotile forms have developed independently among several of the groups. Some of the more common organizational levels, more than one of which may occur in the lifecycle of a species, are

- Colonial: small, regular groups of motile cells
- Capsoid: individual non-motile cells embedded in mucilage
- Coccoid: individual non-motile cells with cell walls
- Palmelloid: nonmotile cells embedded in mucilage
- Filamentous: a string of nonmotile cells connected together, sometimes branching
- Parenchymatous: cells forming a thallus with partial differentiation of tissues

In three lines, even higher levels of organization have been reached, with full tissue differentiation. These are the brown algae,[37]—some of which may reach 50 m in length (kelps)[38]—the red algae,[39] and the green algae.[40] The most complex forms are found among the charophyte algae (see Charales and Charophyta), in a lineage that eventually led to the higher land plants. The innovation that defines these nonalgal plants is the presence of female reproductive organs with protective cell layers that protect the zygote and developing embryo. Hence, the land plants are referred to as the Embryophytes.

CLASSIFICATION

The algae do not represent a formal taxonomic group of organisms, but rather constitute a heterogeneous collection of phyla or divisions with representatives in several kingdoms and having most of the characteristics described above. The divisions are distinguished from each other based on a combination of characteristics, including photosynthetic pigments, starch-like (or other) reserve products, cell covering, and other aspects of cellular organization (e.g., Graham et al., 2008; Lee, 2008). There is little consensus among phycologists as to the exact

number of algal divisions, and the modern methods are likely to change higher classification categories for some years to come.

GROUPS OF FRESHWATER ALGAE

A Cyanobacteria

The cyanobacteria or blue-green algae are prokaryotes without membrane-bound organelles (Table 1; Chapter 3). Other characteristics of this division include unstacked thylakoids, phycobiliprotein pigments, cyanophycean starch, and peptidoglycan matrices or walls. There are 174 genera reported from inland habitats in North America, of which 66 are unicellular, colonial, or pseudofilamentous (Chapter 3) and 108 are filamentous (Chapter 4). However, the taxonomy of this division is currently being revised (Komárek, 2013, Chapter 3), and the number of genera should be considered tentative. Cyanobacteria inhabit the widest variety of freshwater habitats on Earth and can become important in surface blooms in nutrient-rich standing waters (Chapters 2-4 and 20). However, nitrogen-fixing taxa have been shown to be good indicators of N-limited sites, such as streams in California (Stancheva et al., 2013b), as well as in many lakes and reservoirs (Howarth et al., 1988). Some of the cyanobacterial blooms can be toxic to zooplankton and fish, as well as livestock that drink water containing these organisms. Inland cyanobacteria also occur in extreme environments, such as hot springs, saline lakes, and endolithic desert soils and rocks

B Red Algae

Rhodophyta or red algae represent a division that is characterized by chloroplasts that have no external endoplasmic reticulum and unstacked thylakoids, phycobiliprotein pigments, floridean starch, and lack of flagella in all stages (Table 1; Chapter 5). They are predominantly marine in distribution with fewer than 3% of more than 6500 species occurring in truly freshwater habitats (Guiry and Guiry, 2014; Guiry et al., 2014). In North America, 26 genera are recognized in inland habitats (Chapter 5). Author's personal copy 8 Freshwater Algae of North America Freshwater red algae are largely restricted to streams and rivers but also can occur in other inland habitats, such as lakes, hot springs, soils, caves, and even sloth hair (Chapter 5). While many species are indicators of good water quality, a small number, like the chantransia stage of Batrachospermum (formerly Chantransia) macrospora, which can be invasive in relatively polluted waters, due to human activities such as dumping aquarium contents (Kato et al., 2009).

C Green Algae

Chlorophyta and Charophyta, the green algae constitute divisions that have the following set of attributes: chloroplasts with no external endoplasmic reticulum, thylakoids typically in stacks of two to six, chlorophylls a and b as photosynthetic pigments, true starch, and cellulosic walls or scales (Table 1). This is a diverse group in inland habitats of North America that includes 48 flagellated genera (Chapter

6), at least 129 coccoid and non-motile colonies (Chapter 7), 81 filamentous and plantlike genera (Chapter 8), and 49 conjugating genera and desmids (Chapter 9). Some members of the green algae (Charophyeae) are part of a lineage that is ancestral to higher plants

D Euglenoids Photosynthetic Euglenophyta or euglenoids have chloroplasts surrounded by three membranes, thylakoids in stacks of three, chlorophyll a and b as photosynthetic pigments, the ability to store paramylon rather than starch, and a proteinaceous (often spirally arranged) covering (termed a pellicle) (Table 1; Chapter 10). Twelve genera are reported from North American freshwater habitats (Chapter 10). Euglenoids are particularly abundant in the plankton of standing waters rich in nutrients and organic matter, and they can be associated with sediments, fringing higher plants, and leaf litter, although some may dominate in highly acidic environments (Chapters 2 and 10). Some species, like Euglena mutabilis, are pioneering species in severely acid mine drainage-impacted sites with very acidic waters (Ph

Brown Algae Phaeophyceae or brown algae are distinguished by chloroplasts that have four surrounding membranes, thylakoids in stacks of three, fucoxanthin that masks chlorophyll a and c, laminarin as the photosynthetic reserve, and alginates as the wall matrix component. There are six recognized genera of freshwater brown algae, four of which have been collected from freshwater habitats in North America (Chapter 19). Brown algae are predominantly marine in distribution; fewer than 1% of the species are from freshwater. The inland species are benthic, either in lakes or streams, and distribution is quite scattered (Chapter 19). Some species are considered to be possible invaders from brackish or marine habitats, such as Pleurocladia lacustris (Wehr et al., 2013), although others, such as Heribaudiella, appear to be strict freshwater taxa

UNIT III

INTRODUCTION

CONTAMINATION KINDS OF MICROORGANISMS

Spoilage of food can be defined as any visible or invisible change which can makes food or product derived from food unacceptable for human consumption. Major causes of food spoilage are enlisted in Table 6.1.

Spoilage of food not only causes health hazard to the consumer but also cause large economic losses. Spoilage not only leads to loss of nutrients from food but also cause change in original flavor and texture. It is estimated that about 25% of total food produced is spoilt due to microbial activities only despite range of preservation methods available. Thus the spoilage of food is not only a health hazard but also carry lot of economic significance too.

In total, the food spoilage is considered a complex phenomena whereby a combination of microbial and biochemical activities take place. Due to such activities, various types of metabolites are formed which aid in spoilage. The detection of these metabolites help in detection of spoilage.

The ease with which foods are spoiled depend upon factors described. The foods are thus divided into different classes as:

Perishable foods These foods are readily spoilt and require special preservation and storage conditions for use. The foods of this class are mostly used daily such as milk, fruits, vegetables, fish etc.

Semi-perishable foods This class of foods if properly stored can be used for a long duration e.g. potatoes.

Non-perishable foods These foods remain in good form for long duration unless handled improperly. It include sugar, flour etc.

Factors Affecting Microbial Spoilage of Foods

While the spoilage by physical and chemical modes play important role, the microbial spoilage has most significant role. Combination of all these factors is ultimately responsible for overall decay of food. The spoilage of food can occur at different stages of production, processing. Various causes of spoilage at different stages are depicted in (fig. 6.1)

The spoilage of foods due to microbial activity initiates when undesirable microorganism colonize the food. Once colonization is established, the microbial community grow on the food constituents, thereby utilizing them for their metabolism. During the course of such microbial activity, the food become unsuitable for human consumption. Various parameters affect the proliferation of microbes. These include intrinsic to food such as water activity, acidity, oxidation-reduction potential, presence of antimicrobial compounds in food and food structure. Extrinsic parameters such as temperature, humidity and other storage condition aid in spoilage.

Microorganisms in Food

Microorganisms by virtue of their ubiquity and diversity in metabolism are most significant cause of food spoilage. Bacteria and fungi (including yeasts and moulds) are major cause of food spoilage.

Bacteria are round; rod or spiral shaped microorganisms and can grow under a wide variety of conditions. There are many types of bacteria that cause spoilage. Food spoiling bacteria are primarily divided two groups viz. *spore-forming* and *nonspore-forming*. Bacteria generally grow in low acid foods like vegetables and meat.

Yeasts growth causes fermentation which is the result of yeast metabolism. There are two types of yeasts *true* yeast and *false* yeast. *True yeast* metabolizes sugar producing alcohol and carbon dioxide gas. This is known as fermentation. *False yeast* grows as a dry film on a food surface, such as on pickle brine. False yeast occurs in foods that have a high sugar or high acid environment.

Molds grow in filaments forming a tough mass which is visible as `mold growth'. Molds form spores which, when dry, float through the air to find suitable conditions where they can start the growth cycle again.

Mold can cause illness, especially if the person is allergic to molds. Usually though, the main symptoms from eating moldy food will be nausea or vomiting from the bad taste and smell of the moldy food.

Both yeasts and molds can easily grow in high acid foods like fruit, tomatoes, jams, jellies and pickles. Both are easily destroyed by heat. Processing high acid foods at a temperature of 100°C (212°F) in boiling water in can for the appropriate length of time destroys yeasts and moulds.

a) Rod shaped Bacteria

b) Mould

c) Yeast

CONTAMINATION OF CERAELS

INTRODUCTION

• Cereals are one of the most important sources of food (FAO, 2002), which have contributed to human nutrition for millennia. However, cereals are exposed to numerous biotic and abiotic stress factors, from cultivation and throughout their life cycle to processing. • Toxigenic fungi are a major problem in cereal crops as they produce a multitude of toxic metabolites contaminating plants and food products.

ABOUT CEREAL • lThe major cereal crops produced worldwide are wheat (Triticum spp.), rice (Oryza spp.), maize (Zea mays L.), and barley (Hordeum vulgare L.) (USDA, 2013).

• Other cereals include millet, sorghum, rye, oat and triticale .

• Maize ranks first in quantity produced and cultivation area of cereals worldwide, followed by wheat, rice and barley.

• Cereals are important in human nutrition as a source of protein, dietary fiber, and carbohydrates, as well as providing such as, magnesium, zinc, and micronutrients E and B complex -vitamins (McKevith, 2004).

• Cereals are also used to produce oils, starch, flour, sugar, syrup, malt, alcoholic beverages, gluten and renewable energy .

• Indigenous microbiota in cereal grains consists of virus, bacteria, filamentous fungi, yeast, slime moulds and protozoa .

• Cereal grains are exposed to contaminations in the field from several sources (water, composted manure, soil, etc.), during cultivation, harvest, storage, and transport

CEREAL SPOILAGE

Common phytogenic microorganisms include bacteria (e.g. Pseudomonadaceae, Micrococcaceae, Lactobacillaceae and Bacillaceae), yeasts (e.g. Candida, Cryptococcus, Pichia, Sporobolomyces, Rhodotorula, Trichosporon) and filamentous fungi (e.g. Alternaria, Aureobasidium, Cladosporium, Epicoccum, Fusarium, Helminthosporium, Claviceps).

• Additionally, potential secondary infections can occur post-harvest. Grains can be contaminated during cleaning, milling, grading or packaging processes (from residues in containers, equipment, screw-conveyors, etc).

• Common microorganisms infecting grains in storage include xerophilic Aspergillus glaucus group, and Penicillium spp., where the most important parameter for mould germination is the minimum aw of 0.68 (14% moisture) (Lacaet al., 2006; Laitila, 2007; Noots et al., 1999).

• After processing, the main spoilage fungi affecting cereal products belong to the genera Aspergillus, Penicillium, and Fusarium.

• Filamentous fungi are a main safety concern due to the production of mycotoxins accumulated in grains pre- and post-harvest, which are associated with severe health problems.

• **Mycotoxins** can be carcinogenic, mutagenic, genotoxic, teratogenic, neurotoxic, and oestrogenic, including reproductive and developmental toxicity (Fung and Clark, 2004; Jestoi, 2008; Köppen et al., 2010).

• High incidence of mycotoxin infections in cereals have been observed worldwide (Placinta et al., 1999), in different crops and regions (Manthey et al., 2004; Warzecha et al., 2011).

• M**ycotoxins**, such as Fusarium toxins, Alternaria toxins, and the ergot alkaloid groups, are common contaminants of cereal grains (Pleadin et al., 2012; Roscoe et al., 2008; Santos et al., 2012).

• Table shows the most common mycotoxins detected in cereals and its health effects for humans and animals. Over the last two years, contaminations in cereals and bakery products by aflatoxins (48%) and ochratoxin A (OTA) (14%), by Aspergillus species, and deoxynivalenol (DON) (21%) and fumonisins (13%), by Fusarium species, were record (RASFF, 2012).

Changes in major food components during spolage

Change in Carbohydrates

Carbohydrates are used to obtain energy. While monosaccharide are preferred over complex carbohydrates, microorganisms have ability to convert polysaccharides to simpler forms before obtaining energy. The utilization of simple sugar such as glucose vary under aerobic and anaerobic conditions. In aerobic conditions it is converted into carbon dioxide and water through glycolysis and other related pathways. In absence of oxygen, the process yields a number of compounds in different organisms. This process is known as fermentation. These compounds include:

Alcoholic fermentation

It occurs due to yeasts and carbon dioxide and ethanol are the major end products.

