Semester - III

16MBP302 FOOD AND INDUSTRIAL MICROBIOLOGY 4H – 4C

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

This paper adds information about the role of microorganisms in many food, beverage and pharma industries both in production and spoilage processes.

OBJECTIVES

To encode the importance of the role of microorganisms in food industries both in beneficial and harmful ways. To obtain a good understanding of industrial microbiology and become qualified as microbiologist in food and other industries.

UNIT – I

Food and microorganisms – Important microorganisms in food – Fungi, Bacteria; Intrinsic and extrinsic parameters of food affecting microbial growth – sources of contamination of food. Food sanitation – indicators of food safety – Coliform bacteria.

UNIT – II

Food preservation – principles – factors affecting preservation – food preservation using temperature – low temperature food preservation – characteristics of psychrotrophs – high temperature food preservation – characteristics of thermophiles – preservation of foods by drying chemicals and radiation – limitations – commercial application.

UNIT – III

Food borne diseases - food poisoning - food borne infection and intoxication- Food control agencies - microbiological criteria for food, microbial quality control and food laws, Hazard Analysis Critical Control Point (HACCP).

UNIT – IV

History and chronological development of industrial microbiology. Industrially important strains – isolation and preservation. Inoculum development for various fermentation processes - strain development – mutation, recombinant DNA technology and protoplast fusion. Fermentation – submerged, solid state, batch and continuous.

UNIT – V

Fermentor design – scale-up process. Types of fermentors - Tower, cylindroconical, airlift and Components of CSTR. Downstream process – intracellular and extracellular product separation column chromatography, affinity. Production of beverages – beer, vitamins - vitamin B12, Riboflavin, antibiotics.

SUGGESTED READINGS

TEXT BOOKS

1. Banwart, G.J. (2004). *Basic Food Microbiology*. (2nd ed.). CBS Publishers and Distributors New Delhi.
2. Casida, L.E. Jr., (2003). *Industrial Microbiology.* New Age International Publishers, New Delhi.
3. Doyle, M.P., Beuchat, R.L., and Montuille, T.J., (2001). *Food Microbiology – Fundamentals and Frontiers*. ASM press.
4. Frazier, W.C., and Westhoff, D.C., (1995). *Food Microbiology*. Tata McGraw-Hill Publishing Company Limited, New Delhi.
5. Patel, A.H. (2003). *Industrial Microbiology*, Macmillan India Ltd, New Delhi.
6. Shuler, M.L., and Kargi, F., (2005). *Bioprocess Engineering Basic Concepts.* Pearson Education, New Delhi.

REFERENCES

1. Atlas, R.N., and Bartha, R., (2000). *Microbial Ecology - Fundamental and Applications*. (3rd ed.). Redwood City CA. Benjamin/Cumming Science Publishing Co., New Delhi.
2. Gould, G.W. (1996). *New Methods of Food Preservation*. Blackie Academic and Professional, Madras.
3. Jay, J.M. (2000). *Modern Food Microbiology*. CBS Publishers and Distributors, New Delhi.
4. Mansi, E.M.T., and Bryce, C.F.A., (2002). *Fermentation Microbiology and Biotechnology*. Taylor and Francis, New York.
5. Reed, G. (2002). *Presscott and Dunn's Industrial Microbiology*. (5th ed.). CBS Publishers, New Delhi.
6. Stanbury, P.T., and Whittaker, A., (2005). *Principles of Fermentation Technology*. Pergamon Press, NY.
7. Waites, M.J. (2007). *Industrial Microbiology*, Blackwell Publishing. UK.

Lecture plan

FOOD AND INDUSTRIAL MICROBIOLOGY (16MBP302)

SEMESTER – III **4H – 4C**

UNIT I

S. No	Duration	Topic	Reference
1	1	Food and microorganisms	T1: 33-38
2	1	Important microorganisms in food	T1: 39-56,17-33
3	1	Important microorganisms in food – fungi	T1: 39-56,17-33
4		Important microorganisms in food bacteria	T1: 39-56,17-33
5	1	Intrinsic and extrinsic parameters of food affecting microbial growth	R4: 18-45
6	1	Sources of contamination of food	R4: 51-60
7	1	Food sanitation	T1: 479-493
8	1	Indicators of food safety	R4: 303-305
9	1	Coliform bacteria	T1: 56-57
10	1	Unit revision	
Total Hrs: 10			

T1: Frazier, WC and DC. Westhoff, 1995. Food Microbiology, Tata McGraw Hill Publishing company Ltd, New Delhi.

R4: Adams, MR and Mo. Moss, Food Microbiology, Tata Mc Graw Hill Publishing company Ltd, New Delhi.

2017

FOOD AND INDUSTRIAL MICROBIOLOGY

Prepared by – Ms. P. Akilandeswari, Asst Professor, Dept of Microbiology, KAHE.

Unit - 1

Introduction to food and spoilage by microbes

- Most raw foods contain a variety of bacteria, yeasts and molds and may contain plant and animal enzymes as the case may be. Kind and number of micro organisms that will be present on or in food will be influenced by the kind and extent of contamination.
- Because of the environmental conditions, one organisms it spoilage by the first organism allowed to proceed, one or more other kinds of organisms produces secondary spoilage or a further succession of organisms and changes may be involved. Contamination may increase number of micro organisms in the food. Wash water may add surface bacteria in butter, plant equipment may add spoilage organisms to foods during processing. Increased "bio burden" of micro organisms, especially those which cause spoilage, makes preservation more difficult.
- Growth of micro organisms in food obviously will increase number of micro organisms. Pretreatment of foods may remove or destroy some kinds of micro organisms and inactivate part or all of the food enzymes. Washing may remove organisms from the surface or may add some from the wash water. If washing is by means of an antiseptic or germicidal solution, numbers of organisms may be greatly reduced. High temperatures will kill more organisms treatment with rays, ozone, SO2, germicidal vapors will reduce numbers.

Microorganisms important in Food microbiology

Molds:

Mold growth on foods, with its fuzzy or cottony appearance, sometimes colored, is familiar to everyone, and usually food with a moldy or "mildewed" food is considered unfit to eat. Special molds are useful in the manufacture of certain foods or ingredients of foods. Thus, some kinds of cheese are mold-ripened, e.g., blue, Roquefort, Camembert, Brie, Gammelost, etc., and molds are used in making Oriental foods, e.g., soy sauce, miso, sonti, and other discussed later. Molds have been grown as food or feed and are employed to produce products used in foods, such as amylase for bread making or citric acid used in soft chinks.

General characteristics of molds:

The term "mold" is a common one applied to certain multicellular filamentous fungi whose growth on foods usually is readily recognized by its fuzzy or cottony appearance. Colored spores are typical of mature mold of some kinds and give color to part or all of the growth. The thallus, or vegetative body, is characteristic of thallophytes, which lack true roots, stems, and leaves.

Morphological Characterstics:

Hyphae and Mycelium The mold thallus consists of a mass of branching, intertwined filaments called hyphae (singular hypha), and the whole mass of these hyphae is known as the mycelium. Molds are divided into two groups: septate, i.e., with cross walls dividing the hypha into cells; and noncoenocytic, septate with the hyphae apparently consisting of cylinders without cross walls. The non-septate hyphae have nuclei scattered throughout their length and are considered multicellular. Special, mycelial structures or parts aid in the identification of molds. Examples are the rhizoids, or "holdfasts," of *Rhizopus* and *Absidia*, the foot cell in *Aspergillus*, and the dichotomous, or Y-shaped, branching in *Geotrichum*.

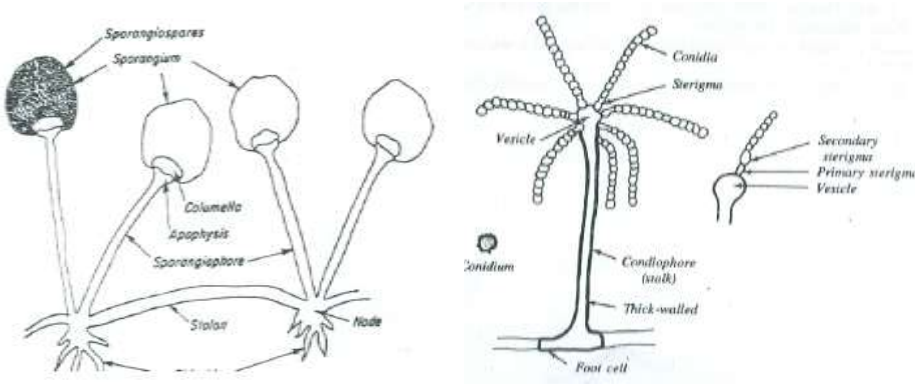
Reproductive Parts or Structures.

Reproduction of molds is chiefly by means of asexual spores. Some molds also form sexual spores. Such molds are termed "perfect" and are classified as either Oomycetes or

Zygomycetes if nonseptate, or Asco-mycetes or Basidiomycetes if septate, in contrast to "imperfect" molds, the Fungi Imperfecti (typically septate), which have only asexual spores.

Asexual Spores

The asexual spores of molds are produced in large numbers and are small, light, and resistant to drying. They are readily spread through the air to alight and start new mold thallus where conditions are favorable. The three principal types of asexual spores are (1) conidia (singular conidium), (2) arthrospores or oidia (singular oidium), and (3) sporan- giospores. Conidia are cut off, or bud, from special fertile hyphae called conidiophores and usually are in the open, i.e., not enclosed in any container, in contrast to the sporangiospores, which are in sporangium (plural sporangia), or sac, at the tip of a fertile hypha, the sporangiophore. Arthrospores are formed by fragmentation of a hypha, so that the cells of the hypha become arthrospores. Examples of these three kinds of spores will be given in the discussion of important genera of molds. A fourth kind of asexual spore, the chlamydospore, is formed by many species of molds when a cell here and there in the mycelium stores up reserve food, swell, and forms a thicker wall than that of surrounding cells.



Sexual Spores: The molds which can produce sexual spores are classified on the basis of the manner of formation of these spores and the type produced. The non septate molds (Phycomycetes) that produce.

1. *Oospores* are termed Oomycetes. These molds are mostly aquatic; however, included in this group are several important plant pathogens. The oospores are formed by the union of a small male gamete and a large female gamete.

2. *Zygospores*: Zygomycetes form zygospores by the union of the tips of two hyphae which often appear similar and which may come from the same mycelium or from different mycelia. Both Oospores and zygospores are covered by a tough wall and can survive drying for long periods.

3. *Ascospores*: The Ascomycetes (septate) form sexual spores known as ascopores, which are formed after the union of two cells from the same mycelium or from two separate mycelia. The ascospores, resulting from cell division after conjugation, are in an ascus, or sac, with usual eight spores per ascus.

4. *Basidiospores*: The Basidiomycetes, which include most mushrooms, plant rusts, smuts, etc.,form a fourth type of sexual spore, the basidiospore.

Physiological characteristics:

Moisture Requirements In general most molds require less available moisture than do most yeasts and bacteria. An approximate limiting total moisture content of a given food for mold growth can be estimated, and therefore it has been claimed that below 14 to 15 percent total moisture in flour or some dried fruits will prevent or greatly delay mold growth.

Classification and identification of molds

Molds are plants of the kingdom Myceteae. They have no roots, stems, or leaves and are devoid of chlorophyll. They belong to the Eumycetes, or true fungi, and are subdivided further to subdivisions, classes, orders, families, and genera.

The following criteria are used chiefly for differentiation and identification of molds:

1 Hyphae septate or non-septate

2 Mycelium clear or dark (smoky)

3 Mycelium colored of colorless

4 Whether sexual spores are produced and the type: oospores, zygospores, or ascospores

6 Characteristics of the spore head

a) Sporangia: size, color, shape, and location

b) Spore heads bearing conidia: single conidia, chains, budding conidia, or masses; shape and arrangement of sterigmata or phialides; gumming together of conidia

7 Appearance of sporangiophores or conidiophores: simple or branched, and if branched the type of branching; size and shape of columella at tip of sporangiophore; whether conidiophores are single or in bundles

8 Microscopic appearances of the asexual spores, especially of conidia: shape, size, color; smooth or rough; one-, two-, or many-celled

9 Presence of special structures (or spores): stolons, rhizoids, foot cells, apo-physis, chlamydospores, sclerotia, etc

Molds of Industrial Importance

Mucor: Mucor are involved in the spoilage of some foods and the manufacture of others. widely distributed species is *M. racemosus*; *M. rouxii* is used in the "Amylo" process for the saccharification of starch, and mucors help ripen some cheese, (e.g., Gammelost) and are used in making certain Oriental foods.

Zygorrhynchus These soil molds are similar to *Mucor* except that the zygo-spore suspensors are markedly unequal in size.

Rhizopus Rhizopus stolonifer, the so-called bread mold, is very common and is involved in the spoilage of many foods: berries, fruits, vegetables, bread, etc.

Absidia: Similar to *Rhizopus*, except that sporangia are small and pear-shaped.

Thamnidium: *Thamnidium elegans* is found on meat in chilling storage, causing "whiskers" on the meat.

Aspergillus: The aspergillus are very widespread. Many are involved in the spoilage of foods, and some are useful in the preparation of certain foods.

Penicillium: P. expansum, the blue-green-spored mold, causes soft rots of fruits. Other important species are *P. digitatum*, with olive, or yellowish-green conidia, causing a soft rot of citrus fruits; *P. italicum*, called the “blue contact mold” with blue green conidia, also rotting citrus fruit; *P.camemberti*, with grayish conidia, useful in the ripening of Camembert cheese; and *P. roqueforti*, with bluish-green conidia, aiding in the ripening of blue cheeses, e.g., Roquefort.

Trichothecium: The common species, *T. roseum*, is a pink mold which grows on wood, paper, fruits such as apples and peaches, and vegetables such as cucumbers and cantaloupes.

Yeasts and yeast like fungi

Like mold, the term "yeast" is commonly used but hard to define. It refers to those fungi which are generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission.

Yeasts may be useful or harmful in foods. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, and surface ripened cheese, and yeasts are grown for enzymes and for food. Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups; molasses, honey, jellies, meats, wine, beer, and other foods.

Bacteria

Morphological characteristics important in food bacteriology

One of the first steps in the identification of bacteria in a food is microscopic examination to ascertain the shape, size, aggregation, structure, and staining reactions of the bacteria present. The following characteristics may be of special significance.

Encapsulation

The presence of capsules or slime may account for sliminess or ropiness of a food. In addition, capsules serve to increase the resistance of bacteria to adverse conditions, such as heat or chemicals. To the organism they may serve as a source of reserved nutrients. Most capsules are polysaccharides of dextrin, dextran, or levan.

Formation of Endospores

Bacteria of the genera Bacillus, Clostridium, Desulfotomaculum, Sporolactobacillus (rods), and Sporosarcina (cocci) share the ability to form endospores. Bacillus - aerobic and some facultative anaerobic and Clostridium - anaerobic. Endospores are formed at an intracellular site, are very refractile, and are resistant to heat, ultraviolet light, and desiccation.

Formation of Cell Aggregates

It is characteristic of some bacteria to form long chains and of others to clump under certain conditions. It is more difficult to kill all bacteria in intertwined chains or sizable clumps than to destroy separate cells.

Cultural characteristics important in food bacteriology:

Bacterial growth in and on foods often is extensive. Pigmented bacteria cause discolorations on the surfaces of foods; films may cover the surfaces of liquids; growth may make surfaces slimy; or growth throughout the liquids may result in undesirable cloudiness or sediment.

Physiological characteristics important in food bacteriology

These changes include hydrolysis of complex carbohydrates to simple ones; hydrolysis of proteins to polypeptides, amino acids, and ammonia or amines; and hydrolysis of fats to glycerol and fatty acids. O-R reactions, which are utilized by the bacteria to obtain energy from foods (carbohydrates, other carbon compounds, simple nitrogen-carbon compounds, etc.), yield such products as organic acids, alcohols, aldehydes, ketones, and gases.

Genera of bacteria important in food bacteriology

Genus *Acetobacter* These bacteria oxidize ethyl alcohol to acetic acid. They are rod-shaped and motile and are found on fruits, vegetables, souring fruits, and alcoholic beverages. They are a definite spoilage problem in alcoholic beverages.

Genus *Aeromonas* These are gram-negative rods with an optimum temperature for growth of 22 to 28 C. They are facultative anaerobes and can be psychrophilic. They are frequently isolated from aquatic environments. A. hydrophila can be a human pathogen; it is also pathogenic to fish, frogs, and other mammals.

Genus *Alcaligenes* As the name suggests, an alkaline reaction usually is produced in the medium of growth. *A. viscolactis*. causes ropiness in milk, and *A. metalcaligenes* gives a slimy growth on cottage cheese. These organisms come from manure, feeds, soil, water, and dust. This genus also contains organisms which were formerly classified in the genus *Achromobacter*.

Genus *Alteromonas* Several former species of *Pseudomonas* are now classified as Alteromonas. They are marine organisms that are potentially important in sea foods.
Genus *Bacillus* The endospores of species of this aerobic to facultative genus usually do not swell the rods in which they are formed. Different species may be mesophilic or thermophilic, actively proteolytic, moderately proteolytic, or non proteolytic, gas-forming or not, and lipolytic or not. In general the spores of the mesophiles, e.g., B. subtilis, are less heat-resistant than spores of the thermophiles. Spores of the obligate thermophiles, e.g., *B. stearothermophilus*, are more resistant than those of facultative thermophiles, e.g., B. coagulans. The actively proteolytic species usually may also sweet-curdle milk; B. cereus is such a species. The two chief acid- and gas-forming species, *B. polymyxa* and *B. macerans*, sometimes are termed "aerobacilli."
Genus *Brevibacterium B. linens* is related to *Arthrobacter globiformis* and may be synonymous.
Genus *Brochotrix* These are gram-positive rods which can form long filamentous like chains that may fold into knotted masses. The optimum temperature for growth is 20 to 250 C, but growth can occur over a temperature range of 0 to 450C depending on the strain.
Genus *Campylobacter* These bacteria were originally classified in the genus vibrio. Several strains of *C. fetus subsp. jejuni* have been associated with gastroenteritis in humans.
Genus *Clostridium* The endospores of species of this genus of anaerobic to microaerophilic bacteria usually swell the end or middle of the rods in which they are formed. Different species may be mesophilic or thermophilic and proteolytic or non-proteolytic. *Clostridium thermosaccharoolyticum* is an example of a saccharolytic obligate thermophile; this organism causes gaseous spoilage of canned vegetables. Putrefaction of foods often is caused by mesophilic, proteolytic species, such as *C. lentoputrescens* and *C. putrefaciens*.
Genus *Corynebacterium* The diphtheria organism, C. diptheriae, may be transported by foods. C. bovis, with the slender, barred, or clubbed rods characteristic of the genus, is commensal on the cow's udder, can be found in aseptically drawn milk, and may be a cause of bovine mastitis.
Genus *Erwinia* The species of this genus are plant pathogens that cause necrosis, galls, wilts, or soft rots in plants and therefore damage the plants and vegetable and fruit products from them. *E.carotovora* is associated with the market disease called "bacterial soft rot." *E. carotovora* subsp. *carotovora* causes rotting in a large number of plants. *E. carotovora subsp. atroseptica* produces a black rot in potatoes. E. carotovora subsp. betavasculorum causes soft rot in sugar beets.
Genus *Escherichia* Found in feces, a predominant gram-negative rod isolated from the intestinal tract of warm-blooded animals and widely distributed in nature. One of the "coliform group," the genus is divided into many biotypes and serotypes, some of which can be pathogenic to humans.
Genus *Flavobacterium* The yellow to orange-pigmented species of this genus may cause discolorations on the surface of meats and be involved in the spoilage of shellfish, poultry, eggs, butter, and milk. Some of the organisms are psychrotrophic and have been found growing on thawing vegetables.
Genus *Klebsiella* Many are capsulated. Commonly associated with the respiratory and intestinal tracts of humans. *K. pneumoniae* is the causative organism for a bacterial pneumonia in humans.
Genus *Lactobacillus* The lactobacilli are rods, usually long and slender, that form chains in most species. They are microaerophilic, (some strict anaerobes are known), are catalase-negative and gram-positive, and ferment sugars to yield lactic acid as the main product.

Factors affecting the growth and survival of micro-organisms in foods.

• *Intrinsic factors*

o Water activity; Bacteria> Yeast> Mold
o Oxygen availability
o pH: Low acid foods, acid foods (4.5 and lower)
o Buffer capacity: change of pH
o Nutrients
o Natural antimicrobial substances
o Microflora

• *Extrinsic factors*

o Temperature: Psychrophiles 12-15C/ Mesophiles 30-54C/ Thermophiles 55-75C
o Relative humidity
o Atmospheric condition: Aerobic/ Anaerobic/ Facultative anaerobic /

Intrinsic parameters:

The parameters of plant and animal tissues that are inherent part of the tissues are referred to as intrinsic parameter. These parameters are as follows:

1. pH:

pH: It is the negative logarithm of the hydrogen ion activity.

$$PH = - \log (a_H) = \log \frac{1}{(a_H)}$$

$$= \log \frac{1}{[H^{-1}]}$$

pH = Hydrogen ion activity
[ ] + H = Hydrogen ion concentration.

Every micro organism has a minimal, a maximal and an optimal pH for growth. Bacteria grow fastest in the pH range 6.0 – 8.0, yeasts 4.5 – 6.0 and filamentous fungi 3.5 – 4.0. Usually between pH 5.0 & 6.0.

Inherent acidity: Some foods have a low pH because of inherent property of the food. Ex: Fruits & vegetables.

Biological acidity: Some foods develop acidity from the accumulation of acid daring fermentation. Ex: curd, sauerkraut, pickles etc. Molds can grow over a wide range of pH vales than the yeast and bacteria. Film yeasts grow well on acid foods such as sauerkraut and pickles. Most yeasts do not grow well in alkaline substrates. Bacteria which are acid formers are favoured by moderate acidity. Active proteolytic
bacteria, can grow in media with a high pH (alkaline.) Ex: Egg white. The compounds that resist changes in pH are important not only for their buffering capacity but also for their ability to be especially effective within a certain pH range.

Vegetable juices have low buffering power, permitting an appreciable decrease in pH with the production of small amount of acid by lactic acid bacteria during the early part of sauerkraut and pickle fermentations. This enables the lactics to suppress the undesirable pectin – hydrolyzing and proteolytic competing organisms. Low buffering power makes for a more rapidly appearing succession of micro-organisms during fermentation than high buffering power. Ex: Milk – High in protein content, act as good buffer. Lactic acid converted to pyruvic acid by glycolytic pathway. Acid again converts to lactic acid by lactic dehydrogenase enzyme. After 5-10 minutes, there will be decreased in pH. Hence the lactic acid bacteria survives and activity slows dawn. Once the acidity increase, yeasts and molds will take upper hand and all the products used by these organisms. The quantity of acid decreases and pH increases to neutral.

Proteolytic bacteria acts on caesin and these proteins are broken down and gives bad smell accompanied by removal of NH3. pH increases and neutral due to deamination. Then lipolytic organisms which utilise the fat present and utilises the short chain fatty acids through hydrolysis which gives still bad smell. Egg white where the pH increases to around 9.2 as CO2 is lost from the egg after laying. Fish spoil more rapidly than meat under chill conditions. The pH of post – rigor mammalian muscle, round 5.6 and it is lower than that of fish (6.2 - 6.5) and this contributes to the longer storage life of meat.

The ability of low pH to restrict microbial growth has been employed since the earliest times in the presentation of foods with acetic and lactic acids. Fruits are acidic than vegetables pH of milk – neutral.

Fruits generally undergo mold and yeast spoilage than vegetables.

Redox potential (Eh): - Oxidation – reduction potential:

Oxygen tension or partial pressure of oxygen about a food and the O R potential or reducing and oxidising power of the food itself, influence the type of organisms which will grow and hence the changes produced in the food. The O R potential of the food is determined by

1. Characteristic O R potential of the original food.
2. The poising capacity i.e., the resistance to change in potential of the food.
3. The oxygen tension of the atmospHere about the food.
4. The access which the atmospHere has to the food.

Head space in an "evacuated" can of food contain low oxygen tension compared to air.

Micro organisms are classified as aerobic, anaerobic, and facultative based on the requirement of O2. Molds – aerobic ,Yeasts – Aerobic and facultative.

- Bacteria – Aerobic, anaerobic and facultative.
- High O - R potential favours aerobes and facultative organisms.
- Low O-R potential favours anaerobic and facultative organisms.

However some aerobes grow at low O-R potential O-R potential of a system is usually written

- Eh and measured and expressed in terms of millivolts (mv).

Highly oxidised substrate would have a positive Eh and a reduced substrate have a negative Eh. Aerobic microorganisms require positive Eh. Ex: *Bacillus, Micrococcus, Pseudomonads, Acinetobacters*. Anaerobic micro organisms required negative Eh. Ex: *Clostridium.* Most fresh plant and animal foods have a low and well poised O – R potential in their interior because plants contain reducing substances like ascorbic acid and reducing sugars where as animal tissues contain –SH (Sulf hydryl) and other reducing groups. As long as the plant or animal cells respire and remain active, they have low level of O-R potential.

Meat could support the aerobic growth of shine forming or souring bacteria at the same time that anaerobic putrefaction was proceeding in the interior. Heating and processing may alter the reducing and oxidising substances of food. Ex: Fruit juices lost reducing substances by their removal during extraction and filtration by their removal during extraction and filtration and therefore have become more favourable for the growth of yeasts.

3. Nutrient content:

Food is required for energy and growth of micro organisms. Carbohydrates especially the sugars are commonly used as an energy source. Complex carbohydrates such as cellulose can be utilized by few organisms and starch can be hydrolysed by any a limited number of organisms. Many organisms cannot use the disaccharide lactose (Milk sugar) and therefore do not grow well in milk. Maltose is not attacked by some yeasts. Some micro organisms hydrolyze pectin of the fruits and vegetables. Limited number of micro organisms can obtain their energy

from fats by producing lipases. Aerobic their energy from fats by producing lipases. Fats are hydrolysed to glycerol and fatty acids. Aerobic micro organisms are more commonly involved in the decomposition of fats than are anaerobic ones and the lipolytic organisms usually are also proteolytic. Hydrolysis products of proteins, peptides and amino acids serve as an energy source for many proteolytic organisms when a better energy source is lacking. Meats are decomposed by

proteolytic sps Ex: *Pseudomonas* sps: Concentration of food in solution increases the osmotic effect and amount of available moisture. Molds & yeasts can grow in the highest concentrations of sugars. Bacteria can grow best in low concentration of sugars.

Micro organisms differ in their ability to use various nitrogenous compounds as a source of nitrogen for growth. Many organisms are unable to hydrolyze proteins and hence cannot get nitrogen from them. Peptides, aminoacids, urea, ammonia and other simpler nitrogenous compounds may be available to some organisms but not to others. These compounds may be used under some environmental conditions but not under other conditions.Ex: Some lactic acid bacteria grow best with polypeptides as nitrogen foods, cannot attack casein. Some microorganisms use fermentable carbohydrates and results in acid production which suppresses the proteolytic bacteria and hence it is called sparing action on the nitrogen compounds.

Many kinds of molds are proteolytic but very few yeasts are actively proteolytic. Proteolytic bacteria grow best at pH values near neutrality and are inhibited by acidity. Carbon for growth may come partly from CO2 and also from organic compounds. Minerals required by microorganisms are always present in low level. Sometimes an essential mineral may be unavailable, lacking or present in insufficient amounts.

Ex: Milk contains insufficient iron for pigmentation of the spores of *Penicillium roqueforti*. Accessory food substances or vitamins needed by the organisms.

Antimicrobial barriers and constituents (or) Inhibitory substances and biological structure:

Inhibitory substances: These originally present in the food or added purposely to prevent growth of micro organisms.

- Freshly drawn milk – Lactenins, anticoliform factors.
- Egg white – Lysozyme
- Cran berries – Benzoic acid
- Short chain fatly acids on animal skin cabbage and other brassicas, garlic, onions and leeks.
- Allicin – Garlic, onion, leeks.
- Phytoalexins are produced by many plants in respone to microbial invasion.
- Antifungal compound phaseolin produced in green beans
- Eugenol – Allspice (pimento), cloves, cinnamon
- Thymol – thyme and oregano
- Cinnamic aldehyde – cinnamon and Cassia
- Inclusion of cinnamon in raisin bread retards mould spoilage.
- Humulones contained in the hop resin and isomers produced during processing, impart the characteristic bitterness of beer.
- Oleuropein – The bitter principle of green olives have antimicrobial properties.
- Lysozyme present in milk, egg is most active against gram positive bacteria.
- Egg – Ovotransferrin, avidin ovolflaroprotein.

- Milk – Lactoferrin
- Ovoflavo protein and avidin in egg white which sequester biotin and riboflavin restricting the growth of those bacteria.

Biological structures of food on the protection of foods against spoilage has been observed.

Ex: 1) Inner parts of healthy tissues of living plants and animals are sterile or low in microbial content.

2) Protective covering on the food like shell on egg, skin on poultry, shell on nuts, rind or skin on fruits and vegetables, artificial coating like plastic or wax.

3) Layers of fat over meat may protect the part of the flesh or scales may protect the outer part of the fish.

Water activity:

Micro organisms have an absolute demand for water. Without water, no growth can occur.

The exact amant of water needed for growth of micro organisms varies. This water requirement is best expressed in terms of available water or water activity (aW).

$$a_w = \frac{Vapour\ pressure\ of\ the\ solution}{Vapour\ pressure\ of\ the\ solvent}$$

aw for pure water is 1.00

For 1.0 m solution of the ideal solute, the aw would be 0.9823. Water activity also defined as the ratio of the partial pressure of water in the atmosphere in equilibrium with the substrate, P, compared with the partial pressure of the atmosphere in equilibrium with pure water at the same temperature, P0.

$$A_w = \frac{P}{P_o} = \frac{1}{100} ERH$$

ERH = Equilibrium relative humidity.

A_w Values	
0.98 and above	Fresh meat, fish, fresh fruits and vegetables, milk, canned vegetables, in brine, canned fruits in light syrup.
0.93 – 0.98	Evaporated milk, tomato paste, processed cheese, bread, canned cured meats, permented sausage, gouda cheese.
0.85 – 0.93	Dried beef, raw ham, aged cheddar cheese, sweetened condensed milk, dry or fermented sausage.
0.60 – 0.85	Dried fruit, flour, cereals, jams & jellies, nuts.
Below 0.60	Chocolate, confectionary, Honey, Biscuits, Crackers, Potato chips, Dried eggs, milk and vegetables.

Water is made unavailable in various ways:

1. Solutes and ions tie up water in solution. Therefore an increase in the concentration of dissolved substances such as sugars and salts effectively dry the material. Water tends to leave the microbial cell by osmosis.
2. Hydrophilic colloids (gels) make water unavailable.
3. Water of crystallization or hydration is usually unavailable to micro organisms. Each micro organisms has a maximal, optimal and minimal aw for growth. Low aw – decrease in the rate of growth of organisms.

Factors that may affect water activity (aw). Requirements of micro organisms include the following.

1. The kind of solute employed to reduce aw. Potassium chloride usually less toxic than NaCl. And less inhibitory than sodium sulphate.
2. The nutritive valve of the culture medium. The better the medium for growth, the lower the limiting aw.
3. Temperature: Most organisms have the greatest tolerance to low aw at about optimal temperatures.
4. Oxygen supply: Growth of aerobes takes place at lower aw in the presence of air than in its absence.
5. pH Most organisms are more tolerant of low aw at PH valves near neutrality than in acid or alkaline media.
6. Inhibitors: The presence of inhibitors narrows the range of aw for growth of micro organisms.

Methods for the control of aw are

1. Equilibrium with controlling solutions
2. Determination of the water – sorption isotherm for the food.
3. Addition of solutes.

Methods for measuring or establishing aw valves of food:

1. Freezing point determinations by Clausius – Clayperson equation.
2. Manometric techniques
3. Electrical devices.

Favourable aw for bacteria to grow in foods – 0.995 to 0.998. They grow best in low concentration of sugar or salt. 3-4% sugar and 1-2% salt may inhibit some bacteria. Molds have optimum aw of 0.98 – 0.99; Mold spores germinate at min aw of 0.62.

Some general conclusions related to water requirement of micro organisms are

1. Each organism has its own characteristic optimal aw.
2. Bacteria require more moisture than yeasts and yeasts more than molds.
   - Minimum aw required for bacteria – 0.91
   - Minimum aw required for yeasts – 0.88
   - Minimum aw required for molds – 0.80
   - Minimum aw required for Halophilic bacteria – 0.75
   - Minimum aw required for Xerophilic fungi – 0.65
   - Minimum aw required for Osmophilic yests – 0.60
3. Micro organisms that can grow in high concentrations of solutes e.g. sugar and salt have low water activity (aw). Osmophilic yeasts grow best in high concentrations of sugar.

Extrinsic parameters (Environmental limitations)

1) Relative humidity: (RH)

Relative humidity and water activity are interrelated. When food commodities having low water activity are stored in an atmosphere of high RH water will transfer from the gas phase

to the food. It may take a very long time for the bulk of the commodity to increase in water activity. Once micro organisms have started to grow and become physiologically active they usually produce water as an end product of respiration. Ex: Grain silos or in tanks in which concentrates and syrups are stored. Storage of fresh fruits and vegetables requires very careful control of relative humidity. It RH is too low, many vegetables will lose water and become flaccid. It is too high then condensation may occur and microbial spoilage may be initiated.

2. Temperature:

Microbial growth can occur over a temperature range from about -8°C up to 100°C. at atmospheric pressure.

- Thermophiles have optimum - 55-75°C
- Mesophile have optimum - 30 -40°C
- Psychrophiles (Obligate psychrophiles) – 12 - 15
- Psychotroph (facultative) – 25-30

Micro organisms can be classified into several physiological groups based on their cardinal temperatures. Low temperature affects the uptake and supply of nutrients to enzyme systems within the cell. Many microgranisms responds to growth at lower temperature by increasing the amount of unsaturated fatty acids in their membrane lipids and that psychrotrophs generally have higher level of unsaturation in a fatty acid decreases its melting point so that membranes containing higher levels of unsaturated fatly acid will remain fluid and hence functional at lower temperatures. As the temperature increases above the optimum, the growth rate declines as a result of denaturation of proteins.

Gaseous atmosphere:

Oxygen comprises 21% of the earth's atmosphere and is the most important gas in contact with food under normal circumstances. The inhibitory effect of CO2 on microbial growth is applied in modified atmosphere packing of food and is an advantage in carbonated mineral waters and soft drinks. Moulds and bacteria are sensitive to CO2 condensation. Some yeasts such as *Bettanomyces* spp how tolerance to high CO2 levels.

Growth inhibition is usually greater under aerobic conditions than anaerobic and the inhibitory effect increases with decrease of temperature, presumably due to the increased solubility of CO2 at lower temperatures. CO2 dissolves in water to produce carbonic acid which decreases PH and partially dissociates into bicarbonate anions and protons. CO2 also affects solute transport, inhibition of key enzymes involving carboxylation, decarboxylation reactions in which CO2 is a reactant and reaction with protein amino groups causing change in their properties and activity.

Chemical change caused by micro organisms:

Different chemical changes are possible because great varieties of organic compounds are present in foods and numerous kinds of micro organisms that can decompose them may grow in the food.

Following changes are observed in foods.

1. Changes in Nitrogenous organic compounds:

Most of the nitrogen in foods is in the form of proteins. Proteins are hydrolysed to polypeptides, simpler peptides or amino acids before they can serve as nitrogenous food for most organisms. Proteinases catalyze the hydrolysis of proteins to peptides gives bitter taste to foods. Peptidases

catalyze the hydrolysis of polypeptides to simpler peptides and finally to amino acids.

$$\text{Proteins} \xrightarrow{\text{Proteinases}} \text{Peptides} \xrightarrow{\text{Peptidases}} \text{Polypeptides}$$

$$\text{Polypeptides} \xrightarrow{\text{Peptidases}} \text{amino acids}$$

Anaerobic decomposition of proteins, peptides or aminoacids result in the production of obnoxious odors called putrefaction. Putrefaction results in foul smelling, sulphur containing products such as hydrogen, methyl and ethyl sulfides and mercaptans, plus ammonia, amines (Ex: histamine, tyramine, piperidine, putrescine and cadaverine), indole, skatole and fatty acids. When micro organisms act on amino acids, they may deaminate them, de-carboxylate them or both. Ex: *Escherichia coli* produces glyoxylic acid, acetic acid, and ammonia from glycine. *Pseudomonas* produces methylamine and CO2 clostridia gives acetic acid, ammonia, methane from alanine these three organisms produces

1) α - Keto acid, ammonia and CO2
2) Acetic acid, ammonia and CO2
3) Propionic acid, acetic acid ammonia and CO2 respectively.

Desulfatomaculum nigrificans an obligate anaerobe, can reduce sulphate to sulphide and produces H2S from cystine.

Changes in Non nitrogenous organic compounds:

Main non nitrogenous foods for micro organisms, mostly used to obtain energy but possibly serving as source of carbon, include carbohydrates, organic acids, aldehydes and ketones, alcohols, glycosides, cyclic compounds and lipids.

Glucose anaerobically decompose to

a) An alcoholic fermentation by yeasts with ethanol and CO2 as the principal products.
b) A simple lactic fermentation as by homo-fermentative lactic acid bacteria.
c) A mixed lactic fermentation by hetero-fermentative lactic acid bacteria with lactic and acetic acids, ethanol, glycerol and CO2 as the chief products.
d) The coli type of fermentation as by coliform bacteria with lactic, acetic formic acids, ethanol, CO2, H2 etc.
e) The propionic acid fermentation by propionic bacterium
f) Butyric – butyl – isopropyl fermentations yields butyric and acetic acids, CO2 & H2.

Organic acids:

Organic acids usually occurring in foods as salts are oxidized by organisms to carbonates, causing medium to become alkaline. Aerobically the organic acids may be oxidized completely to CO2 and water. Saturated fatty acids or ketonic derivatives are degraded to acetic acid.

Other compounds:

Alcohols usually oxidised to the corresponding organic acids.

Ethanol to acetic acid; Acetaldehyde to acetic acid.

Lipids:

Fats are hydrolysed to glycerol and fatty acids by lipase. Phospholipids may be degraded to their constituent phosphate, glycerol, fatty acids and nitrogenous base. Ex: choline

Pectic substances:

Protopectin in plants converted to pectin. Pectin is a water soluble polymer of galacturonic acids. Pectinesterase causes hydrolysis of the methyl ester linkage of pectin to yield pectin acid and methanol.

