PAVENDAR BHARATHIDASAN COLLEGE OF ARTS AND SCIENCE

Unit I

2 Marks

1. Types of staining

Simple and differential staining

2. Name three biochemical test.

Methyl red test, Indole test, catalase test.

3. Define spread plate

Spread plate technique is a method employed to plate a liquid sample for the purpose of isolating or counting the bacteria present in that sample.

4. Define streak plate

Streak plate technique is used for the isolation into a pure culture of the organisms (mostly bacteria), from a mixed population.

5. Methods used in strain improvement

Mutant selection, recombination, rDNA technology

6. Name the phases in microbial growth

Lag phase, log phase, stationary phase and decline phase

5 Marks

1. Isolation of industrially important microbes

- **Selection of source –** water, soil, air, pollutant site, etc
- **Plating technique –** pour plate, spread plate, streak plate, crowded plate technique
- **Identification –** morphology, substrate utilization method

2. Maintenance of industrially important microbes

- After isolation of pure culture, the isolated microbes are allow to store/preserve for future uses
- **Refrigeration –** Pure culture store at 0-4^{o} C in refrigerator or in cold room. This is only for short duration (2 -3 weeks)

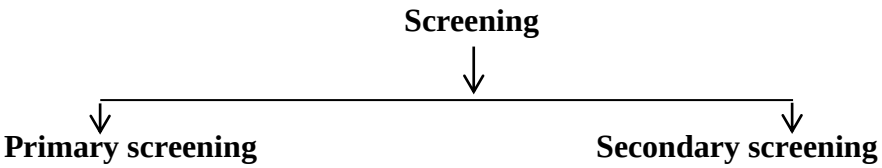
- **Paraffin method –** This is simple method for maintain pure cultures. Sterile paraffin is poured over the bacterial slant culture that prevents the dehydration of the medium.
- **Cryopreservation –** freezing in liquid nitrogen at -196^{o}C in the presence of stabilizing agent
- **Lyophilisation –** The culture is rapidly frozen at very low temperature at -7^{o}C and then dehydrated by vacuum.

3.Microbial growth curve

- Microbes are grown under control conditions
- **Lag phase –** Microbial cells inoculated into the fresh medium and no immediate increase in cell numbers. Cells are synthesizing their own components for survival.
- **Log phase –** Cell growth and division in maximal rate, metabolic activity was high. Cell division continues as long as the availability of nutrients
- **Stationary phase –** Metabolic activity of the bacterial cells were slow down. Depletion of nutrients and accumulation of waste products occurs. Microbial death is equal to microbial growth
- **Decline phase –** Low number of viable cells presence, cell death rate is high, complete depletion of nutrients in the medium.

10 Marks

1. Screening of industrially important microbes



- ✓ Organic acid producing microbes by using dyes
- ✓ Antibiotic producing microbes by crowded plate technique
- ✓ Extracellular metabolite by auxanography technique
- ✓ Enrichment culture technique by defined media

- **Primary screening** – Detection and isolation of microbes of our own interest which can able to produce a desired compound or product

- ✓ Example - Antibiotic producing microbes by crowded plate technique
- ✓ crowded plate technique – Colony showing antibiotic activity are identified by zone of inhibition

- **Secondary screening** – Used to identified the economically valued microbial strains
  - ✓ Antibiotic producing *Streptomyces* sp.
  - ✓ *Streptomyces* sp. was streaked as a narrow band on nutrient agar and the test strain streaked from the edge of plate without touching the *Streptomyces* sp.

2. Strain improvement for increased yield

Purpose of strain improvement – increase the productivity, regulate the enzyme activity

Methods – Mutant selection, recombination and rDNA technology

- ✓ **Mutant selection –** Replica plating technique**,** resistance selection method, substrate utilization method and carcinogenicity test
- ✓ **Recombination –** Transformation, conjugation and transduction
- ✓ **R DNA technology –** cloning of gene of interest production. Ex: production of insulin and interferon, production of ethanol from *E. coli.*

Unit II

2 Marks

1. Types of fermenter

Stirred tank fermenter, airlift fermenter and packed bed fermenter

2. Define fermentation

Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes.

3.Define agitation

Agitation is a means whereby mixing of phases can be accomplished and by which mass and heat transfer can be enhanced between phases or with external surfaces

4.Define sterilization

Sterilization is a process, physical or chemical, that eliminates, removes, kills, or deactivates all forms of life.

