SEMESTER 7
15 APPLIED BIOTECHNOLOGY
Paper Code (BBT 7001 [A])
(Credits: Theory-4, Practicals-2)
THEORY Lectures: 20

h and its maintenance.
g different bioreactors.
ochemistry of different industrial chemicals

tation technology, bioreactors, production optimization, and

n of the course the students should have understood the basics
gy and learnt the concept of different metabolite production by
up.

d Death Kinetics: (10 Lectures)
industrially important microorganisms, Microbial Growth and
rial Fermentation, Air and Media Sterilization. IKS: Contribution

f Bioreactors: (10 Lectures)
es: Analysis of batch, fed-batch and continuous bioreactors,
analysis of mixed populations, specialized bioreactors-pulsed,

fluidized, photo bioreactors, etc. Measurement and Control of bioprocess parameters. Downstream processing, Whole cell immobilization and their industrial applications.

Unit-III Fermentation Technology: (10 Lectures)
Industrial production of chemicals: Ethanol, Acids (citric, acetic and gluconic acid), Solvents (glycerol, acetone, butanol), Antibiotics (penicillin, streptomycin, tetracyclin), Semi-synthetic antibiotics, Amino acids (lysine, glutamic acid), Single cell protein.

Unit-IV Applications of Bioprocess Engineering: (10 Lectures)
Agitation and aeration: requirement in industrial processes, concept of volumetric oxygen transfer coefficient and its determination (Kla). Factors affecting Kla values; Uses of microbes in mineral beneficiation and oil recovery. Introduction to food technology; Elementary idea of canning and packaging, Sterilization and pasteurization of food products.

PRACTICALS

1. To plot Microbial growth curve for shake flask culturing using turbidity method.
2. Prepare a standard curve of reducing sugar by 3,5-Dinitrosalicylic acid method
3. To produce invertase enzyme and find its activity from Baker's Yeast
4. Preparation of standard curve of Ethanol
5. Quantitative estimation of ethanol produced during Yeast fermentation
6. Production of Penicillin and assaying its activity
7. To get familiarized with the lab scale fermenter (bench top fermenter)
8. To determine dissolved oxygen concentration in tap and aerated water.
9. To determine the volumetric transfer coefficient (K la)
10. Estimation of BOD in a given waste water sample.
11. Centrifugation studies during settling of yeast cells.
12. Yeast cell disruption by mechanical methods.

SUGGESTED BOOKS

1. Bioprocess Engineering, Shular M &Kargi F, Prentice Hall
2. Biochemical Engineering Fundamentals, Bailey JE &Ollis DF
3. Bioprocess Engineering Principles, Doran, PM, Academic Press, California

Microbial Enhanced Oil Recovery (MEOR)

An alternative approach for oil recovery is MEOR. MEOR often constitutes the introduction of live microorganisms with essential nutrients into an injection well. When favorable environmental conditions are present in the reservoir, the introduced microbes grow exponentially and their metabolic products mobilize the residual oil