Contamination of foods

Micro organisms from various natural sources act as source of contamination.

- From green plants and fruits
- From animals
- From sewage
- From soil
- From water
- From air
- During handling and processing.

1. From green plants and fruits

Natural surface flora of plants varies with the plant but usually includes species of *Pseudomonas, Alcaligenes, Flavobacterium, Micrococcus,* coliforms and lactic acid bacteria. The no. of bacteria will depend on the plant and its environment and may range from a few hundred or thousand per square centimeter of surface to millions. Ex: Surface of well washed tomato contains 400-700 micro organisms per square centimeter. Outer tissue of unwashed cabbage contain 1 million to 2 million micro organisms. Inner tissues of cabbage contain fewer micro organisms.

2. *From animals:*

Sources of micro organisms from animals include the surface flora, the flora of the respiratory tract, and the flora of the gastro intestinal tract. Hides, hooves, and hair contain micro organisms from soil, manure, feed and water but contain spoilage organisms. Feathers, feet of poultry carry heavy contamination of micro organisms. Skin of many meat animals may contain *Micrococci, Staphylococci* and beta haemolytic
Streptococci. Pig or beef carcasses may be contaminated with salmonellae. Meat from slaughter houses is not frequently associated with human salmonellosis. Many of these diseases have been reduced or eliminated by improvement in animal husbandry, but animal disease causing infections from foods include *Mycobacterium, Coxiella, Listeria, Salmonella* and enteropathogenic *E.Coli* and viruses.

3. From sewage:

When untreated domestic sewage is used to fertilize plant crops, there is a chance that raw plant foods will be contaminated with human pathogens especially those causing gastrointestinal diseases. The use of "night soil" as a fertilizer still persists in some parts of the world. In addition to the pathogens, coliform bacteria, anaerobes, enterococci, other intestinal bacteria and viruses can contaminate the foods from this source. Natural water contaminated with sewage contributes their micro organisms to shell fish, fish, and other seafood.

From soil:

Soil contains greatest variety of micro organisms. They are ready to contaminate the surfaces of plants growing on or in them and the surfaces of animals roaming over the land. Soil dust is whipped up by air currents and soil particles are carried by running water to get into or onto foods. Soil is an important source of heat resistant spore forming bacteria.

From water:

Natural water contain not only their natural flora but also microorganisms from soil and possibly from animals or sewage. Surface waters in streams or pools and stored waters have low microbial content because self purification of quiet lakes and ponds or of running water. Ground waters from springs or wells have passed through layers of rock and soil to a definite level hence most of the bacteria, suspended material have been removed. Kinds of bacteria in natural waters are chiefly of in *Pseudomonas, Chromobacterium, Proteus, Micrococcus, Bacillus, Streptococcus, Enterobacter* and *Escherichia coli*.

From Air:

Air does not contain a natural flora of micro organisms, but accidentally they are present on suspended solid material or in moisture droplets. Micro organisms get into air on dust or lint, dry soil, spray from stream, lakes or oceans, droplets of moisture from coughing, sneezing or talking and growth of sporulating molds on floors, etc. Number of microorganisms in air at any given time depend on factors like amount of movement, sunshine, humidity, location and the amount of suspended dust or spray. No. of micro organisms vary from mountains to dusty air. Less on mountains and more in dusty air. Direct rays from the sun kill micro organisms suspended in air and hence reduce numbers. Dry air contains more organisms than moist air. Number of micro organisms in air may be reduced under natural conditions by sedimentation, sunshine and washing by rain or snow. Filters in ventilating or air conditioning systems prevent the spread of organisms from one part of a plant to another.

During handling and processing:

Additional contamination may come from equipment coming in contact with foods, from packaging materials and from personnel.

Lecture plan

FOOD AND INDUSTRIAL MICROBIOLOGY (16MBP302)

SEMESTER – III **4H – 4C**

UNIT II

S. No	Duration	Topic	Reference
1	1	Introduction to food preservation	T1: 83-86
2	1	Food preservation – principles	T1: 86-89
3	1	Factors affecting food preservation	T1: 90-98
4	1	Food preservation using low temperature and characters of thermophiles	T1: 101-117
5	1	Food preservation using high temperature and characters of thermophiles	T1: 125-138
6	1	Food preservation by drying, and	T1: 143-160
7		Food preservation chemicals	T1: 143-160
8		Food preservation radiation	T1: 143-160
9	1	Limitations and commercial application of food preservation	T1: 161-169
10	1	Unit revision	
Total Hrs: 10			

T1: Frazier, WC and DC. Westhoff, 1995. Food Microbiology, Tata McGraw Hill Publishing company Ltd, New Delhi.

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FOOD AND INDUSTRIAL MICROBIOLOGY

Prepared by – Ms. P. Akilandeswari, Asst Professor, Dept of Microbiology, KAHE.

Unit 2
Food preservation

Introduction

Most foods deteriorate in quality following harvest, slaughter or manufacture, in a manner that is dependent on food type, its composition and storage conditions. The principal quality deterioration reactions of foods may be

Microbiological

The microorganisms present in a food may be contributed by its own natural microflora or from the processing conditions like in the course of harvesting/manufacturing, storage, and transport. In some cases the microflora has no discernible effect on the food quality and food safety while in others, this may affect the quality in several ways like causing food spoilage, food borne illness or food fermentations. While food fermentations are desirable transformations of food but food spoilage, food borne infections and intoxications may result into huge economic losses as in cases where a particular batch of food has been found to be involved in an outbreak of a disease or has low shelf life as desired and hence the complete batch has to be recalled back from the market and destroyed. In developing countries like India, losses due to microbial spoilage have been estimated between 10-25% in various types of foods, which adds to the problems of acute shortage of food supply in these countries.

Enzymatic

Enzymes native to plant and animal tissues or from microorganisms are responsible for changes in the texture, color, smell and appearance of foods e.g. microbial enzymes cause hydrolytic reactions, rancidity and browning in foods, and plant enzymes may cause over ripening of fruits and vegetables rendering them unsuitable for consumption.

Chemical

Chemical reactions like oxidative rancidity, oxidative and reductive discoloration, non enzymatic browning and destruction of nutrients contribute to the deterioration of foods if not stored in a proper environment.

Physical

Physical changes are responsible for loss of texture, flavors and structural damage. The most serious forms of quality deterioration include those due to microorganisms, following the survival and/or growth of spoilage, infectious pathogenic bacteria or the growth of toxinogenic ones. In this chapter we are going to study how these losses due to microbial spoilage of foods can be minimized and how foods are made safe for our consumption. The techniques employed to achieve these targets are called food preservation.

Principles of food preservation

The food preservation methods by which the microbial decomposition of foods can be delayed or prevented include

1. Restrict access of microorganisms to foods (packaging and aseptic packaging),
2. Removal of microorganisms (by filtration or centrifugation),

3. Slow or prevent the growth and activity of microorganisms (reduction in temperature, water activity and pH, removal of oxygen, modified atmosphere packaging and addition of preservatives) and
4. Inactivation of microorganisms (by heat, radiations, high hydrostatic pressures, ultra sound and pulsed electric fields).

These methods usually are also effective against enzymatic activity or chemical reactions in the food, responsible for its self-decomposition. Changes in the requirement of consumers in recent years have included a desire for foods which are more convenient, higher quality, fresher in flavor, texture and appearance, more natural with fewer additives and nutritionally healthier than hitherto. Food industry reactions to these changes have been to develop less severe or minimal preservation and processing technologies with less intensive heating or use of less chemical preservatives. However, minimal technologies tend to result in a reduction in the intrinsic preservation of foods, and may, therefore, also lead to a potential reduction in their microbiological safety. A major trend is to apply these techniques in new combinations, in ways that minimize the extreme use of any one of them, and so improve food product quality. This has formed the basis of hurdle technologies or combination preservation systems proposed by Leistner (2000) that have fostered the development of new routes to food preservation around the world. Thus an ideal method of food preservation has the following characteristics:

1. it improves shelf-life and safety by inactivating spoilage and pathogenic microorganisms,
2. it does not change organoleptic (smell, taste, color, texture, etc.) and nutritional attributes,
3. it does not leave residues,
4. it is cheap and convenient to apply and
5. it encounters no objection from consumers and legislators.

These methods of food preservation are being discussed under two headings:

1. physical methods of preservation and
2. chemical methods of preservation.

Physical methods of food preservation

The foods to be preserved are physically processed or treated in such a way that the metabolic activity of microorganisms and their spores either slowed down or completely arrested. These various physical methods used for the preservation of foods are as follows.

Asepsis

Keeping quality of foods can be increased by introducing as few spoilage organisms as possible i.e., by reducing the amount of contamination. In nature, there are numerous examples of asepsis or removal of microorganisms as a protective factor. The presence of a protective covering surrounding some foods e.g. shells of nuts, shells of eggs, skin of fruits and vegetables and fat on meats and fish, prevents microbial entry and decomposition until it is damaged.

In food industries, contamination is prevented by packaging foods in a wide variety of artificial coverings ranging from a loose carton or wrapping to the hermetically sealed containers of canned foods. Moreover, practicing sanitary methods during the processing and handling of foods reduces total microbial load and thus improves the keeping quality of food. Both flexible

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and rigid packaging materials, alone or in combination with other preservation methods, have been developed to offer the necessary barrier, inactivation, and containment properties required for successful food packaging. Rigid packaging materials such as glass and metal packages are considered absolute barriers, preventing contamination. However, the economic and functional disadvantages of metal and glass have led to the development of flexible packaging materials made from composites of polyester, nylon, polypropylene, polyethylene and polyvinyl. The microbiology of the flexible packaged foods is influenced by the permeability of the packaging material to oxygen, carbon dioxide and water vapor. Packaging with certain additional conditions like controlled atmosphere, modified atmosphere, and vacuum packaging can produce microbiostatic effect, which is more effective with further decrease in storage temperature.

Controlled atmosphere packaging conditions are defined as the alteration of a gaseous atmosphere over a food product regardless of environmental or temperature fluctuations encountered by the product throughout its distribution. This extends the microbial lag phase, depresses microbial and product respiration, and minimizes adverse changes in sensory and textural qualities of stored fruits and vegetables, while inhibiting the growth of certain spoilage organisms.

Vacuum packaging is accomplished by evacuating all the air before sealing, either by inserting a vacuum probe into the neck of the package, or by placing the package into a chamber and evacuating. The absence of oxygen from vacuum packed foods will not only prevent oxidative transformations in both plant or animal tissues and aerobic microbes, but also control oxidative rancidity of fats. Vacuum packaging retards the growth of common aerobic spoilage bacteria such as *Pseudomonas* species, on refrigerated fresh meat, poultry and fish, reducing putrefaction and slime formation. Therefore, It has become the method for packing table-ready meat items. However, it may permit conditions suitable for the growth and toxin production by anaerobic and facultative pathogenic organisms.

Modified atmospheres are generated during packaging by the initial alteration of the gaseous environment in the immediate vicinity of the product. This is achieved by filling the headspace of the food packages by 20-60% carbon dioxide, which will further vary depending on the type of fruits and vegetables and the targeted microorganisms. Modified atmospheres slow down the respiration rate of food as well as microbial growth and reduce the enzymatic degradation. Under these conditions, a variety of spoilage organisms, including *Pseudomonas* spp., *Acinetobacter* spp., and *Moraxella* spp. are inhibited, yet lactic acid bacteria grow slowly.

Removal of microorganisms

The removal of microorganisms is not a very suitable and effective way of food preservation, though it may be helpful under special conditions. Removal may be accomplished by filtration, centrifugation, washing, or trimming. Filtration through a previously sterilized filter made of asbestos pads, sintered glass, diatomaceous earth or similar materials has been used successfully for fruit juices, beer, soft drinks, wine, and water. Centrifugation or sedimentation, generally is not very effective in removing all microorganisms, though is applied for the treatment of water and clarification of milk. The bacteria- removing - centrifuge called bactofuge is used to remove heat-resistant and other bacteria from the milk prior to pasteurization (Fig. 1). This includes the spores of heat resistant bacteria such as *Clostridia sp.* and *Bacillus sp.*, which can remain active in the milk after pasteurization. By using bactofuge, milk has a longer shelf life, better taste, lower bacterial cell counts and reduced impurities.

Food preservation by high temperature

- By destructive effect of heat on microorganisms
- Temperature higher than ambient temperature is applied to food
- By two methods *viz*. pasteurization and sterilization.

Pasteurization:

- Use of heat at 60~80^{0}C for a few minutes for the elimination/ destruction of all disease causing microorganisms, and reduction of potential spoilage organisms
- Commonly used in the preservation of milk, fruit juices, pickles, sauces, beer etc
- Milk Pasteurization - heating the milk at 63^{0}C for 30 min, called low temperature long time (LTLT) process;
- 72^{0}C for 15 sec, called high temperature short time (HTST) process. This process destroys most heat resistant non-spore forming pathogens (Ex. *Mycobacterium tuberculosis*), all yeasts, molds, Gram negative bacteria and most Gram positive bacteria

Organisms surviving pasteurization

- Some organisms survive pasteurization process. The surviving organisms are of two types;
    1. Thermoduric microorganisms
    2. Thermophilic microorganisms
- Thermoduric microorganisms - survive exposure to relatively high temperature but do not grow at these temperatures
- Example: The non-spore forming *Streptococcus* and *Lactobacillus* sp can grow and cause spoilage at normal temperature. So, milk need to be refrigerated after pasteurization to prevent spoilage.
- Thermophilic - not only survive high temperature treatment but require high temperature for their growth and metabolic activities. Example: *Bacillus, Clostridium, Alicyclobacillus, Geobacillus etc.*

Sterilization:

- Sterilization or appertization - destruction of all viable organisms in food as measured by an appropriate enumeration method
- Kills all viable pathogenic and spoilage organisms
- Survivors - non-pathogenic and unable to develop in product under normal conditions of storage
- Thus, sterilized products have long shelf life.
- Commercial sterility - canned foods to indicate the absence of viable microorganisms detectable by culture methods or the number of survivors is so low that they are of no significance under condition of canning and storage
- Foods (solid or semisolid) - packing in cans, sealing and then sterilized
- Liquid foods are sterilized, packed in suitable containers and sealed aseptically
- Temperature and time of sterilization given to a food depends on the nature (pH, physical state, nutritional type etc) of the food being processed

Heat resistance of spores

- Bacterial spores - more heat resistant than vegetative cells. High temperature in canning-spore inactivation .The heat resistance of bacterial endospore is due to their ability to maintain very low water content in the DNA containing protoplast. Presence of calcium

and dipicolonic acid in high concentration in spores helps to reduce cytoplasmic water. Higher the degree of spore dehydration greater will be its heat resistance

Factors affecting heat destruction of microorganisms

Water: Heat resistance of microorganisms increases with decrease in moisture/ water activity and humidity. This is due to faster denaturation of protein in presence of water than air.

Fat: Heat resistance increases in presence of fat due to direct effect of fat on cell moisture. Heat protective effect of long chain fatly acids is better than short chain fatly acids.

Salts: Effect depends on type of salt, concentration used, and other factors. Some salts (sodium salts) have protective effect on microorganisms and others (Ca^{2+} and Mg^{2+}) make cells more sensitive. salts (Ca and Mg) increase water activity, while others (Na^{+}) decrease water activity there by affecting heat sensitivity

Carbohydrtes: Increases heat resistance of microorganisms due to decreased $a_{w.}$ Heat resistance decreases in the order of; sucrose>glucose>sorbitol>fructose>glycerol

PH: Microorganisms are most heat resistant to heat at their optimum pH for growth (about pH 7-0). Increase or decrease in pH reduces heat sensitivity. High acid foods require less heat processing than low acid foods

Proteins: Proteins have protective effect on microorganisms. High protein foods need a higher heat treatment than low protein foods to obtain similar results

Number of microorganisms: Larger the number of microorganisms, higher the degree of heat resistance due to the production of protective substance excreted by bacterial cells, and natural variations in a microbial population to heat resistance

Inhibiting compounds: Heat resistance of most microorganisms decreases in the presence of heat resistant microbial inhibitor such as antibiotic (nisin), sulphur dioxide etc. Heat and inhibiting substances together are more effective in controlling spoilage of foods than either alone

Time and temperature: The longer the heating time, greater the killing effect. But higher the temperature, greater will be the killing effect. As temperature increases, time necessary to achieve the same effect decreases. The size and composition of containers affect heat penetration

Thermal destruction of microorganisms

- The preservative effect of high temperature treatment depends on the extent of destruction of microorganisms
- Certain basic concepts are associated with the thermal destruction of microorganisms include;
  - Thermal death time (TDT)
  - D- value
  - Z- value
  - F- value
  - 12D concept

Thermal death time (TDT):

- TDT - time required to kill a given number of organisms at a specified temperature
- Temperature is kept constant and the time necessary to kill all cells is determined
- Thermal death point is the temperature necessary to kill given number of organisms in a fixed time, usually 10 min. But it is of less importance
- TDT is determined by placing a known number of bacterial cells/spores in sealed containers, heating in a oil bath for required time and cooling quickly

- The number of survivors from each test period is determined by plating on a suitable growth media
- Death is defined as the inability of organism to form viable colonies after incubation.

D-value (Decimal reduction time):

- D-value is the time in minutes required at specified temperature to kill 90% of microorganisms thereby reducing the count by 1 log units
- Hence D – value is the measure of death rate of microorganisms
- It reflects the resistance of an organism to a specific temperature and can be used to compare the relative heat resistance among different organisms/spores
- D-value for the same organism varies depending on the food type
- D -value is lower in acid foods and higher in presence of high proteins
- Example: $D_{250^oF\ (121.1^oC)}$ for *B. stearothermophilus*: 4-5 min
  C. botulinum: 0.1 – 0.2 min.
  - » D_{95^oC} for *B. coagulans*: 13.7 min
    B. licheniformis: 5.1 min.

Z – Value:

- Z-value refers to degrees of Fahrheit required for the thermal destruction curve to drop by one log cycle
- Z value gives information on the relative resistance of an organism for different destruction temperature
- It helps to determine equivalent thermal process at different temperature
- Example: If adequate heat process is achieved at 150^0F for 3 min and Z -value was determined as 10^0F, which means the10^0F rise in temperature reduces microorganisms by 1 log unit
- Therefore, at 140^0F , heat process need to be for 30 min and at 160^0F for 0.3 min to ensure adequate process

F – Value:

- F- value is the better way of expressing TDT. F- is the time in minutes required to kill all spores/vegetative cells at 250^0F (1210^0C)
- It is the capacity of heat process to reduce the number of spores or vegetarian cells of an organism
- F – Value is calculated by

$$F_0 = D_r (\log a - \log b)$$

D_r = Decimal reduction time (D value)
a = initial cell numbers
b = final cell numbers

12D concept:

- 12D concept is used mainly in low acid canned foods (pH >4.6) where *C. botulinum* is a serious concern
- 12D concept refers to thermal processing requirements designed to reduce the probability of survival of the most heat resistant *C. botulinum* spores to 10^{-12}
- This helps to determine the time required at process temperature of 121^o C to reduce spores of *C. botulinum* to 1 spore in only 1of 1 billion containers (with an assumption that each container of food containing only 1 spore of *C. botulinum)*

Protection of Foods with Low Temperatures

- The use of low temperatures to preserve foods is based on the fact that the activities of microorganisms can be slowed at temperatures above freezing and generally stopped at subfreezing temperatures. The reason is that all metabolic reactions of microorganisms are enzyme catalyzed and that the rate of enzyme-catalyzed reactions is dependent on temperature. With a rise in temperature, there is an increase in reaction rate. The temperature coefficient (Q10) may be generally defined as follows:

$$Q_{10} = \frac{\text{(Velocity at a given temp. } +10^{\circ}\text{C)}}{\text{Velocity at T}}$$

- The Q10 for most biological systems is 1.5–2.5, so that for each 10◦C rise in temperature within the suitable range, there is a twofold increase in the rate of reaction. For every 10◦Cdecrease in temperature, the reverse is true.

- **Psychrophile:** This term is now applied to organisms that grow over the range of subzero to 20◦C, with an optimum range of 10–15◦C.

- **Psychrotroph**: is an organism that can grow at temperatures between 0◦C and 7◦C and produce visible colonies (or turbidity) within 7–10 days in this temperature range.

- Because some psychrotrophs can grow at temperatures at least as high as 43◦C, they are, in fact, mesophiles. By these definitions, psychrophiles would be expected to occur only on products from oceanic waters or from extremely cold climes.

Table 3.12 *Cardinal temperatures for microbial growth*

	Temperature (°C)		
Group	*Minimum*	*Optimum*	*Maximum*
Thermophiles	40–45	55–75	60–90
Mesophiles	5–15	30–40	40–47
Psychrophiles (obligate psychrophiles)	−5 to +5	12–15	15–20
Psychrotrophs (facultative psychrophiles)	−5 to +5	25–30	30–35

- **Psychrotrophs include:**

➢ Eurypsychrotroph (eurys, wide or broad):

- Typically do not form visible colonies until sometime between 6 and 10 days. Can grow well at 43◦C. Such as Enterobacter cloacae, Hafnia alvei, and Yersinia enterocolitica .

➢ Stenopsychrotroph (stenos, narrow, little, or close):

- Stenopsychrotrophs typically form visible colonies in about 5 days. Do not grow at 40◦C Such as *Pseudomonas fragi* and *Aeromonas hydrophila* . Psychrotrophs can be distinguished from nonpsychrotrophs by their inability to grow on a nonselective medium at 43◦C in 24 hours, whereas the latter do grow.

- **There are three distinct temperature ranges for low-temperature stored foods:**

1) Chilling temperatures are those between the usual refrigerator (5–7◦C) and ambient temperatures, usually about 10–15◦C. These temperatures are suitable for the storage of certain vegetables and fruits such as cucumbers, potatoes, and limes.

2) Refrigerator temperatures are those between 0◦C and 7◦C (ideally no higher than 40◦F or 4.4◦C).

3) Freezer temperatures are those at or below −18◦C.

PREPARATION OF FOODS FOR FREEZING

- Blanching is achieved either by a brief immersion of foods into hot water or by the use of steam.
- Its primary functions are as follows:

➢ 1. Inactivation of enzymes that might cause undesirable changes during freezing storage

➢ 2. Enhancement or fixing of the green color of certain vegetables

➢ 3. Reduction in the numbers of microorganisms on the foods

➢ 4. Facilitating the packing of leafy vegetables by inducing wilting

➢ 5. Displacement of entrapped air in the plant tissues

➢ Although it is not the primary function of blanching to destroy microorganisms, the amount of heat necessary to effect destruction of most food enzymes is also sufficient to reduce vegetative cells significantly.

- **FREEZING OF FOODS AND FREEZING EFFECTS**
- The two basic ways to achieve the freezing of foods are:

- **Quick (fast) freezing :**

➢ Temperature of foods is lowered to about −20◦C within 30 minutes.

➢ Form small intracellular ice crystals.

- **Slow freezing:**

➢ Temperature of foods is lowered within 3–72 hours.

➢ This is essentially the type of freezing utilized in the home freezer.

➢ Form large extracellular ice crystals.

➢ Crystal growth is one of the factors that limit the freezer life of certain foods, because ice crystals growin size and cause cell damage by disrupting membranes, cell walls, and

internal structures to the point where the thawed product is quite unlike the original in texture and flavor.

- Upon thawing, foods frozen by the slow freezing method tend to lose more drip (drip for meats; leakage in the case of vegetables) than quick-frozen foods held for comparable periods of time.

- **EFFECT OF FREEZING ON MICROORGANISMS**

In considering the effect of freezing on those microorganisms that are unable to grow at freezing temperatures, it is well known that freezing is one means of preserving microbial cultures, with freeze drying being perhaps the best method known.

However, freezing temperatures have been shown to effect the killing of certain microorganisms of importance in foods.

- **The salient facts of what happens to certain microorganisms upon freezing:**

- 1. There is a sudden mortality immediately on freezing, varying with species.
- 2. The proportion of cells surviving immediately after freezing die gradually when stored in the frozen state.
- 3. This decline in numbers is relatively rapid at temperatures just belowthe freezing point, especially about −2◦C, but less so at lower temperatures, and it is usually slow below −20◦C.
- Bacteria differ in their capacity to survive during freezing, with cocci being generally more resistant than Gram-negative rods.
- Of the food-poisoning bacteria, salmonellae are less resistant than Staphylococcus aureus or vegetative cells of clostridia, whereas endospores and food-poisoning toxins are apparently unaffected by low temperatures.
- From the strict standpoint of food preservation, freezing should not be regarded as a means of destroying foodborne microorganisms.
- Low freezing temperatures of about −20◦C are less harmful to microorganisms than the median range of temperatures, such as −10◦C.
- For example, more microorganisms are destroyed at −4◦C than at −15◦C or below.
- Temperatures below −24◦C seem to have no additional effect.
- Food constituents such as egg white, sucrose, corn syrup, fish, glycerol, and undenatured meat extracts have all been found to increase freezing viability, especially of food-poisoning bacteria, whereas acid conditions have been found to decrease cell viability.

Consider some of the events that are known to occur when cells freeze:

- 1. The water that freezes is the so-called free water. Upon freezing, the free water forms ice crystals. Bound water remains unfrozen. The freezing of cells depletes them of usable liquid water and thus dehydrates them
- 2. Freezing results in an increase in the viscosity of cellular matter, a direct consequence of water being concentrated in the form of ice crystals.
- 3. Freezing results in a loss of cytoplasmic gases such as O2 and CO2. A loss of O2 to aerobic cells suppresses respiratory reactions. Also, the more diffuse state of O2 may make for greater oxidative activities within the cell.
- 4. Freezing causes changes in pH of cellular matter. Various investigators have reported changes ranging from 0.3 to 2.0 pH units. Increases and decreases of pH upon freezing and thawing have been reported.
- 5. Freezing effects concentration of cellular electrolytes. This effect is also a consequence of the concentration of water in the form of ice crystals.
- 6. Freezing causes a general alteration of the colloidal state of cellular protoplasm. Many of the constituents of cellular protoplasm such as proteins exist in a dynamic colloidal state in living cells. A proper amount of water is necessary to the well-being of this state.
- 7. Freezing causes some denaturation of cellular.
- 8. Freezing induces temperature shock in some microorganisms. This is true more for thermophiles and mesophiles than for psychrophiles. More cells die when the temperature decline above freezing is sudden than when it is slow.
- 9. Freezing causes metabolic injury to some microbial cells such as certain Pseudomonas spp. Some bacteria have increased nutritional requirements upon thawing from the frozen state and as much as 40% of a culture may be affected in this way.

Effect of Thawing

- Repeated freezing and thawing will destroy bacteria by disrupting cell membranes.
- Also, the faster the thaw, the greater the number of bacterial survivors. Why this is so is not entirely clear.
- It has been pointed out that thawing is inherently slower than freezing and follows a pattern that is potentially more detrimental.

Among the problems attendant on the thawing of specimens and products that transmit heat energy primarily by conduction, are the following:

- 1. Thawing is inherently slower than freezing when conducted under comparable temperature differentials.
- 2. In practice, the maximum temperature differential permissible during thawing is much less than that which is feasible during freezing.

- 3. The time–temperature pattern characteristic of thawing is potentially more detrimental than that of freezing. During thawing, the temperature rises rapidly to near the melting point and remains there throughout the long course of thawing, thus affording considerable opportunity for chemical reactions, recrystallization, and even microbial growth, if thawing is extremely slow.
- It has been stated that microorganisms die not upon freezing but, rather, during the thawing process.
- As to why some organisms are able to survive freezing while others do not, Luyet39 suggested that it is a question of the ability of an organism to survive dehydration and to undergo dehydration when the medium freezes.
- With respect to survival after freeze-drying, Luyet has stated that it may be due to the fact that bacteria do not freeze at all but merely dry up.
- It is fairly well established that the freeze-thaw cycle leads to:

(1) ice nucleation

(2) dehydration,

(3) oxidative damage.

- During thawing, an oxidative burst has been shown to occur and superoxide dismutase (SOD) provides resistance to the deleterious oxidative effects
- Most frozen-foods processors advise against the refreezing of foods once they have been thawed.
- Although the reasons are more related to the texture, flavor, and other nutritional qualities of the frozen product, the microbiology of thawed frozen foods is pertinent.
- Some investigators have pointed out that foods from the frozen state spoil faster than similar fresh products.
- There are textural changes associated with freezing that would seem to aid the invasion of surface organisms into deeper parts
- of the produce and, consequently, facilitate the spoilage process.

Upon thawing, surface condensation of water is known to occur.

- There is also, at the surface, a general concentration of water-soluble substances such as amino acids, minerals, B vitamins, and, possibly, other nutrients.
- Freezing has the effect of destroying many thermophilic and some mesophilic organisms, making for less competition among the survivors upon thawing.
- It is conceivable that a greater relative number of psychrotrophs on thawed foods might increase the spoilage rate.

- Some psychrotrophic bacteria have been reported to have Q10 values in excess of 4.0 at refrigerator temperatures.
- For example, P. fragi has been reported to possess a Q10 of 4.3 at 0◦C.
- Organisms of this type are capable of doubling their growth rate with only a 4–5◦C rise in temperature.
- Although there are no known toxic effects associated with the refreezing of frozen and thawed foods, this act should be minimized in the interest of the overall nutritional quality of the products.
- One effect of freezing and thawing animal tissues is the release of lysosomal enzymes consisting of cathepsins, nucleases, phosphatases, glycosidases, and others.
- Once released, these enzymes may act to degrade macromolecules and thus make available simpler compounds that are more readily utilized by the spoilage biota.

SOME CHARACTERISTICS OF PSYCHROTROPHS AND PSYCHROPHILES

There is an increase in unsaturated fatty acid residues.

- It is known that an increase in the degree of unsaturation of fatty acids in lipids leads to a decrease in the lipid melting point.
- It has been suggested that increased synthesis of unsaturated fatty acids at low temperatures has the function of maintaining the lipid in a liquid and mobile state, thereby allowing membrane activity to continue to function.

This concept, referred to as **the lipid solidification theory**

Psychrotrophs synthesize high levels of polysaccharides.

- From a practical standpoint, increased polysaccharide synthesis at low temperatures manifests itself in the characteristic appearance of low-temperature spoiled meats.
- Slime formation is characteristic of the bacterial spoilage of frankfurters, fresh poultry, and ground beef.
- The coalescence of surface colonies leads to the sliminess of such meats, and no doubt contributes to the increased hydration capacity that accompanies low-temperature meat spoilage.
- This extra polymeric material undoubtedly plays a role in biofilm formation.

- **Pigment production is favored**.
- This effect appears to be confined to those organisms that synthesize phenazine and carotenoid pigments.

- **Some strains display differential substrate utilization.**

THE EFFECT OF LOW TEMPERATURES ON MICROBIAL PHYSIOLOGIC MECHANISMS

Psychrotrophs have a slower metabolic rate.

- The precise reasons as to why metabolic rates are slowed at low temperatures are not fully understood.
- Psychrotrophic growth decreases more slowly than that of mesophilic with decreasing temperatures.
- The temperature coefficients (Q10) for various substrates such as acetate and glucose have been shown by several investigators to be lower for growing psychrotrophs than for mesophiles.
- As noted above, psychrotrophs tend to possess in their membrane lipids that enable the membrane to be more fluid.
- The greater mobility of the psychrotrophic membrane may be expected to facilitate membrane transport at low temperatures.
- In addition, the transport permeases of psychrotrophs are apparently more operative under these conditions than are those of other mesophiles.
- As the temperature is decreased, the rate of protein synthesis is known to decrease, and this occurs in the absence of changes in the amount of cellular DNA.
- One reason may be the increase in intramolecular hydrogen bonding that occurs at low temperatures, leading to increased folding of enzymes with losses in catalytic activity.
- **Psychrotroph membranes transport solutes more efficiently**.
- It has been shown in several studies that upon lowering the growth temperature of mesophiles within the psychrotrophic range, solute uptake is decreased.
- **Some psychrotrophs produce larger cells**.
- Yeasts, molds, and bacteria have been found to produce larger cell sizes when growing under psychrotrophic conditions than under mesophilic conditions.
- On the other hand, psychrotrophic organisms are generally regarded as having higher levels of both RNA and proteins.
- **Flagella synthesis is more efficient.**
- **Psychrotrophs are favorably affected by aeration.**
- It has been commonly observed that plate counts on many foods are higher with incubation at low temperatures than at temperatures of 30◦C and above.

- The generally higher counts are due in part to the increased solubility and consequently, the availability of O2.
- **Some psychrotrophs display an increased requirement for organic nutrients.**
- In one study, the generation times for unidentified aquatic bacterial isolates in low-nutrient media were two to three times longer than in high-nutrient media.

NATURE OF THE LOW HEAT RESISTANCE OF PSYCHROTROPHS/PSYCHROPHILES

- The maximum growth temperatures of bacteria may bear a definite relationship to the minimum temperatures of destruction of respiratory enzymes.
- It has been shown that many respiratory enzymes are inactivated at the temperatures of maximal growth of various psychrotrophic types.
- Thus, the thermal sensitivity of certain enzymes of psychrotrophs is at least one of the factors that limit the growth of these organisms to low temperatures.
- Somewhat surprisingly, the proteinases of many psychrotrophic bacteria found in raw milk are heat resistant.
- The typical raw milk psychrotrophic pseudomonad produces a heat stable metalloproteinase with molecular weight in the 40- to 50-kDa range, which has a D value at 70◦C of 118 minutes or higher.

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Irradiation

The type of radiation of primary interest in food preservation is electromagnetic which includes microwaves, ultraviolet rays, and ionizing radiations.

Microwave radiations

The microwave region of the electromagnetic spectrum occupies frequencies between the infrared (10^9 Hz) and radio frequency (10^{12} Hz) and has relatively low quantum energy. Most food research has been carried out at two frequencies; 915MHz and 2450 MHz. Microwaves are generated using a magnetron, a device first developed in the UK during research into radar during the Second World War. Although microwaves are used both commercially and domestically in domestic microwave ovens and in catering, these have been slow to find industrial applications in food processing. Microwaves have been used to defrost frozen meats before cutting, in blanching of vegetables and fruits, destruction of molds in bread, pasteurization of beer and sterilization of wine.

Microwaves act indirectly on microorganisms through the generation of heat. When food-containing water is placed in a microwave field of 950 MHz, water molecules oscillate back and forth 915 million times/sec creating an intermolecular fraction. This kinetic energy is transmitted to neighboring molecules leading to a rapid rise in temperature throughout the product. This heating effect is responsible for killing microorganisms in food exposed to microwave radiations.

UV Radiation

Ultraviolet light is a powerful bactericidal agent with the most effective wavelength being about 260 nm. It is absorbed by purine and pyrimidine bases causing the production of covalent bonds between adjacent thymine molecules giving thymine dimers. This may prevent the DNA replication in the normal way and disrupt gene functioning by creating new mutants. Although microorganisms have the capacity to repair this DNA damage, extensive damage may cross the limits of DNA repair mechanisms leading to cell death. The resistance of microorganisms to UV is largely determined by their ability to repair such damage. In addition to the repair mechanisms, some organisms such as micrococci also synthesize protective pigments. Generally, the resistance to UV irradiation follows the pattern: Gram-negative < Gram-positive < yeast < bacterial spores < mold spores < viruses. The UV D values for these groups are 3-4, 6-8, 6-10, 8-10, 20-100 and >200 ergs x 10^2 respectively.

High intensity ultraviolet radiation generated by low-pressure mercury vapor lamps is extremely effective in killing microorganisms. The poor penetrating capacity of UV light restricts its use in food applications. UV radiations are able to penetrate only to 300-500 cms in air, 30 cms in water, 0.1 cm in glass and 0.01 cm in milk. Therefore, the practical applications of UV light are limited to surface disinfections and air sterilization such as in hospital theaters, aseptic filling rooms in pharmaceutical industry, in food and dairy industry (in sterile packaging of UHT milk and in bakery to control mold spores). UV radiation is commonly used as an alternative to chlorination in the disinfections of water in water filters installed at homes and offices such as Aqua Guard, Aqua Care etc.

Ionizing Radiations

Ionizing radiations such as X-rays and gamma γ-rays generated by X-ray apparatus and radioisotopes such as cobalt 60 (^{60}Co and ^{137}Cs) respectively are highly effective in killing microorganisms. Since they destroy microorganisms without appreciably raising temperature, the process is termed "cold sterilization." Ionizing radiations can affect the cells directly by interacting with key molecules with in the microbial cell. The main site of damage in cells is the chromosome where hydroxyl radicals cause single and double strand breaks in the DNA molecule as a result of hydrogen removal from deoxyribose sugar. Further cleavage of the molecule occurs by β elimination of phosphate. Ionizing radiations also have indirect inhibitory effect on cell constituents by generating free radicals produced by the radiolysis of water. Free radicals formed from water can combine with each other or oxygen molecules to give powerful oxidizing agents that can damage cell components. Thus in the absence of water and oxygen, radiation doses 2-3 times higher are required to obtain the same lethality.

Death of microorganisms caused by ionizing radiation is logarithmic, producing survivor curves that are similar to those produced by heat. In this case, the number of survivors is plotted against the radiation dose and D values are calculated as the dose required to kill 90% of the population. The radiation dose is currently measured in Gray (Gy), which is equivalent to 1 joule of energy absorbed/kg of material. Microbial resistance to radiation usually decreases in the order viruses > bacterial spores > pigmented mold spores > yeast and molds > Gram-positive bacteria > Gram-

negative bacteria. The most resistance organism is *Micrococcus radiodurans* which has a D value of >30 kGy.

The electromagnetic radiations (gamma rays) emitted from the excited nucleus of ^{60}Co or ^{137}Cs are the cheapest form of radiation for food preservation. Unlike UV light, gamma rays have excellent penetration power so that foods can be packaged and then irradiated to destroy contaminating microorganisms, making it potentially an ideal method of food preservation. Foods are irradiated by using gamma rays in the three following ways:

i) Radappertization

Radappertization is equivalent to radiation sterilization or "commercial sterility" of low acid foods which requires a dose of radiation capable of giving a 12D reduction in the number of spores of *Clostridium botulinum*. As the D value for *C. botulinum* is 3.5 kGy, the dose required will be 42 kGy to achieve 12D kill. The application of radappertization is restricted to only few food products such as bacon as the high doses of radiation may cause color changes and or production of off odors.

ii) Radicidation

Radicidation refers to reduction of the number of viable specific non-sporeforming bacterial pathogens such as *Salmonella* and is equivalent to pasteurization of milk. Irradiation levels of 2-5 kGy are effective in destroying non-sporeforming and non-viral pathogens. The foods such as fresh poultry, cod and red fish, and spices and condiments are preserved by irradiating at these levels.

iii) Radurization

Radurization refers to the enhancement of the keeping quality of a food by causing substantial reduction in the numbers of viable spoilage microorganisms especially gram-negative, non-sporeforming rods by low levels of radiation. Common dose levels are 0.075-2.5 kGy for fresh meats, poultry, seafood, fruits, vegetables, and cereal grains. The shelf life of seafood, fish and shellfish may be extended from two to six folds by radurization.