Lactic fermentation

It is of two types viz. homolactic fermentation where primarily lactic acid is the end product and heterolactic fermentation where along with lactic acid, acetic acid, ethanol, glycerol, carbon dioxide are produced.

Coliform type fermentation

This type of fermentation occur in coliform bacteria. In this process acids such as lactic, acetic, formic. Ethanol, glycerol etc. are produced.

Propionic fermentation

It occurs in propionic bacteria and in it along with propionic acid, succinic acid and carbon dioxide are produced.

Change in Nitrogenous Compounds

Proteins are the major source of nitrogenous compounds in foods. Thus degradation of proteins, include hydrolysis by enzymatic reactions. The source of enzymes can be either microbes or foods own enzymes. Complex proteins are converted into polypeptides, simpler peptides and amino acids. The enzymes involved in conversion of proteins into polypeptide are termed as proteinase while those catalyzing conversion of polypeptides to amino acids are called peptidases. The decomposition of proteins can be aerobic or anaerobic. Usually the anaerobic decomposition of proteins results in obnoxious odors. This process is known as Putrefaction. Along with nitrogenous compounds, other compounds responsible for such smells also include sulfur compounds.

The microbial activity on amino acids cause either deamination (removal of amine group) or decarboxylation (removal of carboxyl group). Major organisms involved in conversion of nitrogenous compounds include *Pseudomonas*, *E. coli*, *Clostridium*, *Desulfotomaculum* etc.

Changes in Lipids

The hydrolysis of lipids is accomplished by lipase enzymes produced by different microorganisms. The major end products include glycerol and fatty acids, which are further used by microorganisms for their metabolism. The process of conversion is known as oxidation. The oxidation of fats is also done by enzymes of food itself. High fat containing foods are prone to such processes.

MICROBIAL SPOILAGE OF CEREALS AND BAKERY FOODS

Introduction

Cereals are important foods which provide bulk of our dietary requirements. They are also source of carbohydrates which are metabolized by body for energy generation. Besides cereals also provide minerals, proteins and vitamins. India produces a large variety of cereals such paddy, wheat, maize, barley millets like, jowar, bajra, ragi. Various types of products are prepared from cereals. Cereal products can be broadly classified into the following groups:

- Whole cereals where only the husk of the grain is removed, e.g. rice, wheat, gram, lentils, etc.
- Milled grain products are made by removing the bran and usually the germ of the seed and then crushing the kernel into various sized pieces. These include wheat flour, maida, semolina (rawa), etc.
- Processed cereals like weaning food, breakfast cereals, etc.
- Ready mixes like cake mix, idli mix, vada mix etc.

The country is self sufficient in grain production and is the second largest rice producer in the world with a 20% share. But due to constantly increasing population there is still a shortfall in cereals. A large amount of these cereals are spoilt every year due to various factors.

Spoilage Factors

The grains are low moisture commodities due to which they are less susceptible to spoilage and have greater shelf-life. The spoilage mainly occurs due to moisture absorption during storage leading to fungal growth at high temperature and humidity. Before bulk packaging and storage, the whole grains are fumigated to reduce microbial load and increase storage period. The factors influencing the quality of cereals are:

Physical

Physical losses are caused by spillages, which occur due to use of faulty packaging materials.

Physiological

Physiological losses include respiration and heating in grains, temperature, humidity and oxygen.

Biological

Biological losses occurs due to micro-organisms, insects, rodents, etc.

The sources of contamination in cereals are:

- Soil
- Air
- Insects
- Natural microflora of harvested grains

Cereal Grains and Flours

At initial stages, the grains are contaminated by *Pseudomonas*, Micrococci, *Lactobacillus* and *Bacillus*. The initial bacterial population may vary from 10^3 to 10^6 per gram while mold population may be more than 10^4 spores per gram.

Due to low moisture content grains and flours usually have long shelf life if these are properly harvested or stored under proper conditions as microbial growth is not supported. If due to any reason they attain moisture, the microbial growth may occur with molds growing at initial stages of moisture while yeasts and bacteria may grow with increasing moisture.

Spoilage of stored grains by molds is attributed to the following factors:

- Type and number of microorganisms
- Moisture content of more than 12-13%
- Storage temperature
- Physical damage

Most common species of molds are *Aspergillus, Rhizopus, Mucor, Fusarium*. A significant aspect of spoilage of molds is production of mycotoxins, which may pose danger to health.

The process of flour making such as washing, milling reduce the microbial content. Moisture content of less than 15% does not allow growth of molds. Most molds and bacteria in flours can grow only above 17% moisture, thus moistening of flours is essential for spoilage by microbes

Spoilage of Bread

Bread is a major product prepared using flours. Dough is prepared from flours which undergo fermentation for which desirable microorganisms must grow. If this fermentation exceeds the required limits, it causes souring. Excessive growth of

proteolytic bacteria reduces the gas holding capacity which is otherwise required for dough rising. Spoilage of bread is usually of two types viz. moldiness and ropiness.

During bread making, it is baked at very high temperature, thereby there are less chances of survival of microorganisms. Thus the contamination usually occurs when cooling is done as well as during packing, handling and from the environment. The molds which are prevalent are *Rhizopus stolonifer* (referred as bread mold), *Penicillium expansum, Aspergillus niger*. *Mucor* and *Geotrichum* also develop.

Ropiness in bread is usually due to bacterial growth and is considered more prevalent in home made breads. The chief causative organism is *Bacillus subtilis* or *B. licheniformis*. These are spore forming bacteria with their spores surviving baking temperatures. These spores can germinate into vegetative cells, once they get suitable conditions as heat treatment activates them. In ropiness, the hydrolysis of bread flour protein (gluten) takes place by proteinases. Starch is also hydrolysed by amylases, which encourage ropiness. The manifestation of ropiness is development of yellow to brown color and soft and sticky surface. It is also accompanied by odor.

Another type of spoilage of bread is chalky bread which is caused by growth of yeast like fungi *Endomycosis fibuligera* and *Trichosporon variable*. This spoilage is characterized by development of white chalk like spots.

An unusual spoilage of bread is Red or Bloody bread, which is due to the growth of bacteria *Serratia marcescens*. This organism produces brilliant red color on starchy foods giving blood like appearance. *Neurospora* and *Geotrichum* may also be involved in imparting pigmentation during spoilage of bread

MICROBIOLOGY OF MEAT, POULTRY AND SEA FOODS

Introduction

The microbiological profile of meat products presented to the consumers is the sum total of the slaughtered animal health, conditions under which it was reared, quality of slaughtering, processing, packaging and conditions under which the meat was stored. Meat pathogens can cause self-limiting human enteric diseases or systemic and fatal infections of the immunocompromised, the elderly and the young. Meat can act as an ideal substrate for microbial proliferation. Major meat associated pathogenic bacteria include *Clostridium perfringens, Staphylococcus aureus, Salmonella spp*, pathogenic strains *of Escherichia coli, Campylobacter spp, Yersinia enterocolitica, Listeria monocytogenes* and *Aeromonas hydrophila*

Microorganisms Associated with Meat During Processing

Meat spoilages indicate (a) color changes (b) textural changes and (c) development of off-flavour or off-odor or slime as a result of microbial growth. *Salmonella* is the primary microbial challenge for poultry. The primary microbial to the beef industry is *Escherichia coli* O157: H7. *Listeria,* which is an adulterant with zero tolerance, is the major problem for ready to eat meat products. Treatment with organic acids, hot water steam carcass pasteurization and steam carcass vacuuming, trisodium phosphate, acidified sodium chlorite, chlorine dioxide, lactoferrins, peroxyacetic acid, sodium lactate, sodium acetate and sodium diacetate, ozone and radiation have been used as microbial decontaminants during meat processing operations. Carcass washing with hot water of 80°C for 10 seconds can reduce microbial loads by 2 logs. Current regulatory policies and inspection in the meat industry include the HACCP (Hazard Analysis Critical Control Point) food safety system with an objective to provide safe food for consumption and prevent chemical, physical and biological hazards.

Gram-negative bacteria (Aerobes)

Neisseriaceae: *Psychrobacter immobilis, P. phenylpyruvica, Acinetobacter* spp., *A. twoffii, A. Johnsonii,*

Pseudomonadaceae: *Pseudomonas fluorescens, P. lundensis, P. fragi, P. putida*

Gram-positive bacteria: *Brochothrix thermosphacta, Kurthia zophii, Staphylococcus spp., Clostridium estertheticum, Clostridium frigidicarnis, Clostridium casigenes, Clostridium algidixylanolyticum* sp. *nov.*

Spoilage

Fresh Meat

In contrast to fruits and vegetables, meats are composed mainly of protein and fats rather than carbohydrates. Water content is 71–76% and therefore moisture is not an issue except for spoilage microbes on cured meats. Muscles of healthy animals do not contain any bacteria or fungi but as soon as animals are slaughtered, meat is exposed to contaminants and good sanitation practices are essential to produce high quality meats. The number of spoilage organisms on meat just after slaughter is a critical factor in determining shelf life. The surface of beef carcasses may contain anywhere from 10^1 to 10^7 cfu/cm2, most of which are psychrotrophic bacteria. Chopping and grinding of meats can increase the microbial load as more surface area is exposed and more water and nutrients become available. A large variety of microbes are commonly found on fresh meat, but different microbes become dominant during

spoilage of different meats depending on pH, composition and texture of processed meats, temperature and packaging atmosphere. *Pseudomonas* spp. is the predominant spoilage bacteria in aerobically stored raw meat and poultry. Once the initial low levels of glucose are depleted by various microbes, *Pseudomonas* has an advantage because it can catabolize gluconates and amino acids more readily than other microbes. Break down of these compounds results in production of malodorous sulfides, ammonia, and amines, including the biogenic amines putrescine and cadaverine. Dark, firm and dry meat with a relatively high pH of 6.0 spoils more rapidly because deamination of amino acids starts earlier. *Shewanella putrefaciens* does not grow on meat at pH<6.0 but can produce sulfides and ammonia even when glucose is still available. These sulfides not only smell bad but also cause color changes in meat, and therefore Shewanella has a high spoilage potential on fresh, high pH meats stored aerobically even when it is not a dominant microbe. *Brochothrix thermosphacta* is often a significant spoilage organism on fresh meat stored aerobically at refrigeration temperatures. *Enterobacteriaceae*, particularly species of *Serratia*, *Enterobacter*, and *Hafnia*, are major causes of spoilage in vacuum-packed, high pH fresh meats. These organisms are facultative anaerobes that produce organic acids, hydrogen sulfide and greening of meats.

Lactic acid bacteria (LAB) grow on meat and poultry packaged under vacuum and modified atmospheres, producing organic acids from glucose by fermentation. This gives rise to aciduric off-odors which may be accompanied by gas and slime formation and greening of meat. However, LAB are weakly proteolytic and so do not produce large amounts of amines or sulfides, and spoilage of meat by LAB is not as offensive. Psychrophilic, anaerobic *Clostridium* spp. are associated with spoilage of vacuum-packaged meats. "Blown pack" meat spoilage is characterized by excessive gas formation with off odors due to formation of butyric acid, butanol and sulfurous compounds. Yeasts and molds grow relatively slowly on fresh meat and do not compete well with bacteria. Therefore, they are a minor component of spoilage flora.

Processed Meat

Addition of sodium chloride, nitrites and/or nitrates, along with various other seasonings, emulsifiers and preservatives to ground or whole muscle meats changes the environment significantly and also the spoilage flora of processed meats. Dried and dry-fermented meats generally do not support microbial growth although process deviations may allow growth of some organisms. Spoilage organisms can grow on fresh and cooked cured meats, so they are best stored chilled, under a vacuum or modified atmosphere. *Pseudomonas* spp. are not usually important causes of spoilage in processed meats because of their sensitivity to curing salts and heat pasteurization and their inability to grow well in meats packed with a vacuum or high carbon dioxide atmosphere. However, when packages have been opened and there has been

insufficient curing, these bacteria may spoil refrigerated processed meats. Some cold- and salt tolerant *Enterobacteriaceae* have been found to cause spoilage in some specific processed meats, such as ham or bacon.

Lactic acid bacteria (LAB) is the group of bacteria primarily associated with spoilage of processed meats. They produce sour off-flavors, gas, slime, and greening, and this spoilage may be more severe than in fresh meat because of the presence of added carbohydrates. Competitive ability of different LAB strains is related to pH and water activity of the meat, cooking and storage temperatures and oxygen and carbon dioxide levels. Sporeformers (*Clostridium* and *Bacillus*) are usually not a spoilage problem in processed meats because of the presence of nitrite and other curing salts. However, faulty cooking/cooling procedures, including long cooling periods and temperature abuse, has allowed growth of these organisms in some cases. Spores of these organisms may be introduced with spices or other ingredients. Yeasts cause some spoilage in processed meats but are generally only important when sulfite is used as a preservative or when meats have been irradiated or are stored aerobically in the cold. Slime may be produced along with vinegary or malty off-odors in some sausages.

Spoilage under aerobic condition

1.) Surface slime, caused by *Pseudomonas acinetobacter, Moraxella alcaligenes Streptococcus, Leuconostuoc, Bacillus* and *Micrococcus.*

2.) Change in colour of meat pigment. The red colour of meat may be changed to shades of green, brown or grey by *Lactobacillus* and *Leconostocs* spp.