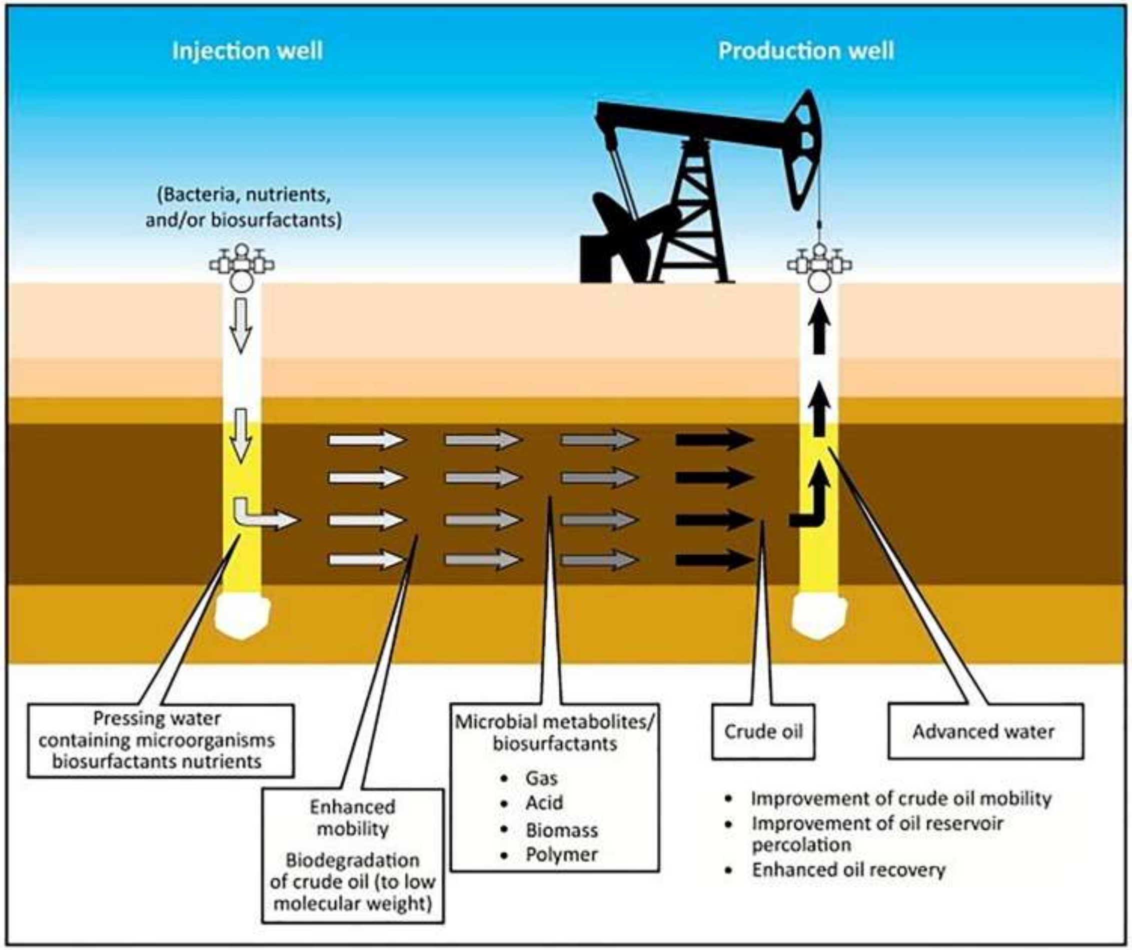
The injected microorganisms can produce a selection of metabolic products which find useful applications in EOR

The growth of the microorganisms and their effects depend on any number of several factors

(i) pressure, porosity and permeability, temperature, pH, dissolved solids, and salinity of the reservoir; (ii) availability of nutrients to the bacteria; (iii) the specific type of microorganisms injected into the reservoir.

- MEOR is believed to be able to extract up to 50% of the residual oil left in a reservoir after primary and secondary recovery processes have been exhausted
- The additional recovery is accomplished by modification of the chemical and physical properties of reservoir rocks and crude oil by the microbial growth and metabolites produced
- **The leading processes** accountable for oil release from porous media. These are: (1) dissolution of inorganic carbonates by bacterial metabolites; (2) generation of bacterial gases which decrease the viscosity of oil, and by that enhancing its flow through the rocks; (3) production of surface-active compounds by some bacteria; and (4) the high affinity of bacteria for solids, which would dislodge oil films from the surface of rocks in the reservoir.

Microbial Enhanced Oil Recovery (MEOR)



TRENDS in Biotechnology

Solvents such as acetone, butanol and propan-2-diol are produced by bacteria of the genera *Clostridium*, *Zymomonas*, and *Klebsiella*. Methane and hydrogen gas are produced by species of bacterial *Clostridium* and *Enterobacter*, as well as by the archaeon *Methanobacterium*.

Fermentation gases can re-pressurize wells, leading to displacement of light crude oil in the well and, thus, facilitating its recovery

Microbial Enhanced Oil Recovery (MEOR)

Microbial products and their application in MEOR.

Product	Application in oil recovery
Biomass	Selective biomass plugging, viscosity reduction, oil degradation, rock wettability alteration
Biosurfactants	Oil emulsification, decrease of interfacial tension, viscosity reduction
Biopolymers	Injectivity profile modification, mobility control
Solvents	Oil dissolution, viscosity reduction
Acids	Permeability increase, emulsification
Gases	Increased pressure, oil swelling, decrease of interfacial tension, viscosity reduction, permeability increase

doi: 10.3389/fmicb.2019.02996

Microbial Enhanced Oil Recovery (MEOR)

More than four hundred MEOR field tests have been conducted in the United States alone. However, the majority were single-well stimulation (also known as microbial "huff and puff") treatments on low-productivity wells; hence, reliable data are sparse.