Not all foods are suitable for irradiation treatment. Softening and discoloration may occur in the case of some fruits. Milk may acquire an unpleasant taste. Certain protein foods are flavor sensitive to irradiation and may develop off-flavors. Another major limitation of irradiation processing of food is its slow acceptance by the consumers, due to a perceived association with radioactivity.

High Hydrostatic Pressures

During high hydrostatic pressure (HHP) processing, foods are subjected to pressures in the range 100 to 1000 MPa (megapascals). High pressures are known to have an antimicrobial effect which appears to be associated with the denaturation of cell proteins and damage to cell membranes. Membrane lipid bilayers have been shown to compress under pressure that alters their permeability. The application of high pressures for food processing is referred to as pascalization. Overall, HHP is very effective in inactivating vegetative cells of microorganisms, but pressure treatment alone does not achieve a substantial inactivation of spores and reduction in activity of certain enzymes. Although, vegetative bacteria, yeast and molds can be reduced by at least one log cycle by 400 MPa applied for 5 min, bacterial endospores can tolerate pressures

as high as 1200 MPa. Therefore, the commercial application of pascalization has been limited to only acid and high acid foods like fruit juices and sauces in which bacterial spores that survive processing are unable to grow. Foods preserved by this technology resemble very much to the fresh product and appears natural to the consumer with none of the negative associations of processes such as heat, irradiation and chemical preservation. Interest in using high-pressure technology to extend the shelf life of low acid foods is increasing by combining this treatment with other food preservation methods.

Relative resistance of microorganisms to HHP is as follows: Bacterial spores > Gram-positives (vegetative cells) > Gram-negatives ≅ yeast and molds.

microorganisms. They do not include substances, which enhance the shelf life of foods by inhibiting a chemical reaction such as rancidity or discoloration. The chemical preservatives may be either intentionally added to the food or may be developed during the growth of microorganisms as in case of some fermentation (lactic acid, acetic acid, bacteriocins etc.). The use of chemical preservatives in foods may allow products to be subjected to less severe heat treatments, resulting in an improvement in product quality and consumer acceptability. While a large number of chemicals have been described that show potential as food preservatives, only a relatively small number are allowed in food products. This is due in large part to the strict rules of safety adhered to by Food and Drug Administration (FDA).

A chemical preservative should have a wide range of antimicrobial activity, should be non-toxic to humans or animals, should be economical, should not have an effect on flavor, taste, aroma of the original food, should not be inactivated by food and should not encourage the development of resistant strains. There are added chemical preservatives which are not defined as such by law such as natural organic acids (lactic, malic, citric etc.), vinegars, sodium chloride, sugars, spices and their oils, wood smoke etc. On the other hand, there are some chemical substances, which are generally recognized as safe (GRAS) for addition to foods such as organic acids and their salts (Propionic, sorbic and benzoic) sodium nitrite, sulfur dioxide and metabisulfites and nisin, a biopreservative. Most of the common antimicrobial additives used in foods and their current allowable levels are presented in Table 2.

Organic Acids and their Salts

Malic, citric, tartaric acids are found naturally in fruits and will inhibit most bacteria. Lactic and acetic acids are produced naturally by microorganisms in amounts sufficient to exert an effect on flavor and the pH of the product, thus potentiating their own action by increasing the proportion of undissociated acid present. Propionic, sorbic, benzoic acids and parabens (*para*-hydroxybenzoic acid esters) are not generally found naturally in foods or produced by microorganisms. There are exceptions, e.g. propionic acid is produced in Swiss cheese by *Propionibacterium spp* and benzoic acid is found in cranberries. These acids are sometime considered to be 'true' chemical preservatives.

Benzoic Acids and Parabens

Benzoic acids and its sodium salts are widely used as antimicrobial compounds in a large number of foods. The antimicrobial activity of benzoate is related to pH, the greatest activity being at low pH values and essentially ineffective at neutral values. This indicates that the antimicrobial activity resides in the undissociated molecule at pH between 2.5 and 4.0. This results in the restriction of benzoic acid and its sodium salts to high acid products such as apple cider, soft drinks, jams, jellies, fruit salads, pickles, tomato catsup. As used in acidic foods, benzoates and their sodium salts act mainly as a mold and yeast inhibitor.

Among parabens, ethyl and methyl parabens are extensively used in foods. Though, these compounds are similar to benzoic acid in their effectiveness, they have an added advantage of being effective at even higher pH values. Because of the esterification of the carboxyl group, the undissociated molecule is retained over a wider pH range exerting inhibitory effect even at neutral pH. This means that they can be used effectively in low and non-acid foods.

Maximum levels of some GRAS chemical food preservatives permitted in foods

Preservatives	Maximum concentrations allowed	Organisms affected	Foods
Benzoic acids	0.1%	Yeasts and molds	Jams, jellies, salad dressings, apple cider, soft drinks, pickles, tomato catsup
Parabens (methyl-, propyl-, and heptyl esters of p-hydroxybenzoic acid)	0.1%	Yeasts and molds	Fruit drinks and beverages, bakery products, salad dressings, apple cider, soft drinks, pickles, tomato catsup
Propionic acid	0.32%	Molds	Bread, cakes, Swiss cheese
Sorbic acid	0.2%	Molds and yeast	Hard cheeses, baked goods, fruit cocktails, syrups, fruit juices, jams and jellies, dried fruits, margarine
SO_2 and sulfites	200-300 ppm	Insects and microorganisms	Wines, molasses, fruit juices, lemon juice, dried fruits (not to be used in meats or other foods containing thiamine)
Nitrites and nitrates	100-120 ppm	Clostridia and molds	Meat and meat products as a meat curing agent
Ethylene and propylene oxide	700 ppm	Yeasts, molds and Clostridia	Fumigants for dried fruits, dried eggs, gelatin, cereals, dried yeast and spices
Ozone	>100 ppm 0.2-0.4 ppm 5-15 ppm	Viruses *Salmonella*, *Pseudomonas* *Botrytis*	Animal sanitation Fish and Poultry Vegetables
Nisin (biopreservative)	100 ppm	Gram+ve spore formers	Processed cheeses, canned fruits and vegetables, condensed milk

In the undissociated form these compounds are soluble in the cell membrane and act apparently as proton ionophores. As such they facilitate proton leakage into the cells thereby increasing the energy output of cells to maintain their usual internal pH. With this disruption in membrane activity, amino acid transport is adversely affected. These compounds have also been found to block the oxidation of glucose and pyruvate at the acetate level. Benzoates have also been found to inhibit the outgrowth of vegetative cells during endospore germination. Maximum concentration of benzoates permitted in foods is 0.1%.

Sorbic Acid

Sorbic acids and their calcium, potassium or sodium salts are permissible in foods at levels not to exceed 0.2%. Like benzoates, they are also most effective at low pH values when present in the undissociated form. These compounds are more effective than sodium benzoate at pH values

between 4.0 and 6.0. Sorbic acid and its salts are used either as a direct antimicrobial additive in foods or as a spray, dip, or coating on packaging materials. These are widely used in cheeses, cheese products, bakery products, beverages, syrups, fruit juices, jellies, jams, pickles and salad dressings. They are active against yeasts, molds and catalase-positive bacteria. Inhibition of mold growth by sorbates is due to the inhibition of the dehydrogenase enzyme system, several other Krebs cycle enzymes and the membrane function impairment affecting the cellular uptake of substrate molecules such as amino acids, phosphate and organic acids. Sorbic acid is also known to inhibit the germination and outgrowth of *C. botulinum* spores.

Propionic acid

Propionic acid and its calcium or sodium salts are permitted in breads, cakes, and certain cheeses as a mold inhibitor to maximum levels of not more than 0.32%. In bread and bread dough it prevents ropiness by inhibiting the rope forming bacilli e.g. *Bacillus subtilis or B. licheniformis*. The mode of action of these compounds on microorganisms is similar to that of benzoates and sorbates. Dissociation tendency of these compounds at high pH values makes them useful preservatives for low acid foods.

Nitrite

Sodium nitrate ($NaNO_3$) and sodium nitrite ($NaNO_2$) are used in curing formulae for meats since they stabilize red meat color, inhibit some spoilage and food poisoning organisms, and contribute to flavor development. In an acid environment nitrite ionizes to nitrous acid that further decomposes to nitric oxide. The nitric oxide co-ordinates to the haem ferrous ion in the muscle pigment myoglobin under reducing conditions converting it to the desirable red pigment nitosomyoglobin. The antibacterial effect of nitrite increases with decreasing pH suggesting that nitrous acid is the active agent. This nitrous acid, being a powerful reducing agent, causes disruption of the cell metabolism and also inhibits the germination and outgrowth of endospores. Nitrite acts as a preservative by inhibiting a wide range of bacteria; including *Clostridium* spp (*C. botulinum* is of particular interest), *Bacillus spp* and *Staphylococcus aureus*. However nitrite is not very effective against lactobacilli or members of the enterobacteriaceae including salmonellae.

Interestingly, it has been shown that the ability of nitrite to inhibit these spore formers in cured, canned, vacuum packed meats and culture media will increase about ten fold if it is added before heating the product. This increased inhibitory activity of nitrite upon heating in a medium is due to the production of a substance referred to as 'Perigo factor'.

It is this Perigo factor that results from the heat processing or smoking of certain meats and fish products containing nitrite that warrants the continued use of nitrite in such products. Nitrite levels of 100 ppm or more in the presence of 3-5% sodium chloride are sufficient to impart an adequate flavor and antibotulinal and antilisterial (against *Listeria monocytogenes*, a bacterial food pathogen) effect in meat products. The only problem with the use of nitrite is their reaction with secondary amines forming nitrosamines that are known to be carcinogenic.

Sulfur Dioxide

Sulfur dioxide (SO_2) and the sodium and potassium salts of sulfite, bisulfite, and metabisulfite have been used as disinfecting agents in wine industry particularly to sanitize wine making equipment and storage vessels and to reduce the normal flora of the grape must. It is also used as an antioxidant to inhibit enzymatic and non-enzymatic browning reactions in some food products. Sulfur dioxide has also been used, in syrups, fruits juices and to treat most light colored dehydrated fruits. The unionized forms of SO_2, which can readily penetrate the cell, have the greatest antimicrobial activity. As a reducing agent it can break disulfide linkages in proteins, and interfere with redox processes. It can also form addition compounds with pyrimidine bases in nucleic acids, sugars and several key metabolic intermediates. However, it has been found to react and destroy the vitamin thiamine present in meat and meat products prohibiting its use in these products.

Sulfur dioxide is active against bacteria, yeasts and molds. Sulfur dioxide, sulfites and metabisulfites are used at 200-300 ppm levels in most of the foods to have their bactericidal effect on all types of microorganisms.

NaCl and Sugars

Both of these preservatives are similar in their mode of action in preserving foods. These compounds tend to tie up moisture and thus exert a drying effect on both food and microorganisms. Salts are added in brine and curing solutions or applied directly to foods to slow down and prevent the activity of food spoilage and pathogenic organisms. The addition of salts has the following effects on food and microorganisms:

1. It causes high osmotic pressure and hence, plasmolysis of cells,
2. It dehydrates foods and microbial cells by drawing out and tying up moisture,
3. It ionizes to yield the chlorine ion, which is harmful to organisms,
4. It reduces the solubility of oxygen in water,
5. It sensitizes the cell against carbon dioxide and
6. It interferes with the action of proteolytic enzymes. The concentration of salt in food varies with the taste of the consumer and type of food. In the absence of refrigeration, salting may effectively preserve fish and other meats.

Sugars such as sucrose exert the same preserving effect, as salt but requires in about six times higher concentrations than salt to affect the same degree of inhibition. The most common uses of sugars as preserving agents are in the making of fruit preserves, candies, chocolates, condensed milk, cakes and pies. The shelf stability of these products is due in large part to the preserving effect of high concentrations of sugar.

Gases

Gases can be used to sterilize materials, which can not withstand the high temperatures of heat sterilization like many organic compounds, volatile food flavors and some plastic material. Gaseous sterilization offers a means for packaging heat sensitive products that only affect airborne surface bacteria but also it can attack the microbial cells after penetrating the porous materials. Some of these gases used to inactivate microorganisms are ethylene oxide, propylene oxide, methyl bromide and formaldehyde.

Ethylene oxide

Ethylene oxide, cyclic ether, is the most commonly used gas for effective sterilization of packaged items, dry products etc., at room temperature because of its good penetration with little damage to materials. The microbicidal action of ethylene oxide gas is directly related to the alkylating activity of cellular enzymes and other proteins. It has been used to sterilize spices, cereals, fruits and dry fruits and dried yeast. However, it is flammable, expensive, and toxic and requires three hours or more for effective sterilization and may alter nutrients and other quality factors of foods.

Ozone

Ozone has recently gained the attention of food and agricultural industries, though it has been used effectively as a primary disinfectant for the treatment of municipal and bottled drinking water for 100 years. In 2001, the Food and Drug Administration (FDA) allowed for the use of ozone as a direct contact-sanitizing agent.

Because of its very high oxidation reduction potential, ozone acts as an oxidant of the constituent elements of cell walls before penetrating inside microorganisms and oxidizing certain essential components e.g., unsaturated lipids, enzymes, proteins, nucleic acids, etc. When a large part of the membrane barrier is destroyed causing a leakage of cell contents, the bacterial or protozoan cells lyse resulting in the destruction of the cell. Most of the pathogenic and food borne microbes are susceptible to this oxidizing effect.

In aqueous solutions, ozone can be used to disinfect equipment, process water, and some food products. It has been used to decontaminate poultry meat, salmon, apples, strawberries and cauliflower. In gaseous form it has been to preserve eggs during cold storage, fresh fruits and vegetables, and fresh fish. Ozone can also be used during the washing of produce before it is packaged and shipped to supermarkets, grocery stores, and restaurants. In food industry, much attention is given to the cleaning and sanitizing operations of food-processing equipment. Water containing low concentrations of ozone can be sprayed onto processing equipment, walls or floors to both remove and kill bacteria or other organic matter that may be present.

The concentrations of ozone, which are large enough for effective decontamination, may change the sensory qualities and colour of some food products, such as meat, milk powder and fish cake due to lipid oxidation. Additionally, microorganisms embedded in product surfaces are more resistant to ozone than those readily exposed to the sanitizer. Ozone is a toxic gas and can cause severe illness, and even death, if inhaled in high quantity. Exposure restrictions to plant operators must be addressed with leak proof system design and process operation.

Biopreservatives

Artificial chemical preservatives are employed to limit the number of microorganisms capable of growing within foods, but increasing consumer awareness of potential health risks associated with some of these substances, has led researchers to examine the possibility of using natural additives. For instance, egg white lysozyme is employed at levels in excess of 100 tones per annum in some cheeses to prevent blowing (gas production) by lysing the vegetative cells of *Clostridium tyrobutyricum*. Activation of the lactoperoxidase system has been shown to be

useful to extend the keeping quality of milk in countries like India where pasteurization is not possible immediately after milking and refrigerated transport systems are poorly developed. Plant derived antimicrobials such as the extracts of herbs and spices are being commonly used in preservation of foods for controlling microorganisms. Microbial products like antibiotics and bacteriocins in particular whether produced by fermenting microorganisms or added from outside are being increasing used in cheese and canned foods. The broad -spectrum antibiotics such as chlorotetracycline (CTC) or oxytetracycline (OTC) were permitted at 5-7μg/g in fish, poultry, shrimps, etc. till 1959. However due to the hazards of the development of resistant strains of pathogens, the potential of hypersensitivity of humans to the antibiotics, the presence of residual antibiotics after cooking, costs and difficulties in monitoring these aspects, the use of these antibiotics in foods was never appreciated.

Bacteriocins produced by lactic acid bacteria (LAB) are a heterogeneous group of antibacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties. The food products that have been targeted for use of bacteriocins or bacteriocin like inhibitory substances include meat and meat products, fish products, dairy products, cereals, fruits and vegetables, and beverages. The bacteriocins can effectively be used to inhibit some gram-positive bacteria, spore-forming bacteria, and food-borne pathogens. The major classes of bacteriocins produced by LAB include:

1. lantibiotics,
2. small heat stable peptides,
3. large heat labile proteins, and
4. complex proteins whose activity requires the association of carbohydrates or lipid moieties.

Out of these, first two groups have received increased attention as food biopreservatives.

The most studied member among lantibiotics is Nisin A, a 34-residue antibacterial peptide that is produced by several strains of *Lactococcus lactis* and strongly inhibits the growth of a wide range of Gram-positive bacteria. This mature peptide displays several unusual features, such as the dehydrated residues dehydroalanine, dehydrobutyrine, lanthionine and β-methyl-lanthionine residues. In Gram-positive bacteria nisin has been shown to act on energized membrane vesicles to disrupt the proton motive force, inhibit uptake of amino acids, and cause release of accumulated amino acids. Nisin A is being used at the concentrations of 100-200 ppm in the preservation of, dairy products such as cheeses and milk, meat products, and fish.

Microgard products are bacteriocins-like inhibitory substances produced by fermenting grade A skim milk with lactic acid bacteria. It has been approved by FDA and widely used as a biopreservative for more than a decade by the Cottage cheese industry. It is antagonistic toward most gram-negative bacteria and some yeasts and molds, but not against gram-positive bacteria.

Lacticin 481 produced by *L. lactis*, lactocin S produced by *Lactobacillus sake* and carnocin U149 produced by *Carnobacterium piscicola* are the other lantibiotics, which are being tried as food biopreservatives.

Class II LAB bacteriocins are small heat stable, non-lanthionine containing membrane-active peptides. Few examples of class II bacteriocins, which have been studied for their antibacterial

effect, are pediocin produced by pediococci (widely applied in the fermentation of meat and vegetables) and leucocin A produced by *Leuconostoc spp*, another LAB found in meat and vegetable fermentations. These peptides are active against broad range of gram-positive bacteria including *Listeria monocytogenes*.

Reuterin is a water-soluble non-proteinaceous product produced by *Lactobacillus reuteri*. It has been described to have antimicrobial effect against certain gram-negative and gram-positive bacteria, yeasts, fungi, and protozoa. It inhibits *Salmonella*, *Shigella*, *Clostridium*, *Staphylococcus*, *Listeria*, and *Trypanosoma*.

Bacteriocins exhibit a very narrow inhibiting spectrum, typically active against only one target microorganism. The bacteriocin activity is not stable and loss occurs when it interacts with food components by binding with food lipids and proteins or being degraded by proteolytic enzymes.

Lecture plan

FOOD AND INDUSTRIAL MICROBIOLOGY (16MBP302)

SEMESTER – III **4H – 4C**

UNIT III

S. No	Duration	Topic	Reference
1	1	Food borne diseases	T1: 404-415
2	1	Food poisoning	T1: 412-426
3	1	Food borne infection and	T1: 423-456
4	1	Food intoxication	T1: 423-456
5	1	Food control agencies	T1: 495-501
6	1	HACCP	T1: 495-501
7	1	Microbiological criteria for food	T1: 505-506
8	1	Microbial quality control	R4: 323-325
9	1	Food laws and hazard analysis	R4: 112-114, 349-358
10	1	Unit revision	
Total Hrs: 10			

T1: Frazier, WC and DC. Westhoff, 1995. Food Microbiology, Tata McGraw Hill Publishing company Ltd, New Delhi.

R4: Adams, MR and Mo. Moss, Food Microbiology, Tata Mc Graw Hill Publishing company Ltd, New Delhi.

2017

FOOD AND INDUSTRIAL MICROBIOLOGY

Prepared by – Ms. P. Akilandeswari, Asst Professor, Dept of Microbiology, KAHE.

Unit 3

FOOD POISONING:

Food poisoning syndrome results from ingestion of water and wide variety of food contaminated with pathogenic microorganisms (bacteria, viruses, protozoa, fungi), their toxins and chemicals. Food poisoning must be suspected when an acute illness with gastrointestinal or neurological manifestation affect two or more persons, who have shared a meal during the previous 72 hours. The term as generally used encompasses both food-related infection and food-related intoxication.

Some microbiologists consider microbial food poisoning to be different from food-borne infections. In microbial food poisoning, the microbes multiply readily in the food prior to consumption, whereas in food-borne infection, food is merely the vector for microbes that do not grow on their transient substrate. Others consider food poisoning as intoxication of food by chemicals or toxins from bacteria or fungi. Consumption of poisonous mushroom leads to mycetism, while consumption of food contaminated with toxin producing fungi leads to mycotoxicosis. Some microorganisms can use our food as a source of nutrients for their own growth. By growing in the food, metabolizing them and producing by-products, they not only render the food inedible but also pose health problems upon consumption. Many of our foods will support the growth of pathogenic microorganisms or at least serve as a vector for their transmission. Food can get contaminated from plant surfaces, animals, water, sewage, air, soil, or from food handlers during handling and processing.

Classification Of Food Poisoning:

I. **Based on symptoms and duration of onset**

a. Nausea and vomiting within six hours (*Staphylococcus aureus, Bacillus cereus*)

b. Abdominal cramps and diarrhoea within 8-16 hours (*Clostridium perfringens, Bacillus cereus*)

c. Fever, abdominal cramps and diarrhoea within 16-48 hours (*Salmonella, Shigella, Vibrio parahemolyticus, Enteroinvasive E.coli, Campylobacter jejuni*)

d. Abdominal cramps and watery diarrhoea within 16-72 hours (*Enterotoxigenic E.coli, Vibrio cholerae*

O1, O139, Vibrio parahemolyticus, NAG vibrios, Norwalk virus)

e. Fever and abdominal cramps within 16-48 hours (*Yersinia enterocolitica*)

f. Bloody diarrhoea without fever within 72-120 hours (*Enterohemorrhagic E.coli O157:H7*)

g. Nausea, vomiting, diarrhoea and paralysis within 18-36 hours (*Clostridium botulinum*)

II. Based on pathogenesis

a. Food intoxications resulting from the ingestion of preformed bacterial toxins. (*Staphylococcus*

aureus, Bacillus cereus, Clostridium botulinum, Clostridium perfringens)

b. Food intoxications caused by noninvasive bacteria that secrete toxins while adhering to the

intestinal wall (*Enterotoxigenic E.coli, Vibrio cholerae, Campylobacter jejuni*)

c. Food intoxications that follow an intracellular invasion of the intestinal epithelial cells. (*Shigella,*

Salmonella)

d. Diseases caused by bacteria that enter the blood stream via the intestinal tract. (*Salmonella typhi,*

Listeria monocytogenes)

Some of the guidelines prevalent in India are listed below:

- Food Safety and Standards Act, 2006 (FSSA)
- Edible Oils Packaging (Regulation) Order, 1998
- Environment (Protection) Act, 1986
- Fruit Products Order, 1955 (FPO)
- Meat Food Products Order, 1973 (MFPO)
- Milk and Milk Product Order, 1992 (MMPO)
- Solvent Extracted Oil, De-oiled Meal and Edible Flour (Control) Order, 1967
- Standards of Weights and Measures Act, 1976
- The Essential Commodities Act, 1955
- The Export (Quality Control and Inspection) Act, 1963
- The Insecticides Act, 1968
- Vegetables Oil Products (Control) Order, 1998
- Prevention of Food Adulteration Act & Rules (PFA Act), 1954

A few of the Food Laws which can be declared voluntarily by the manufacturers of finished products are as follows:

- Agmark Standards (AGMARK)
- Codex Alimentarius Standards

- BIS Standards and Specifications

- Consumer Protection Act, 1986

Food laws and Regulations

- To meet a country's sanitary and phytosanitary requirements, food must comply with the local laws and regulations to gain market access.
- These laws ensure the safety and suitability of food for consumers.
- The requirement of food regulation may be based on several factors such as
- whether a country adopts international norms developed by the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations and the World Health Organization or a country may also has its own suite of food regulations.
- Each country regulates food differently and has its own food regulatory framework.

Food laws in our country

The Indian Parliament has recently passed the *Food Safety and Standards Act, 200*6 that overrides all other food related laws.

Such as;

Prevention of Food Adulteration Act, 1954

Fruit Products Order,1955

Meat Food Products Order ,1973;

Vegetable Oil Products (Control) Order, 1947

Edible Oils Packaging (Regulation) Order 1988

Solvent Extracted Oil, De- Oiled Meal and Edible Flour (Control) Order, 1967,

Milk and Milk Products Order, 1992 etc are repealed after commencement of FSS Act, 2006.

Food Safety and Standards Authority of India (FSSAI)

The Food Safety and Standards Authority of India (FSSAI) has been established under Food Safety and Standards Act, 2006 which consolidates various acts & orders that have hitherto handled food related issues in various Ministries and Departments.

FSSAI has been created for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import to ensure availability of safe and wholesome food for human consumption.

Functions performed by FSSAI

- Framing of Regulations to lay down the Standards and guidelines in relation to articles of food and specifying appropriate system of enforcing various standards.
- Laying down mechanisms and guidelines for accreditation of certification bodies engaged in certification of food safety management system for food businesses.
- Laying down procedure and guidelines for accreditation of laboratories and notification of the accredited laboratories.
- To provide scientific advice and technical support to Central Government and State Governments in the matters of framing the policy and rules in areas which have a direct or indirect bearing of food safety and nutrition .
- Collect and collate data regarding food consumption, incidence and prevalence of biological risk, contaminants in food, residues of various, contaminants in foods products, identification of emerging risks and introduction of rapid alert system.

- Creating an information network across the country so that the public, consumers, Panchayats etc receive rapid, reliable and objective information about food safety and issues of concern.
- Provide training programmes for persons who are involved or intend to get involved in food businesses.
- Contribute to the development of international technical standards for food, sanitary and phyto-sanitary standards.
- Promote general awareness about food safety and food standards

Bureau of Indian Standards (BIS)

- The Bureau of Indian Standards (BIS), the National Standards Body of India, resolves to be the leader in all matters concerning Standardization, Certification and Quality.

Main Activities

- Harmonious development of standardization, marking and quality certification
- To provide new thrust to standardization and quality control.
- To evolve a national strategy for according recognition to standards and integrating them with growth and development of production and exports.
- Certification of Product
- Hallmarking of Gold Jewellery.
- Quality Management System
- Environmental Management Systems
- Occupational Health and Safety Management System
- Food Safety Management System
- Hazard Analysis and Critical Control Points
- Imported Products
- Laboratory Management
- International Activities
- Training Services

AGMARK

- The Directorate of Marketing and Inspection enforces the Agricultural Produce (Grading and Marketing) Act, 1937. Under this Act Grade standards are prescribed for agricultural and allied.
- AGMARK is a Quality Certification Mark .
- It ensures quality and purity of a product.
- It acts as a Third Party Guarantee to Quality Certified.
- Quality standards for agricultural commodities are framed based on their intrinsic quality.
- Food safety factors are being incorporated in the standards to complete in World Trade.
- Standards are being harmonized with international standards keeping in view the WTO requirements. Certification of agricultural commodities is carried out for the benefit of producer/manufacturer and consumer.
- Products available under AGMARK are as follows:-

✓ Pulses
✓ Whole spices & ground spices
✓ Vegetable oils
✓ Wheat Products
✓ Milk products.

- ✓ Other products such as Honey, Compounded asafetida, Rice, Tapioca Sago, Seedless tamarind, Besan (Gram flour)

Fruit Product Order (FPO), 1955

Objective

- The main objective is lay down quality standards to manufacture fruit & vegetable products maintaining sanitary and hygienic conditions in the premises.
- It is mandatory for all manufacturers of fruit and vegetable products including some non fruit products like non fruit vinegar, syrup and sweetened aerated water to obtain a license under this Order.
- Following minimum requirements are laid down in the Fruit Product Order for hygienic production and quality standards:
  - Location and surroundings of the factory
  - Sanitary and hygienic conditions of premises
  - Personnel hygiene
  - Portability of water
  - Machinery & Equipment with installed capacity
  - Quality control facility & Technical staff
  - Product Standards
  - Limits for preservatives & other additives

  Fruit product means any of the following articles, namely
  - Non fruit beverages, syrups and sherbets
  - Vinegar, whether brewed or non-fruit
  - Pickles
  - Dehydrated fruits and vegetables
  - Squashes, crushes cordials, barley water, barreled juice, and ready to serve beverages, fruit nectars or any other beverages containing fruit juices or fruit pulp
  - Jams, jellies and marmalades
  - Tomato products, ketchup and sauces
  - Preserves, candied and crystallized fruit and peel
  - Chutneys
  - Canned and bottled fruits, juices and pulps
  - Canned and bottled vegetables
  - Frozen fruits and vegetables
  - Sweetened aerated water and without fruit juice pr fruit pulp
  - Fruit cereal flakes
  - All unspecified fruit and vegetable products which are considered microbiologically safe and contains only permitted additives within permissible limits.
- Each container in which any fruit product is packed shall specify a code number indicating the lot or the date of manufacture of such fruit product.
- No person can carry on the business of a manufacturer except under and in accordance with the terms of an effective license granted to him under this Order in Form B and shall not use the License number on labels of non-fruit products.FPO mark should be printed on the label with license number.
- The labels shall not contain any statement, claim, design or device which is false or misleading in any particular concerning the fruit products contained n the package or

concerning the quantity or the nutritive value or in relation to the place of origin of the said fruit products.

Meat Food Products Order (MFPO)

Objectives :

- The main objective is to regulate production and sale of meat food products through licensing of manufacturers, enforce sanitary and hygienic conditions prescribed for production of wholesome meat food products, exercise strict quality control at all stages of production of meat food products, fish products including chilled poultry etc.
- Meat & Meat Products are highly perishable in nature and can transmit diseases from animals to human-beings.
- Processing of meat products is licensed under Meat Food Products Order,(MFPO) 1973 which was hitherto being implemented by Ministry of food Processing industries
- Under the provision of MFPO all manufacturers of meat food products engaged in the business of manufacturing, packing, repacking, relabeling meat food products meant for sale are licensed but excluding those manufacturers who manufactures such products for consumption on the spot like a restaurant, hotel, boarding house, snack bar, eating house or any other similar establishment.

Milk and Milk Product order (MMPO)

- ✓ The objective of the order is to maintain and increase the supply of liquid milk of desired quality in the interest of the general public and also for regulating the production, processing and distribution of milk and milk products.
- ✓ As per the provisions of this order, any person/dairy plant handling more than 10,000 liters per day of milk or 500 MT of milk solids per annum needs to be registered with the Registering Authority appointed by the Central Government.
- ✓ In every case where the milk or milk product is packed by the holder of a registration certificate in a tin, barrel, carton or any other container, the registration number shall either be exhibited prominently on the side label of such container or be embossed, punched or printed prominently thereon.

Prevention of Food Adulteration Act, 1954

- The Act was promulgated by Parliament in 1954 to make provision for the prevention of adulteration of food. Broadly, the PFA Act covers food standards, general procedures for sampling, analysis of food, powers of authorized officers, nature of penalties and other parameters related to food.
- It deals with parameters relating to food additives, preservative, colouring matters, packing & labelling of foods, prohibition & regulations of sales etc. The provisions of PFA Act and Rules are implemented by State Government and local bodies as provided in the rules.
- In every case where the milk or milk product is packed **Prevention of Food Adulteration Act, 1954 is repealed from 05.08.2011by the Central Government as per the Food Safety and Standards Act,2006.**
- The act clearly defines “What is meant by Food Adulteration and what is the punishment given to person/manufacturer involved in the crime?
  - The food is considered adulterated if it fulfills any of the below -
  - If food is sub-standard rotten, decomposed or obtained from diseased animal or is insect-infested or is otherwise unfit for human consumption.

- If food contains any other substance which affects, or if the article is so processed as to affect, injuriously the nature, substance or quality thereof
- if the article has been prepared, packed or kept under insanitary conditions whereby it has become contaminated or injurious to health;
- if any constituent of the article has been wholly or in part abstracted so as to affect injuriously the nature, substance or quality thereof.
- if the article contains any poisonous or other ingredient which renders it injurious to health
- if any colouring matter other than that prescribed in respect thereof is present in the article, or if the amounts of the prescribed colouring matter which is present in the article are not within the prescribed limits of variability
- if the article contains any prohibited preservative or permitted preservative in excess of the prescribed limits;
- if the quality or purity of the article fall below the prescribed standard or its constituents are present in.

A few definitions as given by FAO / WHO:

 Codex Alimentarius Commission: The Codex Alimentarius Commission is a subsidiary body of the Food and Agriculture Organization of the United Nations and the World Health Organization. The Commission is entrusted with the elaboration of international standards of food to protect the health of consumers and to ensure fair practices in the food trade.

 Codex Committees: These subsidiary bodies of the Codex Alimentarius Commission include nine general subject committees, fifteen specific commodity committees, six regional coordinating committees and time-limited ad-hoc Intergovernmental Task Forces on specific subjects.

Critical Control Point: A step at which control is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Food Contaminant: Any biological or chemical agent, foreign matter, or other substance not intentionally added to food which may compromise food safety or suitability.

Food Control: A mandatory regulatory activity of enforcement by national or local authorities to provide consumer protection and ensure that all foods during production, handling, storage, processing and distribution are safe, wholesome and fit for human consumption; conform to quality and safety requirements; and are honestly and accurately labelled as prescribed by law.

 Food Hygiene: All conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

 Food Inspection: The examination, by an agency empowered to perform regulatory and/or enforcement functions, of food products or systems for the control of raw materials, processing,

and distribution. This includes in-process and finished product testing to verify that they conform to regulatory requirements.

Good Agricultural Practices (GAP): Practices of primary food producers (such as farmers and fishermen) that are necessary to produce safe and wholesome agricultural food products conforming to food laws and regulations.

 Good Manufacturing Practices (GMP): Conformance with codes of practice, industry standards, regulations and laws concerning production, processing, handling, labelling and sale of foods decreed by industry, local, state, national and international bodies with the intention of protecting the public from illness, product adulteration and fraud.

 HACCP Plan: A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration.

HACCP System: The hazard analysis critical control point system (HACCP) is a scientific and systematic way of enhancing the safety of foods from primary production to final consumption through the identification and evaluation of specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing.

Some establishments may use **Good Manufacturing Practices (GMP)** to reduce the likelihood of certain hazards. GMPs are minimum sanitary and processing requirements. GMPs are fairly broad and general, for example, "*Training: All employees should receive training in personal hygiene."* GMPs are usually not designed to control specific hazards, but are intended to provide guidelines to help establishments produce safe and wholesome products.

- ✓ **Standard Operating Procedures (SOP)** are step-by-step directions for completing important procedures and are usually very specific. SOP may be used to address a specific hazard, for instance, an establishment may have specific preventive maintenance procedures for its processing equipment, which prevent the hazard of metal fragments.
- ✓ **Sanitation SOP (SSOP)** may be considered by establishments to reduce the likelihood of occurrence of some food safety hazards. For example, the SSOP may address washing and sanitizing of knife and hands between carcasses to reduce potential contamination with pathogens.

Product specific GMPs

- thermally processed low-acid canned foods
- acidified foods
- bottled drinking water

GMPs Regulations

21CFR Part 110

- *Subpart A - General Provisions*
- *Subpart B - Building and Facilities*
- *Subpart C - Equipment*
- *Subpart D - [Reserved]*
- *Subpart E - Production and Process Controls*
- *Subpart F - [Reserved]*
- *Subpart G - Defect Action Levels*

GMPs - General Provisions

- provides definitions necessary for *important in understanding implications and applications*

✓ Buildings and Facilities. Buildings must be designed and constructed to facilitate *effective maintenance and sanitation.The* results specified rather than method for achieving detailed expectations in sanitation of operations**.**

✓ The equipment and utensils are *designed and constructed to be easily and properly cleaned*, temperature is measured and recorded by refrigerators and freezers. Also the critical parameters are measured.

✓ Production and Process Controls-

- The end results emphasizes *ensuring that no adulterated food enters marketplace.The terms used subject to variation in interpretation.*
- *The* raw materials and ingredients properly *inspected, analyzed, segregated, stored and handled.*
- manufacturing operations must be monitored
  - *pH, water activity, temperatures*
  - *elimination of metal from product*
- personnel should be trained and aware of GMP requirements

✓ Defect Action Levels

- natural or unavoidable defects may be in food
  - *not harmful at levels present*
  - *present even with GMPs*
- FDA establishes DALs when necessary and possible
- defect level may not be reduced by blending

Thus GMPs are Intended to prevent adulteration. Opportunity for considerable judgment in defining and interpreting regulations. *"spirit" of GMPs is to do what is reasonable and necessary to ensure safe and unadulterated food supply.*

Specific GMPs:

Low acid canned foods

- *Life threatening risk if improperly processed*
- Requires supervision of personnel who have been trained
- Regulations quite detailed for equipment design and operation
- Extensive record keeping requirements

Acidified foods:

- Defined as a low acid food with
  - A_w *greater than 0.85*
  - *acid added to lower pH to 4.6 or lower*
- Product examples
  - *includes beans, cucumbers, cabbage*
  - *excludes carbonated beverages*
- Personnel trained under approved program

Bottled Drinking Water:

- *All water sealed in bottles, packages for human consumption*
- Regulations are general and similar to umbrella GMPs
- Source of water must be approved
- Sanitation, equipment designed, personnel emphasized

Extensive record keeping

What is HACCP?

- The National Advisory Committee on Microbiological Criteria for Food (NACMCF) working group created guidelines and redefined the seven basic principles of HACCP as an effective and rational means of assuring food safety from harvest to consumption.
- The working group published the HACCP principles and application guideline document in August 1997.
- The hazard analysis critical control point system (HACCP) is a scientific and systematic way of enhancing the safety of foods from primary production to final consumption through the identification and evaluation of specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing.
- Under the HACCP regulatory system, establishments assume full responsibility for producing products that are safe for consumers.