3.) Changes in fat. The unsaturated fat in meat gets oxidized by lypolitic bacteria which produce off odours due to hydrolysis of fats and production of aldehydes and acids. This type of spoilage is caused by lypolitic *Pseudomonas*, Achromobacter and yeast.

4.) Surface color change. The red pigment producing bacteria, *Serratia marcescens*, caused red spots on meat. Blue color surface is caused by *Pseudomonas syncyanea* and yellow color is caused by *Micrococcus* species.

5.) Off odor and off taste. Volatile acid like formic, acetic, butyric and propionic acid produce sour odor and *Actinomycetes* produce musty or earthy flavor. Yeast also cause sliminess discoloration and off odor and taste defects.

6.) Aerobic mold also cause spoilage in meat. These are stickiness, whiskers, black-spot, white-spot, green patches off odor and off taste.

7.) Spoialage under anerobic condition.

i) Souring is caused by production of formic, acetic, butyric, lactic, succinic and propionic acid.

ii) Putrefaction. It is caused by decomposition of proteins under anaerobic condition by *Clostridium* species. The foul smell is due to production of hydrogen sulphide, mercaptans, indol, scatol, ammonia and amines.

Poultry Meat

Poultry meat like meat of other animals is also susceptible to contamination by various sources. Contamination of skin and lining of the body cavity take place during various processing operations. The organisms of great importance in poultry are *Salmonella* spp. and *Campylobacter jejuni.* Several Gram negative psychrotropic bacteria *viz*., *Pseudomonas*, *Acenitobacter* and *Flavobacterium* have also been isolated from poultry carasses. Ground turkey also may carry fecal streptococci. It is important to freeze the poultry fast in order to keep it in good condition for several months. Freezing further reduces the number of microorganisms in the poultry meat provided the temperature is maintained quite low (-18 ° C or below).

CONTAMINATION OF FISH

Introduction

Fish are classified as any of the cold-blooded aquatic vertebrates of the super class Pisces typically showing gills, fins and a streamline body. In addition, 'fish' also refers to the flesh of such animals used as food. This super class of vertebrates includes all the bony and cartilaginous finfish, and excludes molluscs and Crustacea. However, some regulatory agencies such as the US Food and Drug Administration (FDA) will include molluscan shellfish, crustaceans, and other forms of aquatic animal as part of their 'fish' definition. In this chapter, "fish" will be used for fresh and seawater finfish. Fish are an important part of a healthy diet since they contain high quality protein, but typically present a low fat percent when compared to other meats. In addition, most fish contain omega 3-fatty acids and other essential nutrients. Although fish is broadly similar in composition and structure to meat there are a number of distinctive features. Protein content in fish fillet varies typically from 16 - 21%. The lipid content, which can be up to 67%, typically fluctuates between 0.2 - 20%, and is mostly interspersed between the muscle fibres.

Spoilage

Fish is a highly perishable food. The spoilage of fish is due to three causes.

1. **Microbiological:** While live fish is bacteriologically sterile, there are large number of bacteria on the surface slime and digestive tracts of living fish. When fish is killed, these bacteria multiply rapidly and attack all tissues. Since the bacteria live on the cold-blooded fish at rather low ocean temperatures, they are adapted to cold and continue to grow even under refrigerator conditions. Growth of micro-organisms and enzymes affect the quality.
2. **Physiological**: Fish struggle when caught and hence all the glycogen stores in the muscle and liver are used up. There is no glycogen left for being converted into lactic acid which helps to increase the pH of the tissues and retard the multiplication of microorganisms.
3. **Biochemical**: The important biochemical change leading to the development of the characteristic fishy off odour is due to the production of trimethylamine by the action of bacterial enzymes on phospholipids and choline present in fish. The fats present in fish are highly unsaturated. By the action of bacterial lipases and lipoxidases, free fatty acids are produced and the fat undergoes oxidative rancidity. This results in additional oxidised and rancid off-odours and off-flavours.

Initial Microflora

Some seafood commodities are inherently more risky than others owing to many factors, including the nature of the environment from which they come, their mode of feeding, the season during which they are harvested, and how they are prepared and served. Fish, mollusks, and crustaceans can acquire pathogens from various sources. All seafood can be susceptible to surface or tissue contamination originating from the marine environment. Bivalve mollusks feed by filtering large volumes of seawater. During this process, they can accumulate and concentrate pathogenic microorganisms that are naturally present in harvest waters, such as vibrios. Contamination of seafood by pathogens with a human reservoir can occur when growing areas are contaminated with human sewage. Outbreaks of seafood-associated illness linked to polluted waters have been caused by calicivirus, hepatitis A virus, and Salmonella enterica serotype Typhi (Desenclos et al, 1991)

Processing and its Effects on the Microflora

Wild finfish are usually caught by net, hook and line, or traps, with very little control over the condition of the fish at the time of death or the duration of the killing process. This contrasts greatly with the meat industry, in which the health of each animal can be assessed prior to

slaughter, and the killing process is designed to minimise stress. However, in recent decades, aquaculture practices have been expanding worldwide, offering better control of fish health prior to, and during harvest. The length of time that set nets have been in the water or the time trawlers' nets are towed, has an effect on the amount of stress and physical damage that the fish will suffer during capture. Physical damage such as loss of scales, bruising and bursting of the gut will increase the number of sites open for bacterial attack and spread. In addition, cortisone levels increase during prolonged stress and can alter the fillet quality (Ahmed F, 1992).

Spoilage

Food spoilage can be considered as any change that renders the product unacceptable for human consumption (Sivertsvik et al, 2004). Spoilage of fish starts upon death due to autoxidation (oxidation of unsaturated lipids), reactions caused by activities of the fish's own enzymes, and metabolic activities of microorganisms present in the fish. Over time, loss of the fresh characteristics may be simply measured by comparative visual and smell analysis. Loss of freshness and spoilage cannot be separated as processes, but it is a commonly held view that loss of freshness is related to autolytic degradation and spoilage is more microbial in origin (Huss, 1994).

Pathogens responsible for seafood Bacteria:

Vibrio species Vibrio organisms are Gram-negative, halophilic bacteria that are widespread and naturally present in marine and estuarine environments. Environmental factors influence their growth, and their numbers are highest when the water is warm. The genus Vibrio includes 30 species, of which at least 14 are recognized as pathogenic in humans. Vibrio infections are acquired through ingestion of contaminated seafood or through exposure of an open wound to seawater. Clinical features most often associated with V. parahaemolyticus infection include watery diarrhea, abdominal cramps, nausea, and vomiting; wound infections and septicemia occur less commonly (Levine et al, 1993).

Salmonella

Salmonellae are Gram-negative bacilli. Approximately 2,500 Salmonella serotypes have been identified, causing a variety of clinical syndromes ranging from asymptomatic carriage to invasive disease. Salmonella most commonly causes acute gastroenteritis, with symptoms including diarrhea, abdominal cramps, and fever. Other clinical manifestations can include enteric fever, urinary tract infections, bacteremia, and severe focal infections. Isolation of Salmonella organisms from cultures of stool, blood, or other clinical samples is diagnostic; isolates are referred to public health laboratories for serotype characterization. Salmonella is a leading cause of food-borne illness, causing approximately 1.4 million illnesses annually in the United States (Mead et al, 1997).

Shigella species

Shigella species are Gram-negative bacilli. Four species have been identified, and clinical presentations vary by species. Clinical manifestations of Shigella infection range from watery, loose stools to more severe symptoms, including fever, abdominal pain, tenesmus, and bloody diarrhea. Complications are rare and include seizures in young children, toxic megacolon, bacteremia, Reiter's syndrome, and hemolytic-uremic syndrome. Diagnosis is made by isolation of Shigella from feces or rectal swabs. Cases occur worldwide, in endemic and epidemic forms. Most cases occur among children aged

Clostridium botulinum

Clostridium botulinum is a spore-forming, anaerobic, Gram-positive bacillus that is widespread in the environment. The bacterium produces a potent neurotoxin under anaerobic, low-acid conditions. Seven types of botulism toxin have been identified; toxin types A, B, and E cause most human illnesses. Food-borne botulism is caused by the ingestion of food contaminated with preformed toxin produced by spores of C. botulinum. Botulism is characterized by an acute, symmetric, descending flaccid paralysis. Early signs and symptoms of botulism often include cranial nerve palsies, with diplopia, ptosis, slurred speech, and difficulty swallowing progressing to descending weakness and paralysis. Symptoms can progress to cause paralysis of the respiratory muscles, requiring ventilatory support. Cases of botulism are rare but serious; an estimated 60 food-borne cases occur each year in the United States. Most cases are sporadic, but food-borne botulism outbreaks are reported each year (Mead, 1999).

Fish Spoilage

Fish is a very perishable, high-protein food that typically contains a high level of free amino acids. The lipid content of the fish is up to 25%. It has very low content of connective tissue, i.e. approximately 3% of the total weight as compared with around 15% in meat. Fish flesh generally contains 15-20% protein and less than 1% carbohydrate. Non-fatty fish such as teleosts cod, haddock and whiting, the fat levels are only about 0.5%, while in fatty fish such as mackerel and herring, levels can vary between 3 and 25%.

Composition of a fish

Water 65 – 80 %

Fat 1 – 20 %

Protein 14 – 20 %

Microbes metabolize these amino acids, producing ammonia, biogenic amines such as putrescine, histamine, and cadaverine, organic acids, ketones, and sulfur compounds. Degradation of lipids in fatty fish produces rancid odors . In addition, marine fish and some freshwater fish contain trimethylamine oxide that is degraded by several spoilage bacteria to trimethylamine (TMA), the compound responsible for fishy off odors. Iron is a limiting nutrient in fish, and this favors growth of bacteria such as *pseudomonas* that produce siderophores that bind iron. Spoilage bacteria differ somewhat for freshwater and marine fish and for temperate and tropical water fish. Storage and processing conditions also affect microbial growth. *Pseudomonas* and *Shewanella* are the predominant species on chilled fresh fish under aerobic conditions. Packing under carbon dioxide and addition of low concentrations of sodium chloride favor growth of lactic acid bacteria and *Photobacterium phosphoreum*. Heavily wet-salted fish support growth of yeasts while dried and salted fish are spoiled by molds. Addition of organic acid select for lactic acid bacteria and yeasts. Pasteurization kills vegetative bacteria but spores of Clostridium and Bacillus survive and may grow, particularly in unsalted fish.

Spoilage of fish and sea foods : Halophilic bacteria like *Serratia*, *Micrococcus*, *Bacillus*, *Alcaligenes* and *Pseudomonas* cause spoilage of salt fish. Shell fish are spoiled by *Acenetobacter*, *Moraxella* and *Vibrio*. Crab meat is spoiled by *PseudomonasAcinetobacter* and *Moraxella* at low temperature and by *Proteus* at high temperature.

Microbial loads in shrimps, oysters, and clams depend on the quality of the water from which they are harvested. If the sewage is drained to water bodies, the microbial quality deteriorates. During handling, fecal coliforms, fecal streptococci, and *S. aureus* may be incorporated into the product. *Salmonella* also is found in oysters possibly due to contaminated water. Seafood also is the source for *Pseudomonas* spp., *C. perfringens*, *L. monocytogenes*, *Vibrio parahemolyticus*, *Salmonella enterica* serovar *enteritidis* and *typhimurium*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and Enteroviruses (Hepatitis A). Smoked salmon and shrimps also are found to carry pathogenic *L. monocytogenes*.

CONCLUSION

Our understanding of foods and pathogens responsible for illness is largely derived from information gained from outbreak investigations. However, many outbreaks likely go unrecognized and uninvestigated. Moreover, the Food-Borne Disease Outbreak Surveillance System is a passive system that relies on voluntary reporting, which may lead to further underestimation of the actual number of outbreaks and illnesses that occur. Outbreak reporting may not be uniform across states, which may be due in part to whether states have dedicated

food-borne disease epidemiologists. Also, outbreaks comprise only a small proportion of all cases of food-borne illness. No information is available on seafood-borne transmission in sporadic cases of infection other than Vibrio illnesses. Enhanced surveillance for food-borne outbreaks began in 1998. As a result, an abrupt increase in reported outbreaks occurred, which should not be interpreted as a true increase in the number of outbreaks. Also, improved laboratory methods and surveillance increased the ability to detect and investigate outbreaks during the study period.

MICROBIAL SPOILAGE OF CANNED FOODS

Introduction

Canning is one of important method of packaging food for long term storage. Normally food is stored in metallic containers along with heat treatment. The heat treatment varies depending upon type of food. There is always a chance that microorganisms may survive if the heat treatment is not proper thereby leading to spoilage of food. Usually the incidences of food spoilage in cans are low. The spoilage of can could be due to biological or chemical reasons or combination of both. The biological spoilage is primarily due to microbial growth while chemical spoilage is due to hydrogen produced due to reaction of acid in food and iron on can. The degree of swelling can also be increased by high summer temperature and high altitudes. Certain other factors such as overfilling, buckling, denting or closing the can while cool may also be responsible for spoilage of foods in cans.

