Semester - III

16MBP302 FOOD AND INDUSTRIAL MICROBIOLOGY 4H – 4C

Instruction Hours / week: L: 4 T: 0 P: 0 **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

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; Coliform bacteria	R4: 303-305; T1: 56-57
9	1	Unit revision	
Total Hrs: 9			

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.

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.

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; HACCP	T1: 495-501; 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: 9			

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.

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.

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.

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

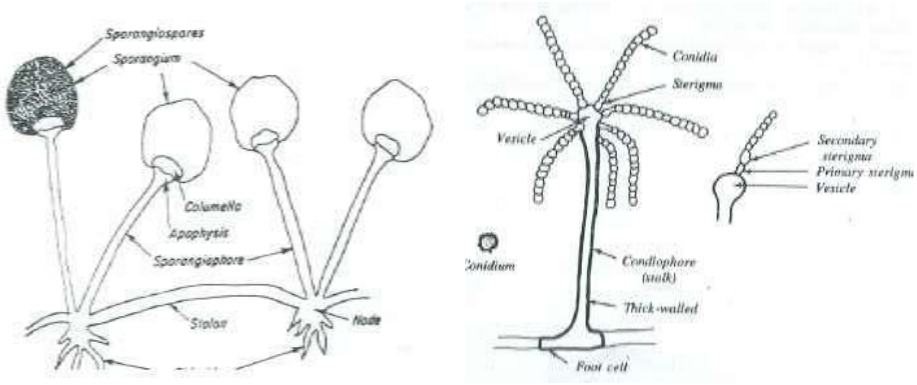
identification of molds. Examples are the rhizoids, or "holdfasts," of *Rhizopus* and *Absidia*, the foot cell in *Aspergillus*, and the dichotomous, or Y-shaped, branching in *Geotrichum*.

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.

Unit I; Possible Questions
Part-A (1 Mark)

Part-B (2 Mark)

1. Define the term mold.
2. What is hyphae?
3. What is mycelium?
4. Name the reproductive parts of mold.
5. What are zygospores?
6. What are basidiospores?
7. What is mucor?
8. How whiskers are caused on meat?
9. Define the term yeast.
10. What is encapsulation?
11. What is redox potential?
12. What are the inhibitory substances present in food?
13. What is water activity?
14. What is relative humidity?

Part-C (8 Marks)

1. Why is E. coli considered as indicator of pollution?
2. What are coliforms?
3. Name several species of pathogenic organisms present in contaminated foods?
4. What are the morphological and cultural characteristics of common fungal contamination present in food?
5. List out the sources of contamination of food and water.
6. Why there is a need of sterilization of food?
7. Name some of the sanitizers used in food industry.
8. What are the preventive measures to control the contamination of food?
9. List the intrinsic and extrinsic factors affecting the food.
10. Note on important characteristics of bacteria, fungi and molds.
11. Name any five natural antimicrobial substances in food that preserve the food.
12. Name the common microflora present in air, water.
13. Explain the enzymes involved in food spoilage by microbes.

Classify the microbes according to the oxygen requirement and temperature and also note on their temperature range

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB COURSE NAME: FOOD AND INDUSTRIAL MICROBIOLOGY

COURSE CODE: 17MBP302 UNIT: I BATCH-2017-2019

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	Cross-contamination of food occurs when	Cleaning and sanitising equipment and benches	Keeping food stored in food-grade containers	Washing hands before handling food	Using food handling gloves for handling money	Using food handling gloves for handling money
2	Which of the following are allergens?	Sources of gluten and Red meat	Fruits and vegetables	Fish and fish products	None of the above	Sources of gluten and Red meat
3	The undesirable change in a food that makes it or human consumption is referred as _____	food decay	food spoilage	food loss	all of the above	food spoilage
4	_____ microorganisms reqire positive Eh values or positive mV O-R potentials	Aerobic	anaerobic	facultative	none of these	Aerobic
5	_____ acid produced by the propionibacteria in swiss cheese is inhibitory to molds	sorbic	acetic	propionic	acetic	propionic
6	Most spoilage bacteria grow at	acidic pH	alkaline pH	neutral pH	any of the pH	neutral pH
7	The microbiological examination of coliform bacteria in foods preferably use	MacConkey broth	violet Red Bile agare	eosine Methylene blue agar	all of these	all of these
8	Which of the following can cause food to be contaminated because of chemical hazards from food handlers?	Hair	Dust	Live insects	Perfume	Perfume
9	_____ is the causative organism for a bacterial pneumonia in human.	*Flavobacterium*	*Escherichia*	*Klebsiella*	*Gluconobacter*	*Klebsiella*
10	The use of indicator microorganisms began with use of *E. coli* testing in _____	soil	plants	water	all of these	water

11	To retard the contamination and other microbial growth in meat is obtained by storing at _____ temperature	10°C	0°C	100°C	-10°C	0°C
12	The percentage of relative humidity is obtained by multiplying by_____.	aw*10	aw*1000	aw*100	aw*0.1	aw*100
13	Many infectious disease agents of animals can be transmitted to people through _____	water	food	soil	juices	food
14	_____ used to fertilize plant crops will be contaminated with human pathogens	sewage	distilled water	mineralized water	none of these	sewage
15	The o-R potential of a system is measured by	mV	mM	aw.	Eh	Eh
16	Which of the following can cause food to be contaminated because of physical hazards from food handlers?	Jewellery	Dust	Rodent droppings	Incorrectly diluted chemicals	Jewellery
17	_____ bacteria oxidize ethylalcohol to acetic acid	Aeromonas	Acetobacter	Alcaligens	Alteromonas	Acetobacter
18	The endospores of _____ do not swell the rods in which they are formed	*Streptococcus*	*Brochotrix*	*Brevibacterium*	*Bacillus*	*Bacillus*
19	_____ is associated with the market disease called bacterial soft rot	*Erwinia*	*Enterobacter*	*Corynebacterium*	*Klebsiella*	*Erwinia*
20	_____ bacteria are those which grow in high concentration of sugars	Halophilic	thermophilic	osmophilic	none of these	osmophilic
21	_____ bacteria grow and cause discoloration on foods high in salt	Halobacterium	Enterobacter	Erwinia	Corynebacterium	Halobacterium
22	The different ACC's between food categories reflect the	expected level of contamination of the raw material	potential for microbial growth during storage	potential shelf life	all of the above	all of the above

23	Yeast and mould count determination requires	nutrient agar	acidified potato glucose agar	MacConkey agar	violet Red Bile agar	acidified potato glucose agar
24	The water requirement of a microorganism is expressed in terms of _____'.	Water action	Water adsorption	Water affinity	Water activity	Water activity
25	A psychrophilic halophile would be a microbe that prefers	cold temperatures and increased amounts of salt	warm temperatures and increased amounts of pressure	cold temperatures and the absence of oxygen	warm temperatures and increased amounts of acid	cold temperatures and increased amounts of salt
26	Many microorganisms cannot use the disaccharide lactose and therefore do not grow well in _____	milk	water	food	sewage	milk
27	_____ yeast is grown with dairy starter cultures to maintain the activity and increase the longevity of the lactic acid bacteria	*Candida sp.*	*Trichosporon*	*Rhodotorula*	*Torulopsis*	*Candida sp.*
28	Which of the following acid will have higher bacteriostatic effect at a given pH?	Acetic acid	Tartaric acid	Citric acid	Maleic acid	Acetic acid
29	Water activity can act as	an intrinsic factor determining the likelihood of microbial proliferation	a processing factor	an extrinsic factor	all of the above	all of the above
30	The culture of Brevibacterium produces _____ pigmentation and helps ripening	orange-red	yellow	black	red	orange - red
31	Pectins are complex _____ that are responsible for cell wall rigidity in vegetables	Proteins	lipids	carbohydrates	vitamins	Carbohydrate

	and fruits					
32	_____ are short rods that are defined as aerobic and facultative anaerobic	*Enterobacter*	*Coliforms*	*Proteus*	*Clostridium*	*Coliforms*
33	The _____ of many meat animals may contain micrococci, Staphylococci, and beta-hemolytic Streptococci	Hair	nail	skin	all of these	skin
34	The microorganism which apparently have no mechanism to tolerate acidic pH _____.	bacteria	fungi	viruses	viruses	fungi
35	_____ is the thermoduric bacteria	*Acenetobacteria*	*Morexella*	*Bacillus*	*Flavobacterium*	*Morexella*
36	Aeromonas grows at an optimum temperature of _____	27 to 37 °C	22 to 28 °C	35 to 37 °C	40 °C	22 to 28 °C
37	The spoilage of meat by microorganism is by_____ process.	Oxidation	Reduction	Decomposition	Precipitation	Oxidation
38	_____ bacteria is found aseptically in drawn milk and cause bovine mastitis	*Corynebacterium*	*Clostridium*	*Campylobacter*	*Enterobacter*	*Corynebacterium*
39	Sugars act as preservatives due to their ability to .	Make water unavailable to organisms	Interfere with the action of proteolytic enzyme	Osmotic effect	Both a and c	Interfere with the action of proteolytic enzyme
40	Preservation affect the growth of microorganism by .	Inhibition	Retardation	Arresting	All of the above	Retardation
41	NaCl can act as	antagonist at optimal concentrations	synergistically if added in excess of optimum level	Both (a) and (b)	None of the above	Both (a) and (b)

42	Which of the bacteria can grow in alkaline pH?	*Lactobacilli*	*Vibrio cholera*	*Salmonella*	*Staphylococcus*	*Vibrio cholera*
43	_____ bacteria are able to grow at commercial refrigeration temperatures	Pschrotropic	halophilics	auttrophic	heterotrophic	Pschrotropic
44	_____ bacteria produce lipase enzyme that hydrolysis fat to fatty acids and glycerol	Saccharolytic	Pectinolytic	lipolytic	proteolytic	lipolytic
45	_____ does not contain a antural flora of microorganisms	soil	air	water	sewage	air
46	Saccharomyces are reclassified by Lodder in the year ____	1985	1978	1982	1984	1984
47	The water activity range of fresh meat and fresh fish was _____	0.93-0.98	0.98 and above	0.60-0.76	below 0.98	0.93-0.98
48	The o-R potential of a system is measured by	mV	mM	aw	Eh	mM
49	_____ has been used as starter culture in fermented sausages	*Photobacterium*	*Pediococcus*	*Propionibacterium*	*Proteus*	*Pediococcus*
50	Truly halophilic bacteria require minimal concentration of dissolved _____ for growth	NaCl2	Hcl	NaNo2	Cacl2	Nacl2
51	There are ___ aspects of water bacteriology that are interested by food microbiologist	2	5	6	4	2
52	Contamination of foods from _____ may be important for sanitary as well as economic reasons	air	soil	water	sewage	air
53	When microbes can use fat as an energy source _____.	absence of sugar molecule	presence of glucose	presence of fructose	Presence of high sugar	absence of sugar molecule
54	The approximate range of bacteria present in fresh vegetable is _____	10^9 -10^7/g	10^3 -10^9/g	10^3 -10^7/g	10^1 -10^7/g	10^3 -10^4/g
55	Cannery cooling water often contain _____	Coliforms	*Aeromonas*	*Klebsiella*	*Clostridium*	Coliforms

56	_____ causes ropiness in milk	Lactobacillus plantarum	Klebsiella pneumonia	Klebsiella oxytoca	Flavobacterium	Klebsiella oxytoca
57	_____ contain the greatest variety of microorganisms f any source of contamination of food	plants	sewage	water	soil	soil
58	Pig and beef carcasses may be contaminated with _____	*Salmonellae*	*Klebsiella*	*E. coli*	*Enterobacter*	*Salmonellae*
59	In fruit juices the growth of the fermentative yeast are favored by _____ pH	4.0-4.5	6.0-6.5	2.0-2.5	3.0-3.5	4.0-4.5
60	The water requirement of a microorganism is expressed in terms of _____	water action	water adsorption	water affinity	water activity	water activity

Unit 2

Syllabus

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

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

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 $_{250\ F\ (121.1\ C)}^{0}$ for *B. stearothermophilus*: 4-5 min

C. botulinum: 0.1 – 0.2 min.

» D $_{95^{o}C}$ 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^{0}F for 3 min and Z -value was determined as 10 ^{0}F, which means the10 ^{0}F rise in temperature reduces microorganisms by 1 log unit
- Therefore, at 140 ^{0}F , heat process need to be for 30 min and at 160 ^{0}F 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 ^{0}F (1210 ^{0}C)
- 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.

Unit-II; Possible Questions

Part- A (1 Marks)

Part- B (2 Marks)

1. What are the principles of food preservation?
2. What are the methods of food preservation?
3. What is blanching?
4. What is pasteurization?
5. Define thermal temperature.
6. What is the use of benzoic acid?
7. Name the inorganic acids and their salts for food preservation?
8. Which chemical preservative is used for fish and meat products?
9. What are antibiotics?
10. What is radiation pasteurization?
11. Write the methods for drying
12. What is sterilization?

Part- C (8 Marks)

1. List and describe the principles upon which methods of food preservation are based.
2. Compare the preservation efficiency by temperature - high/low, which is best.
3. What is the lowest temperature range at which food poisoning bacteria will grow?
4. What is the difference between pasteurization and sterilization?
5. List the chemicals used for food preservation. Name the chemical used for beer and meat preservation.
6. List the factors responsible for food preservation.
7. Outline a procedure suitable for enumeration, isolation and identification of the following groups of microbes from a sample of food: thermophilic, spore formers, coliforms and viruses.
8. What is modified atmosphere packaging?
9. Define asepsis.
10. How will you remove the microbes?
11. What are the types of filters?
12. How do spores survive at high temperature?
13. What is thermal death point?
14. Compare quick and slow freezing.
15. What are the effects of thawing?

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II M.Sc MB COURSE NAME: FOOD AND INDUSTRIAL MICROBIOLOGY

COURSE CODE: 17MBP302 UNIT: II BATCH-2017-2019

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	The concentration of salt used in high protein containing vegetables is _____	4.3-10.3	17.5-20.0	18.6-26.5	19.2-22.2	18.6-26.5
2	_____ is a term used to label foods treated with low level ionizing radiation	Radicidation	radurization	picowaved	radappertization	Picowaved
3	Flavoring etracts such as vanilla and lemon etracts are preserved by their content of ____	sugar	salt	alcohol	ethylene	alcohol
4	Which of the following statements are true about chemical preservatives _____	microbicidal or microstatic	chemical preservatives often hazardous to humans	sodium benzoate is a widely used preservative	all these	All of these
5	The time temperature combination for HTST paterurization of 71.1°C for 15 sec is selected on the basis of	*Coxiella Burnetii*	*E. coli*	*B. subtilis*	*C. botulinum*	*Coxiella Burnetii*
6	_____ contains a large number of olatile compounds that may have bacteriostatic and bactericidal effect	spices	woodsmoke	formaldehyde	alcohol	woodsmoke
7	---------- is a storage method uses bins or boxes for equalization of moisture	sweating	springer	cooling	freezing	springer
8	_____ is used most extensively in the prevention of mold growth and rope development in baked goods	calcium propionate	calcium sorbate	monocholroacetic acid	nitrates	calcium propionate
9	_____ can be dried by a process called explosive puffing	meat	vegetables	fruits	juices	vegetables
10	_____ in 1765 preserved food by heating it in a sealed containers	Spallanzani	Ruiz-Argueso	Rodrigeuz-Navarro	Christophersen	spallanzani
11	Combination of _____ irradiation with	Ultraviolet	infra red	gamma	none of the	ultraviolet

	chilling storage helps preserve foods				above	
12	Which solvent is commonly used to determine fat content	Ethyl alcohol	Hexane	Acetone	Benzene	Hexane
13	During _____ the internal temperature of bread, cake or other bakery products approaches but neve reaches 100 °C	Heating	boiling	baking	all of these	baking
14	Pasteurization is done to kill	Selective microorganism	All the microorganism	Yeast	Yeast and its spores	Selective microorganism
15	Sanitising is_____	Applying detergent to a clean surface	Done before washing	Reducing bacteria by application of heat or chemical	Wiping all surfaces with a clean cloth	Reducing bacteria by application of heat or chemical
16	The simplest dryer is the _____	sun	air	heat	evaporator	evaporator
17	Bacteria which is present in raw or undercooked meat, eggs, sea food and unpasteurized milk is	*E.coli*	*Salmonella*	*Staphylococcus*	cyano bacteria	*salmonella*
18	Milk and curry left over can be turned into sour and spoiled at	high temperature	very low temperature	room temperature	constant temperature	room temperature
19	_____ rays are streams of electrons emitted from radioactive materials	beta	cathode	gamma	X-rays	beta
20	Increase in the concentration of dissolved substances like sugar and salt helps in _____ of the food material	drying	freezing	moistening	thawing	drying
21	Sulfur stinker spoilage of canned food is caused by	*E.coli*	*D. nigrificans*	*Bacillus*	*Clostridium*	*D. nigrificans*
22	Radiation dose in kilograys of ____ inhibits sprouting in potatoes, onions and garlic	0.05-0.15	0.01-0.14	0.05-0.07	0.05-0.11	0.05-0.15

23	Preservation affects the growth of microorganism by	inhibition	retardation	arresting	all the above	retardation
24	Souring of canned meat is caused by _____.	thermoduric cells	thermostatic cells	thermo liable cells	none of the above	thermoduric cells
25	Significant numbers of S. aureus in a food can be determined by examining the food	RNase	thermostable nuclease	protease	thermostable DNase	protease
26	To retard the contamination and other microbial growth in meat is obtained by storing at _____ temperature	10°C	0°C	100°C	-10°C	0°C
27	Gazing at ultraviolet lamps produces irritation of the ____ within few seconds	eye	ear	nose	throat	eye
28	Sugars act as preservatives due to their ability to	make water unavailable to organism's	interfere with the action of proteolytic enzyme	osmotic effect	both a and c	interfere with the action of proteolytic enzyme
29	The minimal pH for the growth of staphylococcus is about ----------	2.5	4.8	2	3.5	4.8
30	_____ alcohol is used as coagulant and enaturizer of cell proteins	methanol	ethanol	butanol	none of these	ethanol
31	The fumes of burning ____ are used to treat light colored dehydrated fruits	sulfur	ethylene	potassium	sodium	sulfur
32	_____ can be used to control bacterial and fungal growth in tapholes of maple tree	paraformaldehyde	benzaldehyde	formaldehyde	all of these	paraformaldehyde
33	Christophersen classified microroganisms on the basis of sensitivity to freezing in the year_____	1984	1989	1973	1981	1973
34	The percentage fat constituent of double	0.5	1.5	3	4.5	1.5

	toned milk is					
35	---------- is mostly used preservative to prevent mold growth	sodium propionate	springer	sorbates	acetate	sodium propionate
36	_____ solvent is poisonous and should not be added to foods	propylene	ethanol	methanol	glycerol	methanol
37	_____ drying is limited to climates with a hot sun and dry atmosphere to fruits	mechanical	solar	freeze	all of these	solar
38	Food should be cooked to which temperature?	5°C	75°C	100°C	60°C	75°C
39	The sclerotia from a species of Penicillium can survive a heat treatment of ____	70 °C	90 to100 °C	50-60 °C	37 °C	90 to100 °C
40	The sodium salt of _____ acid has been used extensively as an antimicrobial agent in foods	propionic	benzoic	sorbic	acetic	benzoic
41	Fruit juice is sterilized by _____.	filteration	freezing	cooling	heating	filteration
42	Pasteurization is a _____	low temperature treatment	steaming treatment	high temperature treatment	low and high temperature treatment	high temperature treatment
43	The reddish liquid comes out from meat on thawing process is called as	drying	wilting	bleeding	leakage	bleeding
44	The spoilage organism bring about the spoilage of meat by	purification	oxidation	decomposition	hydrolysis	decomposition
45	The minimum growth temperature of Bifidobacteria range from	43 to 45	25 to 28	29 to 32	30 to 35	43 to 45
46	_____ acid is used in soft drinks such as colas	phosphoric	benzoic	acetic	sorbic	phosphoric

47	_____ freezing usually refer to freezing in air with only natural air circulation	Sharp	slow	quick	all of these	sharp
48	Jones and Loackhead found enterotoxin forming Staphylococci in _____food	frozen corn	cheese	bread	jam	frozen corn
49	_____ from retail market contain from 0 to2 million bacteria per piece	caramels	jellies	fudges	candies	candies
50	---------- is a storage method uses bins or boxes for equalization of moist	Sweating	Springer	Cooling	Freezing	Springer
51	To retard the contamination and other microbial growth in meat is obtained by storing at _____ temperature	10 °C	0°C	100°C	-10°C	0°C
52	_____organic acid is used in syrups, drinks, jam and jellies	lactic	acetic	propionic	citric	citric
53	Food preservation involves_____	increasing shelf life of food	ensuring safety for human consumption	both a and b	none of these	both a and b
54	97 to 99 % of *E.coli* in air were killed in ____ seconds with a 15 watts lamp	40	10	50	30	10
55	_____ is used as treatment for wrappers use don butter	sodium diacetate	calcium carbonate	sodium nitrate	potassium nitrite	sodium diacetate
56	_____ temperature are more lethal	high freezing	frozen storage	freezing rate	thawing	high freezing
57	About ____ percent of the suspected samples contained viable spores	20	10	30	50	10
58	Sugars act as preservatives due to their ability to	make water unavailable to organism's	interfere with the action of proteolytic enzyme	osmotic effect	both a and c	interfere with the action of proteolytic enzyme
59	_____organic acid is used in syrups, drinks,	lactic	acetic	propionic	citric	citric

	jam and jellies					
60	Sanitising is_____	Applying detergent to a clean surface	Done before washing	Reducing bacteria by application of heat or chemical	Wiping all surfaces with a clean cloth	Reducing bacteria by application of heat or chemical

Unit 3

Syllabus

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).

