

Unit-5

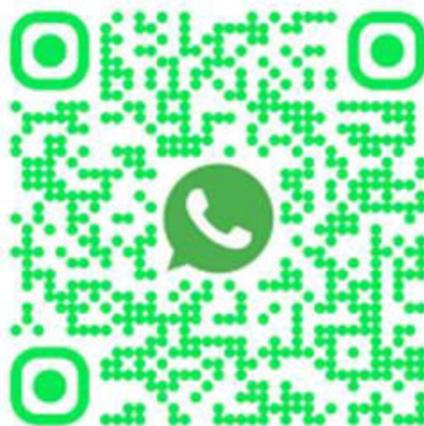
Pharmaceutical Biotechnology

B.Pharma 6th Sem Notes

Unit: 5

- **Fermentation** methods and general requirements, study of media, equipments, sterilization methods, aeration process, stirring.
- Large scale production fermenter design and its various controls.
Study of the production of – Penicillins, citric acid, Vitamin B12, Glutamic acid, Griseofulvin.
- **Blood Products:** Collection, Processing and Storage of whole human blood, dried human plasma, plasma Substitutes.

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Fermentation Technology

Definition:

Fermentation is a metabolic process where microorganisms convert organic substrates into desired products under controlled conditions. In industrial context, it includes both aerobic and anaerobic processes.

Fermentation Methods

A. Based on Culture Method:

1. Batch Fermentation:

- **Definition:** Closed system where