Causes of Spoilage in Cans

Chemical spoilage

The chemical spoilage in most cases is due to production of hydrogen gas produced in can because of action of acid of food on iron of can. This spoilage is termed as Hydrogen swell. It occurs due to following factors:

a) Increased storage temperature.

b) Increased acidity of food

c) Improper exhaust

d) Presence of soluble sulfur and phosphorous compounds

e) Improper timing and lacquering of can at internal surfaces

Biological spoilage

The cause of biological spoilage is microbial activity. In heat treated cans, the growth of microorganisms occur due to:

Leakage of can

It occurs because of manufacturing defects, punctures or rough handling. Bacteria are introduced into can by either in holes or improper seams. In this type, the microorganisms are not usually heat resistant and wide array of organisms had been found to cause spoilage as it is post processing contamination. Microbes may also get entry into can due to cold water, used to cool cans after heat treatment. Leakage may also be responsible for release of vacuum, which can favor the growth of microorganisms. Presence of low heat resistance organisms usually indicates leakage of can.

Various stages of spoilage in can

Microbial Spoilage of Canned Foods

The microbial spoilage of canned food is classified as caused by thermophilic bacteria and mesophilic organisms. Most common spoilages of microbial origin are known as flat sour spoilage, Thermophilic anaerobic (TA) spoilage and putrefaction. These different types are briefly described here.

Spoilage by thermophilic spore forming bacteria

Spoilage by these types of bacteria is most prevalent in under processed heat treated canned foods. Their spores survive the heat treatment and undergo vegetative cell formation and subsequent growth in canned conditions. Major spoilages by these organisms are:

Flat sour spoilage

This is caused by souring bacteria. One characteristic of this spoilage is that ends of can remain flat during souring. Because of this condition, the detection of spoilage from outside is not possible thereby culturing of contents become necessary to detect the type of organisms. Main organisms involved are *Bacillus*, while it occurs more frequently in low acid foods. *Bacillus* spp. has ability to produce acid without gas formation.

TA spoilage

This type of spoilage is caused by thermophilic anaerobe not producing hydrogen sulfide. *Clostridium thermosaccharolyticum* is the main organism involved. It produces acid and gas in foods. Spoiled food produces sour or cheesy smell.

Sulfur stinker spoilage

This type of spoilage occurs in low acid foods and primarily *Desulfotomaculum nigricans* is involved. The spores of these organisms are destroyed at optimal heat treatment, thus presence of this organism usually indicates under processing in terms of heat treatment. It produces hydrogen sulfide which produce typical odour.

Spoilage By Mesophillic Spore formers

Bacillus and Clostridium are involved in this type of spoilage which is usually indicative of under spoilage.

MICROBIAL SPOILAGE OF FRUITS AND FRUIT JUICES

Introduction

Fruits are natural sources of minerals, vitamins besides carbohydrates and other essential substances. Naturally fresh fruits and juices made out of them contain high amount of water thereby making them highly prone to attack by microorganisms. While most of the fruits are naturally provided with coatings and coverings in the form of skins, but these are fragile enough to be easily disturbed by various biological and mechanical factors. Like vegetables, fruits being produce of plants get contaminated through different sources by a variety of microorganisms which may play significant role in their spoilage. These are soil, water, diseased plant, harvesting and processing equipments, handlers, packaging and packing material and contact with spoiled fruits.

Microorganisms Associated with Spoilage in Fruits and Juices

The microorganisms associated with fruits depend on the structure of fruit. The fruits contain different organic acids in varying amounts.The types of acids which are predominately found are citric acid, malic acid and tartaric acid. The low pH of fruits restricts the proliferation of various types of organisms. The pH and type of acids found in different fruits is given in Table 7.1.

Due to the low pH, a large number of microorganisms are restricted to grow on fruits. Fungi are most dominating organisms to grow on fruits because of the ability of yeasts

and molds to grow under acidic conditions. A small number of bacteria which are aciduric (ability to resist acidic conditions) also grow. Also the dry conditions prevailing on the skin and surface do not allow the growth of certain microorganisms. Besides these plants also produce certain antimicrobial components too.

Bacteria

Various groups of bacteria have ability to grow on fruits and its juices. These bacteria by virtue of their diversity in metabolism grow on fruits and produce different types of compounds. The major group of bacteria which are involved are:

- Lactic acid bacteria
- Acetic acid bacteria
- Spore formers

Lactic acid bacteria

The lactic acid bacteria are Gram-positive, catalase negative organisms which can grow under anaerobic conditions. These are rod-shaped (lactobacilli), or cocci (pediococci and leuconostocs) . The homofermentative species produce mainly lactic acid from hexose sugars; the heterofermenters produce one molecule of lactic acid, one molecule of carbon dioxide, and a two-carbon compound, which is usually acetic acid or ethanol or a combination of the two.

Growth of lactic acid bacteria in juices and other fruit products cause the formation of haze, gas, acid, and a number of other changes. Certain heterofermentative lactobacilli lead to slime in cider. The lactobacilli and leuconostocs that are present in citrus juices generate acetylmethylcarbinol and diacetyl, compounds that give the juices an undesirable, buttermilk-like flavor. Some strains, being extremely tolerant to ethanol grow in wines. *Lactobacillus fructivorans* can grow in appetizer and dessert wines containing as much as 20% ethanol. Lactic acid bacteria have the ability to decarboxylate malic acid to lactic acid. This malolactic fermentation is often desirable in high-acid wines because the acidity is reduced and desirable flavors are produced. *Oenococcus oenos* is the most acid and alcohol-tolerant species and often is isolated from wines that are undergoing a malo-lactic fermentation.

Acetic acid bacteria

These are Gram negative, aerobic rods having two genera, viz. *Acetobacter* and *Gluconobacter* . Both of these species oxidize ethanol to acetic acid under acidic condition, *Acetobacter* species can oxidize acetic acid to carbon

dioxide thus, the genus is called as over oxidizer. Because the bacteria are obligate aerobes, juices, wines, and cider are most susceptible to spoilage while held in tanks prior to bottling. Some strains of *Acetobacter pasteurianus* and *Gluconobacter oxydans* produce microfibrils composed of cellulose, which leads to formation of flocs in different fruit juice beverages.

Spore formers

Spores are heat resistant, so role of organisms producing spores is important in heat treated juices and beverages. Variuos spore formers such as *Bacillus coagulans, B. subtilis, B. macerans, B. pumilis, B. sphaericus* , and *B. pantothenticus* have been found to grow in different types of wines. Some of these organisms have also been involved in canned fruits. Spore-forming bacilli that actually prefer a low pH have been responsible for spoilage of apple juice and a blend of fruit juices.

MICROBIAL SPOILAGE OF VEGETABLES

Introduction

Vegetables form an integral part of diet due to their role in providing various types of vital nutrients such as carbohydrates, minerals, vitamins, roughage etc. Vegetables being a part of fresh produce, contain high moisture which makes them highly perishable foods and hence more prone to spoilage. Microorganisms gain entry into vegetables from various sources. These sources include:

- Soil
- Water
- Diseased plant
- Harvesting and processing equipments
- Handlers
- Packaging and packing material
- Contact with spoiled vegetables

The conditions in which vegetables are stored and transported after harvesting also contribute to rate of spoilage. Other than microbial, sources, the spoilage of vegetables can also occur due to the activity of native enzymes.

Types of Spoilage in Vegetables

The microbial spoilage of vegetables is predominately of following types

Spoilage due to pathogens

The plant pathogens which infect stem, leaves, roots, flowers and other parts or the fruit itself.

Spoilage due to saprophytes

Vegetables have general microflora inhabiting them. These organisms under certain conditions can grow on these vegetables and spoil them. The list of these organisms is given in Table 8.1. There are certain secondary invaders which may enter the healthy food or grow after growth of pathogens.

It is well known that plant diseases are mostly caused by fungi. Thus most of the spoilage causing pathogens in vegetables are fungi. Fungi have specific characteristics when spoiling food as it leads to mushy areas which may be water soaked. The fungi produce characteristic spores which may be pigmented. The pigmentation helps in identification of the type of spoilage by fungi. The bacterial diseases too cause spoilage of vegetables but to a lesser extent. Figure 8.1 represent bacterial and fungal diseases of tomato.

The major types of spoilages by pathogens in vegetables

Spoilage in vegetables is largely affected by composition of vegetable. The non acidic foods are thus spoiled by bacterial rot while acidic foods with dry surfaces are more prone to mold spoilage. The product on which organism grows and types of organisms growing largely determine the character of spoilage.

Bacterial Soft Rot

Caused by *Erwinia carotovora* and *Pseudomonas* such as *P. marginalis*. *Bacillus* and *Clostridium* spp. are also implicated.

Breaks down pectin, giving rise to a soft, mushy consistency, sometimes a bad odour and water-soaked apperance.

Vegetables affected- onions, garlic, beans, carrot, beets, lettuce, spinach, potatoes, cabbage, cauliflower, radishes, tomatoes, cucumbers, watermelons.

Some types of spoilage in vegetables by bacteria

Fungal spoilage of vegetables

Penicillium, Cladosporium, Rhizopus, Aspergillus spp. are responsible for various defects in vegetables.

Some types of spoilage in vegetables by fungi are shown in the Figure 8.8 to 8.13.

Gray mold rot – caused by *Botrytis cinera* in vegetables. Favoured by high humidity and warm temperature

CONTAMINATION OF POULTRy

1. Introduction

Poultry meat consumption is steadily increasing worldwide; the last data available indicate it reached 14.2 kg per capita per year [1]. The developed western countries, particularly the United States of America (USA), are the largest consumers, with 49.8 kg per inhabitant per year [1]. The same trend of consumption increase is observed in the European Union (EU) and in countries of the Organization for Economic Co-operation and Development (OECD). Similarly, poultry meat consumption has doubled in France over the past 30 years and has become the second most consumed meat since 2012, reaching more than 26 kg per capita in 2014 (close to the consumption reported for the EU) after pork meat (32.5 kg per capita) [2]. Among poultry meat products, chicken carcasses, cuts, and processed products are the most consumed (~75% of total poultry meat) followed by turkey (~25%) and, to a lesser extent, duck [3]. In France, 60% of the chicken meat is sold as fresh cuts [2], often stored under various modified atmosphere packaging (MAP) [4]. Vacuum packaging, MAP, chilling, or marinades are different practices for ensuring microbial quality during the storage of poultry cuts, and depend on consumer habits and countries

2. Sources of Contamination

While muscles are sterile in healthy living birds, various microbiotas are hosted in the digestive tract, lungs, skin, feathers, etc. In slaughterhouses, the surfaces, air (aerosols), and liquids also encompass bacteria. Therefore, carcasses and cuts after animal killing can be contaminated by animal and slaughterhouse environment microbiota. Figure 1 summarizes the different steps in poultry slaughtering and the associated contamination routes. Although there are some differences between the practices in large-scale commercial slaughterhouses and small-scale slaughtering facilities, the main steps of poultry slaughtering are similar [14]. Compared to the slaughtering process of mammals, the main differences to be noted for poultry slaughtering are (i) the use of a water bath (hot or chilled) at different stages of the process; (ii) the feather removal step, which can be mechanical and is performed differently from removing the skin of mammals; (iii) the small size of birds (compared to cattle or sheep, for example) which has consequences on the ease of carcass manipulation and the mechanization of some processes.

3. Bacterial Contaminants

The literature reports different practices for collecting bacteria from cuts or carcasses (rinsing, swabbing, stomaching) and the use of different media or incubation conditions which may lead

to different results. Nevertheless, to illustrate the diversity of the bacteria targeted in the literature, we list the results (enumerations in log CFU/g) of several studies carried out by cultural methods on chicken meat (Table 1), resulting in a global inventory of the contaminants that can be encountered. Total viable counts represent various bacterial species, increasing during storage, and varying considerably between samples. As an example, we have previously shown that total viable counts from chicken legs sampled after storage at 4 ◦C for 2/3rd of their shelf life varied from 3 to 8 log CFU/g.

4. Spoilage Bacteria

Growth of spoilage bacteria lead to defects in the products and can be responsible for unwanted taste, color, odor, texture, or aspect. There are multiple spoilage mechanisms, and they can result from the production of various metabolites such as volatiles or exopolysaccharides. Once bacteria contaminate meat and constitute the initial microbiota, the storage conditions and the various treatments applied shape the fate of this microbiota. The storage temperature as well as the nature and concentration of the gas used in gas mixtures for packaging are selective for some bacterial populations. Storage at low temperature favors the growth of psychrotrophic and psychrophilic bacteria while CO2 has an inhibitory effect on Pseudomonas spp. Some species can survive throughout the process, such as Shewanella putrefaciens, frequently found on carcasses during the slaughtering process and still present after 14 days of storage under air.