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

- 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.

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 in house.

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.

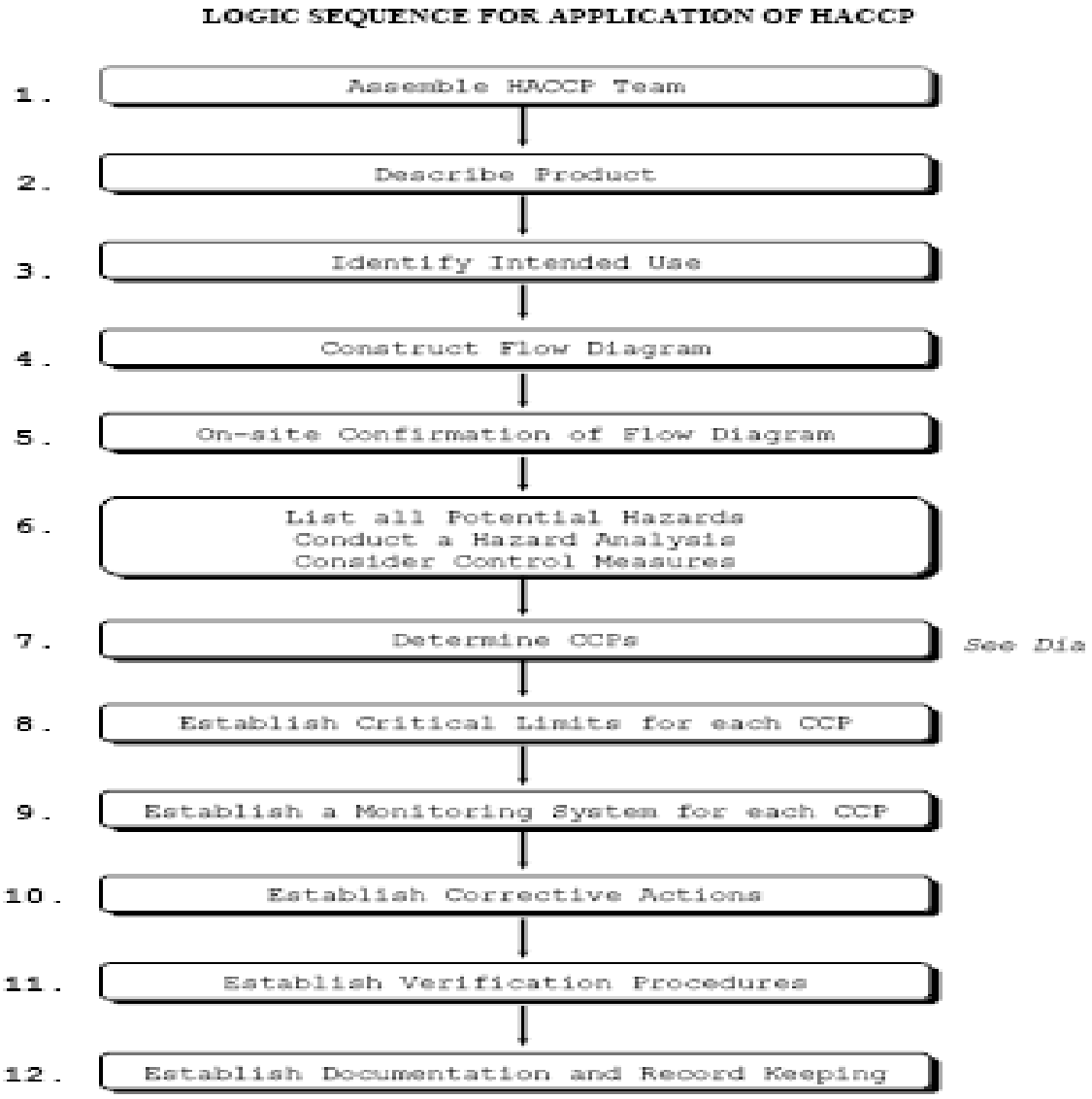
3. Identify intended use

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;

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.

Unit-III; Possible Questions

Part-A (1 Mark)

Part-B (2 Mark)

1. What is food poisoning?
2. What are the two major food-poisoning?
3. What are the seven types of neurotoxins?
4. What is toxin?
5. What are the main sources of botulism?
6. What are the symptoms of botulism?
7. What is enterotoxin?
8. What is Asiatic cholera?
9. Difference between infection and intoxication.
10. Write the food laws in our country.
11. What is food safety?
12. Give expansion for FSSAI.
13. What is AGMARK?
14. What is HACCP?
15. What is bottled drinking water?

Part-C (8 Mark)

1. What are the physiological types of bacteria are most likely to be present when canned food spoils?
2. List the types of microbes involved in spoilage of refrigerated foods with those incriminated in spoilage of canned foods.
3. List several types of food spoilage and name the organism responsible for each instance.
4. Why is milk an excellent bacteriological culture medium?
5. Describe the various types of changes brought about by the microorganisms in foods and name the organism.
6. What are the indicators of food spoilage?
7. List the symptoms of food poisoning.
8. Name the pathogens that cause food poisoning.
9. Differentiate between food poisoning and food intoxication.
10. Give the food laws prevalent in India.
11. Importance of BIS and AGMARK.
12. Functions preferred by FSSAI.
13. Write down the seven principles of HACCP.

14. Benefits of HACCP.
15. Comment on food quality and its importance.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB COURSE NAME: FOOD AND INDUSTRIAL MICROBIOLOGY

COURSE CODE: 17MBP302 UNIT: III BATCH-2017-2019

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	Which of the following toxin causing botulism is less toxic to human beings?	Type A	Type B	Type C	None of these	Type B
2	Which of the following statements are true regarding *Staphylococcus* food poisoning_____	is an enterotoxin	causes gastroenteritis	is produced by Staphylococcus aureus	All of these	All of these
3	Aflatoxin is produced by _____	*Aspergillus sp.*	*Salmonella sp.*	*Fusarium sp.*	*Streptococcal sp.*	*Aspergillus sp.*
4	Which of the following statements are regarding botulinal toxin _____	is a neurotoxin	water soluble exotoxin	is produced by Clostridium botulinum	All of these	is produced by Clostridium botulinum
5	The sore and throat symptom caused by _____ etiologic agent	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Bacillus anthrax*	*E.coli*	*Streptococcus pyogenes*
6	Botulism is caused by the presence of toxin developed by	*Clostridium tyrobutyricum*	*Clostridium sporogenes*	*Clostridium botulinum*	*none of these*	*Clostridium botulinum*
7	The control measure of foods that cause disease by *Vibrio parahaemolyticus* infection is to _____	reheat left over	sanitize equipment	control files	pastuerization	sanitize equipment
8	Salmonellois involves _____	an enterotoxin and exotoxin	an enterotoxin and cytotoxin	is produced by Staphylococcus aureus	All of these	an enterotoxin and cytotoxin
9	The term heat tolerant is a misnomer and refers to growth at ___ temperature	37 °C	40 °C	42 °C	25 °C	42 °C
10	The mold Penicillium islandicum produces _____ toxin	Luteoskyrin	aflatoxin	penicillic acid	roquefortine	Luteoskyrin
11	The major carrier of Salmonellosis are _____	meat and eggs	meat and fish	eggs and fish	eggs and fruits	meat and egs

12	Yersinia enterocolitica is a small _____ shaped bacteria	cocci	chain	rod	bacilli	rod
13	The staphylococcal intoxication refers to presence of	an enterotoxin	neurotoxin	mycotoxin	All of these	an enterotoxin
14	The FDA and USDA cooperative is a _____ surveillance program for dry milk products	*Pseudomonas*	*E. coli*	*Salmonella*	*Vibrio*	*Salmonella*
15	The application of Gamma rays destroys botulism toxin. The dose of gamma rays required for this purpose is	73 Gy	73 Rad	7.3 Mrad	173 Rad	7.3 Mrad
16	The Bacillus cereus causes gasteroenteritis by the production of an exoenterotoxin which is released in food as a result of	cell growth	cell autolysis	cell permeation	cell damage	cell autolysis
17	Nursery epidemics diarrheal disease in infants was implicated in the year _____	1950	1940	1962	1980	1940
18	Botulism is caused by _____	*Clostridium botulism*	*All Clostridium species*	*Clostridium tetanai*	*Clostridium subtilis*	*Clostridium botulinum*
19	The toxin patulin is produced by _____fungi	*Penicillium expansum*	*Fusarium*	*Aspergillus flavus*	*Mucor*	*Penicillium expansum*
20	Miller and Kolurger examined forty environmental isolates of P. shigelloides in the year _____	1987	1982	1980	1986	1986
21	Which of the following is a food infection?	Salmonellois	Botulism	Staphylococcal intoxication	None of these	Salmonellois
22	The symptoms such as nausea and dehydration is caused by _____	*Shigella sonnei*	*Yersinia*	*Arizona*	*E.coli*	*Shigella sonnei*
23	Staphylococcal intoxication is caused by	*Staphylococcus*	*S. cerevisiae*	*S. thermophillus*	none of these	*Staphylococcus*

	the toxin in the food from	*aureus*				*aureus*
24	The etiologic agent of diarrheal syndrome is ____	*Shigellosis*	*Yersiniosis*	*Bacillus cereus*	*Vibrio*	*Bacillus cereus*
25	_____ involves the identification of ingredients and products that have effect on food safety	Hazard analysis	critical control points	fishery service	research and development service	Hazard analysis
26	The term ___ is used to distinguish strains of different antigenetic complements	biovars	serovar	herbivore	none of these	serovar
27	A bacterial food intoxication refers to	illness caused by presence of pathogens	food borne illness caused by the presence of a bacterial toxin formed in food	both (a) and (b)	none of the above	food borne illness caused by the presence of a bacterial toxin formed in food
28	Salmonellois is caused by the	enterotoxin of Salmonella spp	endotoxin of Salmonella spp	neurotoxin of Salmonella spp	exoenterotoxin of Salmonella spp	endotoxin of Salmonella spp
29	Group I *C. botulinum* strains generally includes in	all types of strains (proteolytic)A, B and F	all types of strains (non-proteolytic) E and F	all types of strains (proteolytic)C, D and F	none of the above	all types of strains (proteolytic)A, B and F
30	A_____ refers to food borne illnesses caused by the entrance of bacteria into the body through ingestion of comtaminated food	Food infection	food poisoning	food intoxication	all of these	food infection
31	_____ organism can be isolated from seafoods and sea water	*Vibrio cholerae*	*Vibrio vulnificus*	*Vibrio parahaemolyticus*	All of these	*Vibrio vulnificus*
32	Botulism prevention involves_____	Proper heat sterilization before	addition of chemical	Proper low temperature	All of these	Proper heat sterilization before

		food canning	preservatives	treatment before food canning		food canning
33	Entheropathogenic Escherischia coli infection is involved in ____ foods	vegetables	apple cider	ice creams	cheese	cheese
34	The etiological agent of Arizona infection is ____	*Vibrio*	*E. coli*	*Arizona*	*Streptococcus*	*Arizona*
35	Aeroonas hydrophillia is a gram negative motile rods which are ubiquitous in ____	air	soil	water	land	water
36	The term ___ is used to distinguish strains of different antigenetic complements	biovars	serovar	herbivore	none of these	serovar
37	The method of successful treatment of botulism prior to appearance of botulism symptoms involve administration of	antibiotic	analgesic	antitoxin	antipyretic	antitoxin
38	_____ organism can be isolated from seafoods and sea water	*Vibrio cholerae*	*Vibrio vulnificus*	*Vibrio parahaemolyticus*	All of these	*Vibrio vulnificus*
39	The optimal temperature for growth of Shigellosis is _____	27 ℃	37 ℃	40 ℃	50 ℃	37 ℃
40	The FDA and USDA cooperative is a _____ surveillance program for dry milk products	*Pseudomonas*	*E. coli*	*Salmonella*	*Vibrio*	*Salmonella*
41	_____ is associated with warm blooded animals	*C. jejuni*	*C. botulinum*	*C. perferigens*	*E. coli*	*C. jejuni*
42	Human beings and animals are directly or indirectly the source of the contamination of food with _____	*Salmonella*	*Staphylococcus*	*Bacillus*	*E. coli*	*Salmonella*
43	The food and Drug Administration act was amende in the year ____	1983	1980	1989	1988	1980

44	The _____ virus enters a person through oral route in the fecal contamination of food	Poliomyelitis	Hepatitis	Adeno	Herpes	Hepatitis
45	The mode of transmission of poliomyelitis is _____	food	air	contaminated water	all of these	contaminated water
46	Clostridium perfingens poisoning is associated with_____	meat products	vegetables	canned foods	fish products	meat products
47	Clostridium perfingens poison is an _____	exotoxin	enterotoxin produced during sporulation	endotoxin	enterotoxin produced during vegetative phase	enterotoxin produced during sporulation
48	The pH near ____ favors *C. botulinum*	neutrality	alkalinity	acidic	both b and c	neutrality
49	In the early _____ numerous surveys have been conducted on the detection aflatoxins in foods	1980s	1940s	1950s	1960s	1960s
50	The optimal pH for enteropathogenic E. coli is _____	4.0 to 5.0	7.0 to 7.5	3.0 to 4.0	8.0 to 9.0	7.0 to 7.5
51	The disease gastroenterities caused by C. perfringens was first reported in the year___-	1952	1961	1978	1945	1945
52	Depending on the food and the serotype the _____ values from 0.06 to 11.3 min	D50 C	D40 c	D60 c	D30 c	D60 c
53	Pathogenecity involves the release of a _____ endotoxin which affects the intestinal mucosa	lipopolysaccharides	monosaccharides	polysaccharides	peptidoglycon	lipopolysaccharides
54	Common food poisoning microbes are_____	*Clostridium and Salmonella*	*Clostridium and E. coli*	*E. coli and Salmonella*	*Clostridium and Streptococcus*	*Clostridium and Salmonella*
55	Typhoid fever is caused by _____	*Salmonell*	*Salmonella*	*Salmonella typhi*	*Salmonella*	*Salmonella typhi*

		enteritidis	*infantis*		*typhimurium*	
56	The incubation period of Vibrio parahaemolyticus infection is _____	2-48 hrs	5-24 hrs	40 hrs	37 hrs	2-48 hrs
57	The incubation period of Streptococcus faecalis is _____	5 to 10	2 to 10	2 to 18	8 to 12	2 to 18
58	The growth of Staphylococcus aureus on solid media is usually ____ in color	red	brown	pink	yellow	yellow
59	A_____ refers to food borne illnesses caused by the entrance of bacteria into the body through ingestion of comtaminated food	Food infection	food poisoning	food intoxication	all of these	food infection
60	What is the main type of micro-organism responsible for food poisoning?	Bacteria	Mould	Virus	Parasite	Bacteria

Unit IV

Syllabus

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

History and development of Industrial Microbiology

Use of microbes to obtain a product or service of economic value constitutes industrial microbiology. Any process mediated by or involving microorganisms in which a product of economic value is obtained is called fermentation. The terms industrial microbiology and fermentation are virtually synonymous in their scope, objectives and activities. The microbial product may be microbial cells (living or dead), microbial biomass, and components of microbial cells, intracellular or extracellular enzymes or chemicals produced by the microbes utilizing the medium constituents or the provided substrate.

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		

Microbes have been employed for product generation, e.g., wines, bread, etc., since thousands of years, but these activities were purely art. The science of industrial microbiology is only about 150 years old. The first observations of microorganisms by Leeuwenhoek were published in 1677.

1. Ancient industrial microbiology:

The origin of fermentation are lost in ancient history, perhaps even in prehistory. Fermented foods such as yoghurt, cheese, soya sauce and pickled cabbage have been intentionally produced by man for centuries and in some case millennia. Beer recovered from the pyramids of the ancient Egyptians reveal that the Egyptians were able to produced the fermented brew using almost pure cultures of yeast. In Europe, allowing soil bacteria to nitrify ammonia in horse urine produced nitrates required for gun powder manufacture. The Aztecs of Mexico cultured *Spirulina* for both waste treatment purposes and as a source of protein. In the 20th century, biotechnologists have tried again to produce single celled protein to solve the protein shortage of the world.

2. Industrial Microbiology: Modern era

The father of modern fermentation technology is however Louis Pasteur. This 19th century scientist discovered or perhaps rediscovered that the conservation of sugar to alcohol (in beer and wine production) and the conversion of sugar lactic acid (as occurs in cheese production) were both microbiological conversions and both needed a microbial seed to start them,. In this process, Pasteur disproved the theory of spontaneous generation, discovered that some microbes can grow in the absence of oxygen, and discovered a new way of sterilizing materials, which was subsequently called pasteurization. His discoveries allowed Fresh producers to dramatically improve the quality of two things, which the world still; Fresh wine and French cheese.

Following Pasteur, microbiology and fermentation technology grew hand in hand. The research of the 19th and early 20th century microbiologists such as Winogradsky and Beijerinck have lead the other scientists to isolate new and potentially useful microbes, the development of processes for growing them and new analytical methods.

Wildiers demonstrated in 1901 that yeast required growth factors (vitamins) for growth, especially at low inoculums level; vitamins are used in fermentation even today. In 1929, Alexander Fleming accidentally discovered penicillin produced by *Penicillium* growing as contaminant in a Petri plate of *Staphylococcus*. Fleming developed the technique for assay of antibacterial activity of penicillin using bacteria and showed its low toxicity to man and animals. This was followed by an intensive search for antibiotics during the Second World War leading to the discovery of streptomycin, chloramphenicol, tetracyclines, etc. Later developments have resulted in the use of metabolically blocked mutants of microorganisms, which accumulate large amounts of metabolic intermediates. This was followed by an intensive search for antibiotics during the Second World War leading to the discovery of streptomycin, chloramphenicol, tetracyclines, etc. Later developments have resulted in the use of metabolically blocked mutants of microorganisms, which accumulate large amounts of metabolic intermediates.

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	

3. Fermentation technologies and the world war:

By 1914, Weizmann isolated *Clostridium acetobutylicum*, a bacterium that used inexpensive starch to produce high yield of butyl alcohol and acetone, which is in the production of synthetic rubber. However, World War I broke out in August 1914 and diverted attention away from synthetic rubber towards gunpowder (cordite). As acetone turns out into a solvent for making nitrocellulose and this cordite was acetone. Weizmann was instrumental in making available a source for the creation of acetone and was recruited by Winston Churchill and the British government to set up his microbial fermentation for the production of acetone from corn in London.

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

4. Industrial Microbiology: Present scenario:

The world wars and their aftermath of the 20th Century continued to spur on developments in fermentation technology. Material shortages forced countries to look for biological sources of chemical feed stocks. Scientists found ways of producing or enhancing the production of ethanol and solvents such as acetone and butanol by fermentation. Antibiotic production was also scaled up to meet growing needs derived from diseases and injuries. The designs and technologies developed for improving antibiotic production found application in many other products such as amino acids (e.g. lysine and glutamic acid) and food chemicals e.g. gluconic acid and citric acid. The engineers and the scientist now had to work hand in hand to achieve these improvements. The discipline that we now call Biotechnology was being conceived.