5 Marks

1. Types of fermentation

- Type I fermentation

  When the product is formed directly from the primary metabolism used for energy production.

  Substrate $\longrightarrow$ Product

- Type II fermentation

  Product is produced from the substrate used for primary energy metabolism. The product is produced as a secondary metabolite

  Substrate $\longrightarrow$ Product (as a secondary metabolite)

- Type III fermentation

  Product formation and primary metabolism occur at different time period during the mechanism. Substrate consumption and rapid growth occur in the first phase and the product formed in second phase

3. Difference between Conventional fermentation versus biotransformation

Conventional fermentation

- It is a metabolic process in which microbes produce organic substance through the active enzyme
- It is also known as extraction of energy from carbohydrates in the absence of oxygen

Biotransformation

- It is the change in chemical composition of a component by an organism
- The end product of transformation are generally CO_2, NH_2
- It is also known as mineralization
- Ex: amino acid and nutrients production

10 Marks

1. Explain - Solid substrate and submerged fermentation

Solid substrate

- ✓ Wheat bran or pulp, sweet potato starch was used as culture media.
- ✓ The pH of the medium is adjusted to 4-5 and then sterilized
- ✓ Sterile air is passed and temperature is maintained around 30^{o}C during fermentation
- ✓ The fermentation is stopped after 7-15 days

Submerged process

- ✓ It is a method of manufacturing biomolecules in which enzymes
- ✓ Liquid medium contains all the nutrients
- ✓ It require high volume of oxygen
- ✓ The substrate is operated continuously and the product biomass is harvested simultaneously.

2. Composition of fermentation media

- Designing a media contains all the media component in suitable proportion is called media formulation.
- The designed medium should give maximum amount of yield

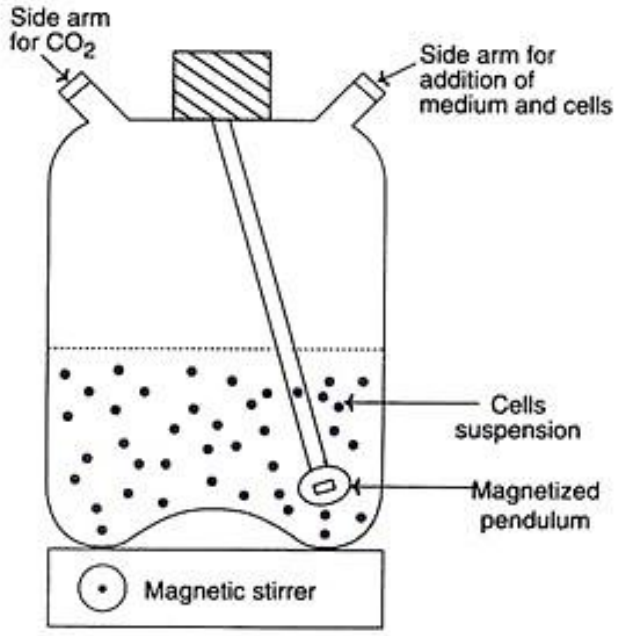
- Component of media – Concentration of each component is important, suitable raw material may give valuable and appropriate product
- Carbon source – Cane molasses, cane juice, glucose, etc.
- Nitrogen source – Ammonium salts, ammonium nitrate, peanut granules
- Minerals – Calcium, potassium, sulphur, zinc, copper, magnesium
- Growth factor – proteins
- pH – pH of the medium should be adjusted to 6-8.

3. Large scale production of a1nimal cell

To increase the scale of a culture depends on the proliferation of cells and is broadly divided into two categories. 1) Scale-up in Suspension and (2) Scale-up in Monolayer.

(i) Scale-up in Suspension

- Scale-up in suspension is the preferred method as it is simpler.
- Scale-up of suspension culture primarily involves an increase in the volume of the culture.
- Small scale generally means the culture capacity less than 2 litres volume.
- It is usually necessary to maintain cell strains in stirred suspension cultures, by agitation (or stirring) of the medium.