5. Variability of Bacterial Contaminants

Regarding Storage Temperature The importance of temperature for bacterial growth can be assessed at different critical points between the slaughtering and the consumption of the product, in particular (i) during carcass handling (the temperature in the processing plants is usually about 10 ◦C); and (ii) during the storage of meat products (with an estimation of a rupture in the cold chain between the time of sale and the consumer's fridge, whose temperature is estimated to be higher than 4 ◦C). The effect of chilling carcasses using chilled air or a cold water-bath on their microbial contaminants has been assessed [69]. Refrigeration by chilled air slows down the development of the total viable count (approximately 1 log) and causes a rapid decrease in temperature. This inhibits the multiplication of Salmonella and Campylobacter, thus chilled-air cooling would be more efficient. However, it is necessary to take the fact that Listeria can grow at this storage temperature into account. The product shelf life can be increased by storage at low temperature and the absence of a break in the cold chain [70]. The shelf life can even be doubled when the temperature is lowered to 3.4 ◦C compared to storage at 8.3 ◦C. Low temperatures delay the growth of Enterobacteriaceae, which can produce sulfuric compounds and the organoleptic deterioration of the meat quality. On the other hand, the growth of psychrotrophic bacteria is enhanced; at 4 and 7 ◦C, the total viable counts develop faster than at 0 ◦C [69]. A faster development of total viable counts is also observed at 10 ◦C when compared to 4 ◦C [45]. Storage at 4 ◦C is damaging for B. thermosphacta and S.

putrefaciens growth after 7, 10 or 14 days whereas A. hydrophila and A. sobria are psychrotrophic bacteria that can develop at low temperature [30]. Smolander et al. (2004) also pointed out, however, that the shelf life of products cannot be lengthened too much by storage at 0 ◦C, because pathogenic agents such as Listeria can multiply at these temperatures.

6.Variability of Bacterial Contaminants in Marinated Chicken and with Various Additives

The definition of marinade varies according to the country [46,75]. Marinades may be composed of a mixture of oil or salt and phosphates (in France and Spain, for instance) or a sauce with oil, organic acids, or spices, essential oil and thickener (Finland, China, and Italy). In all cases, marinades are associated with storage under different MAPs. The combination of oregano essential oil addition at 0.1% or 1% with MAP on the microbiological quality of chicken cuts has been studied [35]. The addition of 0.1% oregano essential oil increased the shelf life by 3–4 days, while the increase provided by the gas mixture (70% CO2−30% N2) was only 2–3 days. The combination of a marinade with oregano essential oil and storage under MAP showed that the two treatments could be added as the shelf life reached more than 20 days with a decrease in the total viable count of 2–3 log CFU/g [35]. In Finland, the consumption of marinated poultry products packaged under MAP is common and the effect of the marinade on their microbial safety has been well documented. The Finnish marinade can be complex as it is composed of acetic acid, honey, glucose, maltodextrin, NaCl, phosphate, rape Microorganisms 2017, 5, 50 9 of 16 seed oil, spices (sweet pepper, curry, black pepper, garlic and turmeric), thickener (guar gum and xanthan gum), and yeast extract [7]. Such marinades may influence the LAB population by favoring the growth of specific species, particularly because of the source of carbohydrates they provide [46]. The MAP commonly used in Finland is composed of 65% N2 and 35% CO2. The marinade favors a LAB psychrotrophic population, not detected in the unmarinated products [46]; especially L. gasicomitatum, also present in spoiled meat and seafood products [13] and in some vegetables associated with marinated fish products [76]. This bacterial species, unable to survive in the digestive tract of the animal, certainly originates from the environment and is adapted to the cold because it can persist throughout the transformation process [46]. As the combination of MAP and marinade favors the emergence of this group of bacteria, it is necessary to understand their mechanism of adaptation to monitor them in such products. It should be noted that the marinade had no effect on Campylobacter [46]. In a study combining the identification of isolates, as well as 16S rDNA gene pyrosequencing and metagenomics an overview of the effect of marinades on broiler fillet strip microbiology was reported [6]. Samples stored at 6 ◦C under MAP (65% N2−35% CO2) with and without marinade were compared. The combination of cultural and molecular methods confirmed that among LAB, marinade favored Leuconostoc and particularly L. gasicomitatum, and decreased B. thermosphacta, Clostridium spp., and Enterobacteriaceae. Among LAB belonging to the genus Carnobacterium, C. divergens was present in higher amounts than C. maltaromaticum, although both species seemed sensitive to marinade, certainly because of the presence of acetic acid.

Conclusion

The poultry meat sector tends to provide ready to eat products, which are safe for the consumer and have a long shelf life. The biological hazards associated with poultry meat production and consumption have been well identified, ranking Campylobacter spp. and Salmonella spp. as a high risk [93]. Such ranking took the severity of the illnesses caused by these pathogens, their impact on human health, the number of cases, and the occurrence of the risk in the poultry meat production chain into account. Consequently, the impact of various treatments (temperature, chemical treatment, marinade, or preservation processes) in reducing pathogens has been investigated. Many studies have also been conducted to test such treatments for extending the shelf life and avoiding spoilage.

UNIT IV

Egg

Freshly laid eggs are generally sterile particularly the inner contents. However the shells get contaminated from the environmental sources such as fecal matter of the bird, beddings, by the handlers and wash water and also the packaging materials in which the eggs are packed. There are several extrinsic and intrinsic mechanisms through which the egg protects itself from the microbial invasion. Waxy shell membrane retards the entry of microorganisms. Further, the shell also prevents the entry of microorganisms. The membranes inside the shell behave as mechanical barriers to the entry of microorganisms. Further lysozymes present in the egg white is effective against Gram positive bacteria and avidin in the egg white forms a complex with biotin, thus making it unavailable for the microorganisms. Also high pH (pH 9-10) of albumin inhibits the microbial growth. Binding of riboflavin by the apo protein and chelation of iron by conalbumin further helps in hindering the growth of microorganisms that might have gained entry inside the egg.

Spoilage of egg

Breaks or cracks in egg shell taking place due to transportation or mechanical damage may allow microorganisms to enter in to the egg yolk and cause spoilage on storage. Eggs on storage may lose moisture and, therefore, weight. The white of the egg becomes thinner and more watery on storage. The major changes in the egg take place due to spoilage organisms. In general the spoilage of eggs is caused by bacteria as compared to molds and can be described as green rot due to the growth of *Pseudomonas fluorescens,* colourless rot due to the growth of *Pseudomonas*, *Acinetobacter* and other species; black rots due to *P roteus, Pseudomonas;* red rots due to *Serratia* spp. and custrad rots due to *Proteus vulgaris* and Pseudomonas *intermedium*. Growth of *Aeromonas* in the egg yolk turns it to black colour and also there is strong putrid odour due to the formation of hydrogen sulphide (H_2S). Storage of eggs in high humid atmosphere may help in growth of several molds on the surface of the egg shell. Molds causing spoilage of eggs include species *of Pencillium*, *Mmucor, Alterneria*

CONTAMINATION OF EGG

Spoilage of eggs is promoted by cracking the eggshell, improper washing, and storage techniques. The most predominate spoilage (rot) of shell eggs is caused by

Gram-negative motile rods: *Pseudomonas*, *Proteus*, *Alcaligenes*, *Aeromonas*, and coliforms. Pasteurized egg products at refrigerated temperatures have limited shelf life, unless additional preservatives are used. The predominant bacteria causing spoilage in pasteurized egg products are psychrotrophic Gram-negative. Dried eggs are not susceptible to microbial spoilage due to low water activity. Examination of an unbroken egg with transmitted light using candle can identify grossly contamination of eggs or rotten egg. A great extent of pasteurization should be included during heat treatment of shell egg since the temperatures required for the killing of microorganisms are close to those at which the egg proteins coagulate. Fumigation of eggs with gaseous ethylene oxide before storage protects eggs against bacterial spoilage.

Eggs are one of nature's most nutritious and economical foods. But it's important that you take care when handling and preparing fresh eggs and egg products.

The inside of eggs that appear normal can contain a germ called *Salmonella* that can make you sick, especially if you eat raw or lightly cooked eggs. Eggs are safe when you cook and handle them properly.

- Consider buying and using pasteurized eggs and egg products, which are widely available.
- Keep eggs refrigerated at 40°F (4°C) or colder at all times. Only buy eggs from stores and suppliers that keep them refrigerated.
- Discard cracked or dirty eggs.

The inside of eggs that appear normal can contain a germ called *Salmonella* that can make you sick, but eggs are safe when you cook and handle them properly.
Poultry may carry bacteria such as *Salmonella*, which can contaminate the inside of eggs before the shells are formed. Egg shells may become contaminated with *Salmonella* from poultry droppings (poop) or the area where they are laid.

- Cook eggs until both the yolk and white are firm. Egg dishes should be cooked to an internal temperature of 160°F (71°C) or hotter.
- Make sure that foods that contain raw or lightly cooked eggs, such as hollandaise sauce, Caesar salad dressing, and tiramisu, are made only with pasteurized eggs.

- Eat or refrigerate eggs and foods containing eggs promptly after cooking. Do not keep eggs or foods made with eggs warm or at room temperature for more than 2 hours, or 1 hour if the temperature is 90°F or hotter.
- Wash hands and items that came into contact with raw eggs—including counter tops, utensils, dishes, and cutting boards—with soap and water.

Wash hands and items that came into contact with raw eggs, including countertops, utensils, dishes, and cutting boards, with soap and water.

Illness from *Salmonella* can be serious and is more dangerous for certain people.

Adults older than 65 years, children younger than 5 years, and people with weakened immune systems, such as those with HIV/AIDS, diabetes, or an organ transplant, may get a more serious illness that can even be life threatening.

In most cases, illness lasts 4-7 days after eating a contaminated food. Symptoms include:

- Diarrhea
- Vomiting
- Fever
- Abdominal cramps

Symptoms typically appear 6 hours to 6 days after eating a contaminated food. Some people can have diarrhea many times a day for several days and the sick person may need to be hospitalized.

SPOILAGE OF MIK

Milk spoilage is an indefinite term and difficult to measure with accuracy. This uncertainty can cause suffering for both milk manufacturers and consumers. Consumers who have been misled by ambiguous expiration dates on milk cartons waste resources by disposing of unspoiled milk or experience discomfort from drinking spoiled milk. Consumers are often unwilling to purchase products close to their inaccurate expiration dates. This consumer behavior has a negative financial impact on milk producers. Inaccurate milk spoilage detection methods also force milk producers to use overly conservative expiration dates in an effort to avoid the legal and economic

consequences of consumers experiencing illness from drinking spoiled milk. Over the last decade, new methods have been researched with the purpose of developing more accurate and efficient means of detecting milk spoilage. These methods include indicators based on pH bacteria counts and gas-sensor arrays. This article explores various methods of spoilage detection designed to prevent such consequences. The respective level of effectiveness of each method is discussed, as well as several further approaches to contain freshness regardless of detection.

2. Current Methods of Milk Spoilage Detection

2.1. Utilizing pH Indicators as a Measure of Spoilage Bacteria growth varies from one species of bacteria to another. While one bacteria species may prosper under certain conditions, another species may weaken. These conditions are interdependent and include nutrient availability, moisture, oxygen levels and the level of other gases, the presence of inhibitors, temperature, and pH [8]. The pH of unspoiled milk is approximately 6.7, a level at which many forms of bacteria thrive [9]. At lower pH levels of 4.0 - 5.0, lactic acid bacteria can grow and produce lactic acid. While these organisms inhibit the growth of many pathogenic bacteria and are also intentionally employed to ferment milk to make other dairy products such as yogurt and cheese, they can also induce undesirable spoilage in certain products. Coliforms, a common form of bacteria, have been an indicator of the presence of pathogens in assessing the contamination of water as well as dairy products [10]. Coliforms can cause rapid spoilage in milk because they ferment lactose with the production of acid and gas, and they can also degrade milk proteins. Escherichia coli is a well-known example of a coliform [11]. Studies have shown that other properties of milk also promote bacteria growth, such as the high availability of moisture and dissolved oxygen which supports both aerobic and facultative anaerobic microorganisms [12]. Temperature is frequently controlled to limit bacteria growth. Extreme heat is lethal to many organisms, such as coliforms, which explains the process of milk pasteurization (63˚C for 30 minutes). Two types of bacteria exist in pasteurized milk: thermoduric bacteria, which are capable of surviving the extreme heat during pasteurization, and bacteria that originate from unsanitary conditions postpasteurization [13]. Psychrotrophs comprise the largest percentage of bacteria in milk and cause spoilage in refrigerator temperatures at or below 7˚C [14]

2.2. Electrical Methods for the Detection of Bacteria

Some traditional methods of detection involve bacterial enumeration, in which spoilage is detected when increased metabolism caused by multiplying bacteria renders a colored solution colorless. The methylene blue reduction test is such an example; however, known flaws of this test include time-consuming and redundant procedures, as well as an inability to discriminate between bacterial types [19]. Lee et al. (2009) sought to improve upon the methylene blue reduction method while maintaining its advantages by supplementing it with an amperometric

sensor. An amperometric sensor, composed of a circuit with a potentiostat and a pair of electrodes, measures current change [20]. Amperometric sensors are small and inexpensive and have been tested in a variety of media to detect changes in bacteria such as E. coli.

3. Comparison of Milk Spoilage Detection Methods

Accuracy, range, usability, speed, and cost are of particular importance when choosing a milk spoilage detection method because these factors impact the feasibility and marketability of packaging products. Table 3 compares available milk spoilage detection methods in terms of those five characteristics:

CONCLUSION

Existing standards for spoilage detection at milk plants are obsolete. Often, plants use rudimentary methods such standard plate count (SPC) to determine and detect bacterial concentrations in post-pasteurized milk. These methods are time-consuming and cumbersome, especially when compared to those used by other food industries. For example, the meat and fish industries have advanced to adopting technologies such as infrared spectroscopy to monitor the quality of the products. Recent research has illuminated a variety of other methods that can streamline the bacterial detection process. Broadly speaking, these methods include detection based on the pH, current change, volatile compounds, and lipid and protein levels.

fats and oils

1. **Flavor reversion**
    1. An objectionable flavour found before the onset of rancidity in refined oils when exposed to UV light, visible light or heating.
    2. The reaction is catalysed by O2 and small amounts of metals such as iron and copper
    3. Fats containing nucleic acid are most susceptible to reversion.
    4. Soya bean oil is mostly subjected to flavor reversion.