In more recent times, threats of diseases, environmental disasters and food shortages has imposed tremendous demand in developed countries for new opportunities in fermentation technology. Today, the fermentation industry is a multi-million dollar industry. The fermentation skills and knowledge are now essential driving force for systematic research into drug/receptor interactions, function of membrane proteins in health and disease, and are powering an unparalleled expansion in our capability to combat serious diseases in the human population, including cancers, degenerative illnesses such as Alzheimer's, and increasingly common complaints of developed societies such as asthma. The new fermentation-derived medicines, including biopharmaceuticals, hold out the prospect of improved specificity of treatment, and decreased side effects. It is truly a revolutionary period in clinical medicine as these new agents manufactured by fermentation routes enter the market. The new fermentation products, therapeutic proteins, antibodies (simple and conjugated) are more cheaper and simple than previous products, and the need to focus upon the fermentation step is now clearer than ever. Basically, the 'quality' of these products (the potency, efficacy, stability and immunogenicity) is determined by the upstream or fermentation stage, so the need for a clear understanding of what happens in that stage, how it can best be monitored, controlled, and carried out in a reproducible fashion, is greater than ever.

Industrial microbiology is a very fast growing area of biotechnology, with ever increasing process and products. With a longer history than any area of biological sciences, industrial microbiology has a longer and brighter future, in the service of mankind, covering important areas of food, agriculture and medicine.

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]

Some important products of fermentation are discussed below:

Microbial biomass: Microbial biomass is produced commercially as single cell protein (SCP) using unicellular algae as species of *Chlorella* or *Spirulina* for human or animal consumption, or viable yeast cells needed for the baking industry.

Primary metabolites: During the log phase or exponential phase microorganisms produce a variety of substances essential for their growth, such as nucleotides, amino acids, lipids, carbohydrates etc or by-products of energy yielding metabolism such as ethanol, acetone, butanol, etc. These products are usually called as primary metabolites and the phase at which it is produced is termed as trophase.

Examples:

Product	Organism	Industrial importance
Citric acid	*Aspergillus niger*	Solvent
Lysine	*Corynebacterium glutamicum*	Nutritional additive
Glutamic acid		Flavour enhancer
Acetone and butanol	*Clostridium acetobutylicum*	Food industry
Riboflavin	*Ashbya gossypii*	Nutritional
Vit B_{12}	*Pseudomonas denitrificans*	

Secondary metabolites: The phase during which products are produced have ni obvious role in the metabolism of the organism and this phase is called as idiophase. The products produced in this phase are called secondary metabolites. Many secondary metabolites are produced as an end product of primary metabolism.

Examples:

Product	Organism	Industrial importance
Penicillin	*P. chrysogenum*	Antibiotics
Steptomycin	*Streptomyces greseus*	
Erythromycin	*Streptomyces erythreus*	
Gresofulvin	*P. griseofulvin*	
Gibberelin	*Gibberella fujikuroi*	Growth hormone

Traditional products

Bread, beer, wine and spirits - *Saccharomyces cerevisiae*
Cheeses, other dairy products - Lactic acid bacteria
Ripening of blue and Camembert-type cheeses - *Penicillium* species
Fermented meats and vegetables - Lactic acid bacteria
Mushrooms - *Agaricus bisporus, Lentinula edodes*
Soy sauce *Aspergillus oryzae, Zygosaccharomyces rouxii*
Sufu (soya bean curd) - *Mucor* species
Vinegar - *Acetobacter* species

Agricultural products

Gibberellins - *Fusarium moniliforme*
Fungicides - *Coniothyrium minitans*
Insecticides - *Bacillus thuringiensis*
Silage - Lactic acid bacteria

Amino acids

l-Tryptophan - *Klebsiella aerogenes*
l-Glutamine - *Corynebacterium glutamicum*
l-Lysine - *Brevibacterium lactofermentum*

Enzymes

α-amylase - *Bacillus subtilis*
β-amylase - *Aspergillus niger*
Amyloglucosidase - *Aspergillus niger*
Glucose isomerase - *Streptomyces olivaceus*
Invertase - *Kluyveromyces* species
Lactase (b-galactosidase) - *Kluyveromyces lactis*
Cellulases - *Trichoderma viride*
Lipases - *Candida cylindraceae*
Pectinases - *Aspergillus wentii*

Fuels and chemical feedstocks

Acetone - *Clostridium* species
Butanol - *Clostridium acetobutylicum*
Ethanol - *Zymomonas mobilis S.cerevisiae*
Glycerol - *Zygosaccharomyces rouxii*
Methane - Methanogenic archaeans

Organic acids

Acetic acid - *Acetobacter xylinum*
Citric acid – *A.niger, Yarrowia lipolytica*
Fumaric acid -*Rhizopus* species
Gluconic acid -*Acetobacter suboxydans*
Itaconic acid -*Aspergillus itaconicus*
Kojic acid -*Aspergillus flavus*
Lactic acid -*Lactobacillus delbrueckii*

Vitamins

Vit B_{12} (cyanocobalamin) - *Pseudomonas denitrificans*
β-Carotene (provitamin A) - *Blakeslea trispora*
Vit C - *Acetobacter suboxydans*
Riboflavin - Recombinant *B.subtilis, Ashbya gossypii*

Pharmaceuticals and related compounds &Hormones

Human growth hormone - Recombinant *Escherichia coli,* Recombinant *Saccharomyces cerevisiae*
Insulin - Recombinant *Escherichia coli,* Recombinant *Saccharomyces cerevisiae*
Cyclosporin - *Trichoderma polysporum*
Interferon - Recombinant *Escherichia coli,* Recombinant *Saccharomyces cerevisiae*
Steroids - *Arthrobacter* species *Rhizopus* species
Vaccines - *Bacillus anthracis, Clostridium tetani,* Recombinant *Escherichia coli, Salmonella typhi*

Polymers

Alginates - *Azotobacter vinelandii*
Cellulose - *Acetobacter xylinum*
Dextran - *Leuconostoc mesenteroides*
Gellan - *Sphingomonas paucimobilis*
Polyhydroxybutyrate - *Ralstonia eutropha*

Single cell protein

Methylococcus capsulatus
Candida utilis
Paecilomyces variotii
Saccharomyces cerevisiae
Fusarium venenatum

Pullulan - *Aureobasidium pullulans*
Scleroglucan - *Sclerotium rolfsii*
Xanthan - *Xanthomonas campestris*
Methylophilus methylotrophus
Kluyveromyces marxianus

Isolation and Screening of Microorganisms

The success of an industrial fermentation process chiefly depends on the microorganism strain used. An ideal producer or economically important strain should have the following characteristics.

1. It should be pure, and free from phage
2. It should be genetically stable, but amenable to genetic modification
3. It should produce both vegetative cells and spores; species producing only mycelium are rarely used
4. It should grow vigorously after inoculation in seed stage vessels
5. Should produce a single valuable product, and no toxic by-products
6. Product should be produced in a short time, e.g., 3 days
7. It should be amenable to long term conservation
8. The risk of contamination should be minimal under the optimum performance conditions

Isolation of Microorganisms

The first step in developing a producer strain is the isolation of concerned microorganisms from their natural habitats. Strategies that are adopted for the isolation of suitable industrial microorganisms from the environment can be divided into 2 types (i) shotgun and (ii) objective.

In shotgun approach, samples of free living microorganisms, biofilms and other microbial communities are collected from animal and plant material, soil, sewage, water and waste stream, particularly from unusual man-mad and natural habitats. These isolates are screened for desirable traits. The alternative is to take a more objective approach by sampling from specific sites where organisms with the desired characteristics are considered to be likely components of the natural microflora.

Alternatively, microorganisms can be obtained as pure cultures from organization, which maintain culture collections.

Culture collection	**Type of microorganisms held**
American Type Culture Collection (ATCC)	All
European Collection of Animal Cell Collection (ECACC)	Animal cell cultures
National Collection of Food Bacteria (NCFB)	Food bacteria
National Collection of Industrial and Marine Bacteria (NCIMB)	Industrial and Marine bacteria
National Collection of Type Cultures (NCTC)	Medical microorganisms
National Collection of Yeast Cultures (NCYC)	Yeast
National Collection of Pathogenic Fungi (NCPF)	Pathogenic fungi
National Collection of Plant Pathogenic Bacteria (NCPF)	Plant pathogenic bacteria
Culture Collection of Algae and Protozoa (Marine) (CCAP)	Algae and Protozoa (Marine)
Culture Collection of Algae and Protozoa (Fresh water) (CCAP)	Algae and Protozoa (Fresh water)

The microorganisms of industrial importance are, generally, bacteria, actinomycetes, fungi and algae. These organisms occur virtually everywhere, e.g., in air, water, soil, surfaces of plants and animals, and plant and animals tissues. But most common sources of industrial microorganisms are soils, and lake and

river mud. Often the ecological habitat from which a desired microorganism is more likely to be isolated will depend on the characteristics of the product desired from it, and of process development. For example, if the objective is to isolate a source of enzymes, which can withstand high temperatures, the obvious place to look will be hot water springs.

A variety of complex isolation procedures have been developed, but no single method can reveal all the microorganisms present in a sample. Many different microorganisms can be isolated by using specialized enrichment techniques, e.g., soil treatment (UV irradiation, air drying or heating at 70 – 120 °C, filtration or continuous percolation, washings from root systems, treatment with detergents or alcohols, pre-inoculation with toxic agents), selective inhibitors (antimetabolites, antibiotics, etc.), nutritional (specific C and N sources), variations in pH, temperature, aeration, etc.
The enrichment techniques are designed for selective multiplication of only some of the microorganisms present in a sample. These approaches however take a long time (20 - 40 days), and require considerable labour and money. The main isolation methods used routinely for isolation from soil samples are: sponging (soil directly), dilution, gradient plate, aerosol dilution, flotation, and differential centrifugation. Often these methods are used in conjunction with an enrichment technique. Microorganisms are used extensively to provide a vast range of products and services. They have proved to be particularly useful because of the ease of their mass cultivation, speed of growth, use of cheap substrates (which in many cases are wastes) and the diversity of potential products.

The most important factor for the success of any fermentation industry is of a production strain. It is highly desirable to use a production strain with the following four characteristics:

I. It should be a high-yielding strain
II. It should have all adoptable characteristics
III. It should not produce undesirable substances
IV. It should be easily cultivated on a large-scale

Screening techniques

Both detection and isolation of high-yielding species from the natural, such as soil, containing a huge microbial population is called screening. The screening process is generally carried out in two stages: (a) primary screening and (b) secondary screening.

(a) Primary Screening

A set of highly selective procedures, which allows the detection and isolation of microorganisms producing the desired metabolite, constitutes primary screening. The process is time consuming and labour intensive since a large number of isolates have to be screened to identify a few potential ones. However, it is most crucial steep since it eliminates the large number of unwanted isolates, which are either non-producers or producers f known compounds.

(i) Crowded plate technique

The crowded plate technique is the simplest screening technique employed in detecting and isolating antibiotic producing microorganisms.

The soil or other source of microorganisms is diluted only to a cell concentration such that agar plats prepared from these dilutions will be crowded with individual colonies. Colonies producing antibiotic activity a indicated by an area of agar sound the colony that is f of growth of the colonies. Such a colony is sub cultured to a similar medium and purified by staking. This technique is used when we are only interested in finding microorganisms capable of producing an antibiotic without regard to what type of microorganisms may b sensitive to antibiotic.

The crowded plate technique has a limited application, since it merely provides information regarding the inhibitory activity of colony against the unwanted microbes that may be resent by chance on the plate. Therefore the technique has been improved upon by introducing the use of a 'test-organism'. In this process, modified agar plates which give well-isolated colonies after incubation are flooded with a suspension of the test organism. Then the plates are subjected to further incubation to allow the growth of the test organism. The formation of inhibitory zones around certain colonies indicates their antibiotic activity.

(ii) Auxanography technique

This technique is rarely applied for detecting microorganisms able to produce growth factors (e.g. amino acids and vitamins) extracellularly. The two major steps of the technique are as under:

(a) Preparation of First Plate

1. A filter paper strip (15 X 12 cm) is put across the bottom of a petri dish in such a way that the ends pass over the edge of the dish
2. A filter, paper disc of petri dish size is placed over paper strip on the bottom of the dish
3. The nutrient agar (45 °C) is poured on the paper disc in the dish and allowed to solidify
4. Microbial source material such as soil, is subjected to dilution such that aliquots on plating will produce well isolated colonies
5. Plating of aliquots diluted soil sample is done

(b) Preparation of Second Plate

1. A minimal medium lacking the growth factor under consideration is seeded with the test organism
2. Tile seeded medium, is poured on the surface of a fresh petri dish
3. The plate is allowed to set

The agar in the first plate, as prepared in step (a), is carefully and aseptically lifted out with the help of tweezers and a spatula and, placed, without inverting, on the surface of the second plate as prepared in major steps (b). Growth factor produced by colonies present on the surface of the first layer of agar can diffuse into the layer of agar containing the test organism. The stimulated growth, of the test organism around the colonies is an indication that they produce growth factor(s) extracellularly.

(iii) Enriched culture technique

This technique was designed by a soil microbiologist, Martinus Beijerinck, to isolate the desired microorganisms from a heterogeneous microbial population present in soil. Either medium or incubation conditions are adjusted so as to favour the growth of the desired microorganisms. On the other hand, unwanted micro-organisms are eliminated, or develop poorly since they do not find suitable growth

conditions in the newly created environment. Today, this technique has become a valuable tool in many screening programmes meant for isolating industrially important strains.

Generally, it consists of the following steps:

1. Nutrient broth containing an unusual substrate (e.g. Cellulose powder) is inoculated with the microbial source material (e.g. soil) and incubated.
2. A small portion of inoculums from step (1) is plated onto a solid medium having the same position. Well isolated colonies appear after incubation.
3. Suspected from plate of step (2) are subcultured on fresh media and they are also subjected to further testing.

An example of screening of enzyme, producing microorganisms may be cited.

Microorganisms excreting alkaline proteases may be detected from the soil as under:

- Selected to serial dilution.
- All soil dilutions are heated at 50 °C for 10 minutes. This treatment kills vegetative cells ,
- The plating of heat treated sample is done by spreading the samples (usually 0·1 ml.) from dilutions on the surface of nutrient agar containing casein at pH 10-12.
- The colonies surrounded by the clear zone are sub-cultured.

(iv) Differential plate technique

The pH indicating dyes may be employed in some screening methods for detecting microorganisms capable of producing organic acids or amines, since a pH indicating dye under colour changes according to its pH. Such dyes (e.g. neutral red, bromophenol blue) are added to the poorly buffered nutrient agar media. The change in the colour of a dye in the vicinity of the colony suggests the capability of colonial cells to produce anionic acids or amines, depending upon the nature of reaction. Such colonies are sub cultured to make stock cultures. Differential plate technique may also be employed in finding out whether microorganisms are capable of certain microbial transformations or not.

The presence of organic acids are also detected by the incorporation of calcium carbonate; indicated by a clear one of dissolved calcium carbonate around the colony.

(b) Secondary Screening

Secondary screening is strictly essential in any systematic screening process intended to isolate industrially useful microorganisms, since primary screening merely allows the detection and isolation of microorganisms that possess potentially interesting industrial application. Moreover, primary screening does not provide much information needed in setting up a new fermentation process. Secondary screening helps in detecting really useful microorganisms in fermentation processes:

The following points associated with the importance of secondary screening

- Provides information whether the product produced by a microorganism is a new one or not which accomplished thin layer, other chromatographic techniques.
- The compound produced by a microorganism under consideration is compared with previously known compounds.

- It gives an idea about the economic position of the fermentation process involving the use of a newly discovered culture. Thus, one may have a comparative study of this process with processes that are already known, so far as the economic status picture is concerned.
- It helps in providing information regarding the product yield potentials of different isolates Thus, this is useful in selecting efficient cultures for the fermentation processes.
- It determines the optimum conditions for growth or accumulation of a product associated with a particular culture.
- It provides information pertaining to the effect of different components of a medium. This is valuable in design in the medium that may be attractive so far as economic consideration is concerned.
- It detects the genetic instability in microbial cultures. This type of information is very important, since microorganisms tending to undergo mutation or alteration in some way may lose their capability for maximum accumulation of the fermentation products.
- It gives information about the number of products produced in a single fermentation additional major or minor products are of distinct value, since their recovery and sale as by-products can markedly improve the economic status of the prime fermentation.
- Chemical, physical and biological properties of a product are also determined during secondary screening. Moreover, it reveals whether a product produced in the culture broth occurs in more than one chemical form.
- It reveals whether the culture is homoferrnentative or hetero fermentative.
- Determination of the structure of the product is done.
- With certain types of products (e.g. antibiotics) determinations of the toxicity for animal, plant and human are made if they are to be therapeutic purposes.

Thus, secondary screening gives answers to many questions that arise during the final sorting out of industrially useful micro-organisms. This is accomplished by performing experiments on **agar plates**, in flasks or small bioreactors containing **liquid media**, or by a combination of these approaches.

A specific example of antibiotic producing *Streptomyces* species may be taken for an understanding of the sequence of events during a screening programme. Those streptomycetes able to produce antibiotics are detected and isolated in a primary screening programme. These streptomycetes exhibiting antimicrobial activity are subjected to an initial secondary screening where their inhibition spectra are determined. A simple, giant-colony', technique is used to do this. Each of the streptomycal isolates is streaked in a narrow band across the centres of the nutritious agar plates. Then, these plates are incubated until growth of a streptomycete occurs. Now, the test organisms are streaked from the edges of the plates but not touching the streptomycete growth. Again, the plates are incubated. At the end of incubation, growth inhibitory zones for each test organism are measured in millimeters. Thus, the microbial inhibition spectrum helps in discarding poor cultures. Ultimately, streptomycete isolates that have exhibited interesting microbial inhibition spectra need further testing. With streptomycetes suspected to produce antibiotics with poor solubility in water, the initial secondary screening is done/in some different way which is out of the scope of this book.

Further screening is carried out employing liquid media in flasks since, such studies give more information than that which can be obtained on agar media. At the same time, it is advisable to use accurate

assay techniques (e.g. paper disc-agar diffusion assay) to exactly determine the amounts of antibiotic present in samples of culture fluids. Thus each of the streptomycete isolates is studied by using several different liquid media in Erlenmeyer flasks provided with baffles. These streptomycete cultures are inoculated into sterilized liquid media. Then, such seeded flasks are incubated at a constant temperature. Usually, such cultures are incubated at near room temperature. Moreover, such flasks are aerated by keeping them on a mechanical shaker, since the growth of streptomycetes and production of antibiotics occur better in aerated flasks than in stationary ones. Samples are withdrawn at regular intervals under aseptic conditions and are tested in a quality control laborator. Important tests to be carried out include:

- Checking for contamination
- Checking of pH
- Estimation of critical nutrients
- Assaying of the antibiotic
- Other determinations, if necessary

Preservation of Microorganisms

There are different methods for microbial preservation. Suitable methods are selected based on:

- Type of microorganism,
- Effect of the preservation method on the viability of the microorganism,
- Frequency at which the cultures are withdrawn,
- Size of the microbial population to be preserved,
- Availability of resources, and
- Cost of the preservation method.

Many methods of preservation for microorganisms have been developed. Here, it is to be noted that there exist different types of micro-organisms (bacteria, viruses, algae, protozoa, yeasts and molds).

The best known of these are the American Type Culture Collection (A.T.C.C.), founded in 1925 and the collection of the Common wealth Mycological Institute (C.M.I.), founded in 1947. Several other countries are developing their own national collections, and there are large collections belonging to industrial concerns as well as specialized government departments.

There are three basic aims in maintaining and preserving the micro-organisms. They are:

(i) To keep cultures alive

(ii) Uncontaminated, and

(iii) As healthy as possible, both physically and physiologically, preserving their original properties until they are deposited in any major collections (i.e. unchanged in their properties).

- **Serial Subculture:**

This is the simplest and most common method of maintaining microbial cultures. Microbes are grown on slants and are transferred to fresh media before they exhaust all the nutrients or dry out. The

drying-up of the medium appeared to encourage good sporulation and the preserved specimen became simply a dried out strand of agar coated with spores which remained viable for a few years at room temperature. For some microbial cultures, no other methods have been found satisfactory, but for the majority of species other methods are available.

There are several factors to be borne in mind while choosing a suitable medium. Solid media should be chosen in preference to liquid media, as growth of a contaminant can be more readily observed.