TABLE 2.

Comparison between microbial huff and puff operation, and bacteria flooding. Adapted from Gao and Zekri (2011).

Microbial huff and puff	Bacteria flooding
Bacteria injected through production tubing	Bacteria injected through injector well
Localized effect near the wellbore	Transport bacteria deep into the reservoir via water flooding
Reservoir shut-in period to allow bacteria to grow	Reservoir shut-in period to allow bacteria to grow
Repeat several times to maximize the gain	Large scale effect
Preferred MEOR option	Involve drilling of injector well unless some are present

MICROBIALLY INDUCED MINERAL BENEFICIATION

Microbe–mineral interactions result in several significant consequences of relevance in mineral beneficiation, namely,

- adhesion of microorganisms to mineral surfaces resulting in biofilm formation,
- biocatalyzed oxidation and reduction reactions, and
- adsorption/chemical interaction of metabolic products.

The end results of such biogenic reactions manifest as mineral formation and conversion, surface modification, dissolution of mineral constituents, as well as biometal accumulation.

Selective leaching, flotation, and flocculation are some of the processes involved in biomineral processing.

The initial adhesion of these organisms on the sulfide mineral substrate is the most vital step in the process of bioprocessing and has been an area of much research. The interaction between the cell and the surface has been shown to be dependent on a number of physical and biochemical parameters. The bacteria are postulated to adhere to the mineral surface directly and utilize cell surface-associated or extracellular biopolymers to catalyze chemical reactions on the surface of the mineral.

The scanning electron micrograph of a biotreated refractory sulfide concentrate in Figure 1 shows the direct attack of A. ferrooxidans, an iron- and sulfur-oxidizing microbe, on the sulfide mineral.

The main components of the cell surface that may contribute to adhesion are LPSs and outer membrane proteins physical parameters like particle size, incubation time, and agitation are important to the overall attachment, the actual attachment mechanism is based on specific and nonspecific interactions with the system.

Biomineral beneficiation

Biomineral beneficiation brought about by microorganisms mediate in a number of surface-chemical and physicochemical phenomena such as the following:

- Alteration of surface chemistry of minerals
- Generation of surface-active chemicals
- Selective dissolution of mineral phases in an ore matrix
- Sorption, accumulation, and precipitation of ions and compounds

Such microbe–mineral interactions release metal ions, which can bind with the organisms by the following ways:

- Extracellular interactions: Involves extracellular polysaccharides, proteins, and acid metabolites
- Cell surface interactions: Binding to cell surfaces through specific functional groups
- Intracellular interactions: Accumulation in cells due to specific transport processes

Several types of autotrophic and heterotrophic bacteria, fungi, yeasts, and algae are implicated in mineral beneficiation processes. However, the role of only a few types of bacteria, namely, Acidithiobacillus ferrooxidans, sulfate-reducing bacteria (Desulfovibrio), Paenibacillus polymyxa, and Mycobacterium phlei, has been so far clearly established in influencing the flotation behavior of various minerals

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Canning:

Canning is a popular method of preserving food items, including fruits, vegetables, meats, and seafood, by sealing them in airtight containers and subjecting them to heat to destroy spoilage-causing microorganisms. The canning process ensures the long-term shelf stability of the products while retaining their nutritional value and flavour.

Concepts of Canning:

1.Preservation through Heat Treatment: Canning involves heating food items in hermetically sealed containers to kill or inactivate microorganisms, including bacteria, yeasts, and moulds. This heat treatment prevents the growth of spoilage and pathogenic microorganisms, thus preserving the food for extended periods.

2.Airtight Sealing: Canning containers, typically made of metal or glass, are sealed tightly to prevent the entry of air and microorganisms. This airtight sealing creates a barrier that keeps the food safe from contamination.

3.High-Temperature Processing: Canned products are subjected to high temperatures during processing. This thermal treatment destroys enzymes that can lead to deterioration and inactivates harmful microorganisms, ensuring the safety and quality of the canned items.

4.Vacuum Sealing: In the canning process, a vacuum is created inside the container, which helps to remove air and reduce the oxygen level. This reduces the risk of oxidative reactions that could lead to food spoilage.

5.Extended Shelf Life: Properly canned products can have a long shelf life, allowing consumers to enjoy seasonal fruits and vegetables throughout the year and reducing food waste.