(ii) Scale-up in Monolayer

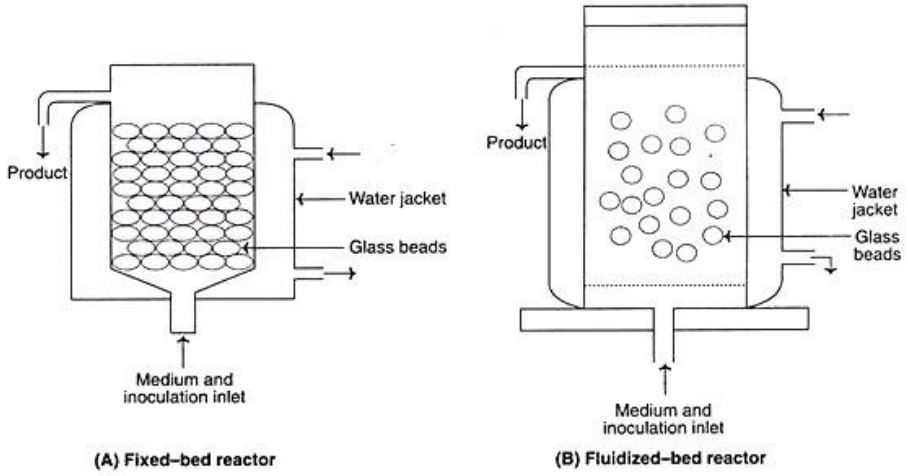
The monolayer culture are anchorage- dependent. Scale-up of monolayer cultures, it is necessary to increase the surface area of the substrate in proportion to the number of cells and volume of the medium. Suspension cultures are preferred as they are simple.

- **The fixed-bed reactor**

  It has a bed of glass beads. The medium is perfused upwards through the bed. The cells are grown on the surfaces of the beads. The products can be collected from the top along with the spent medium.

- **Fluidized-bed reactors**

In a fluidized-bed reactor, the beads are suspended in a stream of medium. These beads are porous in nature, and are made up of ceramics or a mixture of ceramics mixed with natural products such as collagen.



(A) Fixed–bed reactor (B) Fluidized–bed reactor

Unit III

2 Marks

1. Define sterilization

Sterilization is a removal of microbes which interfere in the fermentation process.

2. What is meant by aeration

Aeration is required in the fermentation medium to supply O_2 and remove CO_2.

3. Define agitation

It is a process that uniform the suspension of microbial cells in homogenous microbial medium.

5 Marks

1. Media formulation

- Design a medium contains all the components in suitable proportions.
- Composition of one component in the media changes will affect the concentration of the whole media.
- Complete fractional designing of media – media prepared at the time of process according to the need
- Fractional designing of media – Changing of media composition according to the statistical technique.

2. Aeration and agitation

- Aeration is required in the fermentation medium to supply O_2 and remove CO_2.
- Its facilitate the exchange of gases
- Oxygen is introduced in the bottom of the bioreactor through sprayer
- It is a process that uniform the suspension of microbial cells in homogenous microbial medium.
- Agitation increased the heat of the fluid
- Agitation is done by agitators, impellers, baffles, etc.

3. Control of bioprocess parameters

- Physical parameters – temperature, pressure, flow rates, viscosity and turbidity.
- Chemical parameters – pH, substrate concentration, product concentration, O_2 concentration, waste gases concentration and ionic strength
- Biological parameters – specific enzyme activity, protein concentration, concentration of ATP, DNA/ RNA content.

10 Marks

1.Scale up process

- Pre fermentation stage – isolation, strain improvement and production of improved microbes.
- Screening Method – Primary and secondary screening
- Fermentation process – Batch, continuous and fed batch culture fermentation

2.Scale down process

- Cell disruption – mechanical (Shear force) and non-mechanical (physical, chemical and biological) method.
- Solid liquid separation
- Concentration methods – evaporation, extraction, adsorption, filtration, precipitation
- Purification and drying
- Formulation

Unit IV

2 Marks

1. ***Sedimentation***

The process of particles settling to the bottom the fluid is called sedimentation.

2. ***Centrifugation***

Centrifugation is the process that uses centrifugal force for the separation of two liquids in a mixture.

3. ***Flocculation***

Formation large aggregates of cells are helps to settle down for easy removal. The process of flocculation depends on the nature of cells and the ionic constituents of the medium.

4. ***Filtration***

Filtration is a physical, biological or chemical operation that separates solid matter and fluid from a mixture with a filter medium.