    Prevention of reversion
    5. Hydrogenation
    6. Small amount of linolenic acid prevents reversion.
    7. Metallic activators or sequestrants tie up to iron and copper and prevents revertion in soya oil.

2. **Rancidity**
    1. Occurs mostly in fats containing unsaturated fatty acids.

    Hydrolytic rancidity

2. Occurs due to enzymes that decomposes fat in to free fatty acids (FFA) and glycerol.
3. Butyric acid and caproic acids are the volatile fatty acids, predominately present in butter are responsible for rancid flavour or odour in butter and makes butter inedible.
4. Long chain fatty acids such as stearic, palmitic and oleic acids do not produce rancidity unless oxidation occurs.
5. Heating thoroughly to destroy lipase catalyses hydrolysis of trans fats and prevents hydrolytic rancidity.
6. Certain microorganisms also produces lipase.

Oxidation

7. Unsaturated fats have lipoxygenase and are susceptible to oxidative changes.
8. Highly hydrogenated saturated fatty acids are resistant to oxidation.
9. Hydro peroxides that are formed readily producing smaller volatile substances will give characteristic odours of rancid fat.

Characteristics of Rancidity

10. Undesirable changes in odour, flavour, colour and consistency
11. Inactivates vitamin A & E
12. Oxidative rancidity may be a problem in dry foods containing only small quantities of fat such as prepared cereals.

Prevention of rancidity

13. Storage at refrigerator temperature prevents rancidity.
14. Light coloured glass containers absorb active rays and gives protection against spoilage.
15. Certain shades of green bottles, cora papers, yellow transparent cellophane etc. prevents rancidity.
16. Vacuum packaging
17. Anti-oxidants naturally present in food such as vitamin 'C', β-carotene and vitamin E.
18. Added antioxidants such as
    1. Butylated hydrogen anisole (BHA)
    2. Tertiary butly hydro quivous (TBHQ)
    3. Propyl gallate
19. synergists or sequestering agents – citric acid – bind or chelate the metals and prevents oxidation process.

FACTORS FAVOURING FUNGAL SPOILAGE

Pitt and Hocking (1999) mentioned that a plethora of factors can contribute to the contamination of various milk products by fungi. 1. If the premises of milk processing plants are unsanitary. 2. If pasteurization temperature is inadequately maintained. 3. If the good manufacturing practice is not followed. 4. If the raw materials are of poor quality. 5. If the

equipments and utensils are not properly sterilized. 6. If the preservation is omitted. 7. If the water is unwholesome, unsafe and non-potable. 8. If the sugar, fruits, nuts, and additives are contaminated. 9. If the packaging material is not properly cleaned.

FUNGAL SPOILAGE OF MILK PRODUCTS Fungi are eukaryotic, Gram positive, non-acid fast, heterophilic, non-photosynthetic, osmotrophic, and saprobic living microbe (Pal, 2007). Presently, over 250,000 fungi are present in our environment (Pal, 2007).The fungi are ubiquitous in distribution, and are found in the soil, water, and air (Pal, 2007).The fungi which include moulds and yeasts are responsible for the spoilage of milk and milk products (Pal,2007;Pal and Jadhav,2013). Moulds are filamentous fungi with branching hyphae, multi-cellular, generally aerobic and grow at a pH range of 3 to 8.The spores can tolerate harsh environmental conditions but sensitive to heat treatment. Acremonium, Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Mucor, Penicillium etc., are some of the examples of moulds (Pal,2007).Yeasts are non-filamentous, unicellular, circular to oval shape ,and are generally facultative anaerobe. The common examples of yeasts are Candida, Deberomyces, Hansenula, Kluveromyces, Pichia, Rhodotorula, Saccharomyces,and Trichoporon (Pal,2007). In this context, Pitt and Hocking (1999) reported that 99 yeast species occur in foods including milk products. Moulds are sometimes seen on the surfaces of butter, cheese, khoa and old cream. They often grow in large numbers which are visible as fluffy growth; and discolour the milk products. They are present in the air and therefore, it is easy that milk products (butter) get contaminated by moulds. Mould growth in butter is an indication of high moisture content in it. Furthermore, high acidity of butter favours the growth of moulds. Dark patches inside the butter is suggestive of Cladosporium. Penicillium is the most commonly occurring mould on cheese. Yeasts can produce pink spots on the surface of butter. If the 'ghee' (butter oil) , an Indian milk product, is not properly packed, moulds such as Aspergillus and Penicillium can contaminate it.

Detection of Fungal Spoilage of Dairy Products

The mycological analysis of milk products is conducted using conventional methods as described by APHA (1992). A number of media such as malt extract agar, Sabouraud dextrose agar with chloramphenicol, yeast extract peptone glucose agar, potato dextrose agar and Pal sunflower seed medium can be used to recover fungi from milk and dairy products (Pal,2007; Khalifa et al., 2013; Adebayo et al., 2014). The total fungal count of the food sample is determined by pour plate method employing standard mycological techniques. The fungal counts are expressed as the colony forming units per milliliter (CFU/mL) and propagules and cells/mL (Adebayo et al., 2014).The detailed morphology of fungi is studied in a newly discovered stain designated as "Narayan" (Pal,2004).This stain contains 0.5 ml of methylene blue (3% aqueous solution), 6.0 ml of dimethyl sulfoxide (DMSO), and 4.0 ml of glycerine (Pal, 2004)

MICROBIAL SPOILAGE OF BOTTLE BEVERAGES

Introduction

Soft drinks and fruit juices represent an important market within the food industry. The increasing variety of products being released at a bewildering rate has altered the potential for spoilage problems. Soft drinks are generally nutrientpoor media that are spoiled by relatively few organisms – usually yeasts, and a few acid-tolerant bacteria and fungi. Carbonation shifts the spoilage flora to those organisms tolerant of carbon dioxide. Soft drinks enhanced by the addition of low levels of fruit juice tend to exhibit similar spoilage flora to fruit juices. The use of ever more exotic raw ingredients may lead to the discovery of unusual spoilage organisms in the future. Yeasts in general, and Zygosaccharomyces bailii in particular, remain the key spoilage organisms because of their overall physiology and resistance to organic acid preservatives (Stratford et al., 2000). Microbial problems within soft drinks and fruit juices can be divided into two groups: (1) growth in, and deterioration of, the product by general organisms to produce spoilage; (2) growth in, or contamination of, the product by pathogens to produce food poisoning.

Composition of soft drinks and fruit juices in relation to spoilage

There is a bewildering variety of soft drink and fruit juices for sale, and many methods for their manufacture. Soft drinks can be non-carbonated, carbonated, 280 CHEMISTRY AND TECHNOLOGY OF SOFT DRINKS AND FRUIT JUICES with or without added fruit juice, often with the addition of organic acid preservatives. They can be filled on standard or clean fill lines. Fruit juices, fruit juice concentrates and fruit nectars may be fresh, unpasteurised and clean filled, or pasteurised, then hot, aseptic, or clean filled (Stratford et al., 2000; Stratford and James, 2003). Recent technology using ultra-high pressure has been used to produce 'cold pasteurised' fruit juices. These have the advantage of a fresh juice mouthfeel, but with destruction of pathogens and the majority of spoilage agents, enhancing the shelf life of an essentially fresh product (Mermelstein, 1999; Zook et al., 1999). Simple soft drinks such as orangeade and lemonade are too acidic for the growth of most organisms, so that spoilage is generally by carbonation-resistant species such as Dekkera anomala (Stratford and James, 2003). Yeasts usually require a carbon source such as a hexose sugar, a nitrogen source such as amino acids or ammonium salts, simple salts (phosphate, sulphate, potassium and magnesium ions), trace minerals and vitamins. Some yeasts have particular sugar requirements; for example, Z.bailii and Z.rouxii cannot utilise sucrose (Pitt & Hocking, 1997; Stratford et al., 2000).

Background microbiology – spoilage

Many micro-organisms are found in soft drinks as environmental or raw material contaminants, but relatively few can grow within the acidic and lowoxygen environment. Yeasts are the most significant group of micro-organisms associated with spoilage of soft drinks and fruit juices.

Spoilage will be seen as the growth and production of metabolic byproducts, for example, CO2, acid, and tainting compounds. As noted above, most spoilage is therefore by yeasts and mould species, with yeasts most important, and some spoilage is by acidtolerant bacteria (Hocking & Jensen, 2001; Jay & Anderson, 2001).

Fruit and fruit juices are commonly contaminated with yeasts and moulds, often from insect damage. Fallen fruit should thus be avoided where possible, for all of the risks outlined below. Sugars and sugar concentrates are commonly contaminated with osmophilic yeasts, for example Z.rouxii. Growth is slow in concentrated solutions, but one cell per container of diluted stock is enough to cause spoilage (Davenport, 1996). Flavourings, water and other chemicals are all potential sources of microbial contamination. Process machinery and filling lines are particularly problematic and strict hygiene is essential

Mycotoxins

Mycotoxins are toxic secondary metabolites produced by fungi growing within or on foods. They can be a serious threat to human and animal health (Nagler et al., 2001). Table 11.4 details mycotoxins associated with soft drinks and fruit juice manufacture and raw materials. Patulin is the most common mycotoxin associated with fruit juice, particularly apple juice (Pitt & Hocking, 1997). It commonly occurs if juice is produced from stored apples. Mould growth in infected apples increases with time, raising levels of patulin. The use of windfall apples for juice is also a factor. Avoidance of windfall apples, filtration of juice and pressing quickly after harvest are all methods to reduce the incidence of patulin in juice. Patulin can be destroyed by fermentation to cider or by the addition of ascorbic acid (Marth, 1992). Within Europe, the European Union has set a limit of 50 g/kg for patulin in both apple juice and cider. A recent survey of apple products in Chile found that 28% of samples of juice and concentrate exceeded this limit (Canas & Aranda, 1996)

FOOD BORNE DISEASE

Foodborne illness (also **foodborne disease** and colloquially referred to as **food poisoning**)[1] is any illness resulting from the spoilage of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food,[2] as well as toxins such as poisonous mushrooms and various species of beans that have not been boiled for at least 10 minutes.

Symptoms vary depending on the cause, and are described below in this article. A few broad generalizations can be made. For contaminants requiring an incubation period, symptoms may not manifest for hours to days, depending on the cause and on quantity of consumption. Longer incubation periods tend to cause sufferers to not associate the symptoms with the item consumed, so they may misattribute the symptoms to gastroenteritis, for example.

Symptoms often include vomiting, fever, and aches, and may include diarrhea. Bouts of vomiting can be repeated with an extended delay in between, because even if infected food was eliminated from the stomach in the first bout, microbes, like bacteria (if applicable), can pass through the stomach into the intestine and begin to multiply. Some types of microbes stay in the intestine.

CAUSES

Foodborne illness usually arises from improper handling, preparation, or food storage. Good hygiene practices before, during, and after food preparation can reduce the chances of

contracting an illness. There is a consensus in the public health community that regular hand-washing is one of the most effective defenses against the spread of foodborne illness. The action of monitoring food to ensure that it will not cause foodborne illness is known as food safety. Foodborne disease can also be caused by a large variety of toxins that affect the environment.[3]

Furthermore, foodborne illness can be caused by pesticides or medicines in food and natural toxic substances such as poisonous mushrooms or reef fish.

Most common bacterial foodborne pathogens are:

- *Campylobacter jejuni* which can lead to secondary Guillain–Barré syndrome and periodontitis[6]
- *Clostridium perfringens*, the "cafeteria germ"[7][8]
- *Salmonella* spp. – its *S. typhimurium* infection is caused by consumption of eggs or poultry that are not adequately cooked or by other interactive human-animal pathogens[9][10][11]
- *Escherichia coli O157:H7* enterohemorrhagic (EHEC) which can cause hemolytic-uremic syndrome

Other common bacterial foodborne pathogens are:

- *Bacillus cereus*
- *Escherichia coli*, other virulence properties, such as enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroaggregative (EAEC or EAgEC)
- *Listeria monocytogenes*
- *Shigella* spp.
- *Staphylococcus aureus*
- *Staphylococcal enteritis*
- *Streptococcus*
- *Vibrio cholerae*, including O1 and non-O1
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*
- *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Less common bacterial agents:

- *Brucella* spp.
- *Corynebacterium ulcerans*
- *Coxiella burnetii* or Q fever
- *Plesiomonas shigelloides*

Enterotoxins*[edit]*

See also: Botulism

In addition to disease caused by direct bacterial infection, some foodborne illnesses are caused by enterotoxins (exotoxins targeting the intestines). Enterotoxins can produce illness even when the microbes that produced them have been killed. Symptom appearance varies with the toxin but may be rapid in onset, as in the case of enterotoxins of *Staphylococcus aureus* in which symptoms appear in one to six hours.[12] This causes intense vomiting including or not including diarrhea (resulting in staphylococcal enteritis), and staphylococcal enterotoxins (most commonly staphylococcal enterotoxin A but also including staphylococcal enterotoxin B) are the most commonly reported enterotoxins although cases of poisoning are likely underestimated.[13] It occurs mainly in cooked and processed foods due to competition with other biota in raw foods, and humans are the main cause of contamination as a substantial percentage of humans are persistent

carriers of *S. aureus*.[13] The CDC has estimated about 240,000 cases per year in the United States.[14]

- *Clostridium botulinum*
- *Clostridium perfringens*
- *Bacillus cereus*

Preventing bacterial food poisoning[edit]

Prevention is mainly the role of the state, through the definition of strict rules of hygiene and a public services of veterinary surveying of animal products in the food chain, from farming to the transformation industry and delivery (shops and restaurants). This regulation includes:

- traceability: in a final product, it must be possible to know the origin of the ingredients (originating farm, identification of the harvesting or of the animal) and where and when it was processed; the origin of the illness can thus be tracked and solved (and possibly penalized), and the final products can be removed from the sale if a problem is detected;
- enforcement of hygiene procedures such as HACCP and the "cold chain";
- power of control and of law enforcement of veterinarians.