Canning Standards:

To ensure the safety and quality of canned products, various standards and guidelines are followed:

1. **Good Manufacturing Practices (GMP):** Canning facilities must adhere to GMP, which includes maintaining hygienic conditions, employing trained personnel, and using appropriate equipment.
2. **Heat Processing Time and Temperature:** Specific time and temperature combinations are employed during the heat treatment to achieve adequate sterilization and kill harmful microorganisms.
3. **pH Control:** For certain low-acid foods, such as vegetables and meats, the pH level is adjusted to ensure the safety of the canned products.
4. **Container Integrity:** Cans and jars must be free from defects, such as dents or cracks, to ensure a proper seal and prevent leakage or contamination.
5. **Labelling and Traceability:** Canned products must have accurate and informative labels, including the product name, ingredients, manufacturing date, and storage instructions. Traceability measures are implemented to identify the source of ingredients in case of any quality issues.

Packaging of Canned Products:

Canned products are typically packaged in:

1. **Metal Cans:** Metal cans are commonly used for canning fruits, vegetables, meats, and seafood. They are sturdy, provide excellent protection from light and air, and are easily recyclable.
2. **Glass Jars:** Glass jars are preferred for canning products like jams, jellies, and sauces. They allow for a transparent view of the contents and are reusable for home canning.
3. **Sealing Lids:** Airtight sealing lids, usually made of metal or plastic, are used to close the cans or jars securely.

Types of containers

There are different types of containers that can be used for canning, depending on the food and the design. Some of the common containers are:

- **Steel cans**. These are metal containers that are strong and can handle high heat and pressure. They are coated with tin or chromium to prevent rusting. Steel cans are used for many foods, such as fruits, vegetables, meats, soups, sauces, and drinks.
- **Aluminum cans**. These are metal containers that are light and can also handle high heat and pressure. They are made by shaping a piece of metal into a cylinder with a round end. Aluminum cans are mainly used for drinks, such as soft drinks, juices, beer, and wine.

- **Glass jars**. These are clear containers that can show the color, taste, and nutrients of food. They are made by melting glass into different shapes and sizes. Glass jars are used for foods that need low to medium heat to preserve them, such as jams, pickles, honey, and sauces.
- **Plastic containers**. These are flexible containers that can be made into different shapes and sizes. They are made from different kinds of plastic materials, such as PET, PP, or PS. Plastic containers are used for foods that do not need heat to preserve them, such as salads, dips, spreads, and dairy products.

The process of heat treatment, airtight sealing, and adherence to established standards ensure that canned products retain their nutritional value and taste while remaining free from harmful microorganisms. Proper packaging further contributes to the protection and longevity of canned foods, making them a valuable addition to post-harvest management and enhancing food availability throughout the year.

Packaging of Products

Packaging plays a crucial role in various industries, including food, pharmaceuticals, electronics, and more. It involves designing and creating protective and attractive containers for products, ensuring their safety, preservation, and market appeal. Proper packaging is essential for post-harvest management, value addition, and delivering products to consumers in optimal condition.

Importance of Packaging:

Product Protection: Packaging serves as a protective barrier against physical damage, moisture, light, air, and other external factors that can affect the quality and shelf life of the product.

Preservation and Freshness: Packaging helps preserve the freshness, flavour, and nutritional value of perishable goods like fruits, vegetables, and dairy products by creating a controlled environment.

Safety and Hygiene: Proper packaging ensures that products reach consumers in a safe and hygienic condition, reducing the risk of contamination and ensuring consumer confidence.

Brand Identity and Marketing: Packaging plays a significant role in establishing a brand identity and influencing consumers' purchasing decisions. Attractive and well-designed packaging can enhance a product's market appeal.

Convenience and Portability: Packaging provides convenience in handling, storage, and transportation of products, making it easier for consumers to use and carry them.

Aspects of Packaging: Materials: Packaging materials vary depending on the product type, shelf life, and intended use. Common materials include cardboard, plastic, glass, metal, and flexible packaging like pouches and wrappers.

Design and Graphics: Packaging design includes shape, size, and visual elements like logos, images, and colours that communicate the product's identity and brand message.

Protection and Safety Features: Packaging may include safety features like tamper-evident seals, child-resistant closures, and barrier properties to ensure product integrity and consumer safety.

Labelling and Information: Product labels provide essential information such as product name, ingredients, nutritional facts, manufacturing date, expiry date, and usage instructions.