5. ***Drying***

Drying is an essential component of product formulation. It basically involves the transfer of heat to a wet product for removal of moisture.

6. ***Spray drying***

Spray drying is used for drying large volumes of liquids. In spray drying, small droplets of liquid containing the product are passed through a nozzle directing it over a stream of hot gas.

7. ***Chromatography***

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase

8. ***Electrophoresis***

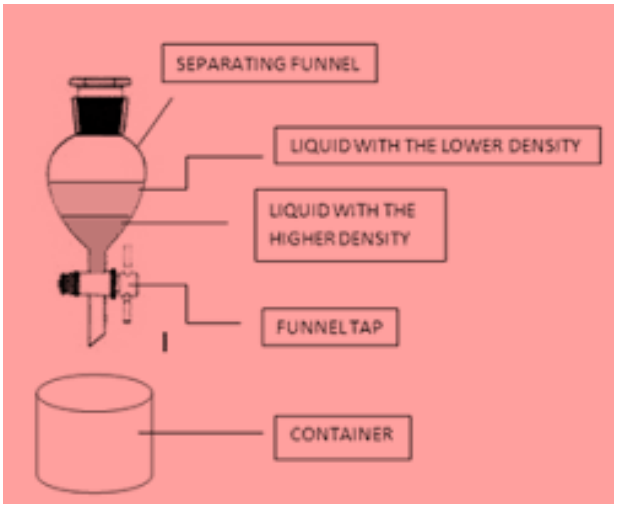
Electrophoresis is the migration of charged molecules under the influence of an electrical field. It was widely used in both analytical and small scale preparative purification of proteins and nucleic acids.

9. **Enzymatic lysis**

Enzymatic lysis is a biological method in which enzymes such as lysozyme, cellulose, protease etc, were used. Most of these enzymes are available commercially and can be used for large scale lysis.

5 Marks

1. **Liquid-liquid extraction**
   - It is a separation process that takes the advantage of the relative solubilities of solute in immiscible solvents.
   - Solute is dissolved more readily and becomes more concentrated in the solvent in which it has a higher solubility.
   - A partial separation occurs when a number of solutes have different relative solubilities in the two solvents used.
   - Solvent should be non-toxic, selective, inexpensive and immiscible with broth and should have a high distribution co-efficient for the product.



2. Electrophoresis

- Electrophoresis is the migration of charged molecules under the influence of an electrical field.
- It was widely used in both analytical and small scale preparative purification of proteins and nucleic acids.
- The component of a sample separate on the basis of their relative electrophoretic mobilities.
- The mobility is a function of charge and molecular weight of the particles.
- There are four basic technique in electrophoresis
  - ✓ Zone electrophoresis
  - ✓ Moving boundary electrophoresis
  - ✓ Isotachophoresis
  - ✓ Isoelectric focusing

3. Chromatography

- It's done to separate those contaminants that resemble the product very closely in physical and chemical properties.
- It includes affinity, size exclusion, reversed phase chromatography, ion-exchange chromatography.
- Chromatography is a laboratory technique for the separation of a mixture.
- The mixture is dissolved in a fluid called the *mobile phase,* which carries it through a structure holding another material called the *stationary phase.*
- The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases.
- **Types -** *Paper chromatography, Thin-layer chromatography (TLC), Gas Chromatography*

4.Crystallization

- Crystallization is a common technique used to purify solids. Two common methods of crystallization are "gradual cooling" and "diffusion".
- **Gradual Cooling -** Gradual cooling involves dissolving the impure solid in a minimum amount of a hot solvent and allowing the resulting solution to cool slowly to room temperature. During the cooling process, pure (or almost pure) crystals form and are then collected by vacuum filtration.
- **Diffusion -** Crystallization by diffusion is an alternative to gradual cooling that does not use heated solvents. This crystallization process is preferable if the desired compound degrades at the elevated temperatures of solvent boiling points.

5. Storage and Packaging

- **Physical protection** – The objects enclosed in the package may require protection from, among other things, mechanical shock, vibration, electrostatic discharge, compression, temperature, etc.
- **Barrier protection** – A barrier to oxygen, water vapor, dust, etc., is often required. Permeation is a critical factor in design. Some packages contain desiccants or oxygen absorbers to help extend shelf life.
- **Containment or agglomeration** – Small objects are typically grouped together in one package for reasons of storage and selling efficiency
- **Information transmission** – Packages and labels communicate how to use, transport, recycle, or dispose of the package or product. With pharmaceuticals, food, medical, and chemical products, some types of information are required by government legislation. Some packages and labels also are used for track and trace purposes.