In August 2006, the United States Food and Drug Administration approved Phage therapy which involves spraying meat with viruses that infect bacteria, and thus preventing infection. This has raised concerns, because without mandatory labelling consumers would not be aware that meat and poultry products have been treated with the spray.[15]

At home, prevention mainly consists of good food safety practices. Many forms of bacterial poisoning can be prevented by cooking food sufficiently, and either eating it quickly or refrigerating it effectively.[2] Many toxins, however, are not destroyed by heat treatment.

Techniques that help prevent food borne illness in the kitchen are hand washing, rinsing produce,[16] preventing cross-contamination, proper storage, and maintaining cooking temperatures. In general, freezing or refrigerating prevents virtually all bacteria from growing, and heating food sufficiently kills parasites, viruses, and most bacteria. Bacteria grow most rapidly at the range of temperatures between 40 and 140 °F (4 and 60 °C), called the "danger zone". Storing food below or above the "danger zone" can effectively limit the production of toxins. For storing leftovers, the food must be put in shallow containers for quick cooling and must be refrigerated within two hours. When food is reheated, it must reach an internal temperature of 165 °F (74 °C) or until hot or steaming to kill bacteria.[17]

Mycotoxins and alimentary mycotoxicoses[edit]

The term alimentary mycotoxicosis refers to the effect of poisoning by mycotoxins through food consumption. The term mycotoxin is usually reserved for the toxic chemical products produced by fungi that readily colonize crops. Mycotoxins sometimes have important effects on human and animal health. For example, an outbreak which occurred in the UK in 1960 caused the death of 100,000 turkeys which had consumed aflatoxin-contaminated peanut meal. In the USSR in World War II, 5,000 people died due to alimentary toxic aleukia (ALA).[18] The common foodborne Mycotoxins include:

- Aflatoxins – originating from *Aspergillus parasiticus* and *Aspergillus flavus*. They are frequently found in tree nuts, peanuts, maize, sorghum and other oilseeds, including corn and cottonseeds.

The pronounced forms of Aflatoxins are those of B1, B2, G1, and G2, amongst which Aflatoxin B1 predominantly targets the liver, which will result in necrosis, cirrhosis, and carcinoma.[19][20] In the US, the acceptable level of total aflatoxins in foods is less than 20 µg/kg, except for Aflatoxin M1 in milk, which should be less than 0.5 µg/kg.[21] The official document can be found at FDA's website.[22][23]

- Altertoxins – are those of alternariol (AOH), alternariol methyl ether (AME), altenuene (ALT), altertoxin-1 (ATX-1), tenuazonic acid (TeA), and radicinin (RAD), originating from *Alternaria* spp. Some of the toxins can be present in sorghum, ragi, wheat and tomatoes.[24][25][26] Some research has shown that the toxins can be easily cross-contaminated between grain commodities, suggesting that manufacturing and storage of grain commodities is a critical practice.[27]

AIR BRONE DISEASE

Each day people are exposed to millions of bioaerosols, including whole microorganisms, which can have both beneficial and detrimental effects. The next chapter in understanding the airborne microbiome of the built environment is characterizing the various sources of airborne microorganisms and the relative contribution of each. We have identified the following eight major categories of sources of airborne bacteria, viruses, and fungi in the built environment: humans; pets; plants; plumbing systems; heating, ventilation, and air-conditioning systems; mold; dust resuspension; and the outdoor environment. Certain species are associated with certain sources, but the full potential of source characterization and source apportionment has not yet been realized. Ideally, future studies will quantify detailed emission rates of microorganisms from each source and will identify the relative contribution of each source to the indoor air microbiome. This information could then be used to probe fundamental relationships between specific sources and human health, to design interventions to improve building health and human health, or even to provide evidence for forensic investigations.

Humans as sources of airborne microorganisms

As humans carry 1012 microorganisms on their epidermis and 1014 microorganisms in their alimentary tract, we might be one of the greatest sources of bioaerosols in the built environment [32]. Respiration and the shedding of millions of skin cells daily contribute to bioaerosols in the built environment. In fact, human occupancy might be the most important factor affecting the total number and community structure of bioaerosols present in the built environment, especially in poorly ventilated or heavily occupied environments [30]. Qian et al.

UNIT V

TREATMENT AND DISPOSAL OF WASTE WATER

Introduction

With increase in demand for milk and milk products, many dairies of different capacities have come up in different places. These dairies collect milk from the producers and then either simply bottle it for marketing, or produce different milk products according to their capacities. Large quantities of waste water originate due to their different operations. The organic substances in the wastes comes either in the form in which they were present in milk or in a degraded form due to their processing. As such, the dairy waste, though biodegradable, are very strong in nature.

Dairy plants process a wide variety of products including milk, cheese, butter, ice cream, yogurt, nonfat dry milk, whey and lactose. The volume and composition of dairy wastes from each plant depends on the types of products produced, waste minimization practices, types of cleaners used and water management in the plant. Because most dairy plants process several milk products, waste streams may vary widely from day to day.

32.2 Sources of Dairy Wastes

The liquid waste from a large dairy originates from the following sections or plants: receiving stations, bottling plant, cheese plant, casein plant, condensed milk plant, dried milk plant, and ice cream plant. The main sources of dairy effluents are those arising from the following:

1. Spills and leaks of products or by-products
2. Residual milk or milk products in piping and equipment before cleaning
3. Wash solutions from equipment and floors
4. Condensate from evaporation processes
5. Pressings and brines from cheese manufacture

Dairy plant operators may choose from a wide variety of methods for treating dairy wastes from their plants. This may range from land application for small plants to operation of biological waste water treatment systems for larger plants. Some dairy plants may pretreat the effluents and discharge them to a municipal waste water treatment plant.

In addition to the wastes from all the above milk processing units, some amount of uncontaminated cooling water comes as waste; these are very often re-circulated.

32.3 Objectives of Treating Dairy Wastes

The objectives of treating dairy wastes are to

a. Reduce the organic content of the waste water,

b. Remove or reduce nutrients that could cause pollution of receiving surface waters or ground water, and

c. Remove or inactivate potential pathogenic microorganisms or parasites.

The level of treatment needed for dairy waste water for each plant is dictated by the environmental regulations applicable to the location of the dairy plant. The Environmental Protection Agency (EPA) establishes general regulations concerning discharges to surface waters and ground water. Each state environmental regulatory agency is responsible for ensuring compliance with those regulations. Each plant must have a discharge permit for each outfall discharging to surface waters. The limits within that permit depend on the flow and type of surface water into which the treated waste water is discharged. If a plant discharges waste water to municipal sewers for treatment, the municipal treatment system may require pretreatment of high-strength wastes to bring the waste load down to domestic sewage strength. This allows for proper treatment of waste water before it is discharged to surface water. For land applications, state regulatory agencies dictate hydraulic loadings and maximum levels of toxic substances that can be spread on each unit of land.

Composition of waste water

Treatment of Milk Wastes

Wastes from processing milk products are almost entirely composed of organic material in solution or colloidal suspension, although some larger suspended solids may be present in waste water from cheese or casein manufacturing plants. Sand and other foreign material is present in limited amounts as a result of floor or truck washes. Because milk waste contains very little suspended matter, preliminary settling of solids does not result in any appreciable reduction of BOD.

However, a screen and grit chamber with 0.95-cm mesh wire screen is recommended to remove large particles to prevent clogging of pipes and pumps in the treatment system. This is especially important, if the waste is to be pumped with high-pressure pumps, as in spray irrigation. After preliminary treatment in the screen and grit

chamber, the waste should be pumped to an equalization tank. With wide variations in waste water flow, strength, temperature, and pH, some reaction time is required to allow neutralization of acid and alkaline cleaning compounds and to allow for complete reaction of residual oxidants from cleaning solutions with organic solids of dairy waste. Ideally, a minimum of 6–12 h of equalization should be provided to allow for waste stabilization. The equilibrated waste can then be treated with one of the following systems or a combination of treatment systems: (a) land application, (b) treatment ponds or lagoons, (c) activated sludge, (d) biological filtration, or (e) anaerobic digestion.

32.6 Treatment Ponds or Lagoons

Dairy plants in rural areas with insufficient farmland available for land application may be able to use ponds or lagoons for economical treatment of dairy wastes. A pond or lagoon normally consists of a shallow basin designed for treatment of dairy wastewater without extensive equipment and controls. The three types of ponds used are aerobic, facultative, and anaerobic.

32.7 Aerobic Ponds

Aerobic ponds are generally 0.5–2.0 m deep, and contents are mechanically mixed and aerated to allow penetration of sunlight necessary for growth of algae. The algae produce oxygen through photosynthesis and use waste products from the bacteria involved in the biological breakdown of milk wastes. At 20°C, a BOD removal of 85% can be experienced with an aeration period of 5 days.

32.8 Anaerobic Ponds

Anaerobic ponds are generally used to pretreat dairy wastes with high protein and fat levels or for stabilizing settled solids. Organic matter is biodegraded and gases such as CH4, CO2, and H2S are produced. To reduce effectively the BOD in anaerobic effluent, an aerobic process must follow to allow aerobic microorganisms to use up the residual breakdown products. The typical retention time for anaerobic treatment ponds ranges from 20 to 50 days.

32.9 Activated Sludge

Activated sludge is one of the most popular methods for treating dairy wastes. The process consists of aerobic oxidation of organic matter to CO_2, H_2O, NH_3, and cell biomass followed by sedimentation of activated sludge. A portion of the activated sludge is returned to the aeration tank to continue the treatment cycle (Figure.32.1.).

Sterilization (Retorting)

Sterilization destroys all pathogenic and spoilage microorganisms in foods and inactivates enzymes by heating. All canned foods are sterilized in a retort (a large pressure cooker) and called commercial sterilization which indicates that no viable organisms are present. This process enables food to have a shelf life of more than two years. Foods that have a pH of more than 4.6, such as meat and most vegetables must undergo severe heating conditions to destroy all pathogens. These foods are heated under pressure to 121°C for varying times. Severe conditions are applied primarly to ensure that *Clostridium botulinum* spores are destroyed during processing. These spores produce the deadly botulinum toxin under anaerobic conditions (that is, where there's no oxygen). The spores are destroyed by heat or are inhibited at pH values of less than 4.6 Therefore, a food with a pH of less than 4.6 that is packaged anaerobically, such as spaghetti sauce, doesn't need to undergo such a severe heat treatment. The destruction of vegetative and sporeforming organism and pathogens is secondary objective of commercially sterilized foods.

Nicolas Appert , a Parisian confectioner by trade, established the heat processing of foods as an industry in 1810. The food product is washed, sorted, and graded and then subjected to steam for three to five minutes. This last process called blanching, destroys many enzymes in the food product and prevents further cellular metabolism. The food is then peeled and cored, and diseased portions are removed. For canning, containers are evacuated and placed in a pressurised steam steriliser, similar to an autoclave at 121°C. This removes especially *Bacillus* and *Clostridium* spores. If canning is defective, foods may become contaminated by anaerobic, bacteria which produce gas. These are species of *Clostridium*, and coliform bacteria (a group of Gram-negative non spore-forming rods which ferment lactose to acid and gas at 32°C in 48 hours).

Canning cooking fruits or vegetables, sealing them in sterile cans or jars, and boiling the containers to kill or weaken any remaining bacteria as a form of pasteurization. High-acid fruits like strawberries require no preservatives to can and holding for only a short boiling cycle, whereas marginal fruits such as tomatoes require longer boiling and addition of other acidic elements. Many vegetables require pressure canning. Food preserved by canning or bottling is at immediate risk of spoilage once the can or bottle has been opened. Lack of quality control in the canning process may allow

ingress of water or micro-organisms. *Clostridium botulinum* produces an acute toxin within the food and may lead to severe illness or death. This organism produces no gas or obvious taste and remains undetected by taste or smell. Food contaminated in this way include Corn, beef and Tuna.

In canning process heat is applied to food that is sealed in a jar in order to destroy any microorganisms that can cause food spoilage. Proper canning techniques stop this spoilage by heating the food for a specific period of time and killing these unwanted microorganisms. During the canning process, air is driven from the jar and a vacuum is formed as the jar cools and seals.