History of HACCP

- Developed by Pillsbury in 1959 as a nontesting approach to assure the safety level required by NASA for foods produced for the space program
- NASA's major concerns • Food crumbs • Foodborne illness
- NASA's Zero Defects program Testing materials
- National Research Council – 1985 • An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients
- Microbiological hazards not controlled by testing
- Recommended using HACCP for food safety assurance
- **National Advisory Committee on Microbiological Criteria for Food (NACMCF) 1988**
- NACMCF proposed 7 principles of HACCP application, Published in 1989;
- 1st. Revision in 1992; 2nd. Revision (latest) in 1997

PRINCIPLES OF THE HACCP SYSTEM

The seven principles of HACCP, which encompass a systematic approach to the identification, prevention, and control of food safety hazards include:

PRINCIPLE 1 Conduct a hazard analysis.

PRINCIPLE 2 Determine the Critical Control Points (CCPs).
PRINCIPLE 3 Establish critical limit(s).
PRINCIPLE 4 Establish a system to monitor control of the CCP.
PRINCIPLE 5 Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
PRINCIPLE 6 Establish procedures for verification to confirm that the HACCP system is working effectively.
PRINCIPLE 7 Establish documentation concerning all procedures and records appropriate to these principles and their application.

APPLICATION

The application of HACCP principles consists of the following tasks as identified in the Logic Sequence
for Application of HACCP (Diagram 1).

1. Assemble HACCP team

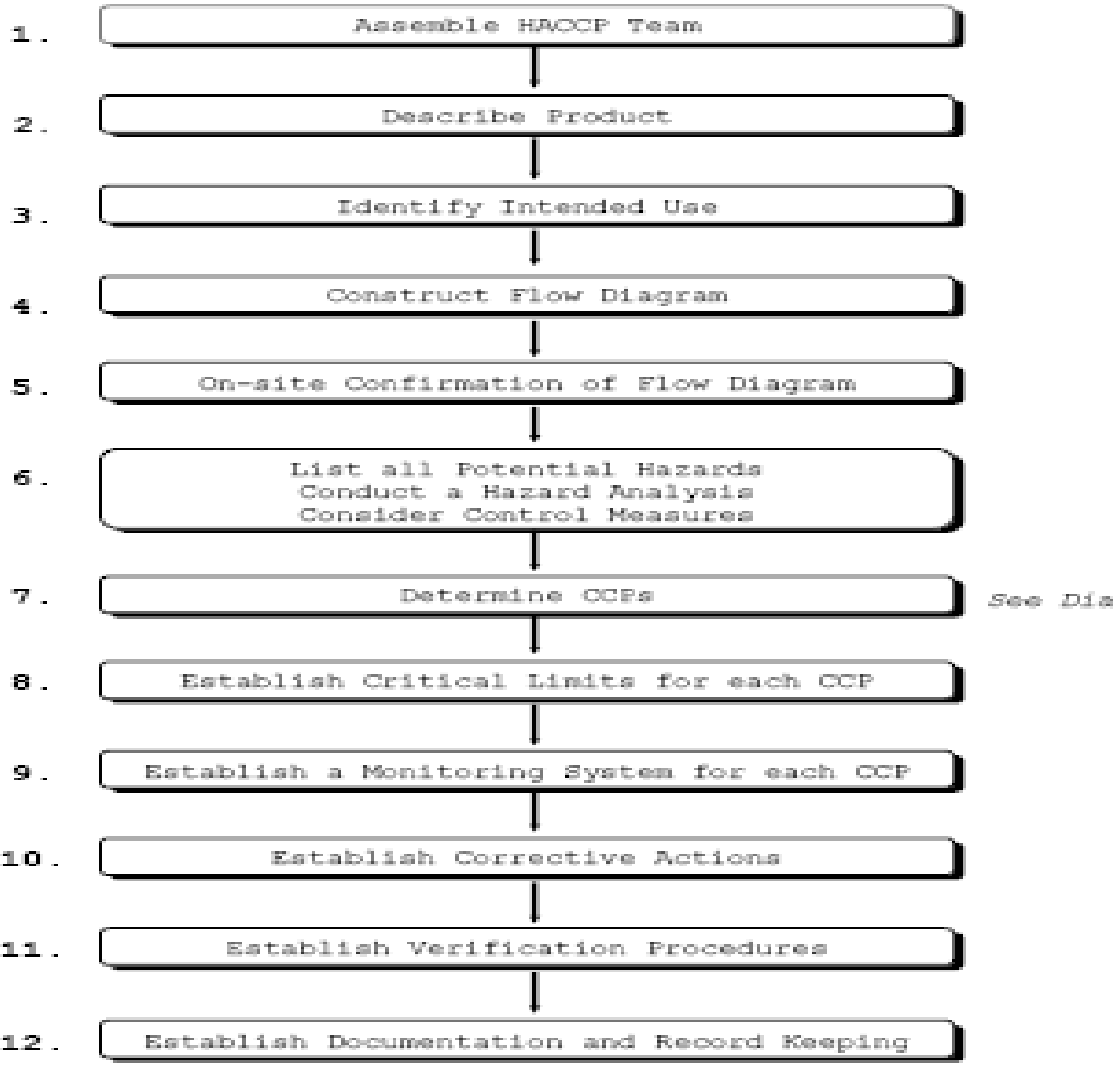
The food operation should assure that the appropriate product specific knowledge and expertise is
available for the development of an effective HACCP plan. Optimally, this may be accomplished by
assembling a multidisciplinary team. Where such expertise is not available on site, expert advice should
be obtained from other sources, such as, trade and industry associations, independent experts, regulatory
authorities, HACCP literature and HACCP guidance (including sector-specific HACCP guides). It may
be possible that a well-trained individual with access to such guidance is able to implement HACCP inhouse.

The scope of the HACCP plan should be identified. The scope should describe which segment
of the food chain is involved and the general classes of hazards to be addressed (e.g. does it cover all
classes of hazards or only selected classes).

2. Describe product

A full description of the product should be drawn up, including relevant safety information such as:
composition, physical/chemical structure (including Aw, pH, etc), microcidal/static treatments (heattreatment,
freezing, brining, smoking, etc), packaging, durability and storage conditions and method of
distribution. Within businesses with multiple products, for example, catering operations, it may be
effective to group products with similar characteristics or processing steps, for the purpose of
development of the HACCP plan.

LOGIC SEQUENCE FOR APPLICATION OF HACCP



3. Identify intended use

The intended use should be based on the expected uses of the product by the end user or consumer. In specific cases, vulnerable groups of the population, e.g. institutional feeding, may have to be considered.

4. Construct flow diagram

The flow diagram should be constructed by the HACCP team (see also paragraph 1 above). The flow diagram should cover all steps in the operation for a specific product. The same flow diagram may be used for a number of products that are manufactured using similar processing steps. When applying HACCP to a given operation, consideration should be given to steps preceding and following the specified operation.

5. On-site confirmation of flow diagram

Steps must be taken to confirm the processing operation against the flow diagram during all stages and
hours of operation and amend the flow diagram where appropriate. The confirmation of the flow diagram should be performed by a person or persons with sufficient knowledge of the processing operation.

6. List all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control identified hazards

The HACCP team should list all of the hazards that may be reasonably expected to occur at each step according to the scope from primary production, processing, manufacture, and distribution until the point of consumption.

The HACCP team should next conduct a hazard analysis to identify for the HACCP plan, which hazards are of such a nature that their elimination or reduction to acceptable levels is essential to the production of a safe food.

In conducting the hazard analysis, wherever possible the following should be included:

• the likely occurrence of hazards and severity of their adverse health effects;
• the qualitative and/or quantitative evaluation of the presence of hazards;
• survival or multiplication of micro-organisms of concern;
• production or persistence in foods of toxins, chemicals or physical agents; and,
• conditions leading to the above.

Consideration should be given to what control measures, if any exist, can be applied to each hazard.

More than one control measure may be required to control a specific hazard(s) and more than one
hazard may be controlled by a specified control measure.

- ✓ A hazard is defined by NACMCF as a biological, chemical or physical agent that is **reasonably likely to occur**, and will **cause illness or injury in the absence of its control**. Establishments must consider all **three types of hazards – biological, chemical, and physical** – at each step of the production process.

7. Determine Critical Control Points

- ✓ A ***critical control point*** is defined as a point, step, or procedure in a food process at which control can be applied, and, as a result, a food safety hazard can be prevented, eliminated, or reduced to acceptable levels. Critical control points are locations in a process at which some aspect of control can be applied to control food safety hazards that have been determined reasonably likely to occur.
- ✓ Examples of CCPs include product temperature, certification of incoming product, microbiological testing, testing for foreign objects such as metal contamination, the chemical concentration of a carcass rinse or spray, and other such parameters.

There may be more than one CCP at which control is applied to address the same hazard. The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree, which indicates a logic reasoning approach. Application of a decision tree should be
flexible, given whether the operation is for production, slaughter, processing, storage, distribution or
other. It should be used for guidance when determining CCPs. This example of a decision tree may not
be applicable to all situations. Other approaches may be used. Training in the application of the decision tree is recommended.

If a hazard has been identified at a step where control is necessary for safety, and no control measure exists at that step, or any other, then the product or process should be modified at that step, or at any earlier or later stage, to include a control measure.

8. Establish critical limits for each CCP

Critical limits (CL) are the parameters that indicate whether the control measure at the CCP is in or out of control. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) states that a CL is **a maximum or minimum value** to which a biological, chemical, or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the occurrence of a food safety hazard.

Critical limits must be specified and validated for each Critical Control Point. In some cases more than one critical limit will be elaborated at a particular step. Criteria often used include measurements of temperature, time, moisture level, pH, Aw, available chlorine, and sensory parameters such as visual appearance and texture.

Where HACCP guidance developed by experts has been used to establish the critical limits, care should be taken to ensure that these limits fully apply to the specific operation, product or groups of products under consideration. These critical limits should be measurable.

9. Establish a monitoring system for each CCP

Monitoring is the scheduled measurement or observation of a CCP relative to its critical limits. The monitoring procedures must be able to detect loss of control at the CCP. Further, monitoring should ideally provide this information in time to make adjustments to ensure control of the process to prevent violating the critical limits. Where possible, process adjustments should be made when monitoring results indicate a trend towards loss of control at a CCP. The adjustments should be taken before a deviation occurs. Data derived from monitoring must be evaluated by a designated person with knowledge and authority to carry out corrective actions when indicated. If monitoring is not continuous, then the amount or frequency of monitoring must be sufficient to guarantee the CCP is in control. Most monitoring procedures for CCPs will need to be done rapidly because they relate to online processes and there will not be time for lengthy analytical testing. Physical and chemical measurements are often preferred to microbiological testing because they may be done rapidly and can often indicate the microbiological control of the product.

All records and documents associated with monitoring CCPs must be signed by the person(s) doing the

monitoring and by a responsible reviewing official(s) of the company.

10. Establish corrective actions

Specific corrective actions must be developed for each CCP in the HACCP system in order to deal with
deviations when they occur.

The actions must ensure that the CCP has been brought under control. Actions taken must also include
proper disposition of the affected product. Deviation and product disposition procedures must be documented in the HACCP record keeping.

The corrective actions consist of:

- ✓ Identifying and eliminating the cause of the deviation,
- ✓ Ensuring that the CCP is under control after the corrective action is taken,
- ✓ Ensuring that measures are established to prevent recurrence, and
- ✓ Ensuring that no product affected by the deviation is shipped.

11. Establish verification procedures

Establish procedures for verification. Verification and auditing methods, procedures and tests, including random sampling and analysis, can be used to determine if the HACCP system is working
correctly. The frequency of verification should be sufficient to confirm that the HACCP system is
working effectively.

Verification should be carried out by someone other than the person who is responsible for performing
the monitoring and corrective actions. Where certain verification activities cannot be performed in
house, verification should be performed on behalf of the business by external experts or qualified third
parties.

Examples of verification activities include:

• Review of the HACCP system and plan and its records;
• Review of deviations and product dispositions;
• Confirmation that CCPs are kept under control.

Where possible, validation activities should include actions to confirm the efficacy of all elements of the
HACCP system.

12. Establish Documentation and Record Keeping

Efficient and accurate record keeping is essential to the application of a HACCP system. HACCP
procedures should be documented. Documentation and record keeping should be appropriate to the
nature and size of the operation and sufficient to assist the business to verify that the HACCP controls
are in place and being maintained. Expertly developed HACCP guidance materials (e.g. sector-specific

HACCP guides) may be utilised as part of the documentation, provided that those materials reflect the
specific food operations of the business.
Documentation examples are:
Hazard analysis;
CCP determination;
Critical limit determination.
Record examples are:
• CCP monitoring activities;
• Deviations and associated corrective actions;
• Verification procedures performed;
• Modifications to the HACCP plan;
An example of a HACCP worksheet for the development of a HACCP plan is attached as Diagram 3.
A simple record-keeping system can be effective and easily communicated to employees. It may be
integrated into existing operations and may use existing paperwork, such as delivery invoices and
checklists to record, for example, product temperatures.

Benefits of HACCP

Although the adoption of HACCP systems worldwide is due primarily to the added food safety protection provided to consumers, there are other benefits to the food industry that can be realized by implementing a successful HACCP system.

a. Formally incorporates food safety principles as integral steps of production processes

HACCP recognition status cannot be completed without a firm commitment by senior management to formally support food safety control measures throughout the production process. The implementation and maintenance of those control measures play a critical role in raising awareness of front line production management and staff of the presence and importance of specific food safety procedures within their process.

b. Increased employees' ownership of the production of safe food

As a sign of this commitment, it is the responsibility of senior management to foster the idea within the facility that food safety is the responsibility of everyone. Through the process of developing and implementing a HACCP system, employees become more aware of food safety and their role in contributing to food safety. This increased knowledge leads to ownership of and pride in the production of a safe food product.

c. Increased buyer and consumer confidence

Establishments that have implemented a HACCP system provide buyers and consumers with a greater degree of confidence that the facility is producing a safe food product. Establishments can demonstrate by showing documents and records that food safety is under control.

d. Maintaining or increasing market access

Market forces continue to drive HACCP implementation throughout the food industry. In many cases, buyer demands and foreign governments require HACCP implementation to maintain market share and/or gain access to previously inaccessible markets. As HACCP systems are accepted worldwide, FSEP helps the Canadian industry to maintain and expand its international markets.

e. Reduced waste

The preventative nature of HACCP allows a company to control costs by minimizing the amount of product requiring rejection or recall, and by focusing resources on areas that have been identified as critical in the manufacture of a safe food product. With the regular monitoring inherent in a HACCP system, establishments become aware of problems earlier and the costs of waste are reduced.

Lecture plan

FOOD AND INDUSTRIAL MICROBIOLOGY (16MBP302)

SEMESTER – III **4H – 4C**

UNIT IV

S. No	Duration	Topic	Reference
1	1	Histroy and chronological development of industrially microbiology	R1: 3-14
2	1	Industrially important strains and preservation	R2: 9-13
3	1	Isolation of industrially important strains	R2: 14-21
4	1	Inoculum development for various fermentation processes	R2: 22-26
5	1	rDNA technology,	R2: 22-26
6	1	Protoplast fusion	R2: 22-26
7	1	Strain development – mutation and	R2: 27-45, 45-50
8	1	Submerged and solid state fermentation	R1: 64-77
9	1	Batch and continuous fermentation	R1: 77-86
10	1	Unit revision	
Total Hrs: 10			

R1: Patel, AH. 2006. Industrial Microbiology, Macmillan India Ltd.

R2: Wulf Cruegar and Anneliese Gueger, 2000. Second edition, Biotechnology. A textbook of industrial Microbiology, Panima Publishing corp, D elhi.

2017

FOOD AND INDUSTRIAL MICROBIOLOGY

Prepared by – Ms. P. Akilandeswari, Asst Professor, Dept of Microbiology, KAHE.

Unit 4

History of Industrial microbiology

Table 1 Dates and events in early biotechnology

Ancient handicraft		
6000 BC	Beer fermentation	
3500 BC	Wine fermentation	
3500 BC	Soja fermentation	
Cheese and bread fermentation		
Fourteenth century	Industrial acetic acid fermentation	
Early period up to 1850		Technical application
Scientific events		
1680 Leeuwenhoek observes microorganisms		
1783 Spallanzani observed protease action		
1793 Lavoisier and		
1810 Gay-Lussac: quantitative chemistry of alcoholic fermentation Gay-Lussac: hypothesis of spontaneous generation		Early eighteenth century: technical beer and wine fermentation; also industrial beer fermentation
1833 Payen and Persoz: diastase (enzyme) characterization		1823 Immobilized bacteria used for acetic acid production
1836 Berzelius: catalysis (including enzymes) [a]		
1837, 1838 Schwann, Cagniard-Latour: living cells as fermentation agents		
1834, 1838 Kützing, Quevenne: hypotheses of spontaneous generation, (see also before, Gay- Lussac); vital factor		
1839 Liebig: chemical decay hypothesis		1840s industrial enzymatic dextrin production (Payen)
1830s Major controversy on fermentation theories		

Table 2 The period from 1850 to 1890 (Scriban 1982, pp.13, 14; Buchholz and Collins 2010, chapters 3 and 4)

Time scientists	Scientific findings, events	Technical progress, industrial innovation
1837/1838 Schwann and Cagniard-Latour	Experimental demonstration of living yeast as agent in alcoholic fermentation	Growing importance of industrial fermentation of beer (production 23 million hL in 1840, Germany) [a]
1850 Rayer and Davaine	Detection of the origin of anthrax and the role of microorganisms in diseases	Technical-scale production of yeast, wine, soy sauce, sake. Industrial-scale beer fermentation in GB
1856–1877 Pasteur	Investigations on fermentation (from 1856 on): Investigations on alcohol fermentation (1858)	
	Studies on spontaneous generation (1859–1862)	1870s: Hansen breeding pure yeast for commercial application; 1874 Christian Hansen's Laboratory
	Detection of anaerobic fermentation (1861)	(Denmark): production of rennet (chymosin) for cheese manufacture
	Studies on wine fermentation, invention of	
	Pasteurisation (1864)	Beer production: 36 million hectolitres in 1873, Germany
	Studies on beer fermentation (1871)	
	Theory of fermentation (1876)	
	Detection of facultative anaerobic fermentation of yeast	New type of industrial beer fermenter (Pasteur; Fig. 1)
1866 Mendel	Heredity laws	
1876 Koch	Work the bacterium leading to anthrax; agar plate method	1895 Wehmer: Lactic acid production
1877-86 Pasteur	Begin of investigations on anthrax (1877)	
1880 Winogradsky	Soil microorganisms: the bacterial nature of nitrification	
1881 Pasteur	Vaccination against anthrax and rabies	

Table 3 The period from 1890 to 1940 (Buchholz and Collins 2010, chapter 4; Roehr 1996)

Time scientists[a]	Scientific findings, events	Technical progress, industrial innovation
1894 E. Fischer	Specificity of enzymes	Enzyme technology expanding (Takadiastase)
1897 Buchner	Fermentation due to enzyme action only	First waste disposal biogas reactor (Bombay)
1900s Buchner	Rersearch on fermentation intermediates	
1905 E. Fischer and others	Research in the nature of proteins	1907 Enzyme technology: Röhm and Haas company (Germany)
1910f Fernbach	Rersearch on fermentation intermediates	
1911f Fernbach and Strange; 1912f Perkin	Microbial formation of acetone and butanol [b]	Fermentation technology expanding: Production of butanol for rubber manufacture [b]
1915f Weizmann	Finding of Clostridium acetobutylicum	War requirements: acetone and butanol production
1915f Connstein and Lüdecke	Glycerol fermentation [b]	Glycerol production for explosives
1916 Thom and Currie	Citric acid fermentation [b]	
1920s		Pfizer: Industrial production of citric acid
1920s and 1930s Embden, Meyerhoff and others	Research on glycolysis	Large-scale industrial yeast production for bakeries
1925, 1930s Sumner, Northrup	Enzyme crystallization	
1928 Fleming	Finding of penicillin action	Large-scale waste water treatment (1928, Essen, Germany)
1933 Reichstein	Sorbitol transformation into L-sorbose	Reichstein process for vitamin C production
End of 1930s Florey and Chain	Resumed research on penicillin	Sterile enzyme fermentation for detergents etc.
1940	Protein structure solved	Peak alcohol production

Table 4 The period from 1940 to 1975 (Buchholz and Poulson 2000; Bud 2007; Buchholz and Collins 2010, chapters 4 and 5)

Time scientists	Scientific findings, events	Technical progress, industrial innovation
End of 1930s Florey and Chain	Resume research on penicillin	
1940	Protein structure solved	
1940s Waksman	Extended research on antibiotics: actinomycin, streptomycin	
1941		USA: penicillin project, due to war requirements
1944		Large-scale industrial penicillin production; Pfizer: deep tank penicillin fermentation
1948 Brotzu and Oxford team	Cephalosporin, broad spectrum antibiotic	
1949	First biochemical engineering symposium	
1952/1953		Production of further antibiotics: Pfizer, Lederle: tetracycline; Eli Lilly: erythromycin
1953 Watson, Crick, Franklin	Structure of DNA	
1950s	Development of immobilized enzymes	Industrial steroid biotransformation (prednisolone)
1958 Gaden (Ed.)	First biotech journal [a]	Expanding waste water treatment due to government requirements
1959 Chain et al. with Beecham	Begin of research on 6-APA	
End of 1960s		Large-scale enzyme processes: detergents, starch processing;
1971		
1972		Industrial production of 6-APA (Bayer, Germany; Beecham GB))
1973 Cohen and Boyer	Gene cloning	Large-scale enzymatic glucose isomerisation
1974	Political level: Germany: DECHEMA-report, followed by other studies on biotechnology in UK, Japan, France	Expanding production of amino and organic acids, vitamins, enzymes in food manufacture
		Failures: SCP production; cellulosics utilization; biosensors[b,c]

Scientific events	Technical application
1944 Avery et al.: chemical nature of chromosomes: DNA	
1950 Chargaff: rule of nucleotide ratios	
1953 Sanger: sequence of insulin	
1953 Watson and Crick: structure of DNA	
	(For technical application up to the 1960s, see Table 4)
1955f Kornberg et al.: enzymatic DNA replication	
1957f Zamecnik and Hoagland: amino acid activation, translation in protein synthesis	
1959 Kendrew: first X-ray enzyme structure	
1960–1961 Jacob and Monod: operon model of gene regulation; concept of mRNA	
1961–1966 Nirenberg, Khorana et al.: genetic code	
1963 Merrifield: solid-phase protein synthesis	
1968 Arber and Linn: restriction endonucleases	
1971f Nathans: southern: DNA separation	1971 Farley, Cape, Glaser: establishment of Cetus, the first Biotech Company
1972 Mertz, Davies: recombinant	1972 Industrial production of 6-amino-penicillanic acid
Berg: first recombinant virus	
Khorana et al.: first chemically synthesized gene	
1973 Cohen, Boyer: recombinant plasmid/microorganism	
	1974 Large-scale production of glucose/fructose syrup
1975f Maxam and Gilbert; Sanger: methods for DNA sequencing	
1975 Köhler and Millstein: monoclonal antibodies	1976 Swanson, Boyer: foundation of second biotech company: Genentec
1975 Asilomar conference (moratorium on recombinant DNA research)	1977f Further New Biotech companies founded
1978 Heffron et al.: directed mutagenesis	1978 Recombinant human insulin (Genentec)
1979 Mayer, Collins and Wagner: recombinant penicillin acylase	
1980 Chakrabarty: first patent for recombinant bacterium	1980f Work on recombinant α-amylase (Novo)
	1982 FDA approval of human insulin (Eli Lilly)
1983f Frank and Blöcker; Carruthers: mechanized DNA synthesis	1982 Large-scale production of recom-binant α-galactosidase (Boehringer Mannheim, D)
1983 Schell and Montagu: first transgenic plant (tobacco)	
	1984 Political level: OTA study; mechanized DNA sequencing
1988 Mullis: polymerase chain reaction (PCR)	1988 Leder, Stewart: patent for transgenic mouse
1990 Start of human genome project [a]	
1994 Stemmer: DNA shuffling	
1995 First complete bacterial genome sequence	
1995f Metabolic engineering [b]	1996 Mass cultivation of recombinant seeds (commercial corn seeds)
1997 First cloned animal: Dolly	
1998 Argonne Structural Genomics Meeting human chromosome 22	1999 Start of CELERA—industrial genome sequencing
2000 First approximate version of the human Genome [a]	1999 Vitamin C via microbial pathway

Specifications of a bioreactor

A typical bioreactor consists of following parts:

Agitator – used for the mixing of the contents of the reactor which keeps the "cells" in the perfect homogenous condition for better transport of nutrients and oxygen to the desired product(s).

Baffle – used to break the vortex formation in the vessel, which is usually highly undesirable as it changes the center of gravity of the system and consumes additional power.

Sparger – In aerobic cultivation process, the purpose of the sparger is to supply adequate oxygen to the growing cells.

Jacket – The jacket provides the annular area for circulation of constant temperature of water which keeps the temperature of the bioreactor at a constant value.

General Requirements and Features of Bioreactors

Bioreactors are required to perform a great number of functions to fulfill many requirements. The requirements are met partly or to a large extent in having incorporated configurationally changes in the classical system of STR or PFRS. As a result several new reactor types have come into existence which are either being tried in prototypes or used in large commercial operations.

1. Stirred Tank Reactors:

Principally, these are upright cylindrical vessels in which air is sparged through the agitated liquid medium. Various types of STRs are in use. Examples include single and multistage STRs and Waldhof reactors. Generally in an STR specific power consumption is high. Comparatively, Waldhof reactors consume less specific power since the center of gravity of the liquid is lower. Gas hold-up is also high since a considerable volume of air is drawn and is finely dispersed through the turbulent agitator zone.

2. Surface Reactors:

In physical configuration these are also cylindrical vessels rotating on the horizontal axis in which air is blown inside the vessel. Examples include the horizontal rotary reactor (HRR), disc reactors and film reactors. In extreme gas-liquid interactions this type of reactor has demonstrated its excellent suitability. It has been suggested that large inequalities in the distribution of DO in STRs, particularly in high viscous non-Newtonian broths, HRRs offer good solution. This reactor is significantly important on account of a number of outstanding performance.

3. Cyclone Reactors:

In this system the cells are circulated around a closed loop to effect oxygen transfer, mixing, and homogeneous cultivation instead of agitation and stirring in a tank. Special advantages are: a high gas exchange, no use of antifoam, no wall growth, and operation suitable for cultivation of aerobic and anaerobic nonpathogenic microorganisms. This reactor can be used for batch and continuous growth of synchronous and asynchronous cultures.

4. Fixed-Film Reactors:

These reactors have a performance behavior similar to that of trickling filters extensively used in waste water treatments. The ideal film thickness should correspond to the penetration depth of the limiting nutrient. Further increase in film thickness leads to no improvement in conversion efficiency.

5. Deep-Shaft Reactors:

The principle of operation is similar to that of the pressure cycle reactor except that all or most of the air is introduced into the down flow tube and no baffles are permitted to rise because of blockage risks. Depth is also considerably greater, normally in the range of 50-150 m. Air introduced in this way is at a lower pressure than if it were introduced at the bottom; this enables savings both in capital and operating costs. Liquid circulation is established by injection of compressed air through a sparger placed at relatively shallow depth in the up flow side. Air is then gradually switched to the down flow side. The gas void age in the top part of the up flow tube continues to balance hydraulic friction losses and net void age resistance in the lower part of the reactor. Bubble contact time, high pressure, and turbulence provide oxygen transfer as high as 3 kg of 0_2/h-m^3. Energy requirement for this high degree of oxygen transfer is about 1 kWh.

6. Immobilized Cell Reactors:

In these reactors, enzymes/cells are either attached by adsorption, chemical bonding (cross- linked or covalently bound), or entrapment on a suitable carrier, or are encapsulated and placed/ packed in different types of vessel configurations to serve as a flow reactor. Reactors with physical adsorption of enzymes or cells encounter practical difficulties because the adsorbed enzyme or cell is weakly bound and is lost rather easily during operation. Gel-entrapped enzyme reactor systems are associated with severe problems of diffusion resistances more exercised by the substrate than the product. Covalently or cross-linked

immobilized reactor systems require mild processing conditions. Large changes in pH and temperature are not permitted. A microencapsulated reactor system is subjected to the requirement of substrate diffusivity across the semi-permeable membrane which contains the enzyme or cells. Despite these disadvantages, the greatest advantage offered by this system is high-productivity.

7. Membrane Reactors:

Membrane reactors are one of the first novel immobilized enzyme reactors. More complex the reactor design is, the more changes of the coefficients and constants will be expected with scale-up or scale-down practices. This underlines the complicated similarity relationships of the standard STR configuration. Although physical functions of bioreactors are determined by the geometry and mechanical inputs, the microbial activities are manifested by the established physiological and morphological picture. Total correlations between physical and mechanical functions against microbial functions are not completely available. The release of substances by the cells into the liquid bulk is generally associated with feedback mechanism controlling the changes in the input variable and thus resulting in an altered regulatory expression in the microbial mass. The release of surface active proteinous materials affects the rheological properties of the liquid medium and thus sets up a chain of changes in the environmental conditions such as air distribution, gas hold-up, diffusion mass transfer, bulk heat transfer, and so forth. These problems change the interrelationships of constants and coefficients of reactor functions. Problems associated with aggregated mycelia in many reactor systems are well known. The oxygen transfer pathway in a typical bioconversion process is shown in Fig. 5.1. Transport of oxygen from air bubbles to the liquid and then to the cell through the liquid pathway poses some serious problems in some particularly immiscible systems.

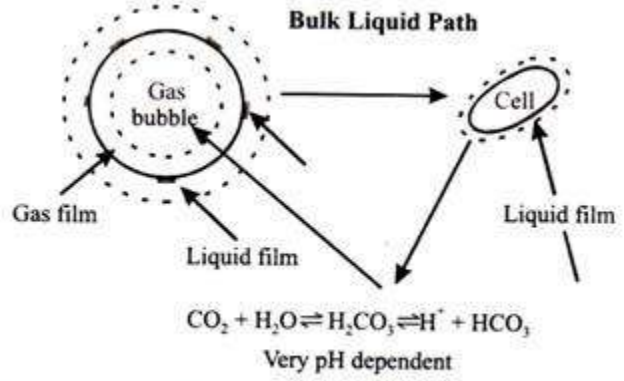


Fig. 5.1 Oxygen and CO_2 pathways in a typical fermentation bioprocessing liquid

It is associated with the sparingly soluble oxygen and the rate-controlling step for oxygen is that from the bubble interface to the bulk liquid. The problem is aggravated by the rheological properties of the liquid. Power requirement in such a system is necessarily high. High and low power input in such systems creates different mixing zones. Creation of mixing zones in turn may result in non-homogeneity in the reaction rates.

Meaning of Fermentation Technology:

Fermentation is the process involving the biochemical activity of organisms, during their growth, development, reproduction, even senescence and death. Fermentation technology is the use of organisms to produce food, pharmaceuticals and alcoholic beverages on a large scale industrial basis.

The basic principle involved in the industrial fermentation technology is that organisms are grown under suitable conditions, by providing raw materials meeting all the necessary requirements such as carbon, nitrogen, salts, trace elements and vitamins.

The end products formed as a result of their metabolism during their life span are released into the media, which are extracted for use by human being and that have a high commercial value. The major products of fermentation technology produced economically on a large scale industrial basis are wine, beer, cider, vinegar, ethanol, cheese, hormones, antibiotics, complete proteins, enzymes and other useful products.

Fermentation Methodology:

Fermentation process is carried out in a container called the fermentor or bioreactor. The design and nature of the fermentor varies depending upon the type of fermentation carried out. Invariably all the fermentors have facilities to measure some of the fermentation parameters like temperature, pressure, pH, elapsed fermentation time, liquid level, mass etc.

The different types of fermentors are:

(1) External recycle airlift fermentor—for producing bacterial biomass, with methanol as substrate.
(2) Internal recycle airlift fermentor—for producing yeast with oil as substrate.
(3) Tubular tower fermentor—Used for making beer, wine, vinegar etc.
(4) Nathan fermentor—used in brewing industry.
(5) Stirred fermentor—used for making antibiotics.

Lecture plan

FOOD AND INDUSTRIAL MICROBIOLOGY (16MBP302)

SEMESTER – III **4H – 4C**

UNIT V

S. No	Duration	Topic	Reference
1	1	Fermentor design and scale up process	R1: 64-70
2	1	Types of fermentor – tower, cylindroconical airlift and components of CSTR	R1: 71-86
3	1	Downstream process – intracellular product separation	R1: 111-119
4	1	Column chromatography	R1: 120-123
5	1	Affinity chromatography	R1: 120-123
6	1	Production of beverages – beer	R1: 162-164
7	1	Production of vitamin B12	R1: 162-164
8	1	Riboflavin production	R1: 165-169
9	1	Antibiotic production	R1: 112-119
10	1	Unit revision	
Total Hrs: 10			

R1: Patel, AH. 2006. Industrial Microbiology, Macmillan India Ltd.

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FOOD AND INDUSTRIAL MICROBIOLOGY

Prepared by – Ms. P. Akilandeswari, Asst Professor, Dept of Microbiology, KAHE.

Unit – 5

Types of Fermentation Processes:

There are three different process of fermentation viz.:

(1) Batch fermentation
(2) Feb-batch fermentation and
(3) Continuous culture.

Batch fermentation:

This term is attributed to that type of fermentation wherein there is change in culture medium, number of microorganisms and the amount of the product produced (i.e. the metabolite or target protein). In batch fermentation six phases of the microbial growth are seen.

(a) Lag phase:

Immediately after inoculation, there is no increase in the numbers of the microbial cells for some time and this period is called lag phase. This is in order that the organisms adjust to the new environment they are inoculated into.

(b) Acceleration phase:

The period when the cells just start increasing in numbers is known as acceleration phase.

(c) Log phase:

This is the time period when the cell numbers steadily increase.

(d) Deceleration phase:

The duration when the steady growth declines.

(e) Stationary phase:

The period where there is no change in the microbial cell number is the stationary phase. This phase is attained due to depletion of carbon source or accumulation of the end products.

(f) Death phase:

The period in which the cell numbers decrease steadily is the death phase. This is due to death of the cells because of cessation of metabolic activity and depletion of energy resources. Depending upon the product required the different phases of the cell growth are maintained. For microbial mass the log phase is preferred. For production of secondary metabolites i.e. antibiotics, the stationary phase is preferred.

Feb-batch fermentation:

In this type of fermentation, freshly prepared culture media is added at regular intervals without removing the culture fluid. This increases the volume of the fermentation culture. This type of fermentation is used for production of proteins from recombinant microorganisms.

Continuous fermentation:

In this type of fermentation the products are removed continuously along with the cells and the same is replenished with the cell girth and addition of fresh culture media. This results in a steady or constant volume of the contents of the fermentor. This type of fermentation is used for the production of single cell protein (S.S.P), antibiotics and organic solvents.

Procedure of Fermentation:

(a) Depending upon the type of product required, a particular bioreactor is selected.
(b) A suitable substrate in liquid media is added at a specific temperature, pH and then diluted.
(c) The organism (microbe, animal/plant cell, sub-cellular organelle or enzyme) is added to it.
(d) Then it is incubated at a specific temperature for the specified time.
(e) The incubation may either be aerobic or anaerobic.
i. Aerobic conditions are created by bubbling oxygen through the medium.
ii. Anaerobic conditions are created by using closed vessels, wherein oxygen cannot diffuse into the media and the oxygen present just above is replaced by carbon dioxide released.
(f) After the specified time interval, the products are removed, as some of the products are toxic to the growing cell or at least inhibitory to their growth. The organisms are re-circulated. The process of removal of the products is called downstream processin

Type # 1. Continuous Stirred Tank Bioreactors:

A continuous stirred tank bioreactor consists of a cylindrical vessel with motor driven central shaft that supports one or more agitators (impellers). The shaft is fitted at the bottom of the bioreactor (Fig. 19.1 A). The number of impellers is variable and depends on the size of the bioreactor i.e., height to diameter ratio, referred to as aspect ratio.

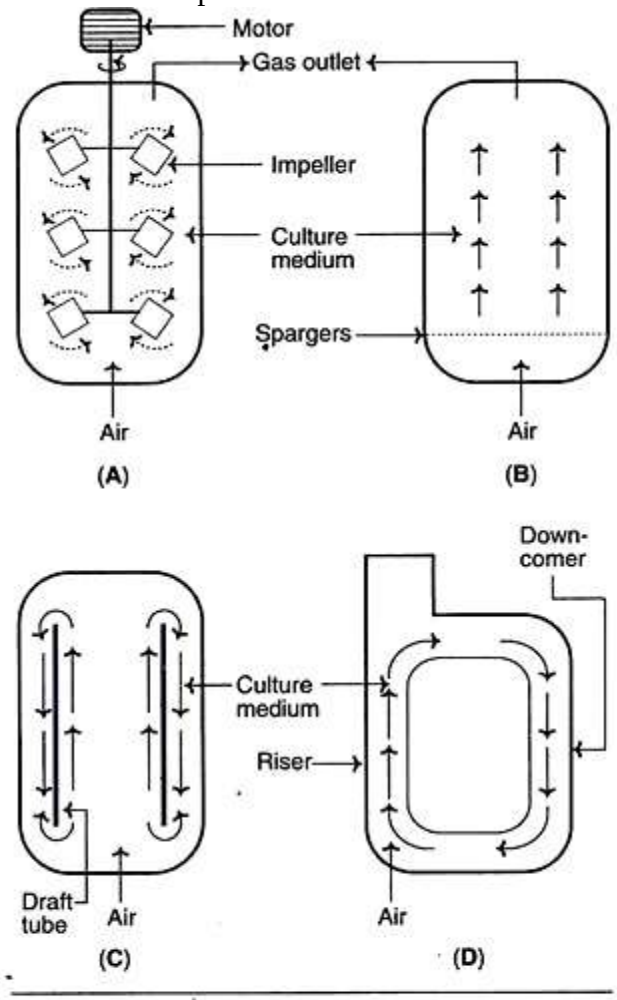


Fig. 19.1 : Types of bioreactors (A) Continuous stirred tank bioreactor (B) Bubble column bioreactor (C) Internal-loop airlift bioreactor (D) External-loop airlift bioreactor.