6.Reverse osmosis

- Reverse osmosis is a more economical operation for concentrating food liquids (such as fruit juices) than conventional heat-treatment processes.
- Its advantages include a lower operating cost and the ability to avoid heat-treatment processes.
- Reverse osmosis is extensively used in the dairy industry for the production of whey protein powders and for the concentration of milk to reduce shipping costs.
- Reverse osmosis (also described as hyperfiltration) is a separation process where the solvent molecules are forced by an applied pressure to flow through a semipermeable membrane in the opposite direction to that dictated by osmotic forces.

7.Crystallization

- Crystallization is a common technique used to purify solids. Two common methods of crystallization are "gradual cooling" and "diffusion".
- **Gradual Cooling -** Gradual cooling involves dissolving the impure solid in a minimum amount of a hot solvent and allowing the resulting solution to cool slowly to room temperature. During the cooling process, pure (or almost pure) crystals form and are then collected by vacuum filtration.
- **Diffusion -** Crystallization by diffusion is an alternative to gradual cooling that does not use heated solvents. This crystallization process is preferable if the desired compound degrades at the elevated temperatures of solvent boiling points.

8.Purification

Protein precipitation is widely used in downstream processing of biological products in order to concentrate proteins and purify them from various contaminants.

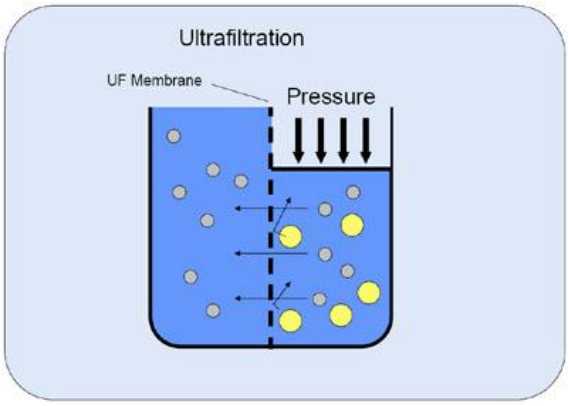
Precipitation by ammonium sulphate

- It is an effect based on the electrolyte–non-electrolyte interaction, in which the non-electrolyte could be less soluble at high salt concentrations.
- It is used as a method of purification for proteins,
- Salting out is the most common method used to precipitate a protein. Addition of a neutral salt,
- Such as ammonium sulfate, compresses the solvation layer and increases protein–protein interactions.
- As the salt concentration of a solution is increased, the charges on the surface of the protein interact with the salt, not the water.

9. Ultrafiltration

- Ultrafiltration (UF) is a membrane filtration process similar to Reverse Osmosis, using hydrostatic pressure to force water through a semi-permeable membrane.
- The pore size of the ultrafiltration membrane is usually 103 - 106 Daltons.
- Ultrafiltration (UF) is a pressure-driven barrier to suspended solids, bacteria, viruses, endotoxins and other pathogens to produce water with very high purity and low silt density.

- Ultrafiltration (UF) is a variety of membrane filtration in which hydrostatic pressure forces a liquid against a semi permeable membrane.
- Suspended solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane.



10 Marks

1. **Treatment of effluent**

Industrial wastewater treatment covers the mechanisms and processes used to treat waters that have been contaminated by anthropogenic activities.

Source of Industrial waste - Agricultural waste, Iron and steel industry, Mines and quarries, Food industry, Chemical industry, Nuclear industry