The time period appropriate for subculture may range from a week to even a few years Under normal conditions cultures have to be re-grown at fairly frequent intervals (e.g. every four, six or eight weeks). With a large collection, this requires much labour. Moreover, there is a risk of occurring hazards, every time a culture is handled. To cut down the frequency of handling of the cultures, it is, therefore desirable to prolong the intervals between subculturing. There are various means to accomplish this (e.g. cold storage and mineral oil storage).

- **Preservation by overlaying cultures with oil:**

This method of preservation is a modification of serial subculture technique. It was first extensively used by Buell and Weston (1947).

This method is cheap and easy, since it does not require special skills or apparatus such as a centri fuge, dessiccator, or vacuum pump. The steps involved in this method are:

i. First of all, inoculation of the agar slant contained in a screw-cap tube with a given culture is practised.
ii. Inoculated agar slant is subjected to incubation until good growth appears.
iii. Using sterial technique, a healthy agar slant culture (from above step) is covered with sterile mineral oil to a depth of about 1 cm above the top of the agar slant. If a short slant of agar is used, less oil is required.
iv. Finally, oiled culture from step (iii) can be stored at room temperature. But better viability is obtained when stored at lower temperatures.

The oil used should be of good quality. British pharmacopoeia medicinal paraffin oil of specific gravity 0·830 to 0·890 is quite satisfactory. Sterilization of oil at the C.M.I is done in McCartney bottles for 15 minutes at 15Ib/in^2

The covering of the culture with oil prevents drying out. The oil allows slow diffusion of gases so growth continues at a reduced rate. This may induce change due to adaption to growth in oil. Some fungus isolates appear stable and survivals of over many years have been obtained at the C.M.I. Others change rapidly, producing a typical culture in a few months (e.g. *Fusarium* species). If the McCartney bottles are used the rubber liners should be removed from the metal caps as the oil tends to dissolve the rubber and this can be toxic to the cultures.

This method has the following advantages:

i. Practically all bacterial species or strains tested live longer under oil than in the control tubes without oil. Some bacterial species have been preserved satisfactorily for many years.

ii. Transplants may be prepared when desired without affecting the preservation of the stock cultures.
iii. The method is especially advantageous when working with unstable variants where occasional transfers to fresh media or growth in mass cultures results in changes in the developmental stages of the strains.
iv. This method also appears to be an ideal method of storage for a busy laboratory with limited funds and a relatively small collection.

- **Lyophilization or freeze-drying:**

Lyophilization is the most satisfactory method of long-term preservation of microorganisms. It is universally used for the preservation of bacteria, viruses, fungi, sera, toxins, enzymes and other biological materials. The process of lyophilization was first applied to microfungi on a large scale by Raper and Alexander in 1945. Lyophilization is perhaps the most popular form of suspended metabolism. It consists of drying cultures or a spore suspension from the frozen state under reduced pressure.

Major steps involved in this technique are:

(i) A cell or spore suspension is prepared in a suitable protective medium
(ii) Using a sterile technique, the suspension from (i) is distributed in small quantities into glass ampoules.
(iii) The ampoules are connected with a high vacuum system usually incorporating a desiccant (e.g. phosphorous pentoxide, silica gel or a freezing trap), and immersed into a freezing mixture of dry ice and alcohol (-70 to -78 °C).
(iv) The vacuum pump is turned on and the ampoules are evacuated till drying is complete, after which they may be sealed off.

Factors affecting the viability of freeze-dried cultures include:
(i) Chemical composition of the protective (suspending) medium;
(ii) Addition of certain compounds to the culture suspension before freeze-drying to give protection to the culture against the toxicity exerted by moisture and oxygen when stored in unsealed ampoules;
(iii) Sealing the ampoules after freeze-drying to stop access of oxygen and moisture;
(iv) Insufficient elimination of oxygen and moisture on the survival rate of freeze-dried culture;
(v) Storage temperature of freeze-dried and sealed cultures.

This method possesses the following advantages:
i. As the ampoules are sealed there is no risk of contamination or infection with mites.
ii. The prepared ampoules are easily stored, they are not readily broken and most species
iii. There is less opportunity for cultures to undergo changes in characteristics (i.e. they remain unchanged during storage period).
iv. Owing to the small size of glass ampoules, hundreds of lyophilized cultures can be stored in a small storage space. In addition to this, the ampoules' small size makes them ideal for postage. It remains viable for many years (more than 20 years in case of many bacterial species).

Checking of viability may be done at long intervals. This may be done when cultures are required or by routine sampling. Usually, many replicates are made so that the material from a constant source can be supplied over a considerable period. It makes the 'Seed Stock' system possible, which is used at the American Type Culture Collection for conserving living reference microbes over long periods.

- **Storage at very low temperatures or Nitrogen Storage**

This method is also called *cryogenic storage*. It is like lyophilization, a satisfactory method for the long-term preservation of microorganisms. The maintenance of microbes is done by suspended metabolism.

Major steps involved in the performance of this method are:

i) The culture is suspended as a cell or a spore suspension, as finely broken-up particles of mycelium, or as a piece of fungus mycelium in a suitable suspending medium (dimethyl sulfoxide / glycerol).

(ii) The suspension as prepared in step (i) is distributed into ampoules (These must be resistant to cold-shock). The manufacturers of ampoules warn us that they must not be overfilled (less than 0·5 ml, being recommended).

(iii) Ampoules filled with a culture suspension are frozen and are hermetically sealed. Freezing can be attained by plunging the ampoules straight into the liquid nitrogen by suspending them over the liquid nitrogen for a short period and then lowering them into the liquid nitrogen, or by controlled cooling. There are many interacting factors concerning the choice of the method of freezing and it will depend largely on the microorganisms to be frozen, the degree of revival required and the apparatus available.

(iv) The frozen ampoules prepared as in (iii) are usually clipped on metal (aluminium) canes, one above the other and six to each cane. The canes, in turn, are packed in metal boxes or canisters (aluminium), which hold about 20 canes. These are perforated to allow the free running of the liquid nitrogen. The cultures are revived by removing from the container, rapidly thawing and culturing them in the usual way.

The method has the following advantages:

I. It is an effective method of preservation
II. No subculturing is required
III. The cultural characteristics remain unchanged. This could be of particular importance while storing highly specialized strains employed in industrial processes
IV. The ampoules are not open to contamination or infection by mites, since they are sealed
V. The living material of a type which would not normally grow in a culture and would not be preserved in a culture collection can be retained in a viable state
VI. The method has also some disadvantages. They are:
    - The method is expensive, since a costly apparatus is required
    - A reliable supply of nitrogen is needed
    - There is a possibility of a minor accident, since the method involves the use of explosive gas (the liquid nitrogen)

Other Methods or Storage for Fungi

Every laboratory cannot afford lyophilization or liquid nitrogen storage. Many technicians lack the time for the periodic transfer to fresh media and they would find oil storage messy. Indeed, in these days, when the safety of the personnel is the first consideration, the spatter made by oily needles when they are sterilized is undesirable. Therefore, various other methods of preservations nave been developed.

- **Soil cultures:** This method is particularly applied for the preservation of sporing microbes (e.g. *Bacillus, Penicillium, Aspergillus* and *Streptomyces).*

Steps involved in this method are listed:

(i) A spore suspension is first prepared which may involve the use of a special medium.

(ii) A mixture of soil (20 %), sand (78 %) and calcium carbonate (2 %) is prepared and distributed into tubes (a few grams per tube). They are sterilized for 8 to 15 hours at 130 °C and then cooled.

(iii) A spore suspension is added to the sterilized loam as prepared in step (ii) and allowed to grow for about 10 days.

(iv) The inoculated tubes as in step (iii) are kept in desiccators under vacuum. The reason behind this is to evaporate the excess water. Then the tubes are sealed.

(v) The culture tubes are stored in a refrigerator at about 5 to 8 °C.

- **Desiccation**

This involves removal of water from the culture. Desiccation is used to preserve actinomycetes **(**a form of fungi-like bacteria) for very long period of time. The microorganisms can be preserved by desiccating on sand, silica gel, or paper strips.

Strain Improvement

After an organism producing a valuable product is identified, it becomes necessary to increase the product yield from fermentation to minimise production costs. Product yields can be increased by

(i) Developing a suitable medium for fermentation,

(ii) Refining the fermentation process and

(iii) Improving the productivity of the strain

Generally, major improvements arise from the last approach; therefore, all fermentation enterprises place a considerable emphasis on this activity. The techniques and approaches used to genetically modify strains, to increase the production of the desired product are called strain improvement or strain development. Strain improvement is based on the following three approaches:

(i) Protoplast fusion,

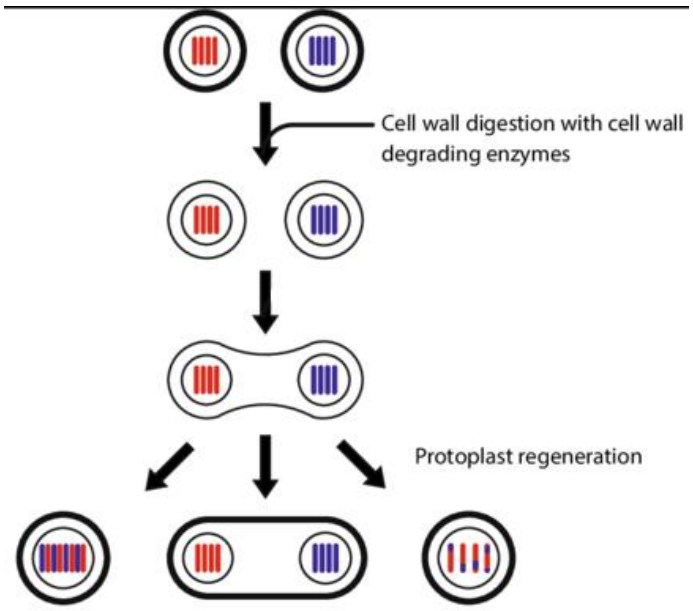
(ii) Mutation,

(iii) Recombinant DNA technology

(i) Protoplast fusion

Protoplasts are the cells of which cell walls are removed and cytoplasmic membrane is the outermost layer in such cells. Protoplast can be obtained by specific lytic enzymes (pectinase, cellulase – plants; lysozyme - bacteria; Novozyme 234 - fungi) to remove cell wall. Protoplast fusion is a physical phenomenon, during fusion, two or more protoplasts come in contact and adhere with one another either spontaneously or in presence of fusion inducing agents. By protoplast fusion it is possible to transfer some useful genes such as disease resistance, nitrogen fixation, rapid growth rate, more product formation rate, protein quality, frost hardiness, drought resistance, herbicide resistance, heat and cold resistance from one species to another.

Protoplast formation in Sterptomyces was first reported by Okanishi and his team in the year 1966. Protoplast fusion has been achieved using filamentous molds, yeasts, Streptomycetes and bacteria.



Methods of protoplast fusion:

Protoplast fusion can be broadly classified into two categories:

(i) Spontaneous fusion: Protoplast during isolation often fuses spontaneously and this phenomenon is called spontaneous fusion. During the enzyme treatment, protoplast from adjoining cells fuses through their plasmodesmata to form multinucleate protoplasts.

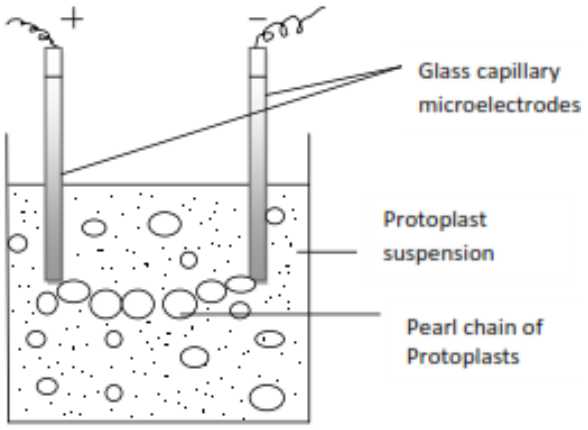
(ii) Induced fusion: Fusion of freely isolated protoplasts from different sources with the help of fusion inducing chemicals agents is known as induced fusion. Normally isolated protoplast do not fuse with each other because the surface of isolated protoplast carries negative charges (-10 mV to -30 mV) around the outside of the plasma membrane, and thus there is a strong tendency in the protoplast to repel each other due to their same charges. So this type of fusion needs a fusion inducing chemicals which actually reduce the electronegativity of the isolated protoplast and allow them to fuse with each others.

The isolated protoplast can be induced to fuse by three ways;

(i) Mechanical fusion: In this process the isolated protoplast are brought into intimate physical contact mechanically under microscope using micromanipulator or perfusion micropipette.

(ii) Chemofusion: Several chemicals have been used to induce protoplast fusion such as sodium nitrate, polyethylene glycol (PEG) and calcium ions (Ca^{2+}). Chemical fusogens cause the isolated protoplast to adhere each other and leads to tight agglutination followed by fusion of protoplast. Chemofusion is a non specific, inexpensive, can cause massive fusion product, can be cytotoxic and non selective and having less fusion frequency.

(iii) Electrofusion: Recently, mild electric stimulation is being used to fuse protoplast. In this two glass capillary microelectrode are placed in contact with the protoplast. An electric field of low strength (10 Kvm^{-1}) gives rise to dielectrophoretic dipole generation within the protoplast suspension. This leads to pearl chain arrangement of protoplasts. Subsequent application of high strength of electric fields (100 Kvm^{-1}) for some microseconds results in electric breakdown of membrane and subsequent fusion.



Protoplast fusion in fungi: Production and regeneration of protoplasts is a useful technique for fungal transformations. Commercial preparation of enzymes which contain mixture of products to digest fungal cell walls used. Novozyme 234 includes enzyme (glucanase and chitinase) mixture is added to rapidly growing fungal tissue suspended in an osmotic buffer (e.g.0.6 mol^{-1} KCl, 1.2 mol^{-1} Sorbitol or 1.2 mol^{-1} $MgSO_4$). The protoplasts and DNA are mixed in presence of 15 % (w/v) PEG 6000 and pH buffer (TRIS HCl). 10 mml^{-1} PEG causes clump formation in protoplasts. At 370 C, grow mycelium on cellophane placed on agar overnight. Incubate with enzyme at 300 C for 1.5 hours in empty Petri plate having KCl, than filter protoplasts, wash protoplast in KCl (Centrifuge and resuspended the pellets). Protoplast fusion frequency in fungi is 0.2 – 2 %.

Protoplast technology for *Streptomyces* species: *Streptomyces* spp also do not have natural means of mating. For obtaining protoplasts from *Streptomyces* lysozyme is used which can break glycan portion of peptidoglycan wall.

Protoplast fusion in bacteria: In bacteria protoplast can be obtained and fusion can be carried out with low frequency in some gram positive organisms. For gram negative bacteria it is possible to obtain protoplast but regeneration is difficult. The procedure is highly efficient and yields up to 80% transformants

(ii) Mutation

Each time a microbial cell divides there is a small probability of an inheritable change occurring. A strain exhibiting such a changed characteristic is termed a mutant and the process giving rise to it, a mutation.

A mutation without a known cause is called Spontaneous mutations. This occurs at low frequency leading to the chemical instability of purine and pyrimidine bases and also due to low level of metabolic errors, or mistakes during the DNA replication. Mutations that results from exposure of organisms to mutagenic agents such as ionizing irradiation, ultraviolet light or various chemicals that react with nucleic acids are called Induced Mutations. Generally, chemical mutagens induce point mutations, whereas ionizing radiations gives rise to large chromosomal abnormalities. Point mutations are simple changes in single base-pairs, the substitution of one base-pair for another, or duplication or deletion of single base-pairs. Point mutations occur at a single point on a chromosome Frame shift mutation, is a kind of mutation caused by the addition or deletion of nucleotides which is not a multiple of three so that the codon is read incorrectly during translation.

In the early stages, selection of spontaneous mutants may be helpful, but induced mutations are the most common sources of improvements. The probability of a mutation occurring may be increased by exposing the culture to a mutagenic agent (physical mutagens are UV rays, X-rays and gamma rays; Chemical mutagens are nitro methyl guanidine, nitrous acid, N-nitroso guanidine, ethyl methyl sulphonate, ethidium bromide etc.). Such an exposure usually involves subjecting the population to a mutagen dose which results in the death of the vast majority of the cells. The survivors of the mutagen exposure may then contain some mutants, the vast majority of which will produce lower levels of the desired product. However, a very small proportion of the survivors may be improved producers. Thus, it is the task of the industrial geneticist to separate the desirable mutants (the superior producers) from the very many inferior types. This approach is easier for strains producing primary metabolites than it is for those producing secondary metabolites, as may be seen from the following examples.

The synthesis of a primary microbial metabolite (such as an amino acid) is controlled such that it is only produced at a level required by the organism. The control mechanisms involved are the inhibition of enzyme activity and the repression of enzyme synthesis by the end product when it is present in the cell at a sufficient concentration. Thus, these mechanisms are referred to as feedback control. It is obvious that a good ‘commercial’ mutant should lack the control systems so that ‘overproduction’ of the end product will result.

Removal of feedback control: The isolation of mutants of *Corynebacterium glutamicum* capable of producing lysine will be used to illustrate the approaches which have been adopted to remove the control systems. The control of lysine synthesis in C. *glutamicum* which it may be seen that the first enzyme in the pathway, aspartokinase, is inhibited only when both lysine and threonine are synthesized above a threshold level. This type of control is referred to as concerted feedback control. A mutant who could not catalyse the conversion of aspartic semialdehyde into homoserine would be capable of growth only in a homoserine-supplemented medium and the organism would be described as a homoserine auxotroph. If such an organism were grown in the presence of very low concentrations of homoserine the endogenous level of threonine would not reach the inhibitory level for aspartokinase control and, thus, aspartate would be converted into lysine which would accumulate in the medium. Thus, aknowledge of the control of the

biosynthetic pathway allows a 'blueprint' of the desirable mutant to be constructed and makes easier the task of designing the procedure to isolate the desired type from the other survivors of a mutation treatment.

Approach	Chief feature	Example/Remark
Mutation		The main approach to strain improvement; produces new alleles of existing genes
Spontaneous Mutations	Occur without any treatment with a mutagen	Used in, the initial stages of strain improvement; also for maintenance of improved strains
Induced Mutations	Induced by chemical (mainly) or physical mutagens	Mutagenesis followed by selection; several cycles employed
Major Mutations	Affect the pattern of metabolite production	Production of 6-demethyl tetracycline in place of tetracycline by *S. aureofaciens*
Minor Mutations	Affect the rate metabolite production	Small gains in each cycle of selection; substantial improvement after several cycles

Many mutations bring about marked changes in a biochemical character of practical interest; these are called major mutations. Some major mutations can be useful in strain improvement.

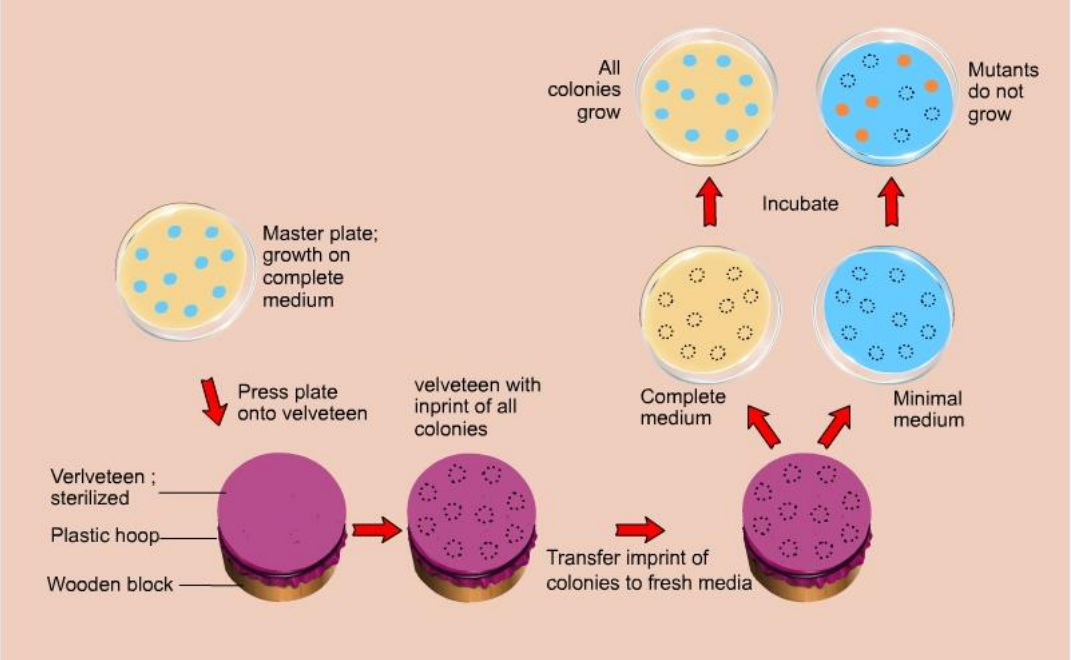
For example, a mutant strain (S-604) of *Streptomyces aureofaciens* produces 6-demethyl tetracycline in place of tetracycline; this demethylated form of tetracycline is the major commercial form of tetracycline. In contrast, most improvements in biochemical production have been due to the stepwise accumulation of so called minor genes. These genes lead to small increases (or decreases) in the antibiotic or other biochemical production, and selection may be expected to result in a 10-15% increase in yield. The selected strains are usually subjected to successive cycles of mutagenesis and selection; after several cycles, a large increase is yield is likely to be obtained. Mutants of *Penicillium chrysogenum* were selected for increased penicillin production; each cycle of selection was preceded by mutagen (chemical) treatment and resulted in only small changes in penicillin yield.