The aspect ratio of a stirred tank bioreactor is usually between 3-5. However, for animal cell culture applications, the aspect ratio is less than 2. The diameter of the impeller is usually 1/3 rd of the vessel diameter. The distance between two impellers is approximately 1.2 impeller diameter. Different types of impellers (Rustom disc, concave bladed, marine propeller etc.) are in use.

In stirred tank bioreactors or in short stirred tank reactors (STRs), the air is added to the culture medium under pressure through a device called sparger. The sparger may be a ring with many holes or a tube with a single orifice. The sparger along with impellers (agitators) enables better gas distribution system throughout the vessel.

The bubbles generated by sparger are broken down to smaller ones by impellers and dispersed throughout the medium. This enables the creation of a uniform and homogeneous environment throughout the bioreactor.

Advantages of STRs:

There are many advantages of STRs over other types. These include the efficient gas transfer to growing cells, good mixing of the contents and flexible operating conditions, besides the commercial availability of the bioreactors.

Type # 2. Bubble Column Bioreactors:

In the bubble column bioreactor, the air or gas is introduced at the base of the column through perforated pipes or plates, or metal micro porous spargers (Fig. 19.1B). The flow rate of the air/gas influences the performance factors —O_2 transfer, mixing. The bubble column bioreactors may be fitted with perforated plates to improve performance. The vessel used for bubble column bioreactors is usually cylindrical with an aspect ratio of 4-6 (i.e., height to diameter ratio).

Type # 3. Airlift Bioreactors:

In the airlift bioreactors, the medium of the vessel is divided into two interconnected zones by means of a baffle or draft tube. In one of the two zones referred to a riser, the air/gas is pumped. The other zone that receives no gas is the down comer. The dispersion flows up the riser zone while the down flow occurs in the down comer. There are two types of airlift bioreactors.

Internal-loop airlift bioreactor (Fig. 11.1C) has a single container with a central draft tube that creates interior liquid circulation channels. These bioreactors are simple in design, with volume and circulation at a fixed rate for fermentation.

External loop airlift bioreactor (Fig. 19.1D) possesses an external loop so that the liquid circulates through separate independent channels. These reactors can be suitably modified to suit the requirements of different fermentations. In general, the airlift bioreactors are more efficient than bubble columns, particularly for more denser suspensions of microorganisms. This is mainly because in these bioreactors, the mixing of the contents is better compared to bubble columns.

Airlift bioreactors are commonly employed for aerobic bioprocessing technology. They ensure a controlled liquid flow in a recycle system by pumping. Due to high efficiency, airlift bioreactors are sometimes preferred e.g., methanol production, waste water treatment, single-cell protein production. In general, the performance of the airlift bioreactors is dependent on the pumping (injection) of air and the liquid circulation.

Two-stage airlift bioreactors:

Two-stage airlift bioreactors are used for the temperature dependent formation of products. Growing cells from one bioreactor (maintained at temperature 30°C) are pumped into another bioreactor (at temperature 42°C). There is a necessity for the two-stage airlift bioreactor, since it is very difficult to raise the temperature quickly from 30°C to 42°C in the same vessel. Each one of the bioreactors is fitted with valves and they are connected by a transfer tube and pump (Fig. 19.2A). The cells are grown in the first bioreactor and the bioprocess proper takes place in the second reactor.

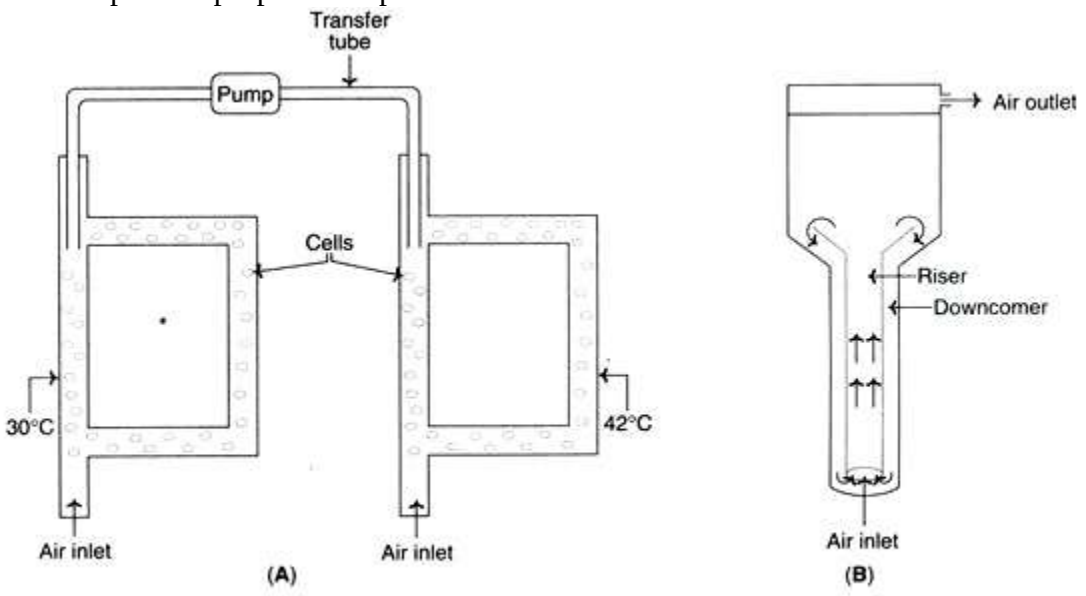


Fig. 19.2 : Types of bioreactors (A) Two–stage airlift bioreactor (B) Tower bioreactor.

Tower bioreactors:

A pressure-cycle fermenter with large dimensions constitutes a tower bioreactor (Fig. 19.2B). A high hydrostatic pressure generated at the bottom of the reactor increases the solubility of O_2 in the medium. At the top of the riser, (with expanded top) reduces pressure and facilitates expulsion of CO_2. The medium flows back in the down comer and completes the cycle. The advantage with tower bioreactor is that it has high aeration capacities without having moving parts.

Type # 4. Fluidized Bed Bioreactors:

Fluidized bed bioreactor is comparable to bubble column bioreactor except the top position is expanded to reduce the velocity of the fluid. The design of the fluidized bioreactors (expanded top and narrow reaction column) is such that the solids are retained in the reactor while the liquid flows out (Fig. 19.3A). These

bioreactors are suitable for use to carry out reactions involving fluid suspended biocatalysts such as immobilized enzymes, immobilized cells, and microbial flocs.

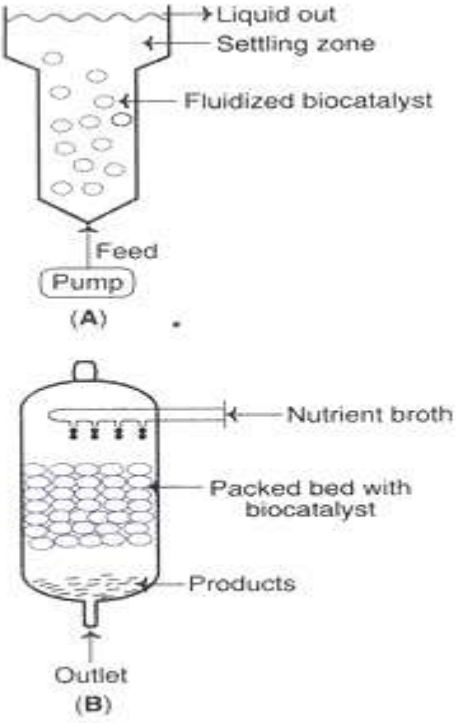


Fig. 19.3 : Types of bioreactors (A) Fluidized bed bioreactor (B) Packed bed bioreactor.

For an efficient operation of fluidized beds, gas is spared to create a suitable gas-liquid-solid fluid bed. It is also necessary to ensure that the suspended solid particles are not too light or too dense (too light ones may float whereas to dense ones may settle at the bottom), and they are in a good suspended state. Recycling of the liquid is important to maintain continuous contact between the reaction contents and biocatalysts. This enable good efficiency of bioprocessing.

Type # 5. Packed Bed Bioreactors:

A bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column constitutes a packed bed bioreactor (Fig. 19.3B). The solids used may be porous or non-porous gels, and they may be compressible or rigid in nature. A nutrient broth flows continuously over the immobilised biocatalyst. The products obtained in the packed bed bioreactor are released into the fluid and removed. While the flow of the fluid can be upward or downward, down flow under gravity is preferred.

The concentration of the nutrients (and therefore the products formed) can be increased by increasing the flow rate of the nutrient broth. Because of poor mixing, it is rather difficult to control the pH of packed bed bioreactors by the addition of acid or alkali. However, these bioreactors are preferred for bioprocessing technology involving product-inhibited reactions. The packed bed bioreactors do not allow accumulation of the products to any significant extent.

Type # 6. Photo-Bioreactors:

These are the bioreactors specialised for fermentation that can be carried out either by exposing to sunlight or artificial illumination. Since artificial illumination is expensive, only the outdoor photo-bioreactors are preferred. Certain important compounds are produced by employing photo-bioreactors e.g., p-carotene, asthaxanthin.

The different types of photo-bioreactors are depicted in Fig. 19.4. They are made up of glass or more commonly transparent plastic. The array of tubes or flat panels constitute light receiving systems (solar receivers). The culture can be circulated through the solar receivers by methods such as using centrifugal pumps or airlift pumps. It is essential that the cells are in continuous circulation without forming sediments. Further adequate penetration of sunlight should be maintained. The tubes should also be cooled to prevent rise in temperature.

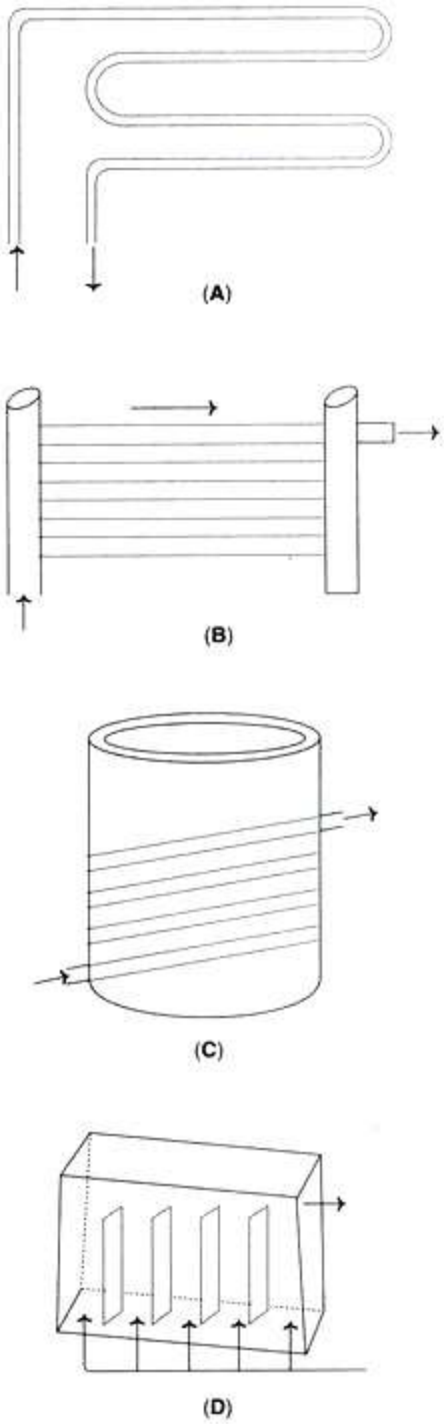


Fig. 19.4 : Types of photobioreactors (A) Continuous run tubular loop (B) Multiple parallel tube (C) Helical wound tubular loop (D) Flat panel configuration.

Photo-bioreactors are usually operated in a continuous mode at a temperature in the range of 25-40°C. Microalgae and cyanobacteria are normally used. The organisms grow during day light while the products are produced during night.

The different types and designs of bioreactors are described. The most common features of a typical bioreactor are diagrammatically represented in Fig. 19.5, and briefly described hereunder.

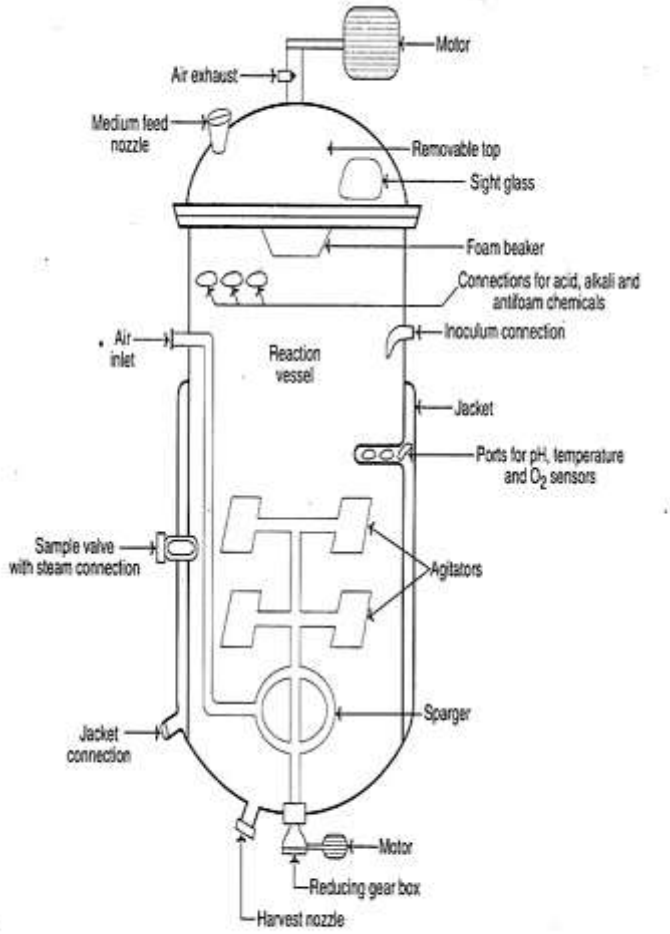


Fig. 19.5 : Diagrammatic representation of a typical bioreactor.

Conventional bioreactors are cylindrical vessels with domed top and bottom. The reaction vessel, surrounded by a jacket, is provided with a sparger at the bottom through which air (or other gases such as CO_2 and NH_3 for pH maintenance) can be introduced.

The agitator shaft is connected to a motor at the bottom. The reaction vessel has side ports for pH, temperature and dissolved O_2 sensors. Above the liquid level of the reaction vessel, connections for acid, alkali, antifoam chemicals and inoculum are located.

The bioreactor is usually designed to work at higher temperature (150-180°C), higher pressure (377-412 kPa). The reaction vessel is also designed to withstand vacuum, or else it may collapse while cooling. The

materials used for the construction of bioreactor must be non-toxic and must withstand the repeated sterilization with high pressure steam.

The bioreactor vessel is usually made up of stainless steel. It should be free from crevices and stagnant areas so that no solids/liquids accumulate. Easy to clean channels and welded joints (instead of couplings) are preferred. Transparent material should be used wherever possible, since it is advantageous to inspect medium and culture frequently.

Operation of a Conventional Bioreactor:

The operation of a bioreactor basically involves the following steps:

1. Sterilization
2. Inoculation and sampling
3. Aeration
4. Control systems
5. Cleaning.

Sterilization:

Aseptic conditions are the basic requirements for successful fermentation. That is the bioreactor and its accessories, the growth medium and the air supplied during fermentation must be sterile.

In situ sterilization:

The bioreactor filled with the required medium is injected with pressurized steam into the jacket or coil surrounding the reaction vessel. The whole system is heated to about 120°C and held at this temperature for about 20 minutes. In situ sterilization has certain limitations. It is not energy- efficient (i.e., energy is wasted) since the bioreactor has to be heated for a long period to rise the temperature of the whole system to 120°C. Prolonged heating may destroy vitamins, besides precipitating the medium components.

Continuous heat sterilization:

In this technique, empty bioreactor is first sterilized by injecting pressurised steam. The medium is rapidly heated to 140°C for a short period, by injecting the pressurised steam. Alternately, the medium can be sterilized by passing through a heat exchanger heated by pressurised steam. Subjecting the medium to high temperature for a short period does not precipitate medium components. Further, there is no energy wastage in continuous heat sterilization method.

Inoculation and Sampling:

The bioreactor with the growth medium under aseptic conditions is ready for inoculation with the production organism. The size of the inoculum is generally 1-10% of the total volume of the medium. A high yielding production strain of the organism taken from a stock culture (lyophilized and stored in a deep freezer or in liquid nitrogen) is used.

During the course of fermentation, samples are regularly drawn from the bioreactor. This is required to check the contamination (if any) and measurement of the product formed.

Aeration:

Aeration of the fermentation medium is required to supply O_2 to the production organisms and remove CO_2 from the bioreactor. The aeration system is designed for good exchange of gases. Oxygen (stored in tanks in a compressed form) is introduced at the bottom of the bioreactor through a sparger.

The small bubbles of the air pass through the medium and rise to the surface. The bioreactor usually has about 20% of its volume as vacant space on the upper part which is referred to as head space. The bioreactor has about 80% working volume. The gases released during fermentation accumulate in the headspace which pass out through an air outlet.

Air-lift system of aeration:

In this type of aeration, sparging of air is done at the bottom of the fermenter. This allows an upward flow of air bubbles. The more is the aeration capacity of the fermenter, the more is the dissolved O_2 in the medium. Further, the aeration capacity of the air-lift system is directly proportional to the airflow rate and the internal pressure.

Oxygen demand refers to the rate at which the growing culture requires O_2. For all the aerobic organisms, the aeration capacity should be more than the oxygen demand or else the growth of the organisms will be inhibited due to oxygen depletion (starvation).

Stirred system of aeration:

The aeration capacity of the medium can be enhanced by stirring. This can be done by using impellers driven by a motor. The aeration capacity of the stirred fermenter is proportional to the stirring speed, rate of air flow and the internal pressure. Stirred fermenters are better suited than air-lift fermenters to produce better aeration capacities.

Control Systems:

It is essential to maintain optimal growth environment in the reaction vessel for maximum product formation. Maximal efficiency of the fermentation can be achieved by continuously monitoring the variables such as the pH, temperature, dissolved oxygen, adequate mixing, nutrient concentration and foam formation. Improved sensors are now available for continuous and automated monitoring of these variables (i.e., on line measurement of pH).

Most of the microorganisms employed in fermentation grow optimally between pH 5.5 and 8.5. In the bioreactor, as the microorganisms grow, they release metabolites into the medium which change pH. Therefore, the pH of the medium should be continuously monitored and maintained at the optimal level.

This can be done by the addition of acid or alkali base (as needed) and a thorough mixing of the fermentation contents. Sometimes, an acid or alkaline medium component can be used to correct pH, besides providing nutrients to the growing microorganisms.

Temperature:

Temperature control is absolutely essential for a good fermentation process. Lower temperature causes reduced product formation while higher temperature adversely affects the growth of microorganisms. The bioreactors are normally equipped with heating and cooling systems that can be used as per the requirement, to maintain the reaction vessel at optimal temperature.

Dissolved oxygen:

Oxygen is sparingly soluble in water (0.0084 g/1 at 25°C). Continuous supply of oxygen in the form of sterilized air is done to the culture medium. This is carried out by introducing air into the bioreactor in the form of bubbles. Continuous monitoring of dissolved oxygen concentration is done in the bioreactor for optimal product formation.

Adequate mixing:

Continuous and adequate mixing of the microbial culture ensures optimal supply of nutrients and O_2, besides preventing the accumulation of toxic metabolic byproducts (if any). Good mixing (by agitation) also creates favourable environment for optimal and homogeneous growth environment, and good product formation. However, excessive agitation may damage microbial cells and increase the temperature of the medium, besides increased foam formation.

Nutrient concentration:

The nutrient concentration in a bioreactor is limited so that its wastage is prevented. In addition, limiting concentrations of nutrients may be advantageous for optimal product formation, since high nutrient concentrations are often associated with inhibitory effect on microbial growth. It is now possible to do on-line monitoring of the nutrient concentration, and suitably modify as per the requirements.

Foam formation:

The media used in industrial fermentation is generally rich in proteins. When agitated during aeration, it invariably results in froth or foam formation that builds in head space of the bioreactor. Antifoam chemicals are used to lower surface tension of the medium, besides causing foam bubbles to collapse. Mineral oils based on silicone or vegetable oils are commonly used as antifoam agents.

Mechanical foam control devices, referred to as mechanical foam breakers, can also be used. Such devices, fitted at the top of the bioreactor break the foam bubbles and the throw back into the fermentation medium.

Cleaning:

As the fermentation is complete, the bioreactor is harvested i.e. the contents are removed for processing. The bioreactor is then prepared for the next round of fermentation after cleaning (technically called turn round). The time taken for turn round referred to as down time should be as short as possible (since it is non-productive). Due to large size of the bioreactors, it is not possible to clean manually. The cleaning of the bioreactors is carried out by using high-pressure water jets from the nozzles fitted into the reaction vessel.

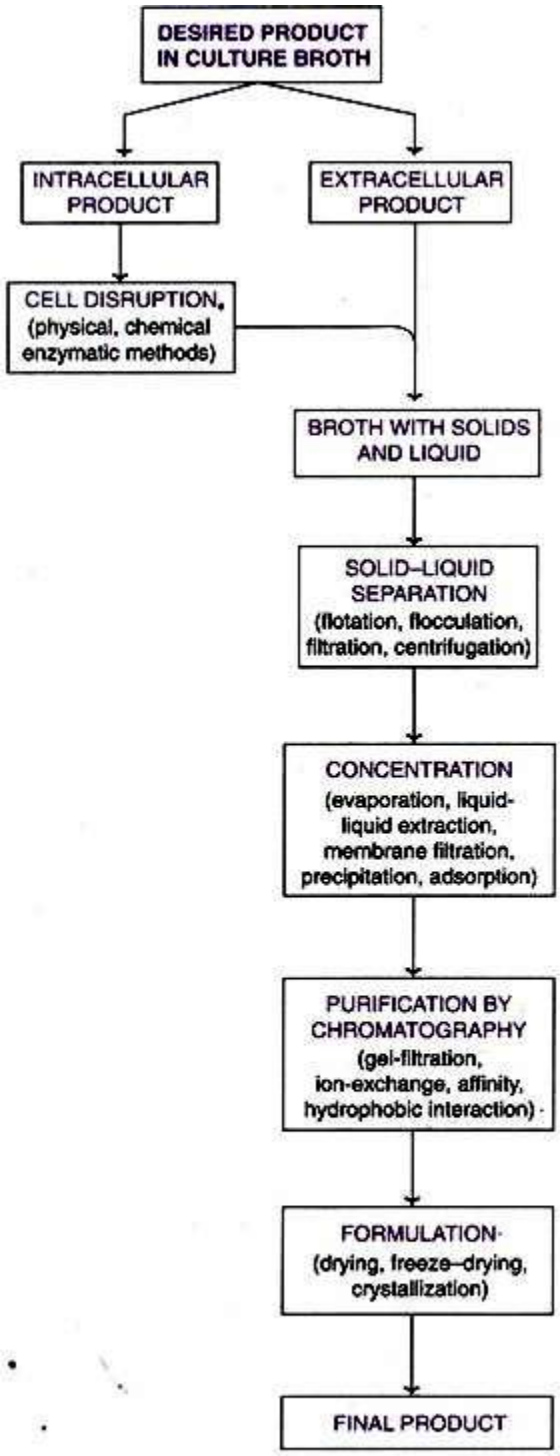
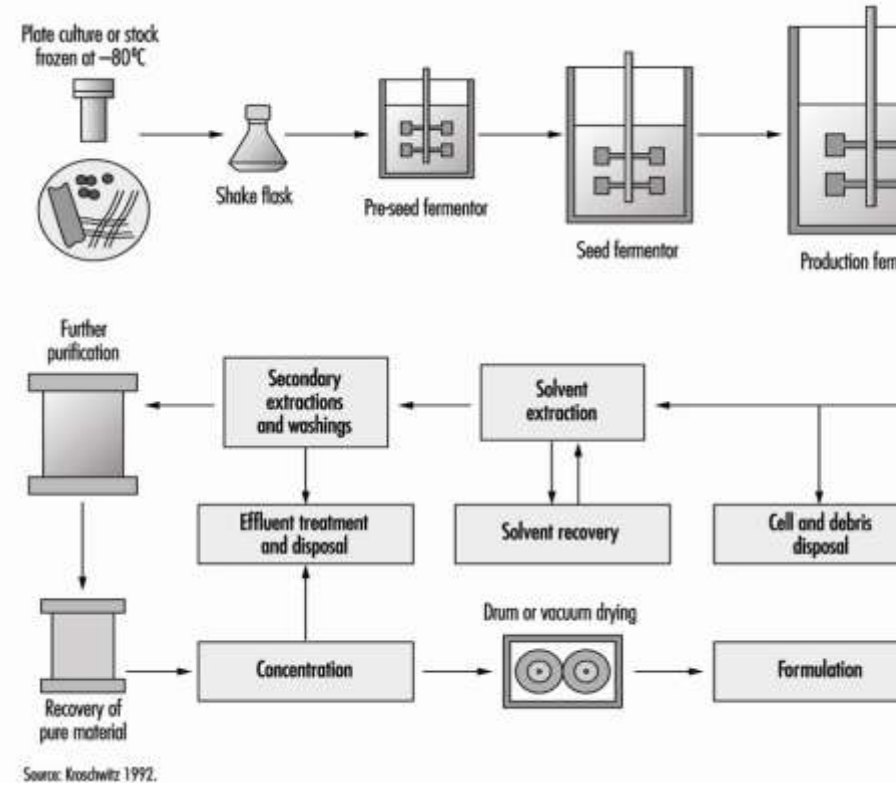


Fig. 20.1 : A summary of the major steps in downstream processing.



In Fig. 20.1, an outline of the major steps in downstream processing is given.

Stage # 1. Solid-Liquid Separation:

The first step in product recovery is the separation of whole cells (cell biomass) and other insoluble ingredients from the culture broth (Note: If the desired product is an intracellular metabolite, it must be released from the cells before subjecting to solid-liquid separation). Some authors use the term harvesting of microbial cells for the separation of cells from the culture medium. Several methods are in use for solid-liquid separation. These include flotation, flocculation, filtration and centrifugation.

Flotation:

When a gas is introduced into the liquid broth, it forms bubbles. The cells and other solid particles get adsorbed on gas bubbles. These bubbles rise to the foam layer which can be collected and removed. The presence of certain substances, referred to as collector substances, facilitates stable foam formation e.g., long chain fatty acids, amines.

Flocculation:

In flocculation, the cells (or cell debris) form large aggregates to settle down for easy removal. The process of flocculation depends on the nature of cells and the ionic constituents of the medium. Addition of flocculating agents (inorganic salt, organic polyelectrolyte, mineral hydrocolloid) is often necessary to achieve appropriate flocculation.

Filtration:

Filtration is the most commonly used technique for separating the biomass and culture filtrate. The efficiency of filtration depends on many factors— the size of the organism, presence of other organisms, viscosity of the medium, and temperature. Several filters such as depth filters, absolute filters, rotary drum vacuum filters and membrane filters are in use.

Depth Filters:

They are composed of a filamentous matrix such as glass wool, asbestos or filter paper. The particles are trapped within the matrix and the fluid passes out. Filamentous fungi can be removed by using depth filters.

Absolute Filters:

These filters are with specific pore sizes that are smaller than the particles to be removed. Bacteria from culture medium can be removed by absolute filters.

Rotary Drum Vacuum Filters:

These filters are frequently used for separation of broth containing 10-40% solids (by volume) and particles in the size of 0.5-10µm. Rotary drum vacuum filters have been successfully used for filtration of yeast cells and filamentous fungi. The equipment is simple with low power consumption and is easy to operate. The filtration unit consists of a rotating drum partially immersed in a tank of broth (Fig. 20.2). As the drum rotates, it picks up the biomass which gets deposited as a cake on the drum surface. This filter cake can be easily removed.

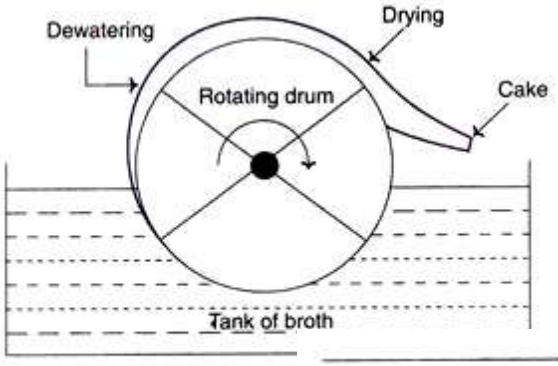


Fig. 20.2 : Diagrammatic re drum vacuu

Membrane Filters:

In this type of filtration, membranes with specific pore sizes can be used. However, clogging of filters is a major limitation. There are two types of membrane filtrations— static filtration and cross-flow filtration (Fig. 20.3). In cross-flow filtration, the culture broth is pumped in a crosswise fashion across the membrane. This reduces the clogging process and hence better than the static filtration.

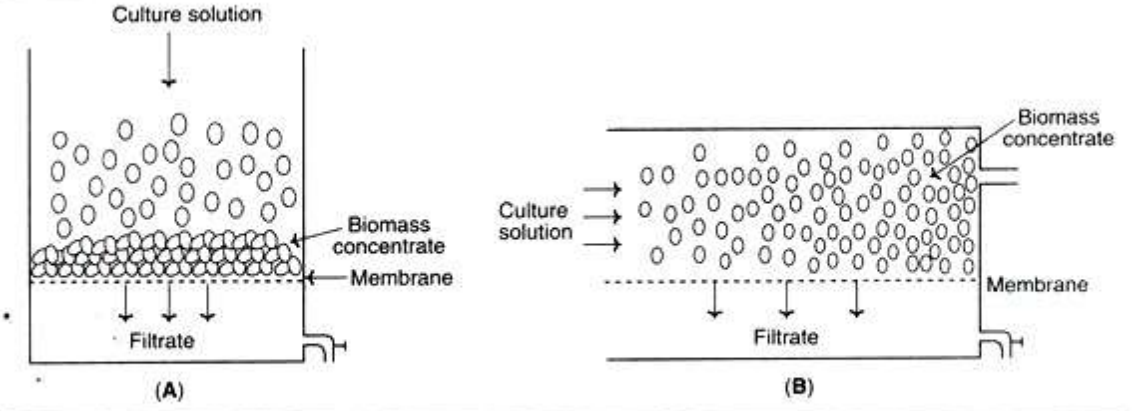


Fig. 20.3 : Filter systems for separation of biomass and culture filtrate (A) Static–flow filtration (B) Cross–flow filtration.

Downstream processing (DSP):

The method by which the products of fermentation are recovered and separated is known as downstream processing. This

forms the major (about 85%) portion of the complete fermentation technology. There are various methods by which DSP is carried out.

First of all, the broth is conditioned i.e. the cells are aggregated and form large clumps, which makes the separation easier. The conditioning is done by heating, freezing, pH change, antigen-antibody reactions etc. Then the conditioned broth is used for the separation of the constituents for which techniques like sedimentation, floatation, filtration, ultra-filtration, centrifugation and micro-filtration are applied.

Types of filtration processes:

There are 3 major types of filtrations based on the particle sizes and other characters (Table 20.1). These are microfiltration, ultrafiltration and reverse osmosis.

Table 20.1 Major types of filtration processes with characteristic features

Type	Sizes of particles separated	Compound or particle separated
1. Microfiltration	0.1–10 μm	Cells or cell fractions, viruses.
2. Ultrafiltration	0.001–0.1 μm	Compounds with molecular weights greater than 1000 (e.g. enzymes).
3. Reverse osmosis (hyperfiltration)	0.0001–0.001 μm	Compounds with molecular weights less than 1000 (e.g. lactose).

Centrifugation:

The technique of centrifugation is based on the principle of density differences between the particles to be separated and the medium. Thus, centrifugation is mostly used for separating solid particles from liquid phase (fluid/particle separation). Unlike the centrifugation that is conveniently carried out in the laboratory scale, there are certain limitations for large scale industrial centrifugation.

However, in recent years, continuous flow industrial centrifuges have been developed. There is a continuous feeding of the slurry and collection of clarified fluid, while the solids deposited can be removed intermittently. The different types of centrifuges are depicted in Fig. 20.4, and briefly described hereunder.

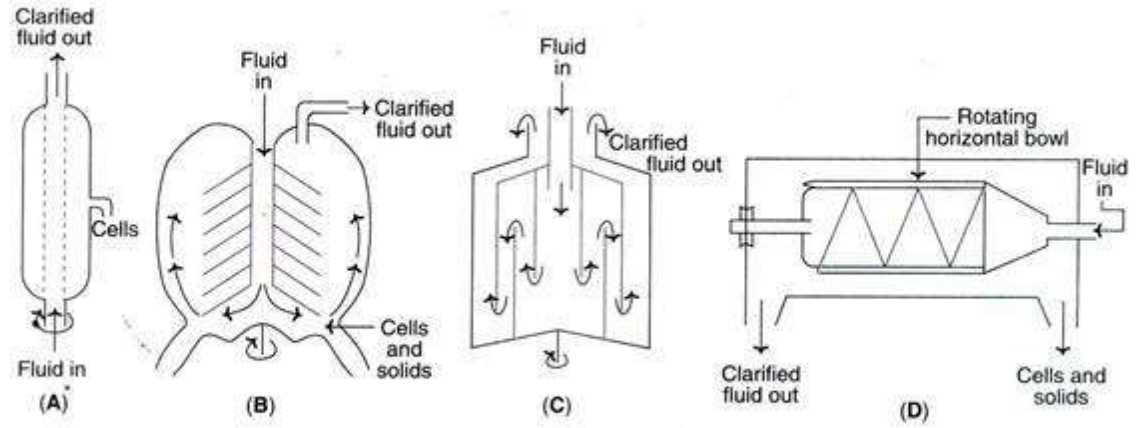


Fig. 20.4 : Centrifuges commonly used in downstream processing (A) Tubular bowl centrifuge (B) Disc centrifuge (C) Multichamber centrifuge (D) Scroll centrifuge (decanter).

Tubular bowl centrifuge (Fig. 20.4A):

This is a simple and a small centrifuge, commonly used in pilot plants. Tubular bowl centrifuge can be operated at a high centrifugal speed, and can be run in both batch or continuous mode. The solids are removed manually.

Disc centrifuge (Fig. 20.4B):
It consists of several discs that separate the bowl into settling zones. The feed/slurry is fed through a central tube. The clarified fluid moves upwards while the solids settle at the lower surface.
Multi-chamber centrifuge (Fig. 20.4C):
This is basically a modification of tubular bowl type of centrifuge. It consists of several chambers connected in such a way that the feed flows in a zigzag fashion. There is a variation in the centrifugal force in different chambers. The force is much higher in the periphery chambers, as a result smallest particles settle down in the outermost chamber.
Scroll centrifuge or decanter (Fig. 20.4D):
It is composed of a rotating horizontal bowl tapered at one end. The decanter is generally used to concentrate fluids with high solid concentration (biomass content 5-80%). The solids are deposited on the wall of the bowl which can be scrapped and removed from the narrow end.
Stage # 2. Release of Intracellular Products:
As already stated, there are several biotechnological products (vitamins, enzymes) which are located within the cells. Such compounds have to be first released (maximally and in an active form) for their further processing and final isolation. The microorganisms or other cells can be disintegrated or disrupted by physical, chemical or enzymatic methods. The outline of different techniques used for breakage of cells is given in Fig. 20.5.

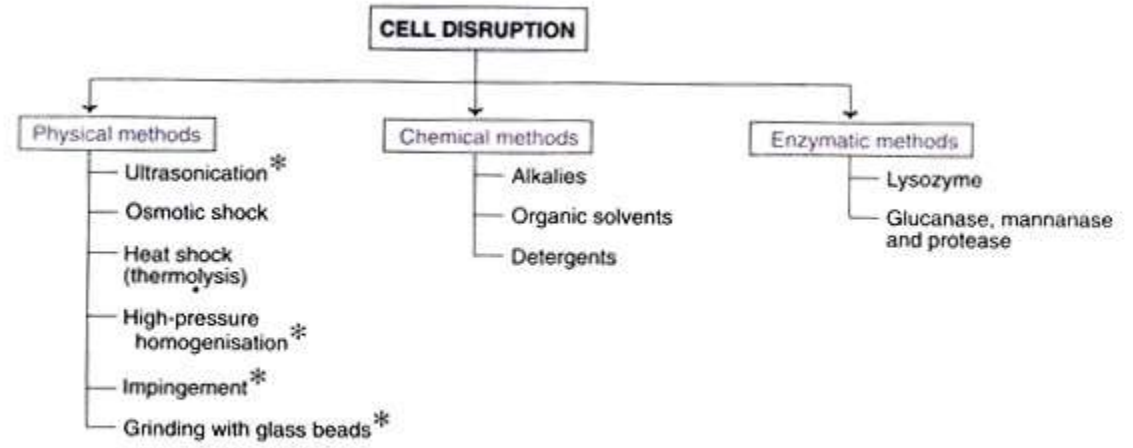


Fig. 20.5 : Major methods for cell disruption to release the intracellular products (indicate mechanical methods while all the remaining are non-mechanical).*

The selection of a particular method depends on the nature of the cells, since there is a wide variation in the property of cell disruption or breakage. For instance, Gram-negative bacteria and filamentous fungi can be more easily broken compared to Gram-positive bacteria arid yeasts.
Cell Disruption:
Physical methods of cell disruption:
The microorganisms or cells can be disrupted by certain physical methods to release the intracellular products.
Ultra sonication:
Ultrasonic disintegration is widely employed in the laboratory. However, due to high cost, it is not suitable for large-scale use in industries.
Osmotic shock:
This method involves the suspension of cells (free from growth medium) in 20% buffered sucrose. The cells are then transferred to water at about 4°C. Osmotic shock is used for the release of hydrolytic enzymes and binding proteins from Gram-negative bacteria.

Heat shock (thermolysis):
Breakage of cells by subjecting them to heat is relatively easy and cheap. But this technique can be used only for a very few heat-stable intracellular products.
High pressure homogenization:
This technique involves forcing of cell suspension at high pressure through a very narrow orifice to come out to atmospheric pressure. This sudden release of high pressure creates a liquid shear that can break the cells.
Impingement:
In this procedure, a stream of suspended cells at high velocity and pressure are forced to hit either a stationary surface or a second stream of suspended cells (impinge literally means to strike or hit). The cells are disrupted by the forces created at the point of contact. Micro fluidizer is a device developed based on the principle of impingement. It has been successfully used for breaking E. coli cells. The advantage with impingement technique is that it can be effectively used for disrupting cells even at a low concentration.
Grinding with glass beads:
The cells mixed with glass beads are subjected to a very high speed in a reaction vessel. The cells break as they are forced against the wall of the vessel by the beads. Several factors influence the cell breakage-size and quantity of the glass beads, concentration and age of cells, temperature and agitator speed. Under optimal conditions, one can expect a maximal breakage of about 80% of the cells.
A diagrammatic representation of a cell disrupter employing glass beeds is shown in Fig. 20.6. It contains a cylindrical body with an inlet, outlet and a central motor-driven shaft. To this shaft are fitted radial agitators. The cylinder is fitted with glass beads. The cell suspension is added through the inlet and the disrupted cells come out through the outlet. The body of the cell disrupter is kept cool while the operation is on.