Sustainability: Eco-friendly and sustainable packaging options, such as biodegradable materials and recycling symbols, are becoming increasingly important to address environmental concerns.

Functional Features: Packaging may include handles, resealable closures, or easy-to-open designs, enhancing convenience for consumers.

Packaging method:

Selection of fruits and vegetables (removal of damage, infected etc fruits and vegetables) → Remove inedible portion before packaging→ Washing in water → Remove adhering water→ Pack loose and fresh

Linners: These are the cushioning materials used to prevent scratches or damage of fruits surface while packing the produce in baskets and wooden boxes. These are newspapers, paddy straw, dry grasses, fruit plant leaves, saw dust etc.

Wrapping: Covering the fruits after harvest with any material in order to improve it post harvest life.

Function of wrapping:

•To provide support and stop entry of foreign materials

•To protect from mechanical and environmental hazards

•To assists sale (retailers and consumers).

Qualities/Characteristics of an ideal package:

•It should be contain the content within it.

•Not affect the flavor of the product packed inside of it.

•Stable performance over large range of temperature

•Adequate compulsive strength and sufficient impact and puncture strength.

•Sufficient thickness of cushioning materials with sufficient ventilation

•Sufficient space for rapid cooling of contents

• Protect the content from oxygen, moisture and light.

•Compatible to the food product

•Protection of the content from adulteration

•Closure characteristics such as opening, sealing, resealing and pouring

•Proper labelling, strong marketing appeal to promote the sale of food product

•Low cost and availability

•Biodegradability and recyclability.

1.Types of Packaging Materials

Paper and Cardboard:

1. **Corrugated Boxes:** Sturdy and versatile, corrugated boxes offer protection and can be customized for different sizes and weights.
2. **Cartons:** Used for food items, cosmetics, and other products, cartons are made from paperboard and often include printed graphics for branding.
3. **Paper Bags:** Lightweight and eco-friendly, paper bags are used for groceries, gifts, and takeout items.

2.Plastics:

1. **PET (Polyethylene Terephthalate):** Used for water bottles, soft drink containers, and food packaging due to its transparency and durability.
2. **HDPE (High-Density Polyethylene):** Commonly used for milk jugs, detergent bottles, and other household products.
3. **LDPE (Low-Density Polyethylene):** Used for flexible packaging like plastic bags and shrink wrap.
4. **PP (Polypropylene):** Used for food containers, yogurt cups, and bottle caps due to its heat resistance and durability.
5. **PS (Polystyrene):** Used for disposable cutlery, CD cases, and foam packaging.
6. **Flexible Plastics:** Used for stand-up pouches, snack packaging, and resealable bags.

1.Glass:

1. **Bottles and Jars:** Commonly used for beverages, sauces, jams, and cosmetics due to its inert nature that doesn't affect flavor or aroma.

2.Metal:

1. **Aluminum Cans:** Widely used for beverages like soda and beer due to their light weight and protection against light and air.
2. **Steel Cans:** Used for canned goods like vegetables, soups, and meats due to their durability and protective properties.

3.Flexible Films:

1. **Polyethylene Films:** Used for items like bread bags, produce bags, and trash bags.
2. **Polypropylene Films:** Used for snack packaging, frozen food bags, and microwaveable packaging.
3. **Vacuum-Sealed Bags:** Used for perishable products to remove air and extend shelf life.

4.Composite Materials:

1. **Tetra Pak:** Combines paper, aluminum, and plastic to create cartons suitable for liquid products like milk, juice, and soups.
2. **Aseptic Packaging:** Combines plastic and metal layers to create shelf-stable packaging for liquids, sauces, and dairy products.

1.Biodegradable and Sustainable Materials:

1. **Bioplastics:** Made from renewable resources like cornstarch or sugarcane, offering an eco-friendly alternative to traditional plastics.
2. **Paper-based Packaging:** Includes compostable trays and containers for food items.
3. **Plant-Based Packaging:** Made from materials like bamboo, palm leaves, or coconut shells, providing sustainable options for various products.

2.Specialty Materials:

1. **Foam Packaging:** Used for fragile items like electronics to provide cushioning and protection.
2. **Bubble Wrap:** Provides protection for delicate items during shipping.
3. **Vacuum-Sealed Pouches:** Used for coffee and perishable goods to preserve freshness.