Water-bath canning and pressure canning are two approved methods of canning.

12.14 Water-Bath Canning

This method sometimes referred to as *hot water canning,* uses a large kettle of boiling water (Figure 12.4). Filled jars are submerged in the water and heated to an internal temperature of 212°F for a specific period of time. This method is used for processing high-acid foods, such as fruit, items made from fruit, pickles, pickled food, and tomatoes.

Fig. 12.4 Water bath canning kettle

12.15 Pressure Canning

Pressure canning uses a large kettle that produces steam in a locked compartment (Figure 12.5). The filled jars in the kettle reach an internal temperature of -240 °C under a specific pressure (stated in pounds) that is measured with a dial gauge or weighted gauge on the pressure-canner cover. A pressure canner should be used for processing vegetables and other low-acid foods, such as meat, poultry and fish.

Fig. 12.5 Pressure canner

12.16 Drying

One of the oldest methods of food preservation is by drying, which reduces water activity sufficiently to delay or prevent bacterial growth. Drying is done to produce concentrated form of foods, inhibits microbial growth and autolytic enzymes, retains most nutrients. Drying can cause loss of some nutrients, particularly thiamine and

vitamin C. Sulphur dioxide is sometimes added to dried fruits to retain vitamin C, but some individuals are sensitive to this substance.

Most types of meat can be dried. This is especially valuable in the case of pig meat, since it is difficult to keep without preservation. Many fruits can also be dried; for example, the process is often applied to apples, pears, bananas, mangos, papaya, and coconut and grapes . Drying is also the normal means of preservation for cereal grains such as wheat, maize, oats, barley, rice, millet and rye. Drying is an excellent way of preserving several of the seasonal fruits for use during the off season. There are several types of dryers which are used. These include: drum dryer, cabinet dryer, tunnel dryer, rotary dryer, spray dryer and solar dryer. The basic methods of drying involves ai r and contac t dr ying under atmospheric pressure . In this cas e the heat is transferred through the food either from heated air or heated surfaces, and the resulting water vapour is removed with the air current . Solar drying, sun drying, drum and spray drying all use this technique.

Advantages of drying are many

i) Long Shelf Life – Since most microorganisms responsible for food spoilage are unable to grow and multiply in the absence of moisture, spoilage due to microbial degradation is limited in dried foods. Furthermore, enzymes which catalyse undesirable changes in foods need moisture to be effective.

ii) Reduced Weight – This results in reduced transportation, storage and shipping costs.

iii) Convenience – The production of convenience items with novelty appeal for niche markets makes drying an attractive option.

iv) Concentration of nutrients – The removal of most of the water from a food results in a highly concentrated source of nutrients.

v) No refrigeration is required for dried products – Savings in energy and storage costs together with the long shelf life provide a lucrative processing alternative for tropical countries.

Disadvantages of Drying

Disadvantages of Drying are few and mainly relate to oxidation, which usually accompanies drying. This results in losses of micronutrients such as carotene and ascorbic acid and minimal loss in protein as a result of browning reactions. Reduced consumer appeal is often linked with the latter. There might also be changes in flavour and texture if drying is not properly controlled, particularly with regard to maximum temperatures.

12.17 Microwave Sterilization

Microwave sterilization is a thermal process. A microwave oven (Figure 12.6) works by passing non ionizing microwave radiation, usually at a frequency of 2.125 GHz (a wavelength of 12.212 cm), through the food. Microwave radiation is between common radio and infrared frequencies. Microwave heating takes place due to the polarization effect of electromagnetic radiation at frequencies between 300 MHz and 300 GHz. It delivers energy to the food package under pressure and controlled temperature to achieve inactivation of bacteria harmful for humans. Most processed foods today are heat treated to kill bacteria. Prolong exposure to high heat often diminishes product quality. Microwaves interact with polar water molecules and charged ions. The friction resulting from molecules aligning in rapidly alternating electromagnetic field generates the heat within food. Since the heat is produced directly in the food, the thermal processing time is sharply reduced. The colour, texture and other sensory attributes of foods processed by microwave sterilization are often better compared with those of conventionally retorted foods while meeting microbial safety requirements. US Federal Communication Commission (FCC) allocates 915 MHz and 21250 MHz bands for industrial and domestic microwave heating applications. The microwave sterilization technology using the combination of 915 MHz microwave and conventional heating to improve heating uniformity. Microwave ovens use electromagnetic radiation to excite water molecules in food. The actual waves penetrate only about 10 inches from the source of the radiation. Within the food, the waves only penetrate 3/12 to 1 inch on all sides. As a result, the actual ovens must be limited in size. Heat is produced within the food by the friction of water molecules, which spreads to the centre of the food by conduction. Small portions are cooked rapidly in microwave ovens. As the quantity of food increases, however, the efficiency is lost.

Microwave heating has also found applications in the food industry, including tempering of frozen foods for further processing, pre-cooking of bacon for institutional use and final drying of pasta products. In those applications, microwave heating demonstrates significant advantages over conventional methods in reducing process time and improving food quality.

The shelf life of a product is determined by its microbiological safety and sensory attributes. In general, microwave sterilization can achieve the same reduction of bacterial population as conventional retorting. Products intended for microwave sterilization are usually packaged in plastic trays or pouches. The ability of plastics to withstand oxygen permeation will affect the organoleptic or sensory acceptance of the product during storage. Normal shelf life expectancy of microwave-sterilized products pre-packaged in plastic containers or pouches is 2-3 years or longer. With innovative plastic technologies coming to the market, the new generations of plastics may increase the expected shelf life even longer.

ELECTRICITY

Pulsed Electric Field (PEF) Processing

PEF utilizes high intensity electric field pulses to inactivate microorganisms mainly in liquid foods at relatively low or moderate temperatures (<60^0C), whilst preserving the fresh flavour, color and integrity of heat sensitive components. A typical PEF food processing unit comprises of a high voltage pulse generator, a treatment chamber, a fluid handling system and control and monitoring devices (Figure 16.2). Depending on the particular PEF systems used, typical PEF treatment parameters include pulsed field intensity of 15-50 kV cm^{-1}, pulse width of 1-5 ms, and pulse frequency of 200-400 Hz (pulses/s). PEF treatment at an electric field intensity greater than a critical threshold of trans-membrane potential of 1 V across the target cells causes irreversible pore formation and destruction of the semi-permeable barrier of the cell membrane and structural changes in enzymes. PEF treated bacterial cells substantially damaged at the cellular level. PEF treatment at up to 25 kV cm^{-1} and 35^0C for 400 ms caused less than 1 log reduction in *E. coli* O157:H7 in apple juice. Examples of pulse field processed foods are apple juice, milk, orange juices, green pea soup etc. PEF processing is restricted to foods that do not contain air bubbles and have low electrical conductivity. PEF is not suitable solid foods can not be pumped. PEF and thermal processing in combination lower the temperature of pasteurization and improve the quality of food.

Ultrasonic Food Processing

Ultrasound is an efficient non-thermal alternative. Ultrasonic cavitation creates shear forces that break cell walls mechanically and improve material transfer. Generally, ultrasound equipment uses frequencies from 20kHz to 10MHz. Higher-power ultrasound at lower frequencies (20–100kHz), which is referred to as ‘‘power ultrasound’’, has the ability to cause cavitation that could be used in food processing to inactivate microorganisms. Low frequency ultrasound refers to pressure waves with a frequency of 20 kHz or more. Ultrasonic waves generate gas bubbles in liquid media, which produce a high temperature and pressure increase when they immediately burst. When the bubbles produced during ultrasonic treatment collapse, the compression/expansion cycles generated are thought to be responsible for cell disruption, microbial and enzyme inactivation in preservation of fruit juices and sauce. Ultrasound has potential to destruction of food borne pathogens like *E.coli, Salmonella, Giargia, poliovirus* etc. This method has application in the preservation

of jam, marmalade or toppings e.g. for ice cream, fruit juices and sauces, meat products and dairy.

Ohmic Heating of Foods

Ohmic heating (sometimes also referred to as Joule heating, electrical resistance heating, direct electrical resistance heating, electro heating, and electro conductive heating) is defined as a process wherein (primarily alternating) electric currents are passed through foods or other materials with the primary purpose of heating them. The heating occurs in the form of internal energy generation within the material. Ohmic heating is an advanced thermal processing method wherein the food material, which serves as an electrical resistor, is heated by passing electricity through it. Electrical energy is dissipated into heat, which results in rapid and uniform heating. Ohmic heating is also called electrical resistance heating, Joule heating, or electro-heating, and may be used for a variety of applications in the food industry. Ohmic heating can be used for heating liquid foods containing large particulates, such as soups, stews, and fruit slices in syrups and sauces, and heat sensitive liquids. The technology is useful for the treatment of proteinaceous foods, which tend to denature and coagulate when thermally processed. At low-frequency (50–60 Hz), electrical charges can build up and form pores across microbial cells and causes death of those microbial cells.

Intense Pulse Light

Pulsed light (PL) is a technique to decontaminate surfaces by killing microorganisms using pulses of an intense broad spectrum, rich in UV-C to near IR (180-1100nm) which is produced using xenon discharge lamp light (Figure 16.3). One pulse is 1-20 flashes/seen for duration of 1μs to 0.15, energy density is 0.01- 50 J/cm^2. PL kills microorganisms using short time high frequency pulses of an intense broad spectrum, rich UV-C light. The germicidal effect of UV light on bacteria is primarily due to the formation of pyrimidine dimers, mainly thymine dimers. The dimer inhibits the formation of new DNA chains in the process of cell replication, thus resulting in the inactivation (inability to replicate, called clonogenic death) of affected microorganisms by UV. On bacterial spores, UV-C treatment results mainly in the formation of the ‘‘spore photoproduct’’ 5-thyminyl-5,6-dihydrothymine, and in single-strand breaks, double-strand breaks and cyclobutane pyrimidine dimers. PL has been used to successfully inactivate *Escherichia coli* O157:H7 on alfalfa seeds and *Aspergillus niger* spores on corn meal. Surface microorganisms are controlled in

various foods. Such as meat products, cheese, baked goods, fish, shrimp, reduction of Pseudomonas on dry cottage cheese, inactivation of spoilage and pathogens in milk.

Plasma Light

Plasma is defined as a neutral ionised gas. It is constituted by particles in permanent interaction. The particles include photons, electrons, positive and negative ions, atoms, free radicals and excited or non-excited molecules (Figure 16.4). Electrons and photons are usually designed as "light" species in contrast to the other constituents defined as "heavy" species. Consequently, the term "plasma" is considered to describe a state of matter in which the heavy species are neutral or ionised particles which result from an energetic transfer to a gas. Non-thermal plasma technology relies on electric discharge into air or liquid to produce energetic atoms, highly reactive radicals, ozone, etc., that can kill microbes in contact. In non-thermal plasma, electric energy is mostly used to generate non-thermal plasma species instead of heat. Therefore, this technology is energy-efficient and will cause minimal heat-induced damages to food products. Potential applications include pasteurization of liquid food products, produce wash, disinfection of processing equipment, plant floors, and packaging materials, city water and wastewater treatment and air pollution control. The primary advantages of plasma processing as a potential tool in the inactivation of microorganisms are (1) minimal thermal denaturation of nutritional and sensory properties, (2) reduced energy requirement for adequate processing, and (3) potential treatment of foods inside a flexible-film package. Non-thermal plasma was capable of killing *Escherichia coli* and *Salmonellae* in liquid foods. At the flow rate of 1000 mL/min, the 5 logs reduction in the bacteria counts has been achieved. This suggests that non-thermal plasma pasteurization can kill the food borne pathogens such as *Escherichia coli* and *Salmonellae* in liquid foods with minimal damage to active ingredients in foods.

Fig. 16.4 Plasma sterilization mechanism

16.8 Oscillating Magnetic Fields (OMF)

OMF applied in the form of pulses reverses the charge for each pulse, and the intensity of each pulse decreases with time to about 10% of the initial intensity. Preservation of foods with OMF involves sealing food in a plastic bag and subjecting it to 1 to 100 pulses in an OMF with a frequency between 5 to 500 kHz at temperatures in the range of 0 to 50^{o}C for a total exposure time ranging from 25 to 100 ms. Frequencies higher than 500 kHz are less effective for microbial inactivation and tend to heat the food material. Magnetic field treatments are carried out at atmospheric pressure and at moderate temperatures. The temperature of the food increases 2-5^{o}C. Exposure to magnetic fields causes inhibition in the growth and reproduction of microorganisms. OMF of intensity of 5 to 50T and frequency of 5 to 500 kHz was applied and reduced the number of microorganisms by at least 2-log cycles. Within the magnetic field of 5-50 T, the amount of energy per oscillation coupled to 1 dipole in the DNA is 10^{-2} to 10^{-3} EV. Inactivation of microorganisms may be based on the theory that the OMF may couple energy into the magnetically active parts of large critical molecules such as DNA. Within 5-50 T range, the amount of energy per oscillation coupled to 1 dipole in the DNA is 10^{-2} to 10^{-3} EV. Several oscillations and collective assembly of enough local activation may result in the breakdown of covalent bonds in the DNA molecule and inhibition of the growth of

microorganisms. Examples of food preserved with OMF milk, yoghurt and orange juice.