A majority of desirable mutants, especially the 'minor gene' mutants, showing increased production are isolated by screening a large number of clones surviving the mutagen treatment; this is called secondary screening. But this approach requires a large amount of work. Therefore, efforts have increasingly focused on developing techniques for the isolation of particular classes of mutants, which are likely to be overproducers. Selective isolation of mutants is carried out using following techniques:

- Replica plating method: This technique is used to isolate auxotrophic mutants.

In this procedure, mutagenized culture (which is a mixture of both mutated and non-mutated bacteria) is spread on an agar plate which acts as a medium of growth for the bacteria. After incubation for some time, both the parent and mutant bacteria begin to grow. This is called the master plate. Using a sterile velvet pad, colonies are transferred from the master plate on to secondary plates (replica plating) that contain media that are capable of sustaining only mutants, and not the non-mutants. After further incubation, replica plates are compared with the master plates, which allows for the identification and isolation of mutant

bacteria from the replica plate. In some cases, it may so happen that only non-mutants are able to grow on the replica plate, and not the mutants. This too helps in the identification of mutants.



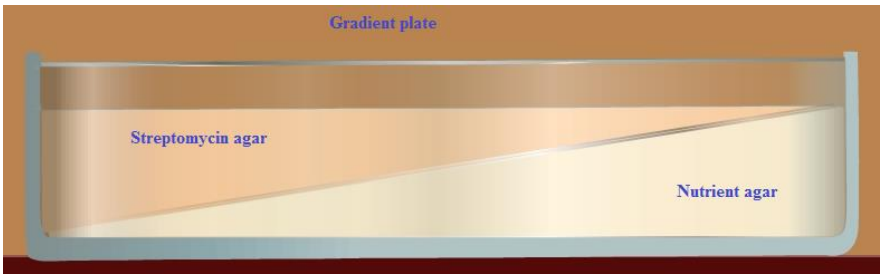
- Penicillin Enrichment Technique:

The isolation of bacterial auxotrophs may be achieved using the penicillin enrichment. Under normal culture conditions an auxotroph is at a disadvantage compared with the parental (wild-type) cells. However, penicillin only kills growing cells and, therefore, if the survivors of a mutation treatment were cultured in a medium containing penicillin and lacking the growth requirement of the desired mutant only those cells unable to grow would survive, *i.e.* the desired auxotrophs. If the cells were removed from the penicillin broth, washed, and resuspended in a medium containing the requirement of the desired auxotroph then the resulting culture should be rich in the required type.

- Resistance selection method or gradient plate method: This method is useful to isolate antibiotic resistant mutants.

An excellent way to determine the ability of organisms to produce mutants that are resistant to antibiotic is to grow them on a gradient plate of a particular antibiotic. The gradient plate consists of two wedges like layers of media: a bottom layer of plain nutrient agar and top layer of antibiotic with nutrient agar. The antibiotic in the top layer, diffuse into the bottom layer producing a gradient of antibiotic concentration from low to high. A gradient plate is made by using Streptomycin in the medium. *E. coli*, which is normally sensitive to Streptomycin, will be spread over the surface of the plate and incubated for 24 to 72 hours. After incubation colonies will appear on both the gradients. The colonies develop in the high concentration are resistant to the action of Streptomycin, and are considered as Streptomycin resistant

mutants. For isolation of antibiotic resistant of gram negative enteric bacteria, the antibiotics commonly used are Rifampicin, Streptomycin, and Erythromycin etc.



- Substrate utilization method: In this method, mutants are isolated using chromogenic substrate.

A synthetic analogue of a natural substrate (food source) which has a dye linked to it is used in the growth medium. For example, the enzyme β-galactosidase, catalyzes the hydrolysis of lactose into its constituent sugars, glucose and galactose. A commonly used chromogenic substrate for β-galactosidase is a special kind of colorless galactose called X-gal, which is a synthetic analogue, containing a dye linked to galactose. The color of the dye is only observed when X-gal is hydrolyzed by β-galactosidase. Bacteria with a functional β-galactosidase gene turn blue on a growth medium containing X-gal, and bacteria that do not produce β-galactosidase stay white.

Recombination (recombinant DNA technology):

Recombination as any process which helps to generate new combinations of genes that were originally present in different individuals. Compared with the use of mutation techniques for the improvement of industrial strains the use of recombination was fairly limited in the early years of improvement programmes. However, techniques are now widely available which allow the use of recombination as a system of strain improvement. *In vivo* recombination may be achieved in the asexual fungi *(e.g. Penicillium chrysogenum,* used for the commercial production of penicillin) using the parasexual cycle. The technique of protoplast fusion has increased greatly the prospects of combining together characteristics found in different production strains.

The application of *in vitro* recombinant DNA technology to the improvement of secondary metabolite formation is not as developed as it is in the primary metabolite field. However, considerable advances have been made in the genetic manipulation of the streptomycetes and the filamentous fungi and a number of different strategies have been devised for cloning secondary metabolite genes.

By employing restriction endonucleases and ligases, investigators can cut and splice DNA at specific sites. Some endonucleases have the ability to cut precisely and generate what are known as “sticky ends.” When different DNA molecules are cut by the same restriction enzyme, they possess similar sticky ends. Through a form of biological “cut and paste” processes, the lower parts of the one DNA is made to stick well onto the upper part of another DNA. These DNA molecules are later ligated to make hybrid molecules. The ability to cut and paste the DNA molecule is the basis of “genetic engineering.” A useful aspect of this cut and paste process involves the use of plasmid, phage, and other small fragments of DNA (vectors) that

are capable of carrying genetic material and inserting it into a host microbe such that the foreign DNA is replicated and expressed in the host. A wide array of techniques can now be combined to isolate, sequence, synthesize, modify, and join fragments of DNA. It is therefore possible to obtain nearly any combination of DNA sequence. The challenges lie in designing sequences that will be functional and useful.

The protocol to modify and improve strains involves the following steps:
a. Isolate the desired gene (DNA fragment) from the donor cells.
b. Isolate the vector (a plasmid or a phage).
c. Cleave the vector, align the donor DNA with the vector, and insert the gene into the vector.
d. Introduce the new plasmid into the host cell by transformation or, if a viral vector is used, by infection.
e. Select the new recombinant strains that express the desired characteristics.

Solid state fermentation (SSF): is defined as the microbial cultivation process in the absence or near absence of free water in the substrate. However, there must be enough moisture present to support cell growth. SSF employs natural raw materials as carbon source such as cassava, barley, wheat bran, sugarcane bagasse, various oil cakes like palm kernel cake, soybean cake, ground nut oil cake, fruit pulps, saw dust, seeds, coffee husk and coffee pulp, tea waste, spent brewing.

Liquid state fermentation (LSF): A controlled fermentation process in which microorganisms are grown in a liquid culture medium.

Submerged fermentation

Submerged fermentation is the cultivation of microorganisms in liquid nutrient broth. Since microbial enzymes are mostly low volume, medium cost products, the production methods using submerged liquid systems have generally relied on bioreactors similar in design and function to those used in antibiotic production processes. This involves growing carefully selected microorganisms (bacteria and fungi) in closed vessels containing a rich broth of nutrients (the fermentation medium) and a high concentration of oxygen. As the microorganisms break down the nutrients, desired product (enzymes or other bioactive compounds) are submerged in a liquid such as alcohol, oil, or nutrient broth. Due to the development of large-scale fermentation technologies, the production of microbial enzymes accounts for a significant proportion of the biotechnology industry's total output. Fermentation takes place in large vessels (fermenter) with volumes of up to 1,000 cubic metres. The fermentation media sterilises nutrients based on renewable raw materials like maize, sugars and soya. Most industrial enzymes are secreted by microorganisms into the fermentation medium in order to break down the carbon and nitrogen sources. Batch-fed and continuous fermentation processes are common. In the batch-fed process, sterilised nutrients are added to the fermenter during the growth of the biomass. In the continuous process, sterilised liquid nutrients are fed into the fermenter at the same flow rate as the fermentation broth leaving the system. This will achieve a steady-state production. Parameters like temperature, pH, oxygen consumption and carbon dioxide formation are measured and controlled to optimise the fermentation process. Firstly, in harvesting enzymes from the fermentation medium one must remove insoluble products, e.g. microbial cells. This is

normally done by centrifugation. As most industrial enzymes are extracellular (secreted by cells into the external environment), they remain in the fermented broth after the biomass has been removed.

Batch Culture Fermentation

This type of fermentation is also called a closed culture system because nutrients and other components are added in specific amounts at the start of the process and are not replenished once the fermentation has started. At the end of the process the product is recovered; then, the fermenter is cleaned, sterilized, and used for another batch process. In the initial stages microorganisms grow at a rapid rate in the presence of excess nutrients but as they multiply in large numbers they use up the nutrients. They also produce toxic metabolites which retard further growth of microorganisms during the later stages of the fermentation process.

Fed-batch Culture Fermentation

In this process the nutrients and substrates are added at the start of the process and at regular intervals after the start. This is called controlled feeding. Inoculum is added to the fermentation vessel when microorganisms are in exponential growth phase. Fed-batch culture is controlled by feed-back control and control without feed-back.

1. Feed-back control– The fermentation process is controlled by monitoring process parameters like dissolved oxygen content, carbon dioxide to oxygen ratio, pH, concentration of substrate, and concentration of the product.
2. Control without feed-back– The substrates and nutrients are added at regular intervals.

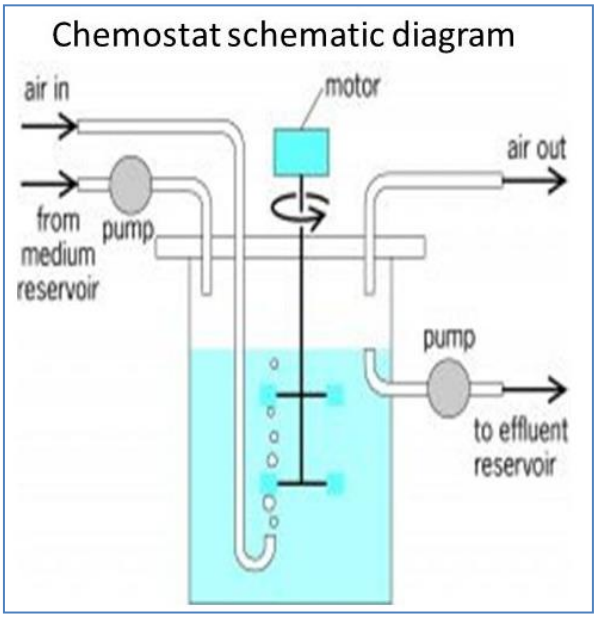
Fed-batch culture requires special equipment such as a reservoir which holds the nutrients, pH modifiers so that they can be added to the fermenter at regular intervals, and pumps to deliver culture medium aseptically to the fermenter.

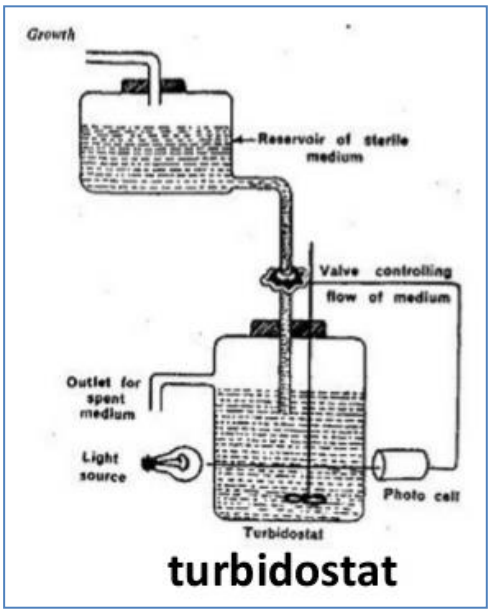
Continuous Fermentation

This method prolongs the exponential growth phase of microbial growth as nutrients are continually supplied and metabolites and other wastes are continually removed thus promoting continual growth of the microorganisms. Continuous culture fermentation is advantageous because of its high productivity. Two control methods are used in continuous culture fermentation, namely, chemostat and turbidostat.

Chemostat: This medium contains excess of all but one of the nutrients which determine the rate of growth of the microorganism. At steady state of the chemostat the rate of input of medium into the fermenter is equal to the rate of output out of the fermenter.

Turbidostat: This medium contains excess of all nutrients so the microbial growth is at its maximum specific growth rate. The system consist of a photoelectric cell which is a turbidity sensor that detects changes in turbidity of the contents in the fermenter and then controls the amount of medium fed to the fermenter.





In other techniques, a fermenter variable, eg. turbidity or pH, will be monitored using an appropriate detector and the liquid flow rate will be automatically adjusted so as to maintain the variable at a constant level.

A turbidostat is a continuous culturing method where the turbidity of the culture is held constant by manipulating the rate at which medium is fed. If the turbidity tends to increase, the feed rate is increased to dilute the turbidity back to its setpoint. When the turbidity tends to fall, the feed rate is lowered so that growth can restore the turbidity to its set point.

The most widespread large scale application of continuous culture reactors is in wastewater treatment. Activated sludge plants, trickle bed filters, anaerobic digester and ponds all operate in an continuous manner. Cell immobilization is also often employed to improve the efficiency of the process. Continuous cultures are well established in the wastewater industry for several reasons:

- Unlike pure culture microbial and animal cell systems, contamination is not a consideration, as the wastewater feed will always contain microorganisms.
- Continuous reactors have long been used in waste treatment and their use is not considered a risk.
- Finally using batch cultures is simply not economically feasible. Wastewater flows are often measured in mega litres per hour and batch reactors simply could not cope with the load.

Unit-IV; Possible questions

PART –A (1 Marks)

PART-B (2 Marks)

1. Name two important discoveries in field of industrial microbiology.
2. Name any four industrially important strains used in industries.
3. Who is the father of industrial microbiology?
4. Name the strategies adopted for the isolation of industrial microorganisms.
5. Mention any four characteristics of industrially important strains.
6. What are the methods of primary screening of industrial important strains?
7. Mention the importance of secondary screening of industrial important strains?
8. Name the agents used for protoplast fusion.
9. What is techniques for isolation of mutant strains
10. Mention any four advantages of using rDNA technology.

PART-C (8 Marks)

1. Discuss important historical developments in industrial microbiology
2. Explain the primary screening methods for strain isolation.
3. Discuss the importance of secondary screening for strain isolation.
4. How to isolate an antibiotic producing organism?
5. Write notes on strain improvement.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB COURSE NAME: FOOD AND INDUSTRIAL MICROBIOLOGY

COURSE CODE: 17MBP302 UNIT: IV BATCH-2017-2019

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	Who is the father of fermentation technology?	Louis Pasteur	Alexander Fleming	Robert Koch	Edward Jenner	Louis Pasteur
2	Who described a lens?	Salvino D Armati	Roger Bacon	Fabri	Leewen Hoek	Roger Bacon
3	The size of paper strip used in Aerocanography was _____.	1.5 *12 cm	2.5*12cm	1.5*10 cm	3*20cm	1.5 *12 cm
4	Enrichment technique was designed by _____.	Beijernick	Pasteur	Bacon	Hooke	Beijernick
5	The medium which doesnot contain particular essential nutrient is called as _____.	Enriched media	Minimal media	Molden agar	Selective media	Minimal media
6	Indicator dye are used in the medium	To detect temp change	To detect nutrient concentration	To detect ph change	To detect aw availability.	To detect ph change
7	Crowded plate technique is used in the detection _____ producing organisms.	Growth factor producers	Aminoacid producers	Antibiotic producers	Organic acid producers.	Antibiotic producers
8	Growth factor producing organisms are identified by using	Growded plate technique	Enrichment technique	Auxanography	Indicator dye method.	Auxanography
9	Chemical stability of the product is identified by _____.	Primary screening	Secondary screening	Enrichment method	Tertiary method.	Secondary screening
10	In serial subculturing method bacteriophages and anaerobic organisms are store at _____.	Solid medium	Minimal medium	Selective medium	Liquid medium	Liquid medium
11	In oil overlaying method paraffin oil is used in specific gravity of _____.	0.743 -0.780	0.801-0.825	0.901-0.925	0.865-0.890	0.865-0.890
12	Oil overlaying method is first used by _____.	Brell	Bacon	Dulaney	Nakayama.	Brell
13	Bacterial species are preserved by oil overlaying method for _____.	10-15 years	15-20 years	5-10 years	5 years.	15-20 years
14	Freeze drying is called as _____.	Lyophilization	Liquefaction	Freezing	Nitrogen storage	Lyophilization
15	Freezing mixture used in lyophilization is _____.	Ice and	Ice and	Only dry ice	Nitrogen and	Ice and alcohol

		methanol	alcohol		ice	
16	Nitrogen storage is also called as _____.	Freezing	Cooling	Cryogenic storage	Lyophilization	Cryogenic storage
17	Fungal species are stored by _____.	Freezing	Soil cultures	Nitrogen storage	Silica gel cultures	Soil cultures
18	Lyophilization is first used by _____.	N.Appert	Davis	Alexander	Pepler	Alexander
19	Among the following which organism was not affected by drying of medium.	Streptomyces	Bacillus	E.coli	Pseudomonas	Streptomyces
20	Organism which does not produce particular product which is require for growth is called _____.	Auxotrophs	Heterotrophs	Autotrophs	Phototrophs	Auxotrophs
21	Control mechanism for the biosynthesis of products are called _____.	Feed back control	Primer control	Negative control	Promoter control	Feed back control
22	End product bind with the enzyme to stop the main reaction is called as _____.	Feed back repression	Feed back inhibition	Feed back control	Positive control	Feed back inhibition
23	End product which stop the synthesis of key enzyme called _____.	Feed back repression.	Feed back control	Feed back inhibition	Primer control	Feed back repression.
24	Each end product controls the pathway in certain percentage is called as _____.	Co-operative control	Sequential control	Multivalent control	Cumulative control	Cumulative control
25	Enzyme which catalyze same reaction but differ in their control characters are called as _____.	Iso enzyme	Holoenzyme	Ortho enzyme	Paraenzyme	Iso enzyme
26	Organism which synthesis all growth factors for their growth is called as _____.	Auxotrophs	Phototrophs	Autotrophs	Protoplast	Phototrophs
27	All the natural isolates of organisms are _____.	Auxotrophs	Phototrophs	Heterotrophs	Autotrophs.	Phototrophs
28	In industries mostly used mutation agent is _____.	UV-rays	EMS	Mustard gas	MNNG	UV-rays
29	Primary metabolites are produced during _____.	Lag phase	Stationary phase	Trophase	Log phase	Stationary phase
30	Among the following which is biotin requiring organism?	*Cornybacterium*	*E.coli*	*Salmonella*	*Bacillus*	*Cornyebacterium*