Choosing the appropriate packaging material depends on factors such as product characteristics, shelf life, transportation requirements, sustainability goals, and consumer preferences. Selecting the right material ensures that products are protected, well-presented, and aligned with branding objectives.

Innovations in Packaging:

Advancements in packaging technology have led to various innovative solutions, such as:

Active Packaging: Incorporating materials that interact with the product to extend shelf life, like oxygen absorbers or moisture control agents.

Smart Packaging: Using technologies like QR codes and NFC tags to provide consumers with additional product information or interactive experiences.

Edible Packaging: Creating packaging materials from edible substances to reduce waste and enhance sustainability.

Modified Atmosphere Packaging (MAP): Adjusting the gas composition inside the package to extend the freshness of perishable foods.

Sterilisation

In sterilisation with moist heat, temperatures generally range from 110 to 120°C with sterilisation times being from 20 - 40 minutes. For example, canned foods are sterilised in an autoclave at about 121˚C for 20 min. Higher temperatures and shorter times may have similar effects (e.g., 134˚C for 3 min.). However, if conditions do not allow the germination of spores, lower temperatures and shorter times can also be applied. For example, with acid fruit juices, jam, or desserts, heating to 80 – 100˚C for 10 min is normally sufficient.

For killing bacterial endospores by dry heat, longer exposure times (e.g. up to 2 hours) and higher temperatures (e.g. 160 – 180˚C) are required than with moist heat.

Solutions containing thermolabile compounds can be sterilised by filtration through mediums such as nitrocellulose membranes, kieselguhr, porcelain, asbestos.

UV irradiation is used to keep rooms partially sterile. Bacteria and their spores are killed quickly, but fungal spores are only moderately sensitive to radiation. Ionising radiation (X ray, gamma radiation) is used to sterilise food and other compact materials. Chemical means may also be applied. Ethylene oxide is used to sterilize food, plastics, glassware, and other equipment.

Generally for sterilisation, the product is canned or bottled and then heat-treated in a steriliser with steam or hot (superheated) water. Sterilisers may be batch or continuous by operated.

BLANCHING

Blanching is used in the processing of fruits and vegetables; its main purpose being deactivate the many enzymes present in the plant materials belonging to this food category. Both pasteurisation and blanching are based on the use of the minimum heat requirement needed to deactivate specific micro-organisms or enzymes, thus minimising any quality changes in the foods themselves.

PASTEURIZATION

The process of pasteurization was named after Louis Pasteur (1960S) who discovered that spoilage organisms could be inactivated in wine by applying heat at temperatures below its boiling point. The process was later applied to milk and remains the most important operation in the processing of milk.

PASTEURIZATION

• Used for milk, liquid eggs, fruit juices and beer. • Destroy pathogens • Reduce microbial load (numbers) • Inactivate enzymes • Extend shelf life

Common milk borne illnesses during that time were: • Typhoid fever • Scarlet fever • Septic sore throat • Diphtheria • Consumption • Diarrheal diseases.