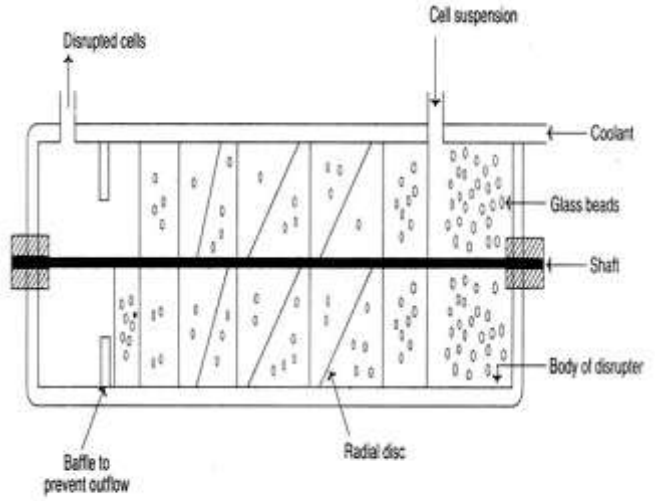


Fig. 20.6 : Diagrammatic representation of a cell disrupter.

Mechanical and non-mechanical methods:
Among the physical methods of cell disruption described above, ultra sonication, high-pressure homogenization, impingement and grinding with glass beads are mechanical while osmotic shock and heat shock are non-mechanical. The chemical and enzymatic methods (described below) are non-mechanical in nature.
Chemical methods of cell disruption:
Treatment with alkalies, organic solvents and detergents can lyse the cells to release the contents.
Alkalies:
Alkali treatment has been used for the extraction of some bacterial proteins. However, the alkali stability of the desired product is very crucial for the success of this method e.g., recombinant growth hormone can be efficiently released from E. coli by treatment with sodium hydroxide at pH 11.
Organic solvents:
Several water miscible organic solvents can be used to disrupt the cells e.g., methanol, ethanol, isopropanol, butanol. These compounds are inflammable; hence require specialised equipment for fire safety. The organic solvent toluene is frequently used. It is believed that toluene dissolves membrane phospholipids and creates membrane pores for release of intracellular contents.
Detergents:
Detergents that are ionic in nature, cationic-cetyl trimethyl ammonium bromide or anionic-sodium lauryl sulfate can denature membrane proteins and lyse the cells. Non-ionic detergents (although less reactive

than ionic ones) are also used to some extent e.g., Triton X-100 or Tween. The problem with the use of detergents is that they affect purification steps, particularly the salt precipitation. This limitation can be overcome by using ultrafiltration or ion-exchange chromatography for purification.

Enzymatic methods of cell disruption:

Cell disruption by enzymatic methods has certain advantages i.e., lysis of cells occurs under mild conditions in a selective manner. This is quite advantageous for product recovery. Lysozyme is the most frequently used enzyme and is commercially available (produced from hen egg white). It hydrolyses β-1, 4-glycosidic bonds of the mucopeptide in bacterial cell walls. The Gram- positive bacteria (with high content of cell wall mucopeptides) are more susceptible for the action of lysozyme.

For Gram-negative bacteria, lysozyme in association with EDTA can break the cells. As the cell wall gets digested by lysozyme, the osmotic effects break the periplasmic membrane to release the intracellular contents. Certain other enzymes are also used, although less frequently, for cell disruption. For the lysis of yeast cell walls, glucanase and mannanase in combination with proteases are used.

Combination of methods:

In order to increase the efficiency of cell disintegration in a cost-effective manner, a combination of physical, chemical and enzymatic methods is employed.

Stage # 3. Concentration:

The filtrate that is free from suspended particles (cells, cell debris etc.) usually contains 80-98% of water. The desired product is a very minor constituent. The water has to be removed to achieve the product concentration. The commonly used techniques for concentrating biological products are evaporation, liquid-liquid extraction, membrane filtration, precipitation and adsorption. The actual procedure adopted depends on the nature of the desired product (quality and quantity to be retained as far as possible) and the cost factor.

Evaporation:

Water in the broth filtrate can be removed by a simple evaporation process. The evaporators, in general, have a heating device for supply of steam, and unit for the separation of concentrated product and vapour, a condenser for condensing vapour, accessories and control equipment. The capacity of the equipment is variable that may range from small laboratory scale to industrial scale. Some of the important types of evaporators in common use are briefly described.

Plate evaporators:

The liquid to be concentrated flows over plates. As the steam is supplied, the liquid gets concentrated and becomes viscous.

Falling film evaporators:

In this case, the liquid flows down long tubes which gets distributed as a thin film over the heating surface. Falling film evaporators are suitable for removing water from viscous products of fermentation.

Forced film evaporators:

The liquid films are mechanically driven and these devices are suitable for producing dry product concentrates.

Centrifugal forced film evaporators:

These equipment evaporate the liquid very quickly (in seconds), hence suitable for concentrating even heat-labile substances. In these evaporators, a centrifugal force is used to pass on the liquid over heated plates or conical surfaces for instantaneous evaporation.

Liquid-Liquid Extraction:

The concentration of biological products can be achieved by transferring the desired product (solute) from one liquid phase to another liquid phase, a phenomenon referred to as liquid-liquid extraction. Besides concentration, this technique is also useful for partial purification of a product.

The efficiency of extraction is dependent on the partition coefficient i.e. the relative distribution of a substance between the two liquid phases. The process of liquid-liquid extraction may be broadly

categorized as extraction of low molecular weight products and extraction of high molecular weight products.

Extraction of low molecular weight products:

By using organic solvents, the lipophilic compounds can be conveniently extracted. However, it is quite difficult to extract hydrophilic compounds. Extraction of lipophilic products can be done by the following techniques.

Physical extraction:

The compound gets itself distributed between two liquid phases based on the physical properties. This technique is used for extraction of non-ionising compounds.

Dissociation extraction:

This technique is suitable for the extraction of ionisable compounds. Certain antibiotics can be extracted by this procedure.

Reactive extraction:

In this case, the desired product is made to react with a carrier molecule (e.g., phosphorus compound, aliphatic amine) and extracted into organic solvent. Reactive extraction procedure is quite useful for the extraction of certain compounds that are highly soluble in water (aqueous phase) e.g., organic acids.

Supercritical fluid (SCF) extraction:

This technique differs from the above procedures, since the materials used for extraction are supercritical fluids (SCFs). SCFs are intermediates between gases and liquids and exist as fluids above their critical temperature and pressure. Supercritical CO_2, with a low critical temperature and pressure is commonly used in the extraction. Supercritical fluid extraction is rather expensive, hence not widely used (SCF has been used for the extraction of caffeine from coffee beans, and pigments and flavor ingredients from biological materials).

Extraction of high molecular weight compounds:

Proteins are the most predominant high molecular weight products produced in fermentation industries. Organic solvents cannot be used for protein extraction, as they lose their biological activities. They are extracted by using an aqueous two-phase systems or reverse micelles formation.

Aqueous two-phase systems (ATPS):

They can be prepared by mixing a polymer (e.g., polyethylene glycol) and a salt solution (ammonium sulfate) or two different polymers. Water is the main component in ATPS, but the two phases are not miscible. Cells and other solids remain in one phase while the proteins are transferred to other phase. The distribution of the desired product is based on its surface and ionic character and the nature of phases. The separation takes much longer time by ATPS.

Reverse miceller systems:

Reverse micelles are stable aggregates of surfactant molecules and water in organic solvents. The proteins can be extracted from the aqueous medium by forming reverse micelles. In fact, the enzymes can be extracted by this procedure without loss of biological activity.

Membrane Filtration:

Membrane filtration has become a common separation technique in industrial biotechnology. It can be conveniently used for the separation of biomolecules and particles, and for the concentration of fluids. The membrane filtration technique basically involves the use of a semipermeable membrane that selectively retains the particles/molecules that are bigger than the pore size while the smaller molecules pass through the membrane pores.

Membranes used in filtration are made up of polymeric materials such as polyethersulfone and polyvinyl di-fluoride. It is rather difficult to sterilize membrane filters. In recent years, micro-filters and ultrafiIters composed of ceramics and steel are available. Cleaning and sterilization of such filters are easy. The other types of membrane filtration techniques are described briefly.

Membrane adsorbers:

They are micro- or macro porous membranes with ion exchange groups and/or affinity ligands. Membrane adsorbers can bind to proteins and retain them. Such proteins can be eluted by employing solutions in chromatography.

Pervaporation:

This is a technique in which volatile products can be separated by a process of permeation through a membrane coupled with evaporation. Pervaporation is quite useful for the extraction, recovery and concentration of volatile products. However, this procedure has a limitation since it cannot be used for large scale separation of volatile products due to cost factor.

Perstraction:

This is an advanced technique working on the principle of membrane filtration coupled with solvent extraction. The hydrophobic compounds can be recovered/ concentrated by this method.

Precipitation:

Precipitation is the most commonly used technique in industry for the concentration of macromolecules such as proteins and polysaccharides. Further, precipitation technique can also be employed for the removal of certain unwanted byproducts e.g. nucleic acids, pigments.

Neutral salts, organic solvents, high molecular weight polymers (ionic or non-ionic), besides alteration in temperature and pH are used in precipitation. In addition to these non-specific protein precipitation reactions (i.e. the nature of the protein is unimportant), there are some protein specific precipitations e.g., affinity precipitation, ligand precipitation.

Neutral salts:

The most commonly used salt is ammonium sulfate, since it is highly soluble, nontoxic to proteins and low-priced. Ammonium sulfate increases hydrophobic interactions between protein molecules that result in their precipitation. The precipitation of proteins is dependent on several factors such as protein concentration, pH and temperature.

Organic solvents:

Ethanol, acetone and propanol are the commonly used organic solvents for protein precipitation. They reduce the dielectric constant of the medium and enhance electrostatic interaction between protein molecules that lead to precipitation. Since proteins are denatured by organic solvents, the precipitation process has to be carried out below 0°C.

Non-ionic polymers:

Polyethylene glycol (PEG) is a high molecular weight non-ionic polymer that can precipitate proteins. It reduces the quantity of water available for protein solvation and precipitates protein. PEG does not denature proteins, besides being non-toxic.

Ionic polymers:

The charged polymers such as polyacrylic acid and polyethyleneimine are used. They form complexes with oppositely charged protein molecules that cause charge neutralisation and precepitation.

Increase in temperature:

The heat sensitive proteins can be precipitated by increasing the temperature.

Change in pH:

Alterations in pH can also lead to protein precipitation.

Affinity precipitation:

The affinity interaction (e.g., between antigen and antibody) is exploited for precipitation of proteins.

Precipitation by ligands:

Ligands with specific binding sites for proteins have been successfully used for selective precipitation.

Adsorption:

The biological products of fermentation can be concentrated by using solid adsorbent particles. In the early days, activated charcoal was used as the adsorbent material. In recent years, cellulose-based adsorbents are employed for protein concentration.

And for concentration of low molecular weight compounds (vitamins, antibiotics, peptides) polystyrene, methacrylate and acrylate based matrices are used. The process of adsorption can be carried out by making a bed of adsorbent column and passing the culture broth through it. The desired product, held by the adsorbent, can be eluted.

Stage # 4. Purification by Chromatography:

The biological products of fermentation (proteins, pharmaceuticals, diagnostic compounds and research materials) are very effectively purified by chromatography. It is basically an analytical technique dealing with the separation of closely related compounds from a mixture. Chromatography usually consists of a stationary phase and mobile phase.

The stationary phase is the porous solid matrix packed in a column (equilibrated with a suitable solvent) on to which the mixture of compounds to be separated is loaded. The compounds are eluted by a mobile phase.

A single mobile phase may be used continuously or it may be changed appropriately to facilitate the release of desired compounds. The eluate from the column can be monitored continuously (e.g. protein elution can be monitored by ultraviolet adsorption at 280 nm), and collected in fractions of definite volumes.

The different types of chromatography techniques used for separation (mainly proteins) along with the principles are given in Table 20.2. A large number of matrices are commercially available for purification of proteins e.g., agarose, cellulose, polyacrylamide, porous silica, cross- linked dextran, polystyrene. Some of the important features of selected chromatographic techniques are briefly described.

Table 20.2 Chromatographic techniques along with the principles for separation of proteins

Chromatography	*Principle*
Gel-filtration (size exclusion)	Size and shape
Ion-exchange	Net charge
Chromatofocussing	Net charge
Affinity	Biological affinity and molecular recognition
Hydrophobic interaction	Polarity (hydrophobicity of molecules)
Immobilized metal-ion affinity	Metal ion binding

Gel-filtration chromatography:

This is also referred to as size-exclusion chromatography. In this technique, the separation of molecules is based on the size, shape and molecular weight. The sponge-like gel beads with pores serve as molecular sieves for separation of smaller and bigger molecules. A solution mixture containing molecules of different sizes (e.g. different proteins) is applied to the column and eluted.

The smaller molecules enter the gel beads through their pores and get trapped. On the other hand, the larger molecules cannot pass through the pores and therefore come out first with the mobile liquid (Fig. 20.7). At the industrial scale, gel-filtration is particularly useful to remove salts and low molecular weight compounds from high molecular weight products.

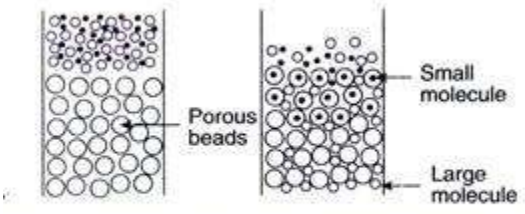


Fig. 20.7 : The principle of gel-filtration chromatography.

Ion-exchange chromatography:

It involves the separation of molecules based on their surface charges. Ion-exchangers are of two types (cation- exchangers which have negatively charged groups like carboxymethyl and sulfonate, and anion-exchangers with positively charged groups like diethylaminoethyl (DEAE). The most commonly used

cation-exchangers are Dowex HCR and Amberlite IR, the anion-exchangers are Dowex SAR and Amberlite IRA.

In ion-exchange chromatography, the pH of the medium is very crucial, since the net charge varies with pH. In other words, the pH determines the effective charge on both the target molecule and the ion-exchanger. The ionic bound molecules can be eluted from the matrix by changing the pH of the eluant or by increasing the concentration of salt solution. Ion-exchange chromatography is useful for the purification of antibiotics, besides the purification of proteins.

Affinity chromatography:

This is an elegant method for the purification of proteins from a complex mixture. Affinity chromatography is based on an interaction of a protein with an immobilized ligand. The ligand can be a specific antibody, substrate, substrate analogue or an inhibitor. The immobilized ligand on a solid matrix can be effectively used to fish out complementary structures.

In Table 20.3, some examples of ligands used for the purification of proteins are given. The protein bound to the ligand can be eluted by reducing their interaction. This can be achieved by changing the pH of the buffer, altering the ionic strength or by using another free ligand molecule. The fresh ligand used has to be removed in the subsequent steps.

Table 20.3 Some examples of ligands used for separation of proteins by affinity chromatography

Ligand	*Type of protein*
Antibody	Antigen
Cofactor	Enzyme
Receptor	Hormone
Hapten	Antibody
Inhibitor	Enzyme
Lectins	Glycoproteins
Heparin	Coagulation factors
Metal ions	Metal ion binding proteins

Hydrophobic interaction chromatography (HIC):

This is based on the principle of weak hydrophobic interactions between the hydrophobic ligands (alkyl, aryl side chains on matrix) and hydrophobic amino acids of proteins. The differences in the composition of hydrophobic amino acids in proteins can be used for their separation. The elution of proteins can be done by lowering the salt concentration, decreasing the polarity of the medium or reducing the temperature.

Stage # 5. Formulation:

Formulation broadly refers to the maintenance of activity and stability of a biotechnological products during storage and distribution. The formulation of low molecular weight products (solvents, organic acids) can be achieved by concentrating them with removal of most of the water. For certain small molecules, (antibiotics, citric acid), formulation can be done by crystallization by adding salts.

Proteins are highly susceptible for loss of biological activity; hence their formulation requires special care. Certain stabilizing additives are added to prolong the shelf life of protein. The stabilizers of protein formulation include sugars (sucrose, lactose), salts (sodium chloride, ammonium sulfate), polymers (polyethylene glycol) and polyhydric alcohols (glycerol). Proteins may be formulated in the form of solutions, suspensions or dry powders.

Drying:

Drying is an essential component of product formulation. It basically involves the transfer of heat to a wet product for removal of moisture. Most of the biological products of fermentation are sensitive to heat, and therefore require gentle drying methods. Based on the method of heat transfer, drying devices may be categorized as contact, convection, radiation dryers. These three types of dryers are commercially available.

Spray drying:

Spray drying is used for drying large volumes of liquids. In spray drying, small droplets of liquid containing the product are passed through a nozzle directing it over a stream of hot gas. The water evaporates and the solid particles are left behind.

Freeze-drying:

Freeze-drying or lyophilization is the most preferred method for drying and formulation of a wide-range of products—pharmaceuticals, foodstuffs, diagnostics, bacteria, viruses. This is mainly because freeze-drying usually does not cause loss of biological activity of the desired product.

Lyophilization is based on the principle of sublimation of a liquid from a frozen state. In the actual technique, the liquid containing the product is frozen and then dried in a freeze-dryer under vacuum. The vacuum can now be released and the product containing vials can be sealed e.g., penicillin can be freeze dried directly in ampules.

Integration of Different Processes:

It is ideal to integrate the fermentation and downstream processing to finally get the desired product. However, this has not been practicable for various reasons. Integration of certain stages in downstream processing for purification of product has met with some success. For instance, protein concentration by extraction into two phase systems combined with clarification and purification can be done together.

Beer Production

Brewing is the production of beer by steeping a starch source (commonly cereal grains, the most popular of which is barley) in water and fermenting the resulting sweet liquid with yeast. It may be done in a brewery by a commercial brewer, at home by a home brewer, or by a variety of traditional methods such as communally by the indigenous peoples in Brazil when making cauim. Brewing has taken place since around the 6th millennium BC, and archaeological evidence suggests that emerging civilizations including ancient Egypt and Mesopotamia brewed beer. Since the nineteenth century the brewing industry has been part of most western economies.

The basic ingredients of beer are water and a fermentable starch source such as malted barley. Most beer is fermented with a brewer's yeast and flavoured with hops. Less widely used starch sources include millet, sorghum and cassava. Secondary sources (adjuncts), such as maize (corn), rice, or sugar, may also be used, sometimes to reduce cost, or to add a feature, such as adding wheat to aid in retaining the foamy head of the beer. The proportion of each starch source in a beer recipe is collectively called the grain bill.

Steps in the brewing process include malting, milling, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging. There are three main fermentation methods, warm, cool and spontaneous. Fermentation may take place in an open or closed fermenting vessel; a secondary fermentation may also occur in the cask or bottle. There are several additional brewing methods, such as barrel aging, double dropping, and Yorkshire Square.

The basic ingredients of beer are water; a starch source, such as malted barley, able to be fermented (converted into alcohol); a brewer's yeast to produce the fermentation; and a flavouring, such as hops, to offset the sweetness of the malt. A mixture of starch sources may be used, with a secondary saccharide, such as maize (corn), rice, or sugar, often being termed an adjunct, especially when used as a lower-cost substitute for malted barley. Less widely used starch sources include millet, sorghum, and cassava root in Africa, potato in Brazil, and agave in Mexico, among others. The amount of each starch source in a beer recipe is collectively called the grain bill.

Hops

Hops are the female flower clusters or seed cones of the hop vine *Humulus lupulus*, which are used as a flavouring and preservative agent in nearly all beer made today. Hops contain several characteristics that brewers desire in beer: they contribute a bitterness that balances the sweetness of the malt; they provide floral, citrus, and herbal aromas and flavours; they have an antibiotic effect that favours the activity of brewer's yeast over less desirable microorganisms; and they aid in "head retention", the length of time that a foamy head will last. The preservative in hops comes from the lupulin glands which contain soft resins with alpha and beta acids. Though much studied, the preservative nature of the soft resins is not yet fully understood, though it has been observed that unless stored at a cool temperature, the preservative nature will decrease. Brewing is the sole major commercial use of hops.

Yeast is the microorganism that is responsible for fermentation in beer. Yeast metabolises the sugars extracted from grains, which produces alcohol and carbon dioxide, and thereby turns wort into beer. In addition to fermenting the beer, yeast influences the character and flavour. The dominant types of yeast used to make beer are *Saccharomyces cerevisiae*, known as ale yeast, and *Saccharomyces pastorianus*, known as lager yeast; *Brettanomyces* ferments lambics,[43] and *Torulaspora delbrueckii* ferments Bavarian weissbier. Before the role of yeast in fermentation was understood, fermentation involved wild or airborne yeasts, and a few styles such as lambics still use this method today. Emil Christian Hansen, a Danish biochemist employed by the Carlsberg Laboratory, developed pure yeast cultures which were introduced into the Carlsberg brewery in 1883, and pure yeast strains are now the main fermenting source used worldwide.

Clarifying agent

Some brewers add one or more clarifying agents to beer, which typically precipitate (collect as a solid) out of the beer along with protein solids and are found only in trace amounts in the finished product. This process makes the beer appear bright and clean, rather than the cloudy appearance of ethnic and older styles of beer such as wheat beers.

Examples of clarifying agents include isinglass, obtained from swimbladders of fish; Irish moss, a seaweed; kappa carrageenan, from the seaweed *Kappaphycus cottonii*; Polyclar (artificial); and gelatin. If a beer is marked "suitable for Vegans", it was generally clarified either with seaweed or with artificial agents, although the "Fast Cask" method invented by Marston's in 2009 may provide another method.

Brewing process

There are several steps in the brewing process, which may include malting, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging.

Malting is the process where barley grain is made ready for brewing. Malting is broken down into three steps in order to help to release the starches in the barley. First, during steeping, the grain is added to a vat with water and allowed to soak for approximately 40 hours. During germination, the grain is spread out on the floor of the germination room for around 5 days. The final part of malting is kilning when the malt goes through a very high temperature drying in a kiln; with gradual temperature increase over several hours.

When kilning is complete, the grains are now termed malt, and they will be milled or crushed to break apart the kernels and expose the cotyledon, which contains the majority of the carbohydrates and sugars; this makes it easier to extract the sugars during mashing. Milling also separates the seed from the husk. Care must be taken when milling to ensure that the starch reserves are sufficiently milled without damaging the husk and providing coarse enough grits that a good filter bed can be formed during lautering. Grains are typically dry-milled with roller mills or hammer mills. Hammer mills, which produce a very fine mash, are often used when mash filters are going to be employed in the lautering process because the grain does not have to form its own filter bed. In modern plants, the grain is often conditioned with water before it is milled to make the husk more pliable, thus reducing breakage and improving lauter speed.

Mashing converts the starches released during the malting stage into sugars that can be fermented. The milled grain is mixed with hot water in a large vessel known as a mash tun. In this vessel, the grain and water are mixed together to create a cereal mash. During the mash, naturally occurring enzymes present in the malt convert the starches (long chain carbohydrates) in the grain into smaller molecules or simple sugars (mono-, di-, and tri-saccharides). This "conversion" is called saccharification. The result of the mashing process is a sugar-rich liquid or "wort", which is then strained through the bottom of the mash tun in a process known as lautering. Prior to lautering, the mash temperature may be raised to about 75–78 °C (167–172 °F) (known as a mashout) to free up more starch and reduce mash viscosity. Additional water may be sprinkled on the grains to extract additional sugars (a process known as sparging).

The wort is moved into a large tank known as a "copper" or kettle where it is boiled with hops and sometimes other ingredients such as herbs or sugars. This stage is where many chemical and technical reactions take place, and where important decisions about the flavour, colour, and aroma of the beer are made. The boiling process serves to terminate enzymatic

processes, precipitate proteins, isomerize hop resins, and concentrate and sterilize the wort. Hops add flavour, aroma and bitterness to the beer. At the end of the boil, the hopped wort settles to clarify in a vessel called a "whirlpool", where the more solid particles in the wort are separated out.

After the whirlpool, the wort is drawn away from the compacted hop trub, and rapidly cooled via a heat exchanger to a temperature where yeast can be added. A variety of heat exchanger designs are used in breweries, with the most common a plate-style. Water or glycol run in channels in the opposite direction of the wort, causing a rapid drop in temperature. It is very important to quickly cool the wort to a level where yeast can be added safely as yeast is unable to grow in very high temperatures, and will start to die in temperatures above 60 °C (140 °F). After the wort goes through the heat exchanger, the cooled wort goes into a fermentation tank. A type of yeast is selected and added, or "pitched", to the fermentation tank. When the yeast is added to the wort, the fermenting process begins, where the sugars turn into alcohol, carbon dioxide and other components. When the fermentation is complete the brewer may rack the beer into a new tank, called a conditioning tank. Conditioning of the beer is the process in which the beer ages, the flavour becomes smoother, and flavours that are unwanted dissipate. After conditioning for a week to several months, the beer may be filtered and force carbonated for bottling, or fined in the cask.

Mashing

Mashing is the process of combining a mix of milled grain (typically malted barley with supplementary grains such as corn, sorghum, rye or wheat), known as the "grain bill", and water, known as "liquor", and heating this mixture in a vessel called a "mash tun". Mashing is a form of steeping, and defines the act of brewing, such as with making tea, sake, and soy sauce. Technically, wine, cider and mead are not brewed but rather vinified, as there is no steeping process involving solids. Mashing allows the enzymes in the malt to break down the starch in the grain into sugars, typically maltose to create a malty liquid called wort. There are two main methods – infusion mashing, in which the grains are heated in one vessel; and decoction mashing, in which a proportion of the grains are boiled and then returned to the mash, raising the temperature. Mashing involves pauses at certain temperatures (notably 45–62–73 °C or 113–144–163 °F), and takes place in a "mash tun" – an insulated brewing vessel with a false bottom. The end product of mashing is called a "mash".

Mashing usually takes 1 to 2 hours, and during this time the various temperature rests activate different enzymes depending upon the type of malt being used, its modification level, and the intention of the brewer. The activity of these enzymes convert the starches of the grains to dextrins and then to fermentable sugars such as maltose. A mash rest from 49–55 °C (120–131 °F) activates various proteases, which break down proteins that might otherwise cause the beer to be hazy. This rest is generally used only with undermodified (i.e. undermalted) malts which are decreasingly popular in Germany and the Czech Republic, or non-malted grains such as corn and rice, which are widely used in North American beers. A mash rest at 60 °C (140 °F) activates β-glucanase, which breaks down gummy β-glucans in the mash, making the sugars flow out more freely later in the process. In the modern mashing process, commercial fungal based β-glucanase may be added as a supplement. Finally, a mash rest temperature of 65–71 °C (149–160 °F) is used to convert the starches in the malt to sugar, which is then usable by the yeast later in the brewing process. Doing the latter rest at the lower end of the range favours β-amylaseenzymes, producing more low-order sugars like maltotriose, maltose, and glucose which are more fermentable by the yeast. This in turn creates a beer lower in body and higher in alcohol. A rest closer to the higher end of the range favours α-amylaseenzymes, creating more higher-order sugars and dextrins which are less fermentable by the yeast, so a fuller-bodied beer with less alcohol is the result. Duration and pH variances also affect the sugar composition of the resulting wort.

Lautering is the separation of the wort (the liquid containing the sugar extracted during mashing) from the grains. This is done either in a mash tun outfitted with a false bottom, in a lauter tun, or in a mash filter. Most separation processes have two stages: first wort run-off, during which the extract is separated in an undiluted state from the spent grains, and sparging, in which extract which remains with the grains is rinsed off with hot water. The lauter tun is a tank with holes in the bottom small enough to hold back the large bits

of grist and hulls. The bed of grist that settles on it is the actual filter. Some lauter tuns have provision for rotating rakes or knives to cut into the bed of grist to maintain good flow. The knives can be turned so they push the grain, a feature used to drive the spent grain out of the vessel. The mash filter is a plate-and-frame filter. The empty frames contain the mash, including the spent grains, and have a capacity of around one hectoliter. The plates contain a support structure for the filter cloth. The plates, frames, and filter cloths are arranged in a carrier frame like so: frame, cloth, plate, cloth, with plates at each end of the structure. Newer mash filters have bladders that can press the liquid out of the grains between spargings. The grain does not act like a filtration medium in a mash filter.

Boiling

After mashing, the beer wort is boiled with hops (and other flavourings if used) in a large tank known as a "copper" or brew kettle – though historically the mash vessel was used and is still in some small breweries. The boiling process is where chemical and technical reactions take place, including sterilization of the wort to remove unwanted bacteria, releasing of hop flavours, bitterness and aroma compounds through isomerization, stopping of enzymatic processes, precipitation of proteins, and concentration of the wort. Finally, the vapours produced during the boil volatilise off-flavours, including dimethyl sulfide precursors. The boil is conducted so that it is even and intense – a continuous "rolling boil". The boil on average lasts between 45 and 90 minutes, depending on its intensity, the hop addition schedule, and volume of water the brewer expects to evaporate. At the end of the boil, solid particles in the hopped wort are separated out, usually in a vessel called a "whirlpool".

Brew kettle or copper

Copper is the traditional material for the boiling vessel, because copper transfers heat quickly and evenly, and because the bubbles produced during boiling, and which would act as an insulator against the heat, do not cling to the surface of copper, so the wort is heated in a consistent manner. The simplest boil kettles are direct-fired, with a burner underneath. These can produce a vigorous and favorable boil, but are also apt to scorch the wort where the flame touches the kettle, causing caramelisation and making cleanup difficult. Most breweries use a steam-fired kettle, which uses steam jackets in the kettle to boil the wort. Breweries usually have a boiling unit either inside or outside of the kettle, usually a tall, thin cylinder with vertical tubes, called a calandria, through which wort is pumped.

Whirlpool

At the end of the boil, solid particles in the hopped wort are separated out, usually in a vessel called a "whirlpool" or "settling tank". The whirlpool was devised by Henry Ranulph Hudston while working for the Molson Brewery in 1960 to utilise the so-called tea leaf paradox to force the denser solids known as "trub" (coagulated proteins, vegetable matter from hops) into a cone in the centre of the whirlpool tank.

Hopback

A hopback is a traditional additional chamber that acts as a sieve or filter by using whole hops to clear debris (or "trub") from the unfermented (or "green") wort, as the whirlpool does, and also to increase hop aroma in the finished beer. It is a chamber between the brewing kettle and wort chiller. Hops are added to the chamber, the hot wort from the kettle is run through it, and then immediately cooled in the wort chiller before entering the fermentation chamber.

Fermenting

Fermentation takes place in fermentation vessels which come in various forms, from enormous cylindroconical vessels, through open stone vessels, to wooden vats. After the wort is cooled and aerated – usually with sterile air – yeast is added to it, and it begins to ferment. It is during this stage that sugars won from the malt are converted into alcohol and carbon dioxide, and the product can be called beer for the first time.

Most breweries today use cylindroconical vessels, or CCVs, which have a conical bottom and a cylindrical top. The cone's aperture is typically around 60°, an angle that will allow the yeast to flow towards the cone's apex, but is not so steep as to take up too much vertical space. CCVs can handle both fermenting and conditioning in the same tank. At the end of fermentation, the yeast and other solids which have fallen to

the cone's apex can be simply flushed out of a port at the apex. Open fermentation vessels are also used, often for show in brewpubs, and in Europe in wheat beer fermentation. These vessels have no tops, which makes harvesting top-fermenting yeasts very easy. The open tops of the vessels make the risk of infection greater, but with proper cleaning procedures and careful protocol about who enters fermentation chambers, the risk can be well controlled. Fermentation tanks are typically made of stainless steel. If they are simple cylindrical tanks with beveled ends, they are arranged vertically, as opposed to conditioning tanks which are usually laid out horizontally. Only a very few breweries still use wooden vats for fermentation as wood is difficult to keep clean and infection-free and must be repitched more or less yearly

Conditioning

After an initial or primary fermentation, beer is *conditioned*, matured or aged, in one of several ways, which can take from 2 to 4 weeks, several months, or several years, depending on the brewer's intention for the beer. The beer is usually transferred into a second container, so that it is no longer exposed to the dead yeast and other debris (also known as "trub") that have settled to the bottom of the primary fermenter. This prevents the formation of unwanted flavours and harmful compounds such as acetylaldehyde.

Kräusening

Kräusening is a conditioning method in which fermenting wort is added to the finished beer. The active yeast will restart fermentation in the finished beer, and so introduce fresh carbon dioxide; the conditioning tank will be then sealed so that the carbon dioxide is dissolved into the beer producing a lively "condition" or level of carbonation. The kräusening method may also be used to condition bottled beer.

Lagering

Lagers are stored at near freezing temperatures for 1–6 months while still on the yeast. The process of storing, or conditioning, or maturing, or aging a beer at a low temperature for a long period is called "lagering", and while it is associated with lagers, the process may also be done with ales, with the same result – that of cleaning up various chemicals, acids and compounds.

Secondary fermentation

During secondary fermentation, most of the remaining yeast will settle to the bottom of the second fermenter, yielding a less hazy product.

Bottle fermentation

Some beers undergo an additional fermentation in the bottle giving natural carbonation. This may be a second or third fermentation. They are bottled with a viable yeast population in suspension. If there is no residual fermentable sugar left, sugar or wort or both may be added in a process known as priming. The resulting fermentation generates CO_2 that is trapped in the bottle, remaining in solution and providing natural carbonation. Bottle-conditioned beers may be either filled unfiltered direct from the fermentation or conditioning tank, or filtered and then reseeded with yeast.

Cask conditioning

Cask ale or cask-conditioned beer is unfiltered and unpasteurised beer that is conditioned (including secondary fermentation) and served from a cask, either pumped up from a cellar via a beer engine (hand pump), or from a tap by gravity. Sometimes a cask breather is used to keep the beer fresh by allowing carbon dioxide to replace oxygen as the beer is drawn off the cask. The term "real ale" as used by the Campaign for Real Ale (CAMRA) refers to beer "served without the use of extraneous carbon dioxide", which would disallow the use of a cask breather.

Filtering

Filtering the beer stabilizes the flavour, and gives beer its polished shine and brilliance. Not all beer is filtered. When tax determination is required by local laws, it is typically done at this stage in a calibrated tank. There are several forms of filters, they may be in the form of sheets or "candles", or they may be a fine powder such as diatomaceous earth, also called kieselguhr. The powder is added to the beer and recirculated past screens to form a filtration bed.

TABLE 20.1 Overview of the Brewing Processing Steps: From Barley to Beer.

Process	Action	Objectives	Time	Temperatur (°C)
Malting				
Steeping	Moistening and aeration of barley	Preparation for the germination process	48 h	12–22
Germination	Barley germination	Enzyme production, chemical structure modification	3–5 days	22
Kilning	Kilning of the green malt	Ending of germination and modification, production of flavoring and coloring substances	24–48 h	22–110
Milling	Grain crushing without disintegrating the husks	Enzyme release and increase of surface area	1–2 h	22
Mashing + wort separation	Addition of warm/hot water	Stimulation of enzyme action, extraction and dissolution of compounds, wort filtration, to obtain the desired fermentable extract as quickly as possible	1–2 h	30–72
Wort boiling	Boiling of wort and hops	Extraction and isomerization of hop components, hot break formation, wort sterilization, enzyme inactivation, formation of reducing, aromatic and coloring compounds, removal of undesired volatile aroma compounds, wort acidification, evaporation of water	0.5–1.5 h	>98
Wort clarification	Sedimentation or centrifugation	Removal of spent hops, clarification (whirlpool, centrifuge, settling tank)	<1 h	100–80
Wort cooling and aeration	Use of heat exchanger, injection of air bubbles	Preparing the wort for yeast growth	<1 h	12–18
Fermentation	Adding yeast, controlling the specific gravity, removal of yeast	Production of green beer, to obtain yeast for subsequent fermentations, carbon dioxide recovery	2–7 days	12–22 (ale) 4–15 (lager)
Maturation and conditioning	Beer storage in oxygen-free tank, beer cooling, adding processing aids	Beer maturation, adjustment of the taste, adjustment of CO_2 content, sedimentation of yeast and cold trub, beer stabilization	7–21 h	−1–0
Beer clarification	Centrifugation, filtration	Removal of yeast and cold trub	1–2 h	−1–0
Biological stabilization	Pasteurization of sterile filtration	Killing or removing of microorganisms	1–2 h	62–72 (past.) −1–0 (filtr.)
Packaging	Filling of bottles, cans, casks, and kegs; pasteurization of small volumes in packings	Production of packaged beer according to specifications	0.5–1.5 h	−1 to room temperature

Kieselguhr filters

Filters that use a powder medium are considerably more complicated to operate, but can filter much more beer before regeneration. Common media include diatomaceous earth and perlite.

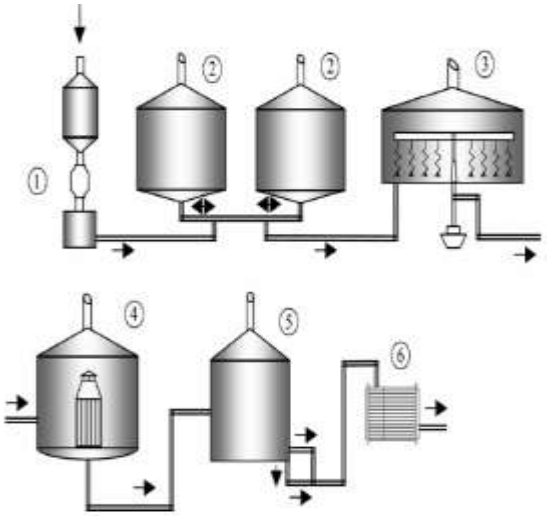


Figure 20.1 Overview of the brewing process.

Production of Antibiotics

Production of antibiotics is a naturally occurring event that thanks to advances in science can now be replicated and improved upon in laboratory settings.