31	Which is used as a inhibitory agent in enrichment method?	Pencillin	Nacl	Calcium	Sulfate	Pencillin
32	Mutant spores are selected by _____.	Enrichment method	Mechanical separation method	Selective method	Serial subculturing method	Mechanical separation method
33	Sandwich method is used to isolate _____.	Mutant spores	Auxotrophs	Mutant vegetative cells	Autotrophs	Mutant spores
34	At the optimum level of biotin C. glutamicum produces _____.	Lysine	Purine	Aspargine	Pyrimidine	Lysine
35	A compound which is similar structure to other compound is called _____.	Isologue	Analogue	Antilogue	Autologue	Analogue
36	Growth below the inhibitory level of analogue is called as _____.	Confluent growth	Synchronous growth	Diauxic growth	Continuous growth	Confluent growth
37	Membrane permeability is altered by changing the concentration of _____.	Fat	Carbohydrate	Phospholipids	Protein	Phospholipids
38	Protoplast fusion is mainly used to improve industrial microbes	Algae	Fungi	Only yeast cells	Bacteria	Fungi
39	Cell without cell wall is called as _____.	Protoplast	Spleroplast	Thermoplast	Periplast	Protoplast
40	Among the following which solution is used for the preparation of protoplast?	Osmotic	Hypertonic	Isotonic	Hyphotonic	Isotonic
41	Different microbial species are fused by _____.	r-DNA technology	Protoplast fusion	Mutation	Plasmid fusion	Protoplast fusion
42	The inoculum level introduced into a production tank is usually _____.	0.5-5%	5 – 25%	20 – 40%	50%	5 – 25%
43	Inoculum media are balanced for _____.	Rapid cell growth	Product formation	Rapid cell growth and not for product formation	Rapid cell growth and product formation.	Rapid cell growth

44	Mutants can be obtained by treating the cells with _____.	UV radiation	Nitrogen mustard	Uranyl nitrate	All the above	UV radiation
45	Primary metabolites are produced during _____.	Lag phase	Stationary phase	Trophophase	Idiophase	Lag phase
46	Secondary metabolites are produced during _____.	Lag phase	Stationary phase	Trophophase	Idiophase	Trophophase
47	_____ are normally operated at high substrate conversion.	CSTF	Air lift fermentor	Tower fermentor	Tubular fermentor	CSTF
48	_____ vessel is used in production of lactic acid and ole.	Wood vessel	Stainless steel	Iron	Glass	Wood vessel
49	Extrachromosomal elements which carry information for synthesis of products is called _____.	Protoplast	Chloroplast	Plasmid	Spheroplast	Plasmid
50	The chemical agent which causes mutation is _____.	Sodium sulfate	Ethyl methyl sulfanate	CaCl2	Ethyl sulfanate	Ethyl methyl sulfanate
51	_____culture where a portion of the culture is harvested at regular intervals and replased by an equal volumeof medium	Fed batch	Batch	Semi continuous	Continuous	Batch
52	_____ culture where medium is fed to the culture resulting in an increases in voloume	Batch	Fed batch	Continuous	Semi continuous	Fed batch
53	_____ is a culture system which contain a intial limited amount of nutrient	Fed batch	Batch	Semi continuous	Continuous	Batch
54	_____-culture is a open system where fresh medium is continuously added	Batch	Continuous	Fed batch	Airlift	Continuous
55	_____ is the organism used in the first truly large scale aseptic fermentation vessels.	*Clostridium acetobutylicum*	*C. perfringens*	*S. cereviseae*	*E.coli*	*Clostridium acetobutylicum*
56	_____ is to provide microorganisms in submerged culture with sufficient oxygen for metabolic requirements.	Aeration	Agitation	Impeller	Baffler	Aeration
57	_____ ensures uniform suspension on microbial cells.	Aeration	Agitation	Sparger	Baffler	Agitation
58	_____device is used to introduce air in fermenter	Sparger	Impeller	Baffler	Turbines	Sparger
59	Aeration and agitation of a liquid medium may lead to the	Acid	Alkali	Foam	Air	Foam

	formation of					
60	Industrial alcohol production can be carried out in very large fermentor upto _____ gallous.	12500	125	25000	100000	12500

Unit V

Syllabus

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.

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:

- Depending upon the type of product required, a particular bioreactor is selected.
- A suitable substrate in liquid media is added at a specific temperature, pH and then diluted.
- The organism (microbe, animal/plant cell, sub-cellular organelle or enzyme) is added to it.
- Then it is incubated at a specific temperature for the specified time.
- The incubation may either be aerobic or anaerobic.
- Aerobic conditions are created by bubbling oxygen through the medium.
- 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.
- 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

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.

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.

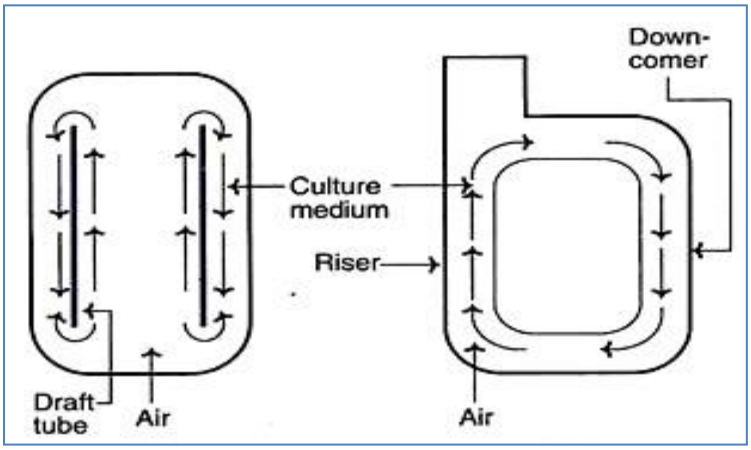
Bubble Column Bioreactors:

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).

Air-lift fermenter: Airlift bioreactors (ALB) are generally classified as pneumatic reactors without any mechanical stirring arrangements for mixing and use the expansion of compressed gas to bring about the mixing.

1. The turbulence caused by the fluid flow ensures adequate mixing of the liquid.
2. The draft tube is provided in the central section of the reactor.
3. The introduction of the fluid (air/liquid) causes upward motion and results in circulatory flow in the entire reactor.
3. Even large fermenters doesn't require internal cooling coils as a jacket can normally provide sufficient heat transfer, due to the rapid movement of fluid within the vessel.
4. The air/liquid velocities will be low and hence the energy consumption is also low.
6. ALBs can be used for both free and immobilized cells.
7. There are very few reports on ALBs for metabolite production.
8. The advantages of Airlift reactors are the elimination of attrition effects generally encountered in mechanical agitated reactors. It is ideally suited for aerobic cultures since oxygen mass transfer coefficient are quite high in comparison to stirred tank reactors.

These fermenters do not have mechanical agitation systems (motor, shaft, impeller blades) but contents are agitated by injecting air from the bottom. Sterile atmospheric air is used if microorganisms are aerobic and "inert gas" is used if microorganisms are anaerobic. This is a gentle method of mixing the contents and is most suitable for fermentation of animal and plant cell cultures since the mechanical agitation produces high shearing stress that may damage the cells. Air-lift fermenters are most widely used for large-scale production of monoclonal antibodies.



Inner loop air lift fermenter Outer loop air lift fermenter

Downstream process

Downstream processing is an essential part of bioprocess technology in that the desired product needs to be isolated, purified and for different end uses. A variety of microorganisms including genetically engineered species are used for the production of desired products. The products formed may be secreted into the broth or may be retained within the cell introducing complexity in the recovery of the product. In view of the complexity, downstream processing involves various techniques and methodologies. Bio products differ greatly in their nature hence different separation principles and mechanisms depending on molecular mass, charge distribution, hydrophobicity, distribution coefficient, structure and immunogenic structure and specific affinity towards other biomolecules becomes necessary for their isolation and purification. The choice of the separation methodology depends to a large extent on the nature of the product, its quantity and the extent of purity required.

The various processes used for the actual recovery of useful products from fermentation or any other industrial process are called downstream processing. The cost of downstream processing (DSP) is often more than 50 % of the manufacturing cost, and there is product loss at each step of DSP. In addition, the product is either present in the cells, in the medium or both. In either case, the concentration of product is usually rather low, and it is generally mixed with other molecules from which it has to be separated. Therefore, the DSP should be efficient, involve as few steps as possible (to avoid product loss), and be cost effective.

Downstream processing involves the following primary steps:

- Fermentation process

- Foam separation
- Primary separation – removal of insoluble products/cell (centrifugation, filtration and sedimentation)
- Cell disruption (physical, chemical and enzymatic)
- Product isolation and separation – solvent extraction, adsorption, aqueous two-phase system and precipitation
- Purification techniques (Chromatography - ion exchange, gel permeation and affinity)
- Membrane separation (micro-filtration, ultra-filtration and reverse phase electrophoresis)
- Product polishing (crystallization, drying and diafiltration).

Foam separation: Initial step in the downstream processing is the removal of foam without loss of cells and products. Whole cells or proteins gets attached to the surface of the air bubbles rising through liquid forming foam and when separated will be lost. To minimize the loss two ways of foam separation has been adapted.

1. Excess foam collected in a separate outlet and the foam is mechanically broken. The cells and protein from the foam is collected for further usage.

2. Materials are made surface active and collected termed as colligends and the surfactants used for that purpose are termed collectors. Addition of surfactants is found to improve the percentage of removal of cells.

Separation of cells and insoluble products

Filtration

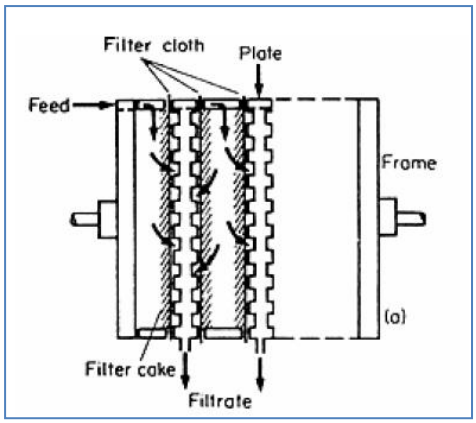
Filtration is defined as the separation of solid in a slurry consisting of the solid and fluid by passing the slurry through a septum called filter medium. For filtration in some cases filter aids (diatomaceous earth) are used to improve porosity and faster flow rate.

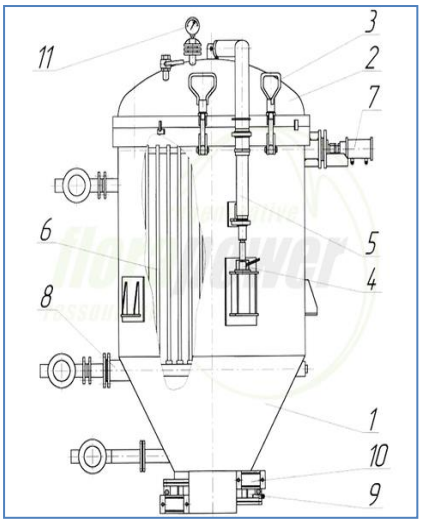
Types of filters: Plate and frame filters, pressure leaf filters, continuous filters, rotary vacuum filtration

1. Batch Filters:

(a) Plate and frame filters: In this plates and frames are arranged alternately assembled on a horizontal framework. Plates are covered with filter clothes and held together by hand screw to prevent leakage between frames. The slurry is fed through the continuous channel by the holes in the corners of the plates and frames. The filtrate passes through the filter cloth or

pad, runs down grooves in the filter plates and is then discharged through outlet taps to a channel.





1. Filter container
2. Container lid
3. Pivot screws
4. Hydr. hoist
5. Lifting arm
6. Filter leafs
7. Pneum. vibrator
8. Drain

Plate and frame filters Pressure leaf filters

(b) Pressure leaf filters: These filters incorporates a number of leaves, each consisting of a metal framework of grooved plates which is covered with a fine wire mesh, or occasionally a filter cloth and often precoated with a layer of cellulose fibers. The slurry is fed into the filter which operated under pressure or by suction with a vacuum pump. There are three types based on the arrangement of filters.

(i) Vertical metal leaf filter – consists of number of vertical porous metal leaves mounted on a hollow shaft in a cylindrical pressure level. The solids from the slurry gradually build up on the surfaces of the leaves and the filtrate is removed from the plates via the horizontal hollow shaft

(ii) Horizontal metal-leaf filter – consists of metal leaves mounted on a vertical hollow shaft within a pressure vessel. Filtration is continued until the cake fills the space between the disc shaped leaves or when the operational pressure has become excessive

(iii) Stacked disc filter – this metafilter consists of a number of precision made rings which are stacked on a fluted rod. The filtrate passes between the discs and is removed through the grooves of the fluted rods, while solids are deposited on the filter coating.

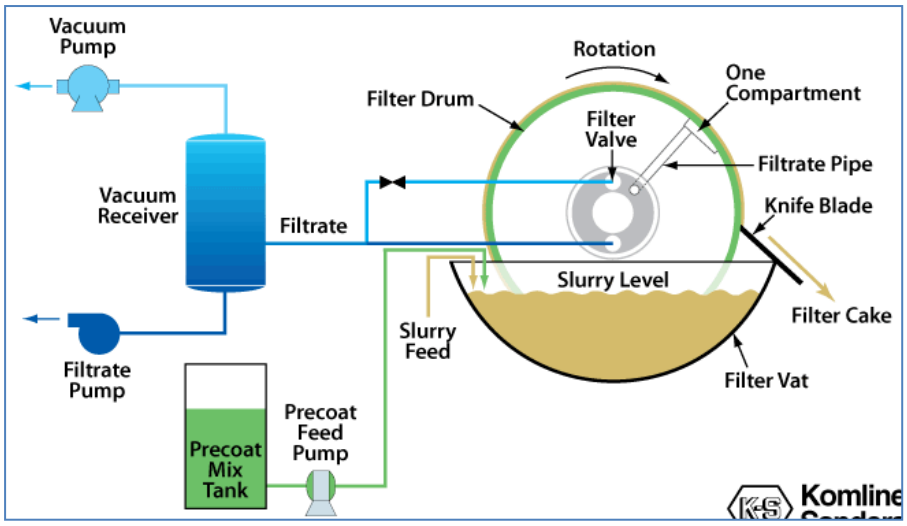
2. Continuous filters

(a) Rotary vacuum filters: Drum covered with diatomaceous earth matter and allowed to rotate under vacuum with half immersed in the slurry tank. Small amount of coagulation agent added to broth and pumped into the slurry tank. As drum rotates in the slurry tank under vacuum thin layer of coagulated particles adhere to drum. The layer thickens to from cake. As the cake portion in the drum comes to the upper region which is not immersed in the liquid it is washed with water and dewatered immediately by blowing air over it. Then before the dried portion is again immersed into the liquid it is cut off from drum by knife. The mechanism of cake discharge is achieved by three ways.

(a) String discharge – Long lengths of string 1.5cm apart are threaded over the drum and round two rollers. The cake is lifted free from the upper part of the drum when the vacuum pressure is released and carried to the small rollers where it falls free

(b) Scraper discharge – by using a knife or scraper positioned accurately to slice off the cake

(c) Scraper discharge with precoating – to avoid blockage of filter cloth in the drum by cells a scraper which is coated with a layer of filter-aid 2 to 10 cm thick.

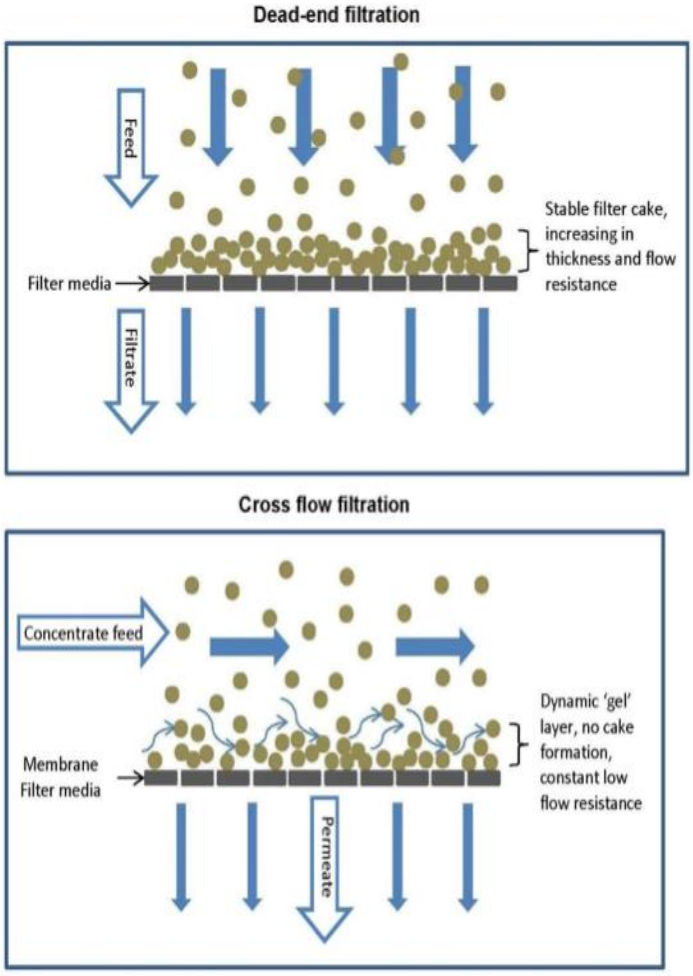


Micro or Ultra Filtration: Filtration of suspended particles can be achieved by either dead end filtration or cross flow filtration.

(a) Dead end filtration – Solution poured over the membrane and the filtrate is collected at the bottom. On prolonged filtration pores become blocked which reduce the filtering capacity.

(b) Cross flow filtration – To prevent the blockage the solution is passed over the membrane. Cell suspension enters laterally and flows over the membrane. The filtrate gets collected at

the bottom whereas the cells are pushed to the opposite end by the continuous flow of suspension which is sent out via an outlet at the opposite end. The liquid is again passed through a tube which recycles back to the flow. Since the cells do not block the pores the filtration process can be performed continuously.



Adsorption on filter aids: Filter aids, are inert incompressible discrete particles of high permeability. Solids such as wood pulp, starch powder, cellulose, inactive carbon, when added as filter aid enhances their filterability. Filter aids absorb small particles, which otherwise clog the filter pores. Filter aids also reduce the compressibility of the accumulated biomass by adsorbing the colloidal particles.

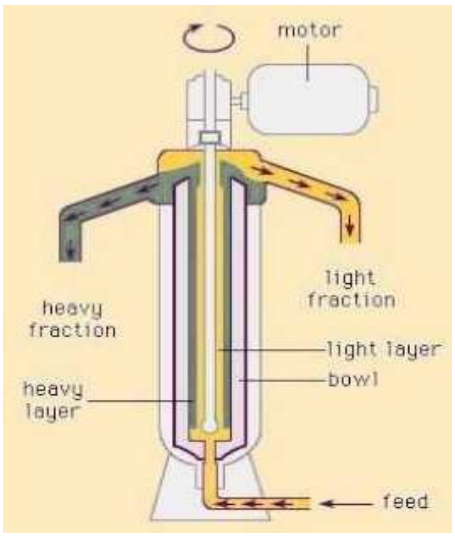
Centrifugation: Centrifugation is a common method used to separate cells from cultured broth. It employs centrifugal force to promote accelerated settling of particles in a solid-liquid mixture. Separation is achieved by means of accelerated gravitational force by rapid rotation. Microorganisms and other cells from the fermented slurry can be removed by using

centrifuge when filtration is not a satisfactory separation method. The particle size that can be separated range from 0.1μm to 100 μm. Separation is based on Stoke's law, which states that the rate of Newtonian viscosity characteristics is proportional to the square of the diameter of the particles which is expressed as,

$$Vg = d2g (\rho P - \rho L) / 18\mu$$

Where, Vg – rate of sedimentation, d – particle diameter, g – gravitational force, ρP – liquid density, ρL – particle density, μ - viscosity

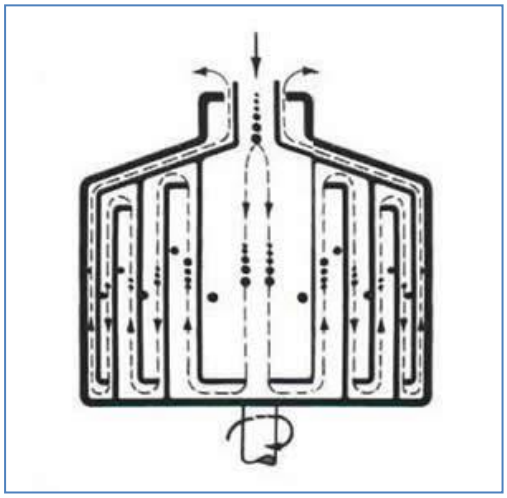
(a) Tubular centrifuges: This is used of separate particle size of 0.1 - 200 μm. This is simple machine made of a tube rotating between bearings at each end. The suspension enters at the bottom of the centrifuge and high centrifugal forces act to separate the solids and liquids. The bulk of solids will adhere o the walls of the bowl, while the liquids exit at the top of the centrifuge. It is employed for light phase/heavy phase liquid separation and solid-liquid phase separation. Application: Pharmaceutical industries, Edible oil industries, chemical and food industries



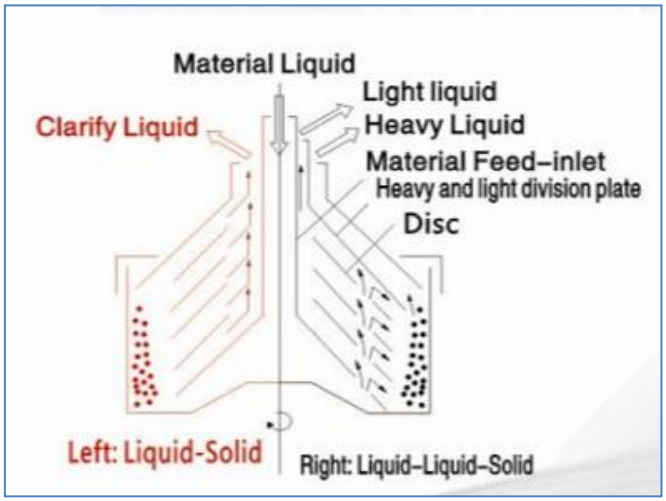
(b) Chamber bowl centrifuge: The chamber bowl centrifuge is a number of tubular bowl arranged co-axially. It has a main bowl containing cylindrical inserts that dived the volume of the bowl into a series of annular chambers, which operate in series. Feed enters the center of the bowl and the suspension passes through each chamber in turn, at increasing distances from the axis. The solids settle onto the outer wall of each chamber and the clarified liquid emerges as an overflow from the largest diameter chamber. This device provides also a classification of the suspended solids: the coarse particles deposit in the inner chamber and

the increasingly fine particle deposit on the subsequent chambers. The removal of sedimented solids requires the stopping of rotation for manual cleaning.