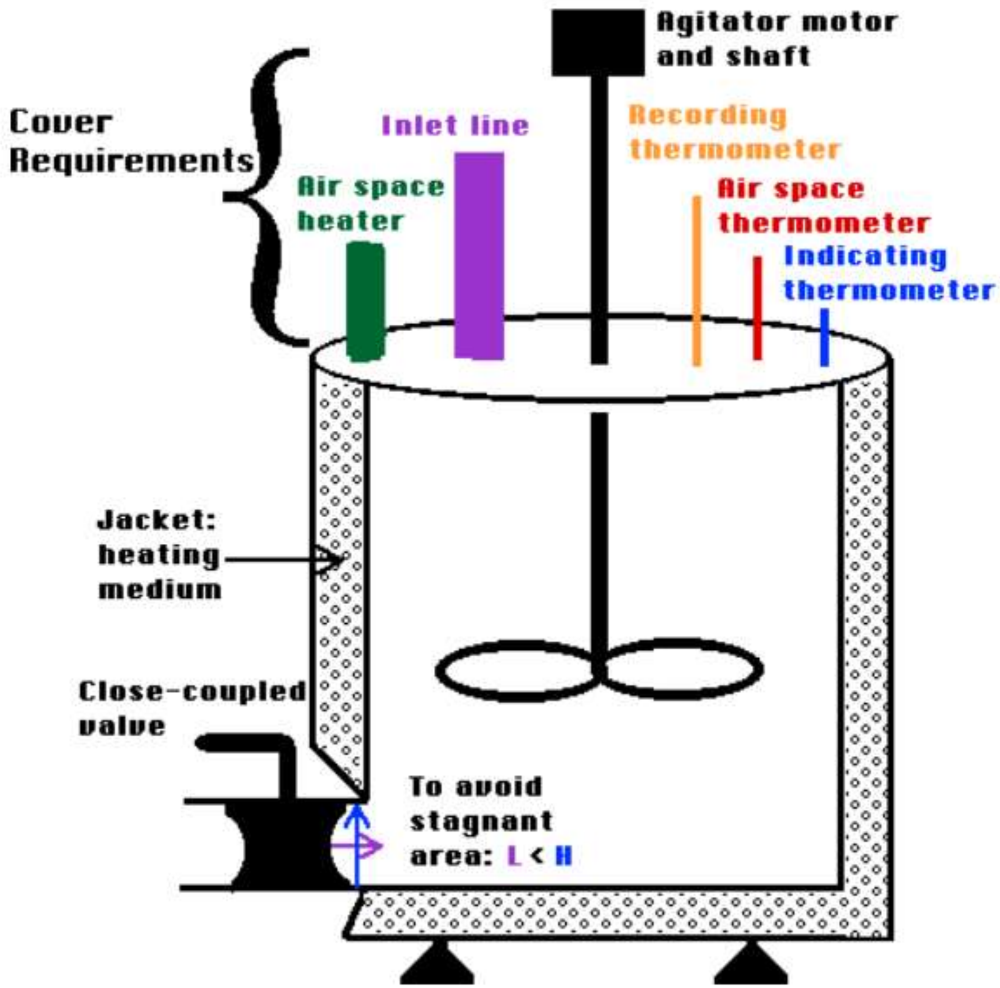
METHODS OF PASTEURIZATION

• Low temperature holding pasteurisation (LTH) Low Temperature Long Time (LTLT), or Batch/Vat Pasteurization • High-temperature, short time (HTST) or continuous flow or Flash Pasteurization • Ultra heat treatment or ultrahigh temperature (UHT)

Batch pasteurization: • Also known as low temperature long time (LTLT) pasteurization. • Heat the milk to 62.8°C for 30 minutes and rapidly cooled to 10°C. • The extendend holding time causes alteration in the milk protein structure and taste.

In the vat the milk is heated and held throughout the holding period while being agitated. The milk may be cooled in the vat or removed hot after the holding time is completed for every particle. • As a modification, the milk may be partially heated in tubular or plate heater before entering the vat. This method has very little use for milk but some use for milk by-products (e.g. creams, chocolate) and special batches.

Batch Pasteurizer



HTST Method: (Flash Pasteurization)

• Heat the milk to between 71.7°C (72°C to 74°C) for 15 (to 20) seconds and then rapidly cooled to 4°C.

Ultra High Temperature (UHT) Pasteurization

• This is a completely closed pasteurization method. The product is never exposed even for a fraction of a second during the entire process. • It involves heating milk or cream to between 135°C to 150°C for one to two seconds, then chilling it immediately and aseptically packaging it in a hermetic (airtight) container for storage.

Disadvantages of high temperature pasteurization

• There is a possibility of alteration of milk proteins. This can affect the properties of such milk when used to make other food products. • High temperatures inactivate the enzymes that protect the product increasing the risk of spoilage. • High temperatures alter the protein structure and imparts a cooked flavor to the milk.

Phoaphatase test

• Alkaline Phosphatase is an enzyme which is naturally present in milk, but is destroyed at a temperature just near to the pasteurization temperature. Alkaline Phosphatase test is used to indicate whether milk has been adequately pasteurised or whether it has been contaminated with raw milk after pasteurisation.

Alkaline phosphatase is a monesterase that catalyzes the hydrolysis of monoesters.

- Alkaline phosphatase is associated with the fat globule of milk, i.e., it is adsorbed to the fat globule membrane surface.
- Alkaline phosphatase (EC 3.1.3.1) is a membrane- bound glycoprotein with sialic acid as sugar moiety. It is a phosphomonoesterase enzyme that catalyzes the hydrolysis of monoesters of phosphoric acid (at alkaline pH), yielding phosphate and the corresponding alco- hol.

HTST Pasteurization plant

HTST Pasteurization plant