Fermentation

Industrial microbiology can be used to produce antibiotics via the process of fermentation, where the source microorganism is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium. Oxygen concentration, temperature, pH and nutrient levels must be optimal, and are closely monitored and adjusted if necessary. As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die. Once the process is complete, the antibiotic must be extracted and purified to a crystalline product. This is easier to achieve if the antibiotic is soluble in organic solvent. Otherwise it must first be removed by ion exchange, adsorption or chemical precipitation.

Semi-synthetic

A common form of antibiotic production in modern times is semi-synthetic. Semi-synthetic production of antibiotics is a combination of natural fermentation and laboratory work to maximize the antibiotic. Maximization can occur through efficacy of the drug itself, amount of antibiotics produced, and potency of the antibiotic being produced. Depending on the drug being produced and the ultimate usage of said antibiotic determines what one is attempting to produce.

An example of semi-synthetic production involves the drug ampicillin. A beta lactam antibiotic just like penicillin, ampicillin was developed by adding an addition amino group (NH_2) to the R group of penicillin. This additional amino group gives ampicillin a broader spectrum of use than penicillin. Methicillin is another derivative of penicillin and was discovered in the late 1950s, the key difference between penicillin and methicillin being the addition of two methoxy groups to the phenyl group. These methoxy groups allow methicillin to be used against penicillinase producing bacteria that would otherwise be resistant to penicillin.

Synthetic

Not all antibiotics are produced by bacteria; some are made completely synthetically in the lab. These include the quinolone class, of which nalidixic acid is often credited as the first to be discovered. Like other antibiotics before it the discovery of nalidixic acid has been chalked up to an accident, discovered when George Lesher was attempting to synthesize chloroquine. However a recent investigation into the origin of quinolones have discovered that a description for quinolones happened in 1949 and that patents were filed concerning quinolones some 5 years before Lesher's discovery.

Strains used for the production

In the earliest years of antibiotic discovery the antibiotics being discovered were naturally produced antibiotics and were either produced by fungi, such as the antibiotic penicillin, or by soil bacteria, which can produce antibiotics including streptomycin and tetracycline.

Microorganisms used in fermentation are rarely identical to the wild type. This is because species are often genetically modified to yield the maximum amounts of antibiotics. Mutation is often used, and is encouraged by introducing mutagens such as ultraviolet radiation, x-rays or certain chemicals. Selection and further reproduction of the higher yielding strains over many generations can raise yields by 20-fold or more. Another technique used to increase yields is gene amplification, where copies of genes coding for enzymes involved in the antibiotic production can be inserted back into a cell, via vectors such as plasmids. This process must be closely linked with retesting of antibiotic production.

Some antibiotics are produced naturally by fungi. These include the cephalosporin producing *Acremonium chrysogenum.* Geldanamycin is produced by Streptomyces hygroscopicus. Erythromycin is produced by what was called Streptomyces erythreus and is now known as *Saccharopolyspora erythraea.* Streptomycin is produced by *Streptomyces griseus*. Tetracycline is produced by *Streptomyces aureofaciens.* Vancomycin is produced by Streptomyces orientalis, now known as *Amycolatopsis orientalis.*

Production of vitamin B12

Vitamin B_{12}, also called **cobalamin**, is a water-soluble vitamin that has a key role in the normal functioning of the brain and nervous system via the synthesis of myelin (myelinogenesis), and the formation of red blood cells. It is one of eight B vitamins. It is involved in the metabolism of every cell of the human body, especially affecting DNA synthesis, fatty acid and amino acid metabolism. No fungi, plants, or animals (including humans) are capable of producing vitamin B_{12}. Only bacteria and archaea have the enzymes needed for its synthesis. Some substantial sources of B_{12} include animal products (shellfish, meat), fortified food products, and dietary supplements. B_{12} is the largest and most structurally complicated vitamin and can be produced industrially through bacterial fermentation synthesis, typically used to manufacture B_{12} for fortified foods and supplements. It can also be produced synthetically via vitamin B_{12} total synthesis.Neither plants nor are animals independently capable of constructing vitamin B_{12}. Only bacteria and archaea have the enzymes required for its biosynthesis. Like all tetrapyrroles, it is derived from uroporphyrinogen III. This porphyrinogen is methylated at two pyrrole rings to give dihydrosirohydrochlorin, which is oxidized to sirohydrochlorin, which undergoes further reactions, notably a ring contraction, to give the corrin ring.

The complete laboratory synthesis of B_{12} was achieved by Robert Burns Woodward and Albert Eschenmoser in 1972, and remains one of the classic feats of organic synthesis, requiring the effort of 91 postdoctoral fellows (mostly at Harvard) and 12 PhD students (at ETH) from 19 nations.

Species from the following genera are known to synthesize B_{12}: *Acetobacterium*, *Aerobacter*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Flavobacterium*, *Lactobacillus*, *Micromonospora*, *Mycobacterium*, *Nocardia*, *Propionibacterium*, *Protaminobacter*, *Proteus*, *Pseudomonas*, *Rhizobium*, *Salmonella*, *Serratia*, *Streptomyces*, *Streptococcus* and *Xanthomonas*.

Industrial production of B_{12} is achieved through fermentation of selected microorganisms.[84] *Streptomyces griseus*, a bacterium once thought to be a yeast, was the commercial source of vitamin B_{12} for many years. The species *Pseudomonas denitrificans* and *Propionibacterium freudenreichii* sub sp. *shermanii* are more commonly used today. These are frequently grown under special conditions to enhance yield, and at least one company, Rhone-Poulenc of France, which has merged into Sanofi-Aventis, used genetically engineered versions of one or both of these species. Since a number of species of Propionibacterium produce no exotoxins or endotoxins and are generally recognized as safe (have been granted GRAS status) by the Food and Drug Administration of the United States, they are presently the FDA-preferred bacterial fermentation organisms for vitamin B_{12} production.

The total world production of vitamin B_{12}, by four companies (the French Sanofi-Aventis and three Chinese companies) is said to have been 35 tonnes in 2008.

Production of vitamin Riboflavin

Riboflavin, also known as **vitamin B_2**, is a vitamin found in food and used as a dietary supplement. As a supplement it is used to prevent and treat riboflavin deficiency and prevent migraines. It may be given by mouth or injection.

Various biotechnological processes have been developed for industrial scale riboflavin biosynthesis using different microorganisms, including filamentous fungisuch as *Ashbya gossypii*, *Candida*

famata and *Candida flaveri*, as well as the bacteria*Corynebacterium ammoniagenes* and *Bacillus subtilis*. The latter organism has been genetically modified to both increase the bacteria's production of riboflavin and to introduce an antibiotic (ampicillin) resistance marker, and is now successfully employed at a commercial scale to produce riboflavin for feed and food fortification purposes. The chemical company BASF has installed a plant in South Korea, which is specialized on riboflavin production using *Ashbya gossypii*. The concentrations of riboflavin in their modified strain are so high, that the mycelium has a reddish/brownish color and accumulates riboflavin crystals in the vacuoles, which will eventually burst the mycelium. Riboflavin is sometimes overproduced, possibly as a protective mechanism, by certain bacteria in the presence of high concentrations of hydrocarbons or aromatic compounds. One such organism is *Micrococcus luteus*(American Type Culture Collection strain number ATCC 49442), which develops a yellow color due to production of riboflavin while growing on pyridine, but not when grown on other substrates, such as succinic acid.

UNIT I

Cross-contamination of food occurs when

Which of the following are allergens?

The undesirable change in a food that makes it or human consumption is re

_____ microorganisms reqire positive Eh values or positive mV O-R pote

_____ acid produced by the propionibacteria in swiss cheese is inhibitory

Most spoilage bacteria grow at

The microbiological examination of coliform bacteria in foods preferably u

Which of the following can cause food to be contaminated because of chem

_____ is the causative organism for a bacterial pneumonia in human.

The use of indicator microorganisms began with use of *E. coli* testing in _

To retard the contamination and other microbial growth in meat is obtained

The percentage of relative humidity is obtained by multiplying by_____.

Many infectious disease agents of animals can be transmitted to people thro

_____ used to fertilize plant crops will be contaminated with human patho

The o-R potential of a system is measured by

Which of the following can cause food to be contaminated because of phys

_____ bacteria oxidize ethylalcohol to acetic acid

The endospores of _____ do not swell the rods in which they are formed

_____ is associated with the market disease called bacterial soft rot

_____ bacteria are those which grow in high concentration of sugars

_____ bacteria grow and cause discoloration on foods high in salt

The different ACC's between food categories reflect the

Yeast and mould count determination requires

The water requirement of a microorganism is expressed in terms of _____

A psychrophilic halophile would be a microbe that prefers

Many microorganisms cannot use the disaccharide lactose and therefore do not grow well in _____

_____ yeast is grown with dairy starter cultures to maintain the activity and

Which of the following acid will have higher bacteriostatic effect at a given

Water activity can act as

The culture of Brevibacterium produces _____ pigmentation and helps ripening

Pectins are complex _____ that are responsible for cell wall rigidity i

_____ are short rods that are defined as aerobic and facultative anaerobic

The _____ of many meat animals may contain micrococci, Staphylococci, a
The microorganism which apparently have no mechanism to tolerate acidic
_____ is the thermoduric bacteria

Aeromonas grows at an optimum temperature of _____
The spoilage of meat by microorganism is by_____ process.

_____ bacteria is found aseptically in drawn milk and cause bovine mastitis
Sugars act as preservatives due to their ability to .
Preservation affect the growth of microorganism by .
NaCl can act as
Which of the bacteria can grow in alkaline pH?
_____ bacteria are able to grow at commercial refrigeration temperatures
_____ bacteria produce lipase enzyme that hydrolysis fat to fatty acids and
_____ does not contain a antural flora of microorganisms
Saccharomyces are reclassified by Lodder in the year ____
The water activity range of fresh meat and fresh fish was _____
The o-R potential of a system is measured by
_____ has been used as starter culture in fermented sausages
Truly halophilic bacteria require minimal concentration of dissolved _____
There are ___ aspects of water bacteriology that are interested by food micr
Contamination of foods from _____ may be important for sanitary as we
When microbes can use fat as an energy source _____.
The approximate range of bacteria present in fresh vegetable is _____
Cannery cooling water often contain _____
_____ causes ropiness in milk
_____ contain the greatest variety of microorganisms f any source of co
Pig and beef carcasses may be contaminated with _____
In fruit juices the growth of the fermentative yeast are favored by _____
The water requirement of a microorganism is expressed in terms of _____
Souring of canned meat is caused by .

UNIT II

The concentration of salt used in high protein containing vegetables is ____
_____ is a term used to label foods treated with low level ionizing radiation
Flavoring etracts such as vanilla and lemon etracts are preserved by their co
Which of the following statements are true about chemical preservatives __
The time temperature combination for HTST paterurization of 71.1°C for 1
_____ contains a large number of olatile compounds that may have bacteric
---------- is a storage method uses bins or boxes for equalization of mois
_____ is used most extensively in the prevention of mold growth and rope
_____ can be dried by a process called explosive puffing
_____ in 1765 preserved food by heating it in a sealed containers
Combination of _____ irradiation with chilling storage helps preserve foo

Which solvent is commonly used to determine fat content
During _____ the internal temperature of bread, cake or other bakery produ
Pasteurization is done to kill
Sanitising is_____
The simplest dryer is the _____
Bacteria which is present in raw or undercooked meat, eggs, sea food and u
Milk and curry left over can be turned into sour and spoiled at
_____ rays are streams of electrons emitted from radioactive materials
Increase in the concentration of dissolved substances like sugar and salt hel
Sulfur stinker spoilage of canned food is caused by
Radiation dose in kilograys of ____ inhibits sprouting in potatoes, onions a
Preservation affects the growth of microorganism by
Souring of canned meat is caused by _____.
Significant numbers of S. aureus in a food can be determined by examining
To retard the contamination and other microbial growth in meat is obtained
Gazing at ultraviolet lamps produces irritation of the ____ within few seco
Sugars act as preservatives due to their ability to
The minimal pH for the growth of staphylococcus is about ----------
_____ alcohol is used as coagulant and enaturizer of cell proteins
The fumes of burning ____ are used to treat light colored dehydrated fruits
_____ can be used to control bacterial and fungal growth in tapholes of m
Christophersen classified microroganisms on the basis of sensitivity to free
The percentage fat constituent of double toned milk is
---------- is mostly used preservative to prevent mold growth
_____ solvent is poisonous and should not be added to foods
_____ drying is limited to climates with a hot sun and dry atmosphere to fr
Food should be cooked to which temperature?
The sclerotia from a species of Penicillium can survive a heat treatment of
The sodium salt of _____ acid has been used extensively as an antimicrob
Fruit juice is sterilized by _____.
Pasteurization is a _____
The reddish liquid comes out from meat on thawing process is called as
The spoilage organism bring about the spoilage of meat by
The minimum growth temperature of Bifidobacteria range from
_____ acid is used in soft drinks such as colas
_____ freezing usually refer to freezing in air with only natural air circula
Jones and Loackhead found enterotoxin forming Staphylococci in _____fo
_____ from retail market contain from 0 to2 million bacteria per piece
---------- is a storage method uses bins or boxes for equalization of mois
To retard the contamination and other microbial growth in meat is obtained
_____organic acid is used in syrups, drinks, jam and jellies
Food preservation involves_____
97 to 99 % of *E.coli* in air were killed in ____ seconds with a 15 watts lan
_____ is used as treatment for wrappers use don butter

_____ temperature are more lethal
About ____ percent of the suspected samples contained viable spores
Sugars act as preservatives due to their ability to
_____organic acid is used in syrups, drinks, jam and jellies
Sanitising is_____
_____ is used most extensively in the prevention of mold growth and rope

UNIT III

Which of the following toxin causing botulism is less toxic to human being
Which of the following statements are true regarding *Staphylococcus* food
Aflatoxin is produced by _____
Which of the following statements are regarding botulinal toxin _____
The sore and throat symptom caused by _____ etiologic agent
Botulism is caused by the presence of toxin developed by
The control measure of foods that cause disease by *Vibrio parahaemolyticu*
Salmonellois involves _____
The term heat tolerant is a misnomer and refers to growth at ___ temperatu
The mold Penicillium islandicum produces _____ toxin
The major carrier of Salmonellosis are _____
Yersinia enterocolitica is a small _____ shaped bacteria
The staphylococcal intoxication refers to presence of
The FDA and USDA cooperative is a _____ surveillance program for dry n
The application of Gamma rays destroys botulism toxin. The dose of gamm
The Bacillus cereus causes gasteroenteritis by the production of an exoente
Nursery epidemics diarrheal disease in infants was implicated in the year _
Botulism is caused by _____
The toxin patulin is produced by _____fungi
Miller and Kolurger examined forty environmental isolates of P. shigelloid
Which of the following is a food infection?
The symptoms such as nausea and dehydration is caused by _____
Staphylococcal intoxication is caused by the toxin in the food from
The etiologic agent of diarrheal syndrome is ____
_____ involves the identification of ingredients and products that have effe
The term ___ is used to distinguish strains of different antigenetic complen
A bacterial food intoxication refers to
Salmonellois is caused by the
Group I *C. botulinum* strains generally includes in
A_____ refers to food borne illnesses caused by the entrance of bacteria int
_____ organism can be isolated from seafoods and sea water
Botulism prevention involves_____
Entheropathogenic Escherischia coli infection is involved in ____ foods
The etiological agent of Arizona infection is ____
Aeroonas hydrophillia is a gram negative motile rods which are ubiquitous
The term ___ is used to distinguish strains of different antigenetic complen
The method of successful treatment of botulism prior to appearance of botu

_____ organism can be isolated from seafoods and sea water
The optimal temperature for growth of Shigellosis is _____
The FDA and USDA cooperative is a _____ surveillance program for dry n
_____ is associated with warm blooded animals
Human beings and animals are directly or indirectly the source of the conta
The food and Drug Administration act was amende in the year ____
The _____ virus enters a person through oral route in the fecal contaminati
The mode of transmission of poliomyelitis is _____
Clostridium perfingens poisoning is associated with_____
Clostridium perfingens poison is an _____
The pH near ____ favors *C. botulinum*
In the early _____ numerous surveys have been conducted on the detection
The optimal pH for enteropathogenic E. coli is _____
The disease gastroenterities caused by C. perfringens was first reported in t
Depending on the food and the serotype the _____ values from 0.06 to 11.3
Pathogenecity involves the release of a _____ endotoxin which affects the
Common food poisoning microbes are_____
Typhoid fever is caused by _____
The incubation period of Vibrio parahaemolyticus infection is _____
The incubation period of Streptococcus faecalis is _____
The growth of Staphylococcus aureus on solid media is usually ____ in col
A_____ refers to food borne illnesses caused by the entrance of bacteria int
What is the main type of micro-organism responsible for food poisoning?
_____ agencies aprove the Good house keeping institute

UNIT IV

Who is the father of fermentation technology?
Who described a lens?
The size of paper strip used in Aerocanography was _____.
Enrichment technique was designed by _____.
The medium which doesnot contain particular essential nutrient is called as _____
Indicator dye are used in the medium
Crowded plate technique is used in the detection _____ producing organisn
Growth factor producing organisms are identified by using
Chemical stability of the product is identified by _____.
In serial subculturing method bacteriophages and anaerobic organisms are store a
In oil overlaying method paraffin oil is used in specific gravity of _____.
Oil overlaying method is first used by _____.
Bacterial species are preserved by oil overlaying method for _____.
Freeze drying is called as _____.
Freezing mixture used in lyophilization is _____.
Nitrogen storage is also called as _____.
Fungal species are stored by _____.
Lyophilization is first used by _____.
Among the following which organism was not affected by drying of medium.

Organism which does not produce particular product which is require for growth
Control mechanism for the biosynthesis of products are called _____.
End product bind with the enzyme to stop the main reaction is called as _____.
End product which stop the synthesis of key enzyme called _____.
Each end product controls the pathway in certain percentage is called as _____
Enzyme which catalyze same reaction but differ in their control characters are cal
Organism which synthesis all growth factors for their growth is called as _____
All the natural isolates of organisms are _____.
In industries mostly used mutation agent is _____.
Primary metabolites are produced during _____.
Among the following which is biotin requiring organism?
Which is used as a inhibitory agent in enrichment method?
Mutant spores are selected by _____.
Sandwich method is used to isolate _____.
At the optimum level of biotin C. glutamicum produces _____.
A compound which is similar structure to other compound is called _____.
Growth below the inhibitory level of analogue is called as _____.
Membrane permeability is altered by changing the concentration of _____.
Protoplast fusion is mainly used to improve industrial microbes
Cell without cell wall is called as _____.
Among the following which solution is used for the preparation of protoplast?
Different microbial species are fused by _____.
The inoculum level introduced into a production tank is usually _____.
Inoculum media are balanced for _____.
Mutants can be obtained by treating the cells with _____.
Primary metabolites are produced during _____.
Secondary metabolites are produced during _____.
_____ are normally operated at high substrate conversion.
_____ vessel is used in production of lactic acid and ole.
Extrachromosomal elements which carry information for synthesis of products is
The chemical agent which causes mutation is _____.
_____culture where a portion of the culture is harvested at regular intervals and r
_____ culture where medium is fed to the culture resulting in an increases in v
_____ is a culture system which contain a intial limited amount of nutrien
_____-culture is a open system where fresh medium is continuously added
_____ is the organism used in the first truly large scale aseptic fermentation
_____ is to provide microorganisms in submerged culture with sufficient oxyger
_____ ensures uniform suspension on microbial cells.
_____device is used to introduce air in fermenter
Aeration and agitation of a liquid medium may lead to the formation of
Industrial alcohol production can be carried out in very large fermentor upto ___
CSTF is expanded as

UNIT V

Wine is fermented by _____.
Asepsis know as ----------
-------is widely used application of asepsis technique

In wine fermentation grapes are crushed and pressed to release the juice, caled
During the production of wine malo-lactic fermentation is used to reduce
In wine making malic acid is degraded by
Wines which have undergone undesirable changes in flavor due to lactic acid bac
Wine defects caused by after fermentations are due to the fermentation of
When wines of low alcohol concentration are exposed to air yeasts develop on th
Chemically beer is sterilized by adding
Bottled beer is sterilized by
Top fermented beers are generally called
In beer making yeast is inoculated into clarified
During beer making, the conversion of the barley starch to sugar is called
After beer has been malted, the germinated grain is added to warm water to give a
Industrial alcohol is produced by fermentation using the micro organism
Wine can be produced by the fermentation of
To prevent the loss of delicate flavors of some wines, the preferred method of r
In wine making secondary fermentations are carried out by
The color and flavor of red wine come from _____.
Beer spoilage is generally caused by which of the following bacteria?
In the beer production, hops is responsible for the _____.
In the beer production, hops is added as _____.
In the ageing process of beer beach wood chips are added to the tanks to provide
The oldest fermented alcoholic beverage is _____.
Which of the following is referred to as bottom yeast in beer making?
Malt adjuncts are additional provided in the medium for brewing _____.
Mass cultivation of algae for SCP is usually carried out under photosynthetic con
Hydrocarbon of interest in the bacterial SCP production is
Riboflavin fermentation employs media containing _____.
Species of Candida involved in riboflavin fermentation are extremely sensitive to
Fermentation medium used in riboflavin production employing candida need nc
Semi- synthetic penicillin are prepared from _____.
Strains of Aspergillus orzyae are used for the production of amylase by
Baker's yeast is produced from the strains of ‘
Daily requirement of vitamin B12 from human beings is _____.
Cyanocobalamine consisting of a molecule of _____ linked to a nucleotide.
Vitamin B12 is manufactured by _____ culture process.
Vitamin B12 production using Streptomyces olivaceus NRRLB _____ strain
In Bennet's agar the composition of N-2- Amine A is _____ g/lit
Cobalt increases the growth of _____.
Temperature of _____ in the production tank is satisfactory during fermentat
Stabilization of mask is practiced by reducing the pH and adding reducing agents
Sterilization of air is done by passing it through columns filled with _____.
_____ are the antifoam agents used to suppress the foam formation.
The yield of cobalamine are usually in the range of _____mg/ litre.
Adsorbing agent used in packed clumns are _____.
Betaine is added to medium to furnish _____ groups.
Size of fermentors used in riboflavin production ranges from _____ gal.

Precipitation of riboflavin by addition of _____.
High- yielding strain in the manufacture of an antibiotic is achieved by _____
_____ strains is used for the production of Penicillin.
_____ act as carbon source.
Removal of mycelium is done by _____.
A major ingradient of penicillin production media is
The outstanding example of traditional microbial fermentation product is
Industrially important Antibiotic producing organisms shall be isolated by
Industrial alchohol will be produced by using starter culture
In the industrial production of streptomycin, the secondary metabolite or byprodu
Penicilin is commercially produced by
_____ fermenter is called as elongated non-mechanicaliy stirred fermenter
_____ fermentor is a gas tight baffled rise tube connected to a down comer tub
_____ chromatography separates according to the affinity of the protein, for
_____ chromatography is a powerful and highiy selective purification techr
Microbial cells and other insoluable materials are normally separated from the ha
Salts such as ammonium and ----------are used for the discover of protein
Dextrans can be precipated out of a broth by the addition of _____.
_____ method is used for large scale enzyme purification
_____ is used to carry out microbiological process on batch basis.
_____ is an established and final purification of a diverse range of compour
A combined sparger and agitator may be used in ---------fermenter
Small lab fermentor are in the size range of
Larger fermentor range from _____ gallous.
the temperature of _____ in the production tanks is satisfactory during fermen

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF MICROBIOLOGY
FOOD AND INDUSTRIAL MICROBIOLOGY

OPTION 1	**OPTION 2**
Cleaning and sanitising equipment a	Keeping food stored in food-grade co
Sources of gluten and Red meat	Fruits and vegetables
food decay	food spoilage
Aerobic	anaerobic
sorbic	acetic
acidic pH	alkaline pH
MacConkey broth	violet Red Bile agare
Hair	Dust
Flavobacterium	*Escherichia*
soil	plants
10°C	0°C
aw*10	aw*1000
water	food
sewage	distilled water
mV	mM
Jewellery	Dust
Aeromonas	Acetobacter
Streptococcus	*Brochotrix*
Erwinia	*Enterobacter*
Halophilic	thermophilic
Halobacterium	Enterobacter
expected level of contamination of th	potential for microbial growth during
nutrient agar	acidified potato glucose agar
Water action	Water adsorption
cold temperatures and increased amo	warm temperatures and increased am
milk	water
Candida sp.	*Trichosporon*
Acetic acid	Tartaric acid
an intrinsic factor determining the lik	a processing factor
orange-red	yellow
Proteins	lipids
Enterobacter	*Coliforms*

Hair	nail
bacteria	fungi
Acenetobacteria	*Morexella*
27 to 37 °C	22 to 28 °C
Oxidation	Reduction
Corynebacterium	*Clostridium*
Make water unavailable to organisms	Interfere with the action of proteolyti
Inhibition	Retardation
antagonist at optimal concentrations	synergistically if added in excess of o
Lactobacilli	*Vibrio cholera*
Pschrotropic	halophilics
Saccharolytic	Pectinolytic
soil	air
1985	1978
0.93-0.98	0.98 and above
mV	mM
Photobacterium	*Pediococcus*
NaCl2	Hcl
2	5
air	soil
absence of sugar molecule	presence of glucose
10^9 -10^7/g	10^3 -10^9/g
Coliforms	*Aeromonas*
Lactobacillus plantarum	Klebsiella pneumonia
plants	sewage
Salmonellae	*Klebsiella*
4.0-4.5	6.0-6.5
water action	water adsorption
Thermoduric cells	Thermostatic cells
4.3-10.3	17.5-20.0
Radicidation	radurization
sugar	salt
microbicidal or microstatic	chemical preservatives often hazardo
Coxiella Burnetii	*E. coli*
spices	woodsmoke
sweating	springer
calcium propionate	calcium sorbate
meat	vegetables
Spallanzani	Ruiz-Argueso
Ultraviolet	infra red

Ethyl alcohol	Hexane
Heating	boiling
Selective microorganism	All the microorganism
Applying detergent to a clean surface	Done before washing
sun	air
E.coli	*Salmonella*
high temperature	very low temperature
beta	cathode
drying	freezing
E.coli	*D. nigrificans*
0.05-0.15	0.01-0.14
inhibition	retardation
thermoduric cells	thermostatic cells
RNase	thermostable nuclease
10°C	0°C
eye	ear
make water unavailable to organism'	interfere with the action of proteolyti
2.5	4.8
methanol	ethanol
sulfur	ethylene
paraformaldehyde	benzaldehyde
1984	1989
0.5	1.5
sodium propionate	springer
propylene	ethanol
mechanical	solar
5°C	75°C
70 °C	90 to100 °C
propionic	benzoic
filteration	freezing
low temperature treatment	steaming treatment
drying	wilting
purification	oxidation
43 to 45	25 to 28
phosphoric	benzoic
Sharp	slow
frozen corn	cheese
caramels	jellies
Sweating	Springer
10 °C	0°C
lactic	acetic
increasing shelf life of food	ensuring safety for human consumpti
40	10
sodium diacetate	calcium carbonate

high freezing	frozen storage
20	10
make water unavailable to organism'	interfere with the action of proteolyti
lactic	acetic
Applying detergent to a clean surface	Done before washing
calcium propionate	calcium sorbate

Type A	Type B
is an enterotoxin	causes gastroenteritis
Aspergillus sp.	*Salmonella sp.*
is a neurotoxin	water soluble exotoxin
Streptococcus pyogenes	*Staphylococcus aureus*
Clostridium tyrobutyricum	*Clostridium sporogenes*
reheat left over	sanitize equipment
an enterotoxin and exotoxin	an enterotoxin and cytotoxin
37 °C	40 °C
Luteoskyrin	aflatoxin
meat and eggs	meat and fish
cocci	chain
an enterotoxin	neurotoxin
Pseudomonas	*E. coli*
73 Gy	73 Rad
cell growth	cell autolysis
1950	1940
Clostridium botulism	*All Clostridium species*
Penicillium expansum	*Fusarium*
1987	1982
Salmonellois	Botulism
Shigella sonnei	*Yersinia*
Staphylococcus aureus	*S. cerevisiae*
Shigellosis	*Yersiniosis*
Hazard analysis	critical control points
biovars	serovar
illness caused by presence of pathoge	food borne illness caused by the pres
enterotoxin of Salmonella spp	endotoxin of Salmonella spp
all types of strains (proteolytic)A, B a	all types of strains (non-proteolytic) I
Food infection	food poisoning
Vibrio cholerae	*Vibrio vulnificus*
Proper heat sterilization before food	addition of chemical preservatives
vegetables	apple cider
Vibrio	*E. coli*
air	soil
biovars	serovar
antibiotic	analgesic

Vibrio cholerae	*Vibrio vulnificus*
27 °C	37 °C
Pseudomonas	*E. coli*
C. jejuni	*C. botulinum*
Salmonella	*Staphylococcus*
1983	1980
Poliomyelitis	Hepatitis
food	air
meat products	vegetables
exotoxin	enterotoxin produced during sporulat
neutrality	alkalinity
1980s	1940s
4.0 to 5.0	7.0 to 7.5
1952	1961
D50 C	D40 c
lipopolysaccharides	monosaccharides
Clostridium and Salmonella	*Clostridium and E. coli*
Salmonell enteritidis	*Salmonella infantis*
2-48 hrs	5-24 hrs
5 to 10	2 to 10
red	brown
Food infection	food poisoning
Bacteria	Mould
Commercial	State

Louis Pasteur	Alexander Fleming
Salvino D Armati	Roger Bacon
1.5 *12 cm	2.5*12cm
Beijernick	Pasteur
Enriched media	Minimal media
To detect temp change	To detect nutrient concentration
Growth factor producers	Aminoacid producers
Growded plate technique	Enrichment technique
Primary screening	Secondary screening
Solid medium	Minimal medium
0.743 -0.780	0.801-0.825
Brell	Bacon
10-15 years	15-20 years
Lyophilization	Liquefaction
Ice and methanol	Ice and alcohol
Freezing	Cooling
Freezing	Soil cultures
N.Appert	Davis
Streptomyces	Bacillus

Auxotrophs	Heterotrophs
Feed back control	Primer control
Feed back repression	Feed back inhibition
Feed back repression.	Feed back control
Co-operative control	Sequential control
Iso enzyme	Holoenzyme
Auxotrophs	Phototrophs
Auxotrophs	Phototrophs
UV-rays	EMS
Lag phase	Stationary phase
Cornybacterium	*E.coli*
Pencillin	Nacl
Enrichment method	Mechanical separation method
Mutant spores	Auxotrophs
Lysine	Purine
Isologue	Analogue
Confluent growth	Synchronous growth
Fat	Carbohydrate
Algae	Fungi
Protoplast	Spleroplast
Osmotic	Hypertonic
r-DNA technology	Protoplast fusion
0.5-5%	5 – 25%
Rapid cell growth	Product formation
UV radiation	Nitrogen mustard
Lag phase	Stationary phase
Lag phase	Stationary phase
CSTF	Air lift fermentor
Wood vessel	Stainless steel
Protoplast	Chloroplast
Sodium sulfate	Ethyl methyl sulfanate
Fed batch	Batch
Batch	Fed batch
Fed batch	Batch
Batch	Continuous
Clostridium acetobutylicum	*C. perfringens*
Aeration	Agitation
Aeration	Agitation
Sparger	Impeller
Acid	Alkali
12500	125
Continous stirred tank fermentor	Continuous solid tank fermentor
Bacteria	Yeast
Keeping out from microbes	Filteration
Centrifugation	Heating.

Must	Grape juice
Alkalinity	Acidity
Sulfur bacteria	Acetic acid bacteria
Propionic acid	Acetic acid
Citric acid	Vinegar
Budding	Sugar
Benzoic acid	n-heptyl p-hydroxy benzoate
Pasteurization	Decolorization
Ales	Alcohol
Juice	Ales
Crushing	Malting
Wort	Malt
Bacillus	Saccharomyces cerevisiae
Berries	Honey
Filtration	Addition of so2
Saccharomyces	Lactic acid bacteria
The grapes skin and seed	Artificial additives
Lactic acid bacteria	Aceticacid bacteria
Aromatic and pungent character	Preservative action
An adjunct	A carbonating agent
Surface of the yeast	Desired flavor
Beer	Whisky
Saccharomyces cerevisiae	Saccharomyces bayanus
. Protein	Starch
Fed batch	Bio-processing
Methanol	Ethanol
Sugar and lipid	Protein and lipid
Manganese	Iron
At low nutrient concentration	At low humidity
5-A PA	4 – APA
Mobile	Log
Aspergillus niger	Pseudomonas
0.05mg/day	0.001mg/day
Cobalt	Cyanide
Batch	Continuous

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4

1127
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Streptomyes olivaceus	S. griseus
75° F	80° F
Sodium citrate	Sodium sulphite
Activated charcoal	Dry charcoal
Soyabean oil	Cord-linee oil
2 – 3 mg	1 – 2 mg
TEMED	Methylene blue
Methyl	Ethyl
1000 – 10000 gals	10000 – 100000 gals

Dithionite	Trithionite
Backward mutation	Secondary screening
penicillium chrysogenum	P. notatum
Lactose	Maltose
Radiation	Filtration
Corn meal	Corn steep liquor
Vinegar	Penicillin
Disk plate method	Direct plate method
Top yeast	Middle yeast
Vitamin – B12	Vitamin – C
P.notatum	P.chrysogenum
Tower	Airlift
Tower	Airlift
Adsorption	Affinity
Adsorption	Affinity
Filteration	Centrifugation
NH3SO4	Na2SO4
Methanol	Ethanol
Liquidshear	Solidshear
Batch fermentor	Continuous fermentor
Drying	Crystallization
Laboratary	Tower
1-21+	0.5-11+
5000-10000	100-1000
80°F	70°F

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OPTION 3	OPTION 4	ANSWER
Washing hands before handling food	Using food handling gloves for handling money	Using food
Fish and fish products	None of the above	Sources of
food loss	all of the above	food spoila
facultative	none of these	Aerobic
propionic	acetic	propionic
neutral pH	any of the pH	neutral pH
eosine Methylene blue agar	all of these	all of these
Live insects	Perfume	Perfume
Klebsiella	*Gluconobacter*	*Klebsiella*
water	all of these	water
100°C	-10°C	0°C
aw*100	aw*0.1	aw*100
soil	juices	food
mineralized water	none of these	sewage
aw.	Eh	Eh
Rodent droppings	Incorrectly diluted chemicals	Jewellery
Alcaligens	Alteromonas	Acetobacte
Brevibacterium	*Bacillus*	*Bacillus*
Corynebacterium	*Klebsiella*	*Erwinia*
osmophilic	none of these	osmophilic
Erwinia	Corynebacterium	Halobacterium
potential shelf life	all of the above	all of the a
MacConkey agar	violet Red Bile agar	acidified p
Water affinity	Water activity	Water acti
cold temperatures and the absence of o	warm temperatures and increased amounts of acid	cold tempe
food	sewage	milk
Rhodotorula	*Torulopsis*	*Candida sp*
Citric acid	Maleic acid	Acetic acid
an extrinsic factor	all of the above	all of the a
black	red	orange - red
carbohydrates	vitamins	Carbohydr
Proteus	*Clostridium*	*Coliforms*

skin	all of these	skin
viruses	viruses	fungi
Bacillus	*Flavobacterium*	*Morexella*
35 to 37 °C	40 °C	22 to 28 °C
Decomposition	Precipitation	Oxidation
Campylobacter	*Enterobacter*	*Coryneb acterium*
Osmotic effect	Both a and c	Interfere w
Arresting	All of the above	Retardatio
Both (a) and (b)	None of the above	Both (a) a
Salmonella	*Staphylococcus*	*Vibrio cho*
auttrophic	heterotrophic	Pschrotrop
lipolytic	proteolytic	lipolytic
water	sewage	air
1982	1984	1984
0.60-0.76	below 0.98	0.93-0.98
aw	Eh	mM
Propionibacterium	*Proteus*	*Pediococc*
NaNo2	Cacl2	Nacl2
6	4	2
water	sewage	air
presence of fructose	Presence of high sugar	absence of
10^3 -10^7/g	10^1 -10^7/g	10^3 -10^4/g
Klebsiella	*Clostridium*	Coliforms
Klebsiella oxytoca	Flavobacterium	Klebsiella
water	soil	soil
E. coli	*Enterobacter*	*Salmonell*
2.0-2.5	3.0-3.5	4.0-4.5
water affinity	water activity	water activ
Thermo liable cells	None of the above	Thermodu
18.6-26.5	19.2-22.2	18.6-26.5
picowaved	radappertization	Picowaved
alcohol	ethylene	alcohol
sodium benzoate is a widely used pres	all these	All of thes
B. subtilis	*C. botulinum*	*Coxiella B*
formaldehyde	alcohol	woodsmok
cooling	freezing	springer
monocholroacetic acid	nitrates	calcium pr
fruits	juices	vegetables
Rodrigeuz-Navarro	Christophersen	spallanzan
gamma	none of the above	ultraviolet