(c) Disc centrifuge: The simplest design is a closed bowl, containing the disk stack, with any solids present collecting at the outer part of the bowl, from which they have to be removed manually after stopping rotation. The solids are discharged from the bowl by a number of methods, including the basic use of nozzles, which are open continuously, allowing a thick slurry to discharge. In the more complicated design valved nozzles open automatically when the solid depth in the bowl reaches a certain value, and then close again when most of the solids have been discharged. In the most complicated design the bowl is opened: its shell splits circumferentially for a short period, with the opening also controlled by solids depth in the bowl.

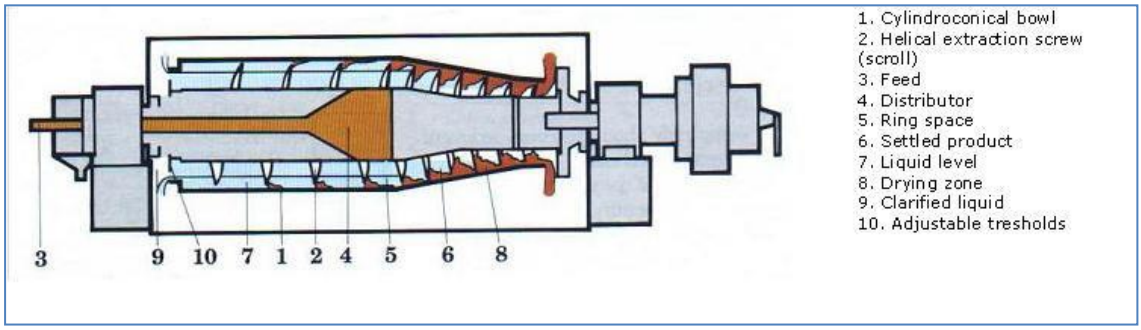


Chamber bowl centrifuge



Disc centrifuge

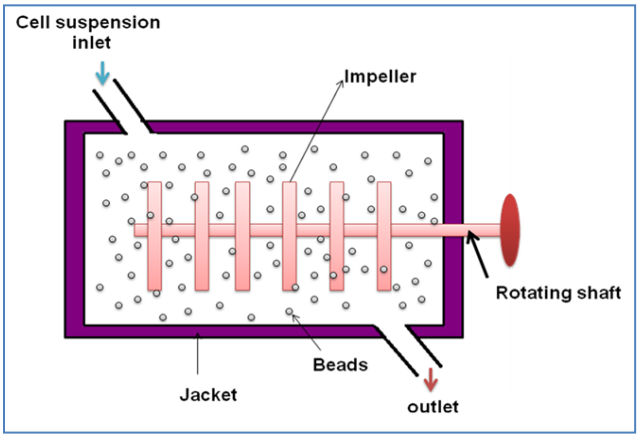
(d) Decanter centrifuge: This used for continuous handling of slurry. This sedimentation centrifuge is designed to handle significant solid concentration in feed suspension. It consists of horizontal cylindrical bowl rotating at a high speed, with a helical extraction screw placed co-axially. The screw perfectly fits the internal contour of the bowl, only allowing clearance between bowl and scroll. The differential speed between the screw and scroll provides the conveying motion to collect and remove solids that accumulate at the bowl wall.



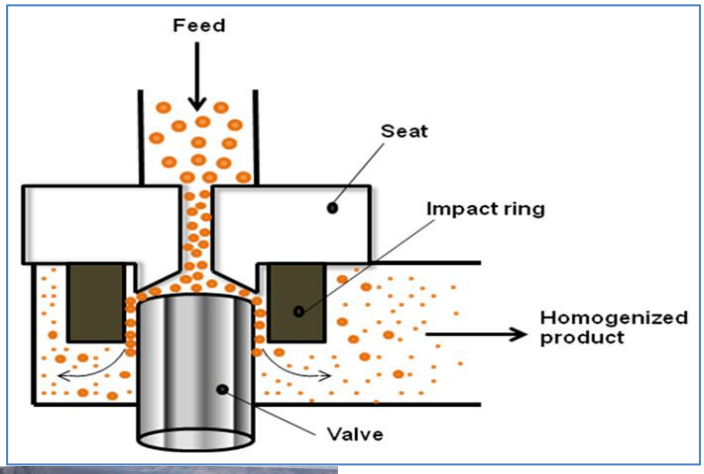
Cell disruption

(a) Physical methods / Mechanical methods: In Ultrasonic vibrators (sonicators) the ultrasound waves of frequencies greater than 20 KHz/s ruptures the cell wall by a phenomenon known as cavitation. The passage of ultrasound waves creates alternating areas of compression and rarefaction which change rapidly. The cavities formed in the areas of rarefaction rapidly collapses as the area changes to one of compression. The bubbles produced in the cavities collapse creating shock waves which disrupt cell walls.

In laboratory, grinding with a bead mill or a waring blender may be used. Waring blender is particularly effective with animal cells and tissues as well as with mycelial organisms. In industrial scale, cell disruption is carried out using a bead mill or high pressure homogenizer. Vertical or horizontal bead mill consists of a grinding cylinder with a central shaft fitted with a number of impellers and driven by motor. The cell suspension is pumped into the cylinder and cell disruption occurs due to shear forces produced between velocity gradients because of the rotary motion of cells and beads. In addition, collision between beads and cells and grinding of cells between rolling beads also contribute to the disruptive forces. High pressure homogenization consists of a high pressure positive displacement pump couple to an adjustable discharge valve with a restricted orifice. The cell suspension is pumped through the homogenizing valve at 200 – 1000 atmospheric pressure depending on microbes and cell concentration. Cell disruption occurs due to stress due to impingement, normal stress during passage through narrow channel and shear stress due to pressure drop.



In High pressure homogenization, the cell suspension is drawn through a valve into a pump cylinder. Then it is forced under pressure of up to 1500 bar, through a narrow annular gap and discharge valve, where the pressure drops to atmospheric. Cell disruption is achieved due to the sudden drop in pressure upon the discharge, causing the cells to explode. This method is one of the most widely known and used methods. It is mostly used for yeast cells. It is a vital unit in the dairy production industry, for milk homogenization. By operating the press at higher pressures, the number of passes of the slurry through it can be decreased in order to obtain the desired degree of disruption. However, the operating pressure may be limited due to the deactivation of certain heat-sensitive proteins, which may increase the number of passages required.



Non mechanical physical methods: Osmotic shock is physiologic dysfunction caused by a sudden change in the solute concentration around a cell, which causes a rapid change in the movement of water across its cell membrane which ultimately results in bursting. Freezing thawing cycles cause loss of membrane integrity and cell wall is ruptured. In Thermolyis the heat inactivates the organism by disrupting the cell walls without affecting the products. The effect of heat shock depends on pH, ionic strength, and presence of chelating or sequestering agents such as EDTA.

(b) Chemical methods: Chemical treatment for disrupting cell includes alkali, organic solvent, detergents and chaotropic agents. Alkali acts on the cell wall and results in saponification of membrane lipids. Alkali such as NaOH addition alters the pH and affects the integrity of the cell membrane. It is carried out at pH range of 11 to 12 for about 20 to 30 min. Organic solvents (alcohols, dimethyl sulfoxide, methyl ethyl ketone or toluene) results in disruption of cell wall. Cell wall absorbs the solvent resulting in swelling and rupture of cell wall. At low concentration the cell wall is not ruptured but the permeability is increased. Product stability should be considered when choosing the solvent. Eg. Toluene is used for

Agrobacterium radiobacter, Ethyl acetate used for yeast and Dimethyl sulfoxide (DMSO) for plant cell wall. Other organic solvents used are benzene, chlorobenzene, xylene, cumeme, octanol, etc.

Detergents permeabilize cells by solubilising cell membranes. They are amphipathic in nature capable of interacting with both water and lipids. Eg. Anionic detergents such as Sodium dodecyl sulphate (SDS), sodium sulphonate; Cationic detergents such as cetyltrimethyl ammonium bromide (CTAB); Non-ionic detergents such Triton X100. Chaotropic agents disrupt the structure of water making it less hydrophilic and weaken the hydrophobic interactions and increase permeability.

(c) Biological methods: Enzymatic digestion is involved in two stages (i) cell wall disruption resulting in the release of cell wall proteins leaving the protoplast intact and (ii) digestion of organelle membrane to release the organelle proteins. Digestion may be achieved by hydrolyzing cell walls by specific enzymes such as Lysozyme. Hydrolyzing cell wall by combination of enzymes (1,3-glucanase, 1,6-glucanase, mannanase, chitinase etc) may be also used for digestion in cases like plants cell wall. The cell wall of yeast and fungi differs significantly from the cell wall bacteria. One commonly used enzyme mixture for degradation of cell wall of yeast and fungi is Zymolyase. It has for example β-1,3-glucanase and β-1,3-glucan laminaripentao-hydrolase activities (Zymolyase | Yeast lytic enzyme). In addition, the enzymes that are commonly used for degradation of cell wall of yeast and fungi include different cellulases, pectinases, xylanases and chitinases.

Product Separation

Solvent extraction or Liquid-liquid extraction

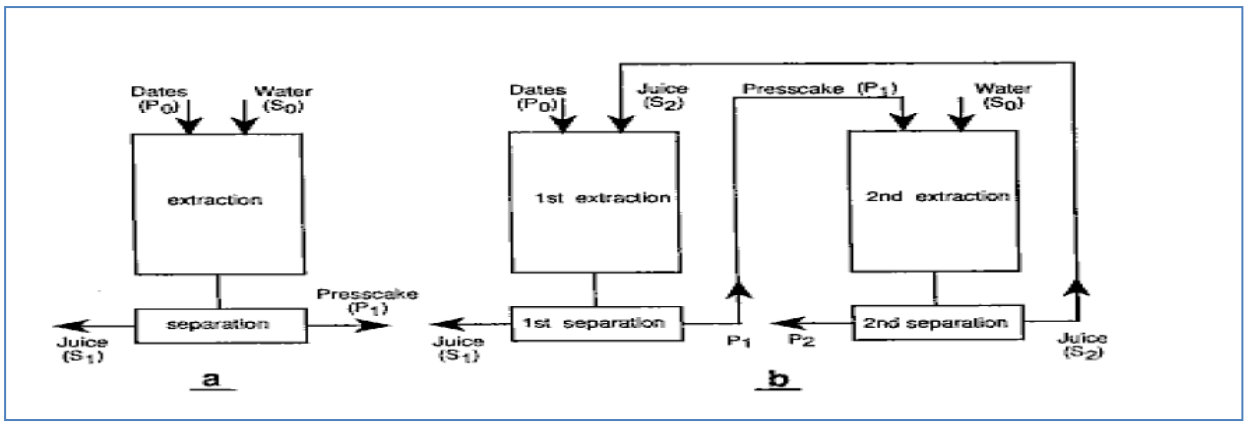
It is a classical method for recovery as well as concentration of various products. Solvent extraction has several advantages such as selectivity of extraction directly from broth or reaction medium, reduction in product loss as the product is just transferred to a second phase and easy scale up. Solvent extraction involves extraction of compound in a liquid phase to another liquid. The solute originally present in aqueous phase gets partitioned in both the phases. The distribution between the two immiscible liquids and solubility in two liquids decide the efficacy of extraction. The choice of solvent selection was based on dielectric point. The dielectric constant is a measure of the degree of molar polarization of a compound. An increase dielectric pole increases the polarity of the solute.

$D = C/C_0$

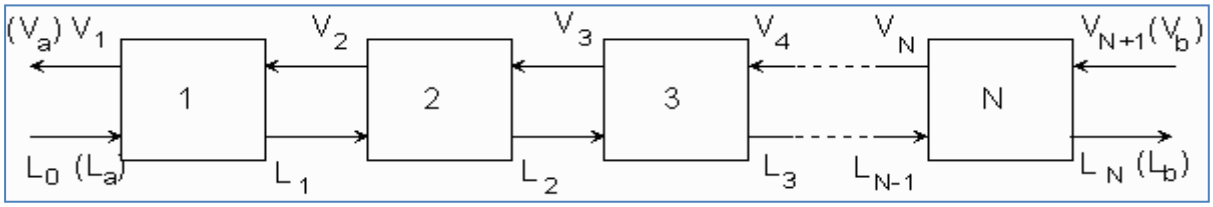
Where, D – dielectric constant, C – electrostatic capacity of a condenser containing the substance V between the plates, C_0 – electrostatic capacity of the same condenser when completely evacuated. The final choice of solvent is influenced by partition coefficient (k).

k = concentration of solute in extract / concentration of solute in raffinate High value of k required single stage extraction and low value k requires multistage extraction. In single stage batch extraction, the aqueous feed is mixed with the organic solvent and after equilibration; the extract phase containing the desired solute is separated out for further processing. In some cases, a single stage extraction may not be enough and multi stage process is required wherein fresh volume of solvent is contacted with the raffinate.

Continuous extraction can be carried out by co-current or counter current methods. In Cocurrent extraction there are n mixer vessels in line and the raffinate goes from vessel 1 to vessel n. Fresh solvent is added to each stage and the extracting solvent pass through the cascade in the same direction. At every stage the extract is recovered. In Counter current extraction the extracted raffinate passes from vessel 1 to vessel n while the product-enriched solvent is flowing from vessel n to vessel 1. This is the most efficient method of extraction.



(a) Single stage and (b) double stage extraction process



Counter current extraction process

Extraction is achieved by three mechanisms *viz.*, physical extraction, dissociative extraction and selective extraction. Physical extraction involves preferential dissolution of the desired solute in a chosen organic solvent. Dissociative extraction involves the modification of the physical property of the solute to increase the solubility in organic phase. For example for extraction of organic acids pH is adjusted below *pK* value enhancing dissociation and thus

extraction. Selective extraction involves modifying the solute solubility through ion pair or complex or adducts formation. For example long chain aliphatic amines are used for citric acid extraction. Solvent recovery after extraction process Solvent recovery after extraction process is essential one which is usually done by distillation. The distillation is performed in three stages (i) evaporation of solvent into vapour phase, (ii) vapour-liquid separation and (iii) condensation to collect solvent.

Aqueous two phase extraction

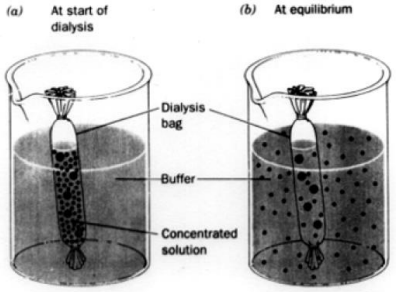
The basic principle involves differential partitioning of solute in two immiscible phases. Phase separation occurs when hydrophilic polymers are added to an aqueous solution. At low concentration of polymers, homogenous solution is formed but at discrete concentration rise, two immiscible phases are formed using two aqueous phases with incompatible polymers such as PEG and dextran. Eg. PEG water / dextran water and PEG water/K-phosphate water, PEG phosphates. Homogenates are prepared with the two incompatible polymers after which mixer – phase separation is done by keeping idle. The bottom phase and top phase separated. Then the soluble and nonsoluble substances are separated by ultra filtration and product recovered. The retentate may be recycled for further recovery.

Precipitation - by decreasing the solubility of the solutes the solute can be separated by precipitation Solubility of the particle can be changed by,

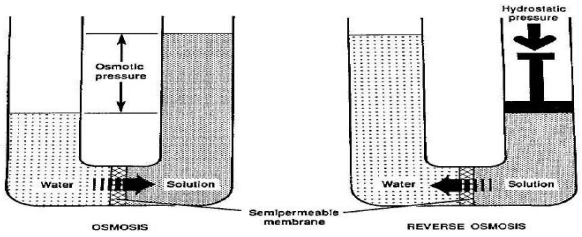
1) Salting out – by increasing ionic strength by adding salts as ammonium sulphate, disodium sulphate
2) Solubility reduction at low temperature – by adding organic solvents at low temperature
3) Solvent precipitation – adding salt, pH adjustment and low temperature
4) Isoelectric precipitation – by the changing the pH to isoelectric pH (no charge in proteins)
5) Use of electrolytes – ionic polymers (ionic polysaccharides), non ionic polymer (dextrans)

Adsorption - This refers to the binding of molecules or particles to a surface or adsorption of solutes from liquid media onto solids. Adsorption occurs due to van der Waals' force, strong ionic bonds. There are different types of solid – liquid contactors to facilitate adsorption *viz.*, packed bed, moving bed, fluidized bed, and agitated vessel contactors. Packed beds are adsorbent bed packed and liquid sent through it. In Moving bed, adsorbent solid is continuously supplied, after adsorbing get removed from vessel. Fluidized bed is in which adsorbent solid is suspended in liquid. Packed and moving beds are widely used.

Dialysis is a membrane separation used to remove low molecular weight solutes (organic acids, inorganic ions). Dialysis is the movement of molecules by diffusion from high concentration to low concentration through a semi-permeable membrane. Only those molecules that are small enough pass through the membrane pores and reach equilibrium with the entire volume of solution in the system. Once equilibrium is reached, there is no further net movement of the substance because molecules keep moving through the pores in and out of the dialysis unit at the same rate. Factors affecting dialysis: dialysis buffer volume, buffer composition, number of buffer changes, time, temperature and particle size. Membranes containing convoluted pores, not the tube-like pores often found in traditional dialysis tubing.



Reverse osmosis is a process where the solvent molecules are forced by an applied pressure to flow through a semi permeable membrane in the opposite direction. Reverse osmosis from low to high by applying pressure uses membrane (pore size 0.0001 – 0.001 μm) permeable to water but not dissolved salts of low molecular weight. This method is applicable to concentrate smaller molecules



Supercritical fluid extraction

Supercritical fluid extraction involves the dissolution power of super critical fluids *ie.* fluids above their critical temperature and pressure. Critical temperature is defined as the temperature above which a distinct liquid phase cannot exist regardless of pressure. The vapour pressure of the substance at its critical temperature is called the critical pressure. Alternately, pressure and temperature required to liquefy a gas are critical temperature and pressure. At temperature and pressure above but close to the critical point a substance exists

as a supercritical fluid. For example Carbon dioxide, NO, SO_2 are used in extraction of β-carotene, vanilla, vegetable oil, etc.

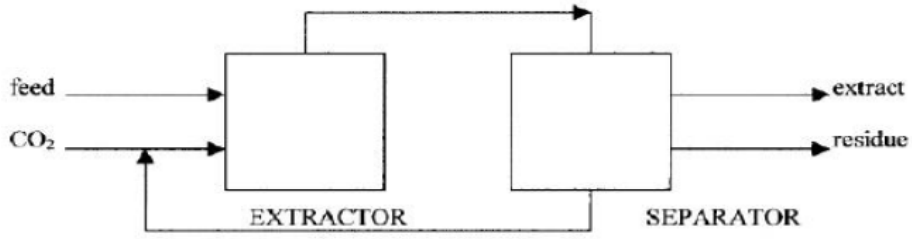


Fig: Supercritical fluid extraction process. CO2 is fluidized and used for extraction

Concentration: Some concentration of the product may occur during the extraction step. Further concentration may be achieved by: (i) evaporation, (ii) membrane filtration, (iii) ion exchange methods and (iv) adsorption methods.