Process

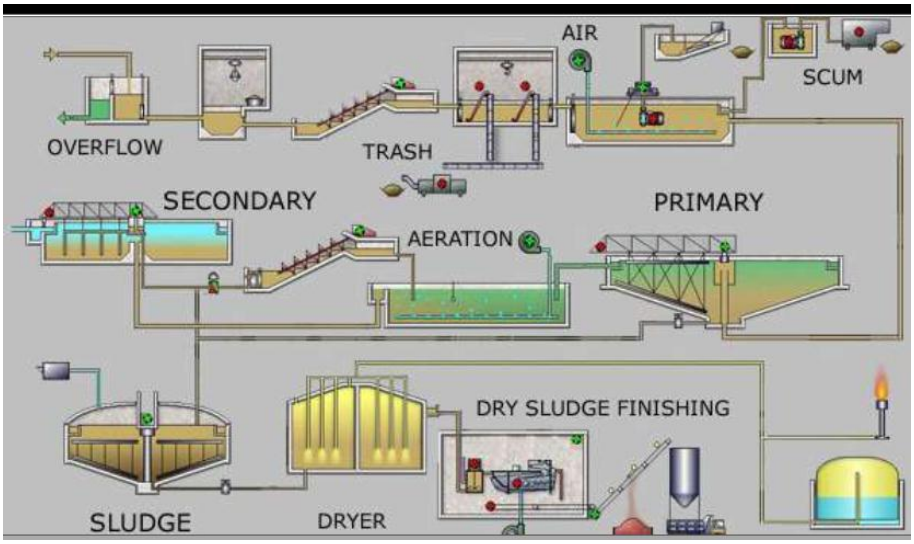
1. **Pre-treatment** - removes materials that can be easily collected from the raw waste water before they damage or clog the pumps and skimmers of primary treatment clarifiers (trash, tree limbs, leaves, etc.)

2. Grit removal - Sand or grit channel or chamber where the velocity of the incoming wastewater is adjusted to allow the settlement of sand, grit, stones, and broken glass. These particles are removed because they may damage pumps and other equipment.

3. Primary treatment - consists of temporarily holding the sewage in a quiescent basin where heavy solids can settle to the bottom while oil, grease and lighter solids float to the surface.

4. Secondary treatment - It removes dissolved and suspended biological matter. Secondary treatment is typically performed by indigenous, water-borne micro-organisms in a managed habitat.

5. Tertiary treatment - To allow rejection into a highly sensitive or fragile ecosystem. Treated water is sometimes disinfected chemically or physically



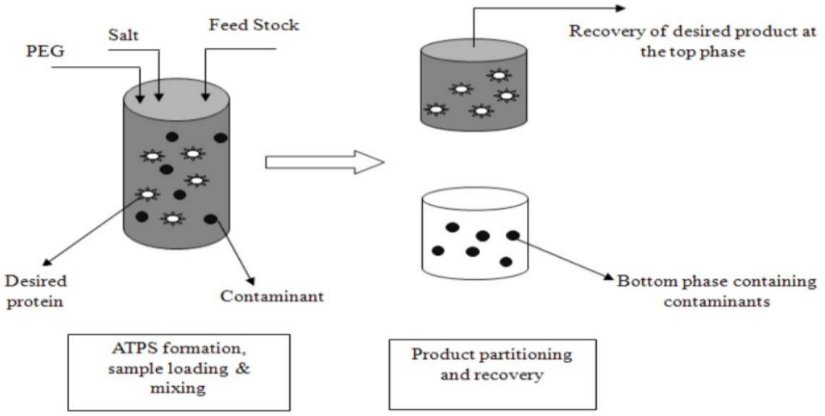
2. Product Extraction process

Solvent extraction

- Separation process for isolating the constituents of a liquid mixture.
- Liquid–liquid extraction (LLE), also known as solvent extraction and partitioning
- It's a method to separate compounds or metal complexes.
- It is also widely used in the production of fine organic compounds, the processing of perfumes, the production of vegetable oils and biodiesel.