Acetone	Benzene	Hexane
baking	all of these	baking
Yeast	Yeast and its spores	Selective r
Reducing bacteria by application of he	Wiping all surfaces with a clean cloth	Reducing l
heat	evaporator	evaporator
Staphylococcus	cyano bacteria	*salmonell*
room temperature	constant temperature	room temp
gamma	X-rays	beta
moistening	thawing	drying
Bacillus	*Clostridium*	*D. nigrific*
0.05-0.07	0.05-0.11	0.05-0.15
arresting	all the above	retardation
thermo liable cells	none of the above	thermodur
protease	thermostable DNase	protease
100°C	-10°C	0°C
nose	throat	eye
osmotic effect	both a and c	interfere w
2	3.5	4.8
butanol	none of these	ethanol
potassium	sodium	sulfur
formaldehyde	all of these	paraforma
1973	1981	1973
3	4.5	1.5
sorbates	acetate	sodium pr
methanol	glycerol	methanol
freeze	all of these	solar
100°C	60°C	75°C
50-60 °C	37 °C	90 to100 °
sorbic	acetic	benzoic
cooling	heating	filteration
high temperature treatment	low and high temperature treatment	high tempe
bleeding	leakage	bleeding
decomposition	hydrolysis	decomposi
29 to 32	30 to 35	43 to 45
acetic	sorbic	phosphori
quick	all of these	sharp
bread	jam	frozen cor
fudges	candies	candies
Cooling	Freezing	Springer
100°C	-10°C	0°C
propionic	citric	citric
both a and b	none of these	both a and
50	30	10
sodium nitrate	potassium nitrite	sodium dia

freezing rate	thawing	high freezi
30	50	10
osmotic effect	both a and c	interfere w
propionic	citric	citric
Reducing bacteria by application of he	Wiping all surfaces with a clean cloth	Reducing l
monocholroacetic acid	nitrates	calcium pr
Type C	None of these	Type B
is produced by Staphylococcus aureus	All of these	All of thes
Fusarium sp.	*Streptococcal sp.*	*Aspergillu*
is produced by Clostridium botulinum	All of these	is produce
Bacillus anthrax	*E.coli*	*Streptococ*
Clostridium botulinum	*none of these*	*Clostridiu*
control files	pastuerization	sanitize eq
is produced by Staphylococcus aureus	All of these	an enteroto
42 °C	25 °C	42 °C
penicillic acid	roquefortine	Luteoskyri
eggs and fish	eggs and fruits	meat and e
rod	bacilli	rod
mycotoxin	All of these	an enteroto
Salmonella	*Vibrio*	*Salmonell*
7.3 Mrad	173 Rad	7.3 Mrad
cell permeation	cell damage	cell autoly
1962	1980	1940
Clostridium tetanai	*Clostridium subtilis*	*Clostridiu*
Aspergillus flavus	*Mucor*	*Penicillium*
1980	1986	1986
Staphylococcal intoxication	None of these	Salmonell
Arizona	*E.coli*	*Shigella so*
S. thermophillus	none of these	*Staphyloco*
Bacillus cereus	*Vibrio*	*Bacillus ce*
fishery service	research and development service	Hazard ana
herbivore	none of these	serovar
both (a) and (b)	none of the above	food borne
neurotoxin of Salmonella spp	exoenterotoxin of Salmonella spp	endotoxin
all types of strains (proteolytic)C, D an	none of the above	all types o
food intoxication	all of these	food infect
Vibrio parahaemolyticus	All of these	*Vibrio vuli*
Proper low temperature treatment befo	All of these	Proper hea
ice creams	cheese	cheese
Arizona	*Streptococcus*	*Arizona*
water	land	water
herbivore	none of these	serovar
antitoxin	antipyretic	antitoxin

Vibrio parahaemolyticus	All of these	*Vibrio vuli*
40 °C	50 °C	37 °C
Salmonella	*Vibrio*	*Salmonella*
C. perferigens	*E. coli*	*C. jejuni*
Bacillus	*E. coli*	*Salmonella*
1989	1988	1980
Adeno	Herpes	Hepatitis
contaminated water	all of these	contamina
canned foods	fish products	meat produ
endotoxin	enterotoxin produced during vegetative phase	enterotoxi
acidic	both b and c	neutrality
1950s	1960s	1960s
3.0 to 4.0	8.0 to 9.0	7.0 to 7.5
1978	1945	1945
D60 c	D30 c	D60 c
polysaccharides	peptidoglycon	lipopolysa
E. coli and Salmonella	*Clostridium and Streptococcus*	*Clostridiu*
Salmonella typhi	*Salmonella typhimurium*	*Salmonella*
40 hrs	37 hrs	2-48 hrs
2 to 18	8 to 12	2 to 18
pink	yellow	yellow
food intoxication	all of these	food infect
Virus	Parasite	Bacteria
Federal	Private	Private

Robert Koch	Edward Jenner	Louis Past
Fabri	Leewen Hoek	Roger Bacc
1.5*10 cm	3*20cm	1.5 *12 cm
Bacon	Hooke	Beijernick
Molden agar	Selective media	Minimal m
To detect ph change	To detect aw availability.	To detect p
Antibiotic producers	Organic acid producers.	Antibiotic p
Auxanography	Indicator dye method.	Auxanograp
Enrichment method	Tertiary method.	Secondary s
Selective medium	Liquid medium	Liquid med
0.901-0.925	0.865-0.890	0.865-0.89(
Dulaney	Nakayama.	Brell
5-10 years	5 years.	15-20 years
Freezing	Nitrogen storage	Lyophilizat
Only dry ice	Nitrogen and ice	Ice and alc
Cryogenic storage	Lyophilization	Cryogenic s
Nitrogen storage	Silica gel cultures	Soil cultur
Alexander	Pepler	Alexander
E.coli	Pseudomonas	Streptomyc

Autotrophs	Phototrophs	Auxotrophs
Negative control	Promoter control	Feed back c
Feed back control	Positive control	Feed back i
Feed back inhibition	Primer control	Feed back r
Multivalent control	Cumulative control	Cumulative
Ortho enzyme	Paraenzyme	Iso enzyme
Autotrophs	Protoplast	Phototroph
Heterotrophs	Autotrophs.	Phototroph
Mustard gas	MNNG	UV-rays
Trophase	Log phase	Stationary p
Salmonella	*Bacillus*	*Cornyebact*
Calcium	Sulfate	Pencillin
Selective method	Serial subculturing method	Mechanical
Mutant vegetative cells	Autotrophs	Mutant spo
Aspargine	Pyrimidine	Lysine
Antilogue	Autologue	Analogue
Diauxic growth	Continuous growth	Confluent
Phospholipids	Protein	Phospholip
Only yeast cells	Bacteria	Fungi
Thermoplast	Periplast	Protoplast
Isotonic	Hyphotonic	Isotonic
Mutation	Plasmid fusion	Protoplast f
20 – 40%	50%	5 – 25%
Rapid cell growth and not for product forr	Rapid cell growth and product formation.	Rapid cell g
Uranyl nitrate	All the above	UV radiati
Trophophase	Idiophase	Lag phase
Trophophase	Idiophase	Trophopha
Tower fermentor	Tubular fermentor	CSTF
Iron	Glass	Wood vess
Plasmid	Spheroplast	Plasmid
CaCl2	Ethyl sulfanate	Ethyl meth
Semi continuous	Continuous	Batch
Continuous	Semi continuous	Fed batch
Semi continuous	Continuous	Batch
Fed batch	Airlift	Continuous
S. cereviseae	*E.coli*	*Clostridium*
Impeller	Baffler	Aeration
Sparger	Baffler	Agitation
Baffler	Turbines	Sparger
Foam	Air	Foam
25000	100000	12500
Cell suspended tank fermentor	Continuous solid type fermentor	Continous s
Parasite	Virus	Yeast
Removal of microbes	Heating	Keeping ou
Cooling	Packaging	Packaging

Rust	Extract		Must
Neutral	All the above		Acidity
Lactic acid bacteria	Zymomonas		Lactic acid
Formic acid	Lactic acid		Lactic acid
Both a and b	Residual sugar		Residual su
Film foaming	None		Film foami
Acetic acid	Alcohol		n-heptyl p-h
Chemicals	Washing		Pasteurizati
Wine	All the above		Ales
Wort	None		Wort
Icing	Pressing		Malting
juice	Extraction		Wort
Zymomonas	Pseudomonas		Saccharom
Grapes	All the above		All the abo
Pasteurization	All the above		Filtration
Aceticacid bacteria	Zymomonas	b. Cyanide	Lactic acid
The yeasts used	Yeast fermentation		The grapes
Zymomonas anaerobia	All the above		All the abo
Stabilizing effect	All the above		All the abo
A seasoning agent	All the above	b. Continuc	A seasoning
Adjuant	Enzymes		Surface of
Wine	Brandy		Beer
Pseudomonas	Saccharomyces uvarum		Saccharom
Lipids	Amino acids	b. 1127	Starch
Filtration	Sewage treatment	d. 1141	Sewage tre
Acetic acid	Propionic acid		Methanol
Organic acid and inorganic acid	Organic and inorganic salts		Sugar and
Calcium	Magnesium		Iron
At low temperature	At low PH		At low PH
6 – APA	3 - APA		6 – APA
Lag	Stationary		Stationary
Saccharomyces cereviciae	Bacillus		Bacillus
0.002mg/day	0.5mg/day		0.001mg/da
Cobinamide	Cupric oxide		Cobinamid
Submerged	Tubular fermentation		Submerged
1161 3	1141 5		1125 2
S. oryzae	P. notatum		Streptomy
85° F	63° F		80° F
Ammonium sulphate	Sodium thiosulphate		Sodium su
Liquid paraffin	Melted charcoal		Activated c
Palm oil	Cedar-wood oil		Soyabean o
2 - 4 mg	2 – 5 mg		1 – 2 mg
Bentorite	Mica		TEMED
Propyl	Calloxyl		Methyl
1000 – 100000 gals	500 – 10000 gals		10000 – 10

Mononthionite	thionite	Dithionite
Sequential genetic isolation	Revertant mutant	Sequential
A. niger	A. flavus	penicillium
Dextrose	Fructose	Lactose
Extraction	Sonication	Filtration
Cane steep liquor	None of these	Corn steep
Citric acid	Tetracyclin	Vinegar
Serial dilution method	Crowded plate method	Crowded pl
Bottom yeast	Feeder yeast	Bottom yea
Vitamin – B6	Ethanol	Vitamin – I
P.citrinum	P.roquefortii	P.chrysoger
Cylindraconical	Deep jet	Tower
Cylindraconical	Deep jet	Airlift
Ion exchange	Column	Adsorption
Ion exchange	Column	b. Affinity
a or b	Sedimentation	a or b
CaCl2	Ca	Na2SO4
Butanol	Alcohol	Methanol
Ultrasonication	Freezethawing	Liquidshea
Fed batch fermentor	Semi continuous fermentor	Batch ferme
Filteration	HPLC	Crystallizat
Airlift	Batch	Laboratary
1-101+	5-101+	1-21+
1000-5000	d. 10000-20000	5000-10000
90°F	100°F	

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KARPAGAM ACADEMY OF HIGHER EDUCATION

(Established Under Section 3 of UGC Act, 1956)

Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

(For candidates admitted from 2009, onwards)

M. Sc., DEGREE INTERNAL EXAMINATION July – 2017

MICROBIOLOGY

FOOD AND INDUSTRIAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**

Date:

Part - A (20 X 1 = 20 Marks)

1. The fumes of burning ____ are used to treat light colored dehydrated fruits
   a) Sulfur b) Ethylene
   c) Potassium d) Sodium
2. The percentage fat constituent of double toned milk is_____
   a) 0.5 b) 1.5
   c) 3 d) 4.5
3. The water requirement of a microorganism is expressed in terms of _____
   a) Water action b) Water adsorption
   c) Water affinity d) Water activity
4. Sugars act as preservatives due to their ability to_____
   a) Make water unavailable to organism b) Interfere with proteolytic enzyme
   c. Osmotic effect d) Diffusion effect
5. The O-R potential of a system is measured by_____
   a) mV b) mM
   c) aw d) Eh
6. The simplest dryer is the _____
   a) Sun b) Air
   c) Heat d) Evaporator
7. Radiation dose in kilograms of _____ inhibits sprouting in potatoes, onions and garlic
   a) 0.05-0.15 b) 0.01-0.14
   c) 0.05-0.07 d) 0.05-0.11
8. Preservation affects the growth of microorganism by _____
   a) Inhibition b) Retardation
   c) Arresting d) Antisepsis
9. _____ bacteria are able to grow at commercial refrigeration temperatures
   a) Pschrotropic b) Halophilics
   c) Autotrophic d) Heterotrophic
10. Contamination of foods from _____ may be important for sanitary as well as economic reasons
    a) Air b) Soil
    c) Water d) Sewage
11. When microbes can use fat as an energy source _____
    a) Presence of sugar molecule b) Presence of glucose
    c) Presence of fructose d) Presence of high sugar

12. Pasteurization is done to kill_____

a) Selective microorganism b) All the microorganism

c) Yeast d) Yeast and its spores

13. Sanitising is_____

a) Applying detergent to a clean surface b) Done before washing

c) Reducing bacteria by application of heat d) Wiping with a clean cloth

14. _____ is used extensively in the prevention of mold growth and rope development in baked goods

a) Calcium propionate b) Calcium sorbate

c) Monocholroacetic acid d) Nitrates

15. The time temperature combination for HTST paterurization of 71.1°C for 15 sec is selected on the basis of_____

a) *Coxiella Burnetii* b) *E. coli*

c) *B. subtilis* d) *C. botulinum*

16. In the early _____ numerous surveys have been conducted on the detection aflatoxins in foods

a) 1980s b) 1940s

c) 1950s d) 1960s

17. During _____ the internal temperature of bread, cake or other bakery products approaches but never reaches 100 °C

a) Heating b) Boiling

c) Baking d) Baking

18. Most spoilage bacteria grow at_____

a) Acidic pH b) Alkaline pH

c) Neutral pH d) Any of the pH

19. The use of indicator microorganisms began with use of E. coli testing in _____

a) Soil b) Plants

c) Water d) Air

20. Souring of canned meat is caused by_____

a) Thermoduric cells b) Thermostatic cells

c) Thermo liable cells d) Halophile

Part - B Answer all the questions (3 X 2 = 6 Marks)

21. What is oomycetes?

22. Define water activity.

23. Differentiate between sterilization and pasteurization.

Part - C Answer all the questions (3 X 8 = 24 Marks)

24. a) Explain the sources of contamination of food.

(or)

b) Explain the extrinsic parameters of food affecting microbial growth.

25. a) Brief note on preservation of food using chemicals.

(or)

b) Write notes on important bacteria in food microbiology.

26. a) Write notes on Food control agencies.

(or)

b) Explain about food borne intoxication.

Reg. No._____

[16MBP302]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Established Under Section 3 of UGC Act, 1956)

Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

(For candidates admitted from 2009, onwards)

M. Sc., DEGREE INTERNAL EXAMINATION September – 2017

MICROBIOLOGY

FOOD AND INDUSTRIAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**

Date:

Part - A (20 X 1 = 20 Marks)

1. Cell without cell wall is called as _____
   a) Protoplast b) Spleroplast
   c) Thermoplast d) Periplast
2. _____ fermentor is normally operated at high substrate conversion.
   a) CST fermentor b) Air lift
   c) Tower d) Tubular
3. _____ is a culture system which contain a intial limited amount of nutrient
   a) Fed batch b) Batch
   c) Semi continuous d) Continuous
4. _____ ensures uniform suspension on microbial cells.
   a) Aeration b) Agitation
   c) Sparger d) Baffler
5. _____ fermentor is a gas tight baffled rise tube connected to a down comer tube.
   a) Tower b) Airlift
   c) Cylindraconical d) Deep jet
6. Precipitation of riboflavin by addition of _____
   a) Dithionite b) Trithionite
   c) Mononthionite d) Thionite
7. Penicilin is commercially produced by_____
   a) *P. notatum* b) *P. chrysogenum*
   c) *P. citrinum* d) *P. roquefortii*
8. _____ is an established and final purification of a diverse range of compounds.
   a) Drying b) Crystallization
   c) Filtration d) HPLC
9. Primary metabolites are produced during _____.
   a) Lag phase b) Stationary phase
   c) Trophase d) Log phase
10. Among the following which is biotin requiring organism?
    a) *Cornyebacterium* b) *E.coli*
    c) *Salmonella* d) *Bacillus*
11. A combined sparger and agitator may be used in ---------fermenter
    a) Laboratory b) Tower
    c) Airlift d) Batch

12. In wine fermentation grapes are crushed and pressed to release the juice is called
    a) Must b) Grape juice
    c) Rust d) Grape extract
13. -------is widely used application of asepsis technique
    a) Centrifugation b) Heating
    c) Cooling d) Packaging
14. Secondary metabolites are produced during _____
    a) Lag phase b) Stationary phase
    c) Trophophase d) Idiophase
15. Mutant spores are selected by _____method.
    a) Enrichment b) Mechanical
    c) Selective d) Serial subculture
16. Organism which does not produce particular product which is required for growth is called _____
    a) Auxotrophs b) Heterotrophs
    c) Autotrophs d) Phototrophs
17. Freezing mixture used in lyophilization is _____.
    a) Ice and methanol b) Ice and alcohol
    c) Only dry ice d) Nitrogen and ice
18. Chemically beer is sterilized by adding_____.
    a) Benzoic acid b) n-heptyl p-hydroxy benzoate
    c) Acetic acid d) Alcohol
19. Vitamin B12 production using *Streptomyces olivaceus* NRRLB _____ strain.
    a) 1125 b) 1127
    c) 1161 d) 1141
20. Riboflavin fermentation employs media containing _____
    a) Sugar and lipid b) Protein and lipid
    c) Organic and inorganic acid d) Organic and inorganic salts

Part - B Answer all the questions (3 X 2 = 6 Marks)

21. What is HACCP?
22. What is downstream processing?
23. What are the types of fermentation process?

Part - C Answer all the questions (3 X 8 = 24 Marks)

24. a. Discuss on strain isolation and development

(or)

b. Detail on batch fermentation with diagram.

25. a. Discuss on recombinant DNA technology for strain development.

(or)

b. Detail on submerged and solid-state fermentation.

26. a. Detail on riboflavin production.

(or)

b. Write briefly about the production of beer?

FOOD AND INDUSTRIAL MICROBIOLOGY [16MBP302]

CIA – I

Key Answer

Part - A (20 X 1 = 20 Marks)

1 – a, 2 – b, 3 – b, 4 – b, 5 – b, 6 – d, 7 – a, 8 – b, 9 – a, 10- a, 11 – d, 12 – a, 13 – c, 14 – a, 15 – a, 16 – d, 17 – d, 18 – c, 19 – c, 20 – a.

Part - B Answer all the questions (3 X 2 = 6 Marks)

21. **Oomycetes**

These molds are mostly aquatic; however, included in this group are several important plant pathogens. The oospores are formed by the union of a small male gamete and a large female gamete.

22. **Water activity.**

Micro organisms have an absolute demand for water. Without water, no growth can occur.
The exact amant of water needed for growth of micro organisms varies. This water requirement is best expressed in terms of available water or water activity (aW).

23. **Sterilization and pasteurization.**

Use of heat at 60~80^{0}C for a few minutes for the elimination/ destruction of all disease causing microorganisms, and reduction of potential spoilage organisms

Sterilization or appertization - destruction of all viable organisms in food as measured by an appropriate enumeration method.

Part - C Answer all the questions (3 X 8 = 24 Marks)

24. a**) Sources of contamination of food.**

From green plants and fruits

Natural surface flora of plants varies with the plant.

Pseudomonas, Alcaligenes, Flavobacterium, Micrococcus, coliforms and lactic acid bacteria.

From animals

Sources of microorganisms from animals include the surface flora, the flora of the respiratory tract, and the flora of the gastro intestinal tract.

Hides, hooves, and hair contain microorganisms from soil, manure, feed and water but contain spoilage organisms.

From soil

Soil contains greatest variety of micro organisms. They are ready to contaminate the surfaces of plants growing on or in them and the surfaces of animals roaming over the land.

Pseudomonas, Chromobacterium, Proteus, Micrococcus, Bacillus, Streptococcus, Enterobacter and *Escherichia coli*.

From Air

Air does not contain a natural flora of micro organisms, but accidentally they are present on suspended solid material or in moisture droplets.

Yeasts especially asporogenous chromogenic ones are found in most samples of air

During handling and processing

Additional contamination may come from equipment coming in contact with foods, from packaging materials and from personnel.

24. b) **Extrinsic parameters of food affecting microbial growth.**

Relative humidity (RH)

Ex: Grain silos or in tanks in which concentrates and syrups is stored.

Storage of fresh fruits and vegetables requires very careful control of relative humidity.

Temperature

Microbial growth can occur over a temperature range from about -8°C up to 100°C at atmospheric pressure.

Thermophiles have optimum: 55-75°C

Mesophile have optimum: 30 -40°C

Psychrophiles (Obligate psychrophiles): 12 – 15 °C

Psychotroph (facultative): 25-30 °C

Gaseous atmosphere.

25 a) **Preservation of food using chemicals.**

Organic acids and their salts:

Several organic acids and their salts are common preservatives as they have marked microbiostatic and microbicidal action.

Benzoic acid and benzoate are used for the preservation of vegetables. Sodium benzoate is used in the preservation of jellies, jams, fruit juice and other acid foods.

Salicylic acid and salicylates are used as preservatives of fruits and vegetables in place of benzoate. However, it is considered to be deleterious to health of consumer.

Sorbic acid is recommended for foods susceptible to spoilage fungi,

e.g., it inhibits mold growth in bread. Foods prepared by fermentation processes, e.g. milk products etc. are preserved mainly by lactic, acetic and propionic acids.

Flavoring extracts of vanilla, lemons are preserved in 50-70% alcohol as it coagulates cell proteins.

Inorganic acids and their salts:

Most common among the inorganic acids and their salts are, sodium chloride, hypochlorites, sulphurous acids and sulphites, sulphurdioxide, nitrate and nitrite.

Sodium chloride

Sodium chloride produces high osmotic pressure and therefore causes destruction of many microorganisms by plasmolysis.

Hypochlorites

The hypochlorous acid liberated by these salts is an effective germicide. It is oxidative in its action. The commonly used forms are sodium and calcium hypochlorites. Drinking water or water used for washing foods may be dissolved with hypochlorites.

Sulphurous acids and Sulphites

Sulphurous acids and sulphites are added to wines as preservatives.

Sulphur dioxide

Sulphur dioxide has a bleaching effect desired in some fruits, and also suppresses the growth of yeast and molds.

Nitrates and Nitrites

Nitrates and nitrites produce an inhibitory effect on bacterial growth and are used usually together in meat and fish preservation and for retention of red-colour of the meat.

Nitrate is changed to nitrous acid which reacts with myoglobin to give nitric oxide myoglobin.

25 b) **Important bacteria in food microbiology.**

Bacteria

Genus *Acetobacter*

These bacteria oxidize ethyl alcohol to acetic acid.

Genus *Aeromonas*

These are gram-negative rods with an optimum temperature for growth of 22 to 28 °C. They are frequently isolated from aquatic environments.

Genus *Alcaligenes*

As the name suggests, an alkaline reaction usually is produced in the medium of growth. *A. viscolactis* causes ropiness in milk, and *A. metalcaligenes* gives a slimy growth on cottage cheese.

Genus *Alteromonas*

Several former species of *Pseudomonas* are now classified as *Alteromonas*. They are marine organisms that are potentially important in sea foods.

Genus *Bacillus*

The endospores of species of this aerobic to facultative genus usually do not swell the rods in which they are formed. e.g., *B. subtilis*

Genus *Brevibacterium*

B. linens are related to *Arthrobacter globiformis* and may be synonymous.

Genus *Brochotrix*

These are gram-positive rods which can form long filamentous like chains that may fold into knotted masses.

Genus *Campylobacter*

These bacteria were originally classified in the genus *Vibrio*. Several strains of *C. fetus* subsp. *jejuni* have been associated with gastroenteritis in humans.

Genus *Clostridium*

The endospores of species of this genus of anaerobic to microaerophilic bacteria usually swell the end or middle of the rods in which they are formed. *Clostridium thermosaccharoolyticum* is an example of a saccharolytic obligate thermophile

Genus *Corynebacterium*

The diphtheria organism, *C. diptheriae*, may be transported by foods.

Genus *Erwinia*

The species of this genus are plant pathogens that cause necrosis, galls, wilts, or soft rots in plants and therefore damage the plants and vegetable and fruit products from them. *E. carotovora* is associated with the market disease called "bacterial soft rot.

Genus *Escherichia*

Found in feces, a predominant gram-negative rod isolated from the intestinal tract of warm-blooded animals and widely distributed in nature.

Genus *Flavobacterium*

The yellow to orange-pigmented species of this genus may cause discolorations on the surface of meats and be involved in the spoilage of shellfish, poultry, eggs, butter, and milk.

Genus *Klebsiella*

Commonly associated with the respiratory and intestinal tracts of humans. *K. pneumoniae* is the causative organism for a bacterial pneumonia in humans.

Genus *Lactobacillus*

The lactobacilli are rods, usually long and slender, that form chains in most species.

26 a) **Food control agencies**

A mandatory regulatory activity of enforcement by national or local authorities to provide consumer protection and ensure that all foods during production, handling, storage, processing and distribution are safe, wholesome and fit for human consumption; conform to quality and safety requirements; and are honestly and accurately labelled as prescribed by law.

Food Hygiene: All conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

Food Inspection: The examination, by an agency empowered to perform regulatory and/or enforcement functions, of food products or systems for the control of raw materials, processing, and distribution. This includes in-process and finished product testing to verify that they conform to regulatory requirements.

Good Agricultural Practices (GAP): Practices of primary food producers (such as farmers and fishermen) that are necessary to produce safe and wholesome agricultural food products conforming to food laws and regulations.

Good Manufacturing Practices (GMP): Conformance with codes of practice, industry standards, regulations and laws concerning production, processing, handling, labelling and sale of foods decreed by industry, local, state, national and international bodies with the intention of protecting the public from illness, product adulteration and fraud.

HACCP Plan: A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration.

26 b) **Food borne intoxication.**

There are two major food-poisonings or food-intoxications caused by bacteria. These are: Botulism and Staphylococoal poisoning.

Botulism

Botulism is caused by the ingestion of food containing the neurotoxin (toxin that affects the nervous system) produced by Clostridium botulinum.

There are 7 types (type A,B,C, D,E,F,G) of these neurotoxins recognized on the basis of serological specificity. The neurotoxin of C. botulinum is a protein. It has been purified and crystallized and is so powerful that only a does as low as 0.01 mg is said to be fatal to human being. The toxin is absorbed mostly in the small intestine and paralyzes the involuntary muscles of the body.

Source

The main sources of botulism are canned meat, fish, string beans, sweet corn, beets and other low medium acid foods.

Temperature is considered to be the most important factor in determining whether toxin production will take place and what the rate of production will be. Various strains of C. botulinum types A and B vary in their temperature requirements; a few strains grow at 10 to 11 °C. However, the lowest temperature for germination of spores of most of the strains is 15 °C and maximum of 48 °C.

Symptoms

Symptoms generally occur within 12 to 36 hours after consumption of the spoiled food. Early symptoms are digestive disturbances followed by nausea, vomiting, diarrhea together with

dizziness and headache. Double vision may occur early and there may be difficulty in speaking. Mouth may become dry, throat constricted; tongue may get swollen, and coated. Involuntary Muscles become paralyzed and paralysis spreads to the respiratory system and to the heart. Death normally results from respiratory failure.

Prevention

Canned food should be properly processed by using approved heat processes.

Avoiding food that has been cooked but not well heated. Raw foods, frozen foods thawed and held at room temperature should be avoided. Gassy and spoiled canned foods should be rejected. Boiling of suspected food for at least 15 minutes.

Treatment:

Successful treatment is by the administration of polyvalent antitoxin in the early stages of infection. Once the symptoms appear the fails to prove useful.

FOOD AND INDUSTRIAL MICROBIOLOGY [16MBP302]

CIA – II

Key Answer

Part - A (20 X 1 = 20 Marks)

1 – a, 2 – b, 3 – b, 4 – b, 5 – b, 6 – a, 7 – b, 8 - b, 9 – a, 10 – a, 11 – a, 12 – a, 13 – d, 14 – c, 15 – b, 16 – a, 17 – b, 18 – b, 19 – a, 20 – a.

Part - B Answer all the questions (3 X 2 = 6 Marks)

21. **HACCP**

The hazard analysis critical control point system (HACCP) is a scientific and systematic way of enhancing the safety of foods from primary production to final consumption through the identification and evaluation of specific hazards and measures for their control to ensure the safety of food.

22. **Downstream processing**

The method by which the products of fermentation are recovered and separated is known as downstream processing. This forms the major (about 85%) portion of the complete fermentation technology. There are various methods by which DSP is carried out.

23. **Types of fermentation process**

(1) Batch fermentation
(2) Feb-batch fermentation and
(3) Continuous culture.

Part - C Answer all the questions (3 X 8 = 24 Marks)

24. a. **Strain isolation and development**

Inoculation and Sampling:

The bioreactor with the growth medium under aseptic conditions is ready for inoculation with the production organism. The size of the inoculum is generally 1-10% of the total volume of the medium. A high yielding production strain of the organism taken from a stock culture (lyophilized and stored in a deep freezer or in liquid nitrogen) is used.

During the course of fermentation, samples are regularly drawn from the bioreactor. This is required to check the contamination (if any) and measurement of the product formed.

Aeration of the fermentation medium is required to supply O_2 to the production organisms and remove CO_2 from the bioreactor. The aeration system is designed for good exchange of gases. Oxygen (stored in tanks in a compressed form) is introduced at the bottom of the bioreactor through a sparger.

The small bubbles of the air pass through the medium and rise to the surface. The bioreactor usually has about 20% of its volume as vacant space on the upper part which is referred to as head space. The bioreactor has about 80% working volume. The gases released during fermentation accumulate in the headspace which pass out through an air outlet.

Control Systems:

It is essential to maintain optimal growth environment in the reaction vessel for maximum product formation. Maximal efficiency of the fermentation can be achieved by continuously monitoring the variables such as the pH, temperature, dissolved oxygen, adequate mixing, nutrient concentration and foam formation. Improved sensors are now available for continuous and automated monitoring of these variables (i.e., on line measurement of pH).

Most of the microorganisms employed in fermentation grow optimally between pH 5.5 and 8.5. In the bioreactor, as the microorganisms grow, they release metabolites into the medium which change pH. Therefore, the pH of the medium should be continuously monitored and maintained at the optimal level.

This can be done by the addition of acid or alkali base (as needed) and a thorough mixing of the fermentation contents. Sometimes, an acid or alkaline medium component can be used to correct pH, besides providing nutrients to the growing microorganisms.

Temperature:

Temperature control is absolutely essential for a good fermentation process. Lower temperature causes reduced product formation while higher temperature adversely affects the growth of microorganisms. The bioreactors are normally equipped with heating and cooling systems that can be used as per the requirement, to maintain the reaction vessel at optimal temperature.

Dissolved oxygen:

Oxygen is sparingly soluble in water (0.0084 g/1 at 25°C). Continuous supply of oxygen in the form of sterilized air is done to the culture medium. This is carried out by introducing air into the bioreactor in the form of bubbles. Continuous monitoring of dissolved oxygen concentration is done in the bioreactor for optimal product formation.

Adequate mixing:

Continuous and adequate mixing of the microbial culture ensures optimal supply of nutrients and O_2, besides preventing the accumulation of toxic metabolic byproducts (if any). Good mixing (by agitation) also creates favourable environment for optimal and homogeneous growth environment, and good product formation. However, excessive agitation may damage microbial cells and increase the temperature of the medium, besides increased foam formation.

Nutrient concentration:

The nutrient concentration in a bioreactor is limited so that its wastage is prevented. In addition, limiting concentrations of nutrients may be advantageous for optimal product formation, since high nutrient concentrations are often associated with inhibitory effect on microbial growth. It is now possible to do on-line monitoring of the nutrient concentration, and suitably modify as per the requirements.

Foam formation:

The media used in industrial fermentation is generally rich in proteins. When agitated during aeration, it invariably results in froth or foam formation that builds in head space of the bioreactor. Antifoam chemicals are used to lower surface tension of the medium, besides causing foam bubbles to collapse. Mineral oils based on silicone or vegetable oils are commonly used as antifoam agents.

Mechanical foam control devices, referred to as mechanical foam breakers, can also be used. Such devices, fitted at the top of the bioreactor break the foam bubbles and the throw back into the fermentation medium.

Cleaning:

As the fermentation is complete, the bioreactor is harvested i.e. the contents are removed for processing. The bioreactor is then prepared for the next round of fermentation after cleaning (technically called turn round). The time taken for turn round referred to as down time should be as short as possible (since it is non-productive). Due to large size of the bioreactors, it is not possible to clean manually. The cleaning of the bioreactors is carried out by using high-pressure water jets from the nozzles fitted into the reaction vessel.

24 b) **Batch fermentation.**

This term is attributed to that type of fermentation wherein there is change in culture medium, number of microorganisms and the amount of the product produced (i.e. the metabolite or target protein). In batch fermentation six phases of the microbial growth are seen.

(a) Lag phase:

Immediately after inoculation, there is no increase in the numbers of the microbial cells for some time and this period is called lag phase. This is in order that the organisms adjust to the new environment they are inoculated into.

(b) Acceleration phase:

The period when the cells just start increasing in numbers is known as acceleration phase.

(c) Log phase:

This is the time period when the cell numbers steadily increase.

(d) Deceleration phase:

The duration when the steady growth declines.

(e) Stationary phase:

The period where there is no change in the microbial cell number is the stationary phase. This phase is attained due to depletion of carbon source or accumulation of the end products.

(f) Death phase:

The period in which the cell numbers decrease steadily is the death phase. This is due to death of the cells because of cessation of metabolic activity and depletion of energy resources. Depending upon the product required the different phases of the cell growth are maintained. For microbial mass the log phase is preferred. For production of secondary metabolites i.e. antibiotics, the stationary phase is preferred.

25. a) **Recombinant DNA technology for strain development.**

Inoculation and Sampling:

The bioreactor with the growth medium under aseptic conditions is ready for inoculation with the production organism. The size of the inoculum is generally 1-10% of the total volume of the medium. A high yielding production strain of the organism taken from a stock culture (lyophilized and stored in a deep freezer or in liquid nitrogen) is used.

During the course of fermentation, samples are regularly drawn from the bioreactor.

Aeration of the fermentation medium is required to supply O_2 to the production organisms and remove CO_2 from the bioreactor.

The small bubbles of the air pass through the medium and rise to the surface.

Control Systems:

It is essential to maintain optimal growth environment in the reaction vessel for maximum product formation.

Most of the microorganisms employed in fermentation grow optimally between pH 5.5 and 8.5. In the bioreactor, as the microorganisms grow, they release metabolites into the medium which change pH. Therefore, the pH of the medium should be continuously monitored and maintained at the optimal level.

Temperature:

Temperature control is absolutely essential for a good fermentation process. Lower temperature causes reduced product formation while higher temperature adversely affects the growth of microorganisms. Dissolved oxygen:

Oxygen is sparingly soluble in water (0.0084 g/1 at 25°C). Continuous supply of oxygen in the form of sterilized air is done to the culture medium.
Adequate mixing:
Continuous and adequate mixing of the microbial culture ensures optimal supply of nutrients and O_2, besides preventing the accumulation of toxic metabolic byproducts.
Nutrient concentration:
The nutrient concentration in a bioreactor is limited so that its wastage is prevented.
Foam formation:
The media used in industrial fermentation is generally rich in proteins. Mineral oils based on silicone or vegetable oils are commonly used as antifoam agents.
Cleaning:
As the fermentation is complete, the bioreactor is harvested i.e. the contents are removed for processing.

25 b) Submerged and solid-state fermentation.

Fermentation process is carried out in a container called the fermentor or bioreactor. The design and nature of the fermentor varies depending upon the type of fermentation carried out. Invariably all the fermentors have facilities to measure some of the fermentation parameters like temperature, pressure, pH, elapsed fermentation time, liquid level, mass etc.
The biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria and fungi are known as fermentation. In the way of metabolic breakdown they are capable to release several additional compounds distant. from usual products of fermentation such as carbondioxide and alcohol. These additional compounds are called as secondary metabolites. Since these posses biological activity, they are also known as bioactive compounds and produced in large quantities in solid state fermentation while other compounds are extracted from submerged fermentation. So based on the substrate used during fermentation they are classified into solid state fermentation and submerged fermentation.
SSF has existed for several centuries. The common solid substrates mainly used are wheat bran, rice, rice straw, hay, fruit and vegetable wastes, coconut coir and synthetic media bagasse and paper pulp and advantage over these substrates are nutrient rich, easily recycled.

26 a) **Riboflavin production**
Riboflavin, also known as vitamin B_2, is a vitamin found in food and used as a dietary supplement. As a supplement it is used to prevent and treat riboflavin deficiency and prevent migraines. It may be given by mouth or injection.
Various biotechnological processes have been developed for industrial scale riboflavin biosynthesis using different microorganisms, including filamentous fungi such as *Ashbya gossypii*, *Candida famata* and *Candida flaveri*, as well as the bacteria *Corynebacterium ammoniagenes* and *Bacillus subtilis*. The chemical company BASF has installed a plant in South Korea, which is specialized on riboflavin production using *Ashbya gossypii*. The concentrations of riboflavin in their modified strain are so high, that the mycelium has a reddish/brownish color and accumulates riboflavin crystals in the vacuoles,

which will eventually burst the mycelium. Riboflavin is sometimes overproduced, possibly as a protective mechanism, by certain bacteria in the presence of high concentrations of hydrocarbons or aromatic compounds. One such organism is *Micrococcus luteus*(American Type Culture Collection strain number ATCC 49442), which develops a yellow color due to production of riboflavin while growing on pyridine, but not when grown on other substrates, such as succinic acid.

26 b) **Production of beer**

Brewing is the production of beer by steeping a starch source (commonly cereal grains, the most popular of which is barley) in water and fermenting the resulting sweet liquid with yeast.

The basic ingredients of beer are water and a fermentable starch source such as malted barley. Most beer is fermented with a brewer's yeast and flavoured with hops. Steps in the brewing process include malting, milling, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging.

Clarifying agent

Some brewers add one or more clarifying agents to beer, which typically precipitate (collect as a solid) out of the beer along with protein solids.

Brewing process

There are several steps in the brewing process, which may include malting, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging.

Mashing

Mashing is the process of combining a mix of milled grain (typically malted barley with supplementary grains such as corn, sorghum, rye or wheat), known as the "grain bill", and water, known as "liquor", and heating this mixture in a vessel called a "mash tun".

Brew kettle or copper

Copper is the traditional material for the boiling vessel, because copper transfers heat quickly and evenly, and because the bubbles produced during boiling, and which would act as an insulator against the heat, do not cling to the surface of copper, so the wort is heated in a consistent manner.

Whirlpool

At the end of the boil, solid particles in the hopped wort are separated out, usually in a vessel called a "whirlpool" or "settling tank".

Hopback

A hopback is a traditional additional chamber that acts as a sieve or filter by using whole hops to clear debris (or "trub") from the unfermented (or "green") wort, as the whirlpool does, and also to increase hop aroma in the finished beer.

Fermenting

Fermentation takes place in fermentation vessels which come in various forms, from enormous cylindroconical vessels, through open stone vessels, to wooden vats.

Conditioning

After an initial or primary fermentation, beer is *conditioned*, matured or aged, in one of several ways, which can take from 2 to 4 weeks, several months, or several years, depending on the brewer's intention for the beer.