(a) Evaporation. It is generally used in cases of solvent extraction usingvarious devices, *e.g.*, continuous flow evaporators, falling film evaporators, thin film evaporators, centrifugal thin film evaporators and spray-dryers. Efficient arrangements must be made for recovery of the evaporated solvent to reduce costs. For low grade products, often evaporation of the whole broth is undertaken using a spray-drier.

(b) Membrane Filtration. It generally achieves both concentration and separation of the products usually based on the size of molecules. The different processes of membrane filtration are: microfiltration, ultrafiltration, reverse osmosis and electrodialysis. Micro and ultra-filtration work as sieves and separate molecules of different sizes, but reverse osmosis can separate molecules of similar size. Microfiltration can be used for cell separation as well.

(c) Ion Exchange Resins. These are polymers having firmly attached ionizable groups (anions or cations) which ionize under a suitable environment. These may be solid, *e.g.*, dextran, cellulose, polyamine, acrylate etc., or liquid, e.g., a solvent carrying a functional group like phosphoric acid mono or diester etc. Solid ion exchangers may be used in two ways: (i) they may be packed in columnsor (ii) they may be added to the extract and removed by decantation. Liquid ion exchangers dissolve only in nonaqueous solvent carrier and the separation is similar to liquid-liquid extraction. Some antibiotics are recovered directly from the whole broth using ion exchange resins. The product is recovered from the ion exchangers by ion displacement; this also regenerates the ion exchanger.

(d) Adsorption Resins. These are porous polymers without ionization. Most compounds are adsorbed to the resins in non-ionized state. The porosity of the resin determines the surface available for adsorption. These resins may be apolar (e.g.,styrene-divinyl beneze), polar (e.g., sulfoxide, amide etc.), or semipolar (e.g., acrylic ester). The products are recovered from such resins by solvent (organic) extraction, changed pH etc.

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 proc
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.

Kieselguhr filters

Filters that use a powder medium are considerably more complicated to operate, but can filter much more beer before regeneration.

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*, *Coryne bacterium*, *Flavobacterium*, *Lactobacillus*, *Micromonospora*, *Mycobacterium*, *Nocardia*, *Propionibacteriu m*, *Protaminobacter*, *Proteus*, *Pseudomonas*, *Rhizobium*, *Salmonella*, *Serratia*, *Streptomyces*, *Streptococcu s* 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 – 5; Possible Questions

Part-A (1 Mark)

Part-B (2 Mark)

1. Write the types of fermentation.
2. What is lag phase?
3. What is batch fermentation?
4. What is continuous stirred tank bioreactor?
5. What is sparger?
6. Write the advantages of continuous stirred tank bioreactor?
7. Define tower bioreactor.
8. What is fluidized bed bioreactor?
9. Write the types of conventional bioreactor.
10. What is sterilization?
11. What is aeration?
12. Define dissolved oxygen.
13. What is flotation?
14. Write the types of filters.
15. What is multi chamber centrifuge?
16. What is thermolysis?
17. Write the chemical methods of cell distruption.

Part-C (8 Marks)

1. What is the alcohol content of beer?
2. Name the organism employed for beer production.
3. Name the organism employed for riboflavin production.
4. Name the organism employed for vitamin B12.
5. Name the penicillin producer.
6. What is the mode of action of penicillin?
7. Principles of affinity chromatography.
8. How is beer sterilized?
9. How are drugs purified?
10. Upstream processing and downstream processing.
11. Define LSTR.
12. Advantages of packed bed reactors and photo bioreactors.
13. Give examples of intracellular and extracellular product separation.
14. Methods of filtration and filters.
15. Name the types of fermentor used in beer production.
16. List the methods of drying.
17. What are the precipitating agents used for precipitation?

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB COURSE NAME: FOOD AND INDUSTRIAL MICROBIOLOGY

COURSE CODE: 17MBP302 UNIT: V BATCH-2017-2019

Sl. No	Question	Option A	Option B	Option C	Option D	Correct Ans
1	Wine is fermented by _____.	Bacteria	Yeast	Parasite	Virus	Yeast
2	Asepsis know as ----------	Keeping out from microbes	Filteration	Removal of microbes	Heating	Keeping out from microbes
3	-------is widely used application of asepsis technique	Centrifugation	Heating.	Cooling	Packaging	Packaging
4	In wine fermentation grapes are crushed and pressed to release the juice, caled	Must	Grape juice	Rust	Extract	Must
5	During the production of wine malo-lactic fermentation is used to reduce	Alkalinity	Acidity	Neutral	All the above	Acidity
6	In wine making malic acid is degraded by	Sulfur bacteria	Acetic acid bacteria	Lactic acid bacteria	Zymomonas	Lactic acid bacteria
7	Wines which have undergone undesirable changes in flavor due to lactic acid bacteria are said to have a	Propionic acid	Acetic acid	Formic acid	Lactic acid	Lactic acid
8	Wine defects caused by after fermentations are due to the fermentation of	Citric acid	Vinegar	Both a and b	Residual sugar	Residual sugar
9	When wines of low alcohol concentration are exposed to air yeasts develop on the surface	Budding	Sugar	Film foaming	None	Film foaming
10	Chemically beer is sterilized by adding	Benzoic acid	n-heptyl p-hydroxy benzoate	Acetic acid	Alcohol	n-heptyl p-hydroxy benzoate
11	Bottled beer is sterilized by	Pasteurization	Decolorization	Chemicals	Washing	Pasteurization
12	Top fermented beers are generally called	Ales	Alcohol	Wine	All the above	Ales
13	In beer making yeast is inoculated into clarified	Juice	Ales	Wort	None	Wort
14	During beer making, the conversion of the barley starch to sugar is called	Crushing	Malting	Icing	Pressing	Malting

15	After beer has been malted, the germinated grain is added to warm water to give an extract called	Wort	Malt	juice	Extraction	Wort
16	Industrial alcohol is produced by fermentation using the micro organism	Bacillus	Saccharomyces cerevisiae	Zymomonas	Pseudomonas	Saccharomyces cerevisiae
17	Wine can be produced by the fermentation of	Berries	Honey	Grapes	All the above	All the above
18	To prevent the loss of delicate flavors of some wines, the preferred method of removing residual microorganism from wine is	Filtration	Addition of so2	Pasteurization	All the above	Filtration
19	In wine making secondary fermentations are carried out by	Saccharomyces	Lactic acid bacteria	Aceticacid bacteria	Zymomonas	Lactic acid bacteria
20	The color and flavor of red wine come from _____.	The grapes skin and seed	Artificial additives	The yeasts used	Yeast fermentation	The grapes skin and seed
21	Beer spoilage is generally caused by which of the following bacteria?	Lactic acid bacteria	Aceticacid bacteria	Zymomonas anaerobia	All the above	All the above
22	In the beer production, hops is responsible for the _____.	Aromatic and pungent character	Preservative action	Stabilizing effect	All the above	All the above
23	In the beer production, hops is added as _____.	An adjunct	A carbonating agent	A seasoning agent	All the above	A seasoning agent
24	In the ageing process of beer beach wood chips are added to the tanks to provide _____.	Surface of the yeast	Desired flavor	Adjuant	Enzymes	Surface of the yeast
25	The oldest fermented alcoholic beverage is _____.	Beer	Whisky	Wine	Brandy	Beer
26	Which of the following is referred to as bottom yeast in beer making?	Saccharomyces cerevisiae	Saccharomyces bayanus	Pseudomonas	Saccharomyces uvarum	Saccharomyces uvarum
27	Malt adjuncts are additional provided in the medium for	. Protein	Starch	Lipids	Amino acids	Starch

	brewing _____.					
28	Mass cultivation of algae for SCP is usually carried out under photosynthetic condition in plants.	Fed batch	Bio-processing	Filtration	Sewage treatment	Sewage treatment
29	Hydrocarbon of interest in the bacterial SCP production is	Methanol	Ethanol	Acetic acid	Propionic acid	Methanol
30	Riboflavin fermentation employs media containing _____.	Sugar and lipid	Protein and lipid	Organic acid and inorganic acid	Organic and inorganic salts	Sugar and lipid
31	Species of Candida involved in riboflavin fermentation are extremely sensitive to traces of _____.	Manganese	Iron	Calcium	Magnesium	Iron
32	Fermentation medium used in riboflavin production employing candida need not be sterilized since the sterilization is carried out _____.	At low nutrient concentration	At low humidity	At low temperature	At low PH	At low PH
33	Semi- synthetic penicillin are prepared from _____.	5-A PA	4 – APA	6 – APA	3 - APA	6 – APA
34	Strains of Aspergillus orzyae are used for the production of amylase by culture.	Mobile	Log	Lag	Stationary	Stationary
35	Baker's yeast is produced from the strains of '	Aspergillus niger	Pseudomonas	Saccharomyces cereviciae	Bacillus	Bacillus
36	Daily requirement of vitamin B12 from human beings is _____.	0.05mg/day	0.001mg/day	0.002mg/day	0.5mg/day	0.001mg/day
37	Cyanocobalamine consisting of a molecule of _____ linked to a nucleotide.	Cobalt	Cyanide	Cobinamide	Cupric oxide	Cobinamide
38	Vitamin B12 is manufactured by _____ culture process.	Batch	Continuous	Submerged	Tubular fermentation	Submerged
39	Vitamin B12 production using Streptomyces olivaceus NRRLB _____ strain.	1125	1127	1161	1141	1125

40	In Bennet's agar the composition of N-2- Amine A is _____ g/lit	4	2	3	5	2
41	Cobalt increases the growth of _____.	Streptomyes olivaceus	S. griseus	S. oryzae	P. notatum	Streptomyes olivaceus
42	Temperature of _____ in the production tank is satisfactory during fermentation.	75° F	80° F	85° F	63° F	80° F
43	Stabilization of mask is practiced by reducing the pH and adding reducing agents _____.	Sodium citrate	Sodium sulphite	Ammonium sulphate	Sodium thiosulphate	Sodium sulphite
44	Sterilization of air is done by passing it through columns filled with _____.	Activated charcoal	Dry charcoal	Liquid paraffin	Melted charcoal	Activated charcoal
45	_____ are the antifoam agents used to suppress the foam formation.	Soyabean oil	Cord-linee oil	Palm oil	Cedar-wood oil	Soyabean oil
46	The yield of cobalamine are usually in the range of _____mg/ litre.	2 – 3 mg	1 – 2 mg	2 - 4 mg	2 – 5 mg	1 – 2 mg
47	Adsorbing agent used in packed clumns are _____.	TEMED	Methylene blue	Bentorite	Mica	TEMED
48	Betaine is added to medium to furnish _____ groups.	Methyl	Ethyl	Propyl	Calloxyl	Methyl
49	Size of fermentors used in riboflavin production ranges from _____ gal.	1000 – 10000 gals	10000 – 100000 gals	1000 – 100000 gals	500 – 10000 gals	10000 – 100000 gals
50	Precipitation of riboflavin by addition of _____.	Dithionite	Trithionite	Mononthionite	thionite	Dithionite
51	High- yielding strain in the manufacture of an antibiotic is achieved by _____.	Backward mutation	Secondary screening	Sequential genetic isolation	Revertant mutant	Sequential genetic isolation
52	_____ strains is used for the production of Penicillin.	penicillium chrysogenum	P. notatum	A. niger	A. flavus	penicillium chrysogenum
53	_____ act as carbon source.	Lactose	Maltose	Dextrose	Fructose	Lactose

54	Removal of mycelium is done by _____.	Radiation	Filtration	Extraction	Sonication	Filtration
55	A major ingradient of penicillin production media is	Corn meal	Corn steep liquor	Cane steep liquor	None of these	Corn steep liquor
56	The outstanding example of traditional microbial fermentation product is	Vinegar	Penicillin	Citric acid	Tetracyclin	Vinegar
57	Industrially important Antibiotic producing organisms shall be isolated by	Disk plate method	Direct plate method	Serial dilution method	Crowded plate method	Crowded plate method
58	Industrial alchohol will be produced by using starter culture	Top yeast	Middle yeast	Bottom yeast	Feeder yeast	Bottom yeast
59	In the industrial production of streptomycin, the secondary metabolite or byproducts is	Vitamin – B12	Vitamin – C	Vitamin – B6	Ethanol	Vitamin – B12
60	Penicilin is commercially produced by	P.notatum	P.chrysogenum	P.citrinum	P.roquefortii	P.chrysogenum

Reg. No. : ----------

[17MBP302]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

M.Sc. DEGREE EXAMINATION, AUGUST 2018

DEPARTMENT OF MICROBIOLOGY

I INTERNAL TEST – THIRD SEMESTER

FOOD AND INDUSTRIAL MICROBIOLOGY

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

Date / Session:

Part A

Multiple Choice Questions: No 1 to 20 **20x1 = 20 marks**

1. Which of the following is called 'bread mold'?
   A. *Mucor rouxii* B. *Trichothecium roseum*
   C. *Rhizopus stolonifer* D. *Thanmidium elegans*
2. Which of the common microflora found in water
   A. *Micrococcus* B. *E.coli*
   C. *Chromobacterium* D. All the above
3. Which of the following are beneficial molds?
   (i) *Penicillium digitatum* (ii) *Penicillium camemberti*
   (iii) *Penicillium italicum* (iv) *Penicillium roqueforti*
   A. (i) and (iii) B. (ii) and (iii)
   C. (i) and (iv) D. (ii) and (iv)
4. Bacteria grow fastest in the pH range ____, yeast ____ and filamentous fungi ____
   A. 5.0-7.0, 4.5-7.0 and 3.5-4.0 B. 6.0-7.0, 4.5-6.0 and 3.5-4.0
   C. 6.0-7.0, 5.0-6.0 and 3.5-4.0 D. 6.5-7.0, 3.8-7.0 and 4.6-5.0
5. Total moisture below _____ percent in dried fruits and flour will prevent or greatly delay mold growth.
   A. 15 to 17 B. 13 to 15
   C. 14 to 15 D. 12 to 14
6. Unicelled microbes grown as a source of proteins are called as _____
   A. Single cell proteins B. Unicelled proteins
   C. Microbial proteins D. None of these
7. The O-R potential of a system is measured by _____
   A. mV B. Eh
   C. mV D. aw
8. The water requirement of a microorganism is expressed in terms of _____
   A. Water action B. Water activity
   C. Water adsorption D. Water affinity
9. Which of the following yeast are involved in discolouration of food?
   A. *Rhodotorula* sp. B. *Saccharomyces uvarum*
   C. *Pichia* sp. D. *Schizosaccharomyces pombe*
10. _____ shows tolerance to high carbon dioxide levels.
    A. *Alternaria* sp. B. *Zygosaccharomyces* sp.
    C. *Candida* sp. D. *Bettanomyces* spp.
11. _____ causes gaseous spoilage of canned foods
    A. *C.jejuni* B. *C.lentoputrescens*
    C. *C.thermosaccharoolyticum* D. *A.hydrophila*
12. Pasteurization is employed to kill_____
    A. Bacteria B. Fungi

C. Selective microorganism D. All the microorganisms

13. _____ is associated with the market disease called 'Bacterial soft rot'

A. *Alcaligenes* sp. B. *Erwinia* sp.

C. *Clostridium* sp. D. *Campylobacter* sp.

14. Which of the following is NOT an intrinsic factor in food spoilage?

A. pH B. Nutrient content

C. Temperature D. Moisture content

15. Preservation affects the growth of microorganism by _____

A. Antisepsis B. Inhibition

C. Retardation D. Arresting

16. _____ bacteria are able to grow at commercial refrigeration temperatures

A. Autotrophic B. Halophilics

C. Heterotrophic D. Pschrotropic

17. Louis Pasteur established the modern era of food microbiology in 1857 when he showed that microorganisms cause _____ spoilage.

A. Beer B. Fruit juice

C. Milk D. Wine

18. What is the concentration of Nisin A used for the preservation of _____ and _____ products

A. 100-200 ppm, dairy and meat B. 150-250 ppm, milk and fish

C. 100-200 ppm, dairy and juice D. 110-280 ppm, dairy and meat

19. Which of the following bio preservative found in an egg?

A. Benzoic acid B. Lysozyme

C. Eugenol D. Allicin

20. _____ is an alternative to chlorination in the disinfection of water in water filters.

A. X rays B. microwave radiation

C. UV radiation D. Gamma rays

Part B

Answer all the questions **3x2 = 6 marks**

21. What are coliforms?

22. Define water activity

23. What are the effects of thawing?

Part C

Answer all the questions **3x8 = 24 marks**

24. A. Write notes on important molds, yeast and bacteria in food microbiology

Or

B. Explain the intrinsic and extrinsic parameters of food affecting microbial growth

25. A. Explain the sources of contamination of food

Or

B. Brief note on preservation of food using temperature

26. A. List out the characteristics of thermotrophs and psychrotrophs

Or

B. Brief note on preservation of food using radiation

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Reg. No. : ----------

[17MBP302]

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
SECOND INTERNAL ASSESSMENT, OCTOBER 2018
THIRD SEMESTER
MICROBIOLOGY
FOOD AND INDUSTRIAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**
Date: 08 /10/2018 [AN] **Class: II M.Sc. MB**

PART A – (20 x 1 = 20 marks)
Answer all the questions

1. Salmonellois is caused by the _____ of Salmonella spp.
   a) enterotoxin b) endotoxin c) exoenterotoxin d) exfoliative
2. The optimal temperature for growth of Shigellosis is _____.
   a) 27 °C b) 37 °C c) 40 °C d) 50 °C
3. The FDA and USDA cooperative is a _____ surveillance program for dry milk products.
   a) Pseudomonas b) E. coli c) Salmonella d) Vibrio
4. A_____ refers to food borne illnesses caused by the entrance of bacteria into the body through ingestion of contaminated food.
   a) Food infection b) food poisoning c) food intoxication d) Food illness
5. What is the main type of micro-organism responsible for food poisoning?
   a) Bacteria b) Mould c) Virus d) Parasite
6. The pH near ____ favors *C. botulinum*.
   a) alkalinity b) neutrality c) acidic d) base
7. Who is the father of fermentation technology?
   a) Louis Pasteur b) Alexander Fleming c) Robert Koch d) Mutualism
8. Who described a lens?
   a) Salvino D Armati b) Roger Bacon c) Fabri d) Leewen Hoek
9. The medium which doesnot contain particular essential nutrient is called as_____.
   a) Enriched media b) Minimal media c) Molden agar d) Selective media
10. Indicator dye are used in the medium to detect _____
    a) temp change b) nutrient concentration c) ph change d) aw availability
11. Crowded plate technique is used in the detection _____ producing organisms.
    a) Growth factor b) Aminoacid c) Antibiotic d) Organic acid
12. Freeze drying is called as _____.
    a) Lyophilization b) Liquefaction c) Freezing d) Nitrogen storage
13. Nitrogen storage is also called as _____.

a) Freezing b) Cooling c) Cryogenic storage d) Lyophilization

14. Organism which does not produce particular product which is require for growth is called _____.

a) Auxotrophs b) Heterotrophs c) Autotrophs d) Phototrophs

15. In industries mostly used mutation agent is _____.

a) UV-rays b) EMS c) Mustard gas d) MNNG

16. Wine is fermented by _____.

a) Bacteria b) Yeast c) Parasite d) Virus

17. Top fermented beers are generally called_____.

a) Ales b) Alcohol c) Wine d) Arrack

18. In beer making yeast is inoculated into clarified_____.

a. Juice b) Ales c) Wort d) Bile

19. Penicilin is commercially produced by_____.

a. P.notatum b) P.chrysogenum c) P.citrinum d) P.roquefortii

20. Removal of mycelium is done by _____.

a) Radiation b) Filtration c) Extraction d) Sonication

PART B – (03 x 02 = 06 marks)

Answer all Questions

21. Define fermentation?
22. Comment on food borne infection.
23. Expand the term HACCP.

PART B – (3 x 08 = 24 marks)

Answer all questions choosing either a (or) b. (All questions carry equal marks)

24. Write notes on microbial quality control. **(OR)**

    Outline the importance of HACCP.

25. Discuss in detail about the industrially important strains isolation and preservation. **(OR)**

    Give a note on the various types of fermentation**.**

26. Outline briefly about the types of fermentor. **(OR)**

    State the protocol of beer production.

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