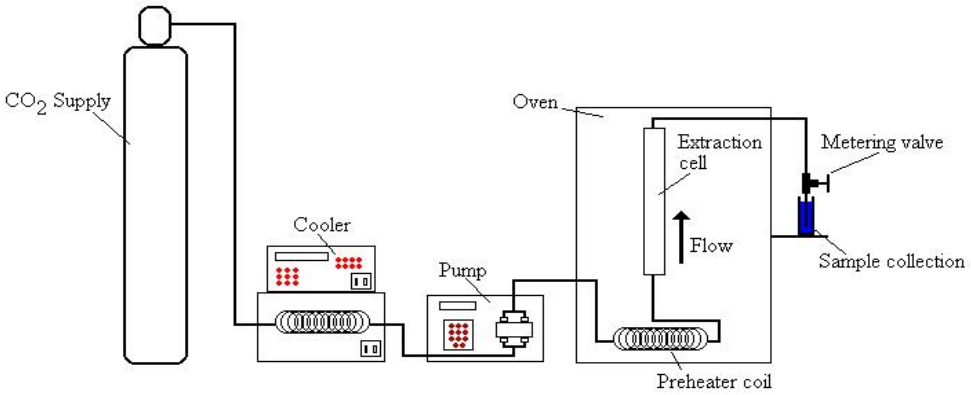
Aqueous two phase separation

- Aqueous two-phase extraction has applications in biochemical processes involving protein separation and purification.
- It includes two different immiscible polymers or polymer and salt systems for protein recovery.
- The two phases are mostly composed of water and non-volatile components, thus eliminating volatile organic compounds.
- PEG (Poly Ethylene Glycol) dextran system – The components is mixed with PEG



Supercritical fluid extraction

- Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent.
- Extraction is usually from a solid matrix, but can also be from liquids.
- The dissolved material is swept from the extraction cell into a separator at lower pressure, and the extracted material settles out.
- The CO_2 can then be cooled, re-compressed and recycled, or discharged to atmosphere



Unit V

2 Marks

1. Name some fermented foods

Pickle, curd, yogurt, cheese and bread

2. Source for ethanol production

Sugarcane, barley, potatoes, corn, fruit, wheat and sunflower.

3. Bacteriocins

Bacteriocins are ribosomally synthesized proteins by bacterial strains during their stationary phase.

4. Single cell protein

Protein derived from a culture of single celled organisms used a food supplement.

5. Preservation

It's a method to arrest the microbial growth

5 Marks

1. Food ingredients used in fermentation

- Salt – Common table salt contains iodine which act as a best fermenting agent. A combination of salt and water is called brine which is used to ferment foods.
- Water – Purified water was used as a fermenting agent in some cases
- Sugar – Sugar is a best source for microbial growth which enhance the fermentation

2. Role of Microbes in pickling

- *E. aerogens* – produce carbon dioxide which create anaerobic environment in the medium
- *Lactobacillus* sp. – produce lactic acid that softens the substance.
- Salt – inhibits the growth of undesirable microbes

3. Role of Microbes producing colors and flavors

- Colors have number of beneficial properties like anti cancerous, immune suppressive, antibiotics, anti-proliferative, etc.
- Microbial pigment are more significant when compare with chemicals.
- *Staphylococcus aureus* - zeaxanthin
- *Monascus roseus* - canthaxanthin

4. Role of Microbes in alcoholic beverages

- Alcoholic beverages are highly produced by microbes in large scale level
- Production of alcoholic beverages is a process that involves the active participation of microbe, most often yeast are used
- Yeast are majorly used for alcohol production like beer and other alcoholic beverages
- *Saccharomyces cerevisiae* were highly used
- It ferments the sugar present in the sources
- Ex: Wine from grapes, beer from barley.

6. Role of Microbes

- Role in pickle production - *E. aerogens* – produce carbon dioxide which create anaerobic environment in the medium. *Lactobacillus* sp. – produce lactic acid that softens the substance.
- Role in producing colors and flavors - Microbial pigment are more significant when compare with chemicals.*Staphylococcus aureus* – zeaxanthin, *Monascus roseus* - canthaxanthin
- Role in producing alcoholic beverages - Yeast are majorly used for alcohol production like beer and other alcoholic beverages. *Saccharomyces cerevisiae* were highly used that ferments the sugar present in the sources. Ex: Wine from grapes, beer from barley.

10 Marks

1. Bacteriocins production from Lactic acid bacteria

- **Definition –** Bacteriocins are ribosomally synthesized proteins by bacterial strains during their stationary phase. It is secondary metabolites. In 1925, the first bacteriocin called colicin was originally identified asa an antimicrobial protein produced by *E. coli*.

- **Types –** Class I, Class II, Class III and Class IV

- **Isolation –** Isolated from milk products, serially diluted, isolation done by plating methods (pour plate, streak plate, allow to grow in differential media).

    - ✓ **Application -** Safer preservative than synthetic agents, More accurate efficacy, Good acceptance from consumers and less toxic

    - ✓ **Advantage -** Inhibit the bacterial growth, Used as a natural preservative in food industry, Used as a disease control agent in agriculture, Used in pharmaceutical industry – Lantibiotics, Colicins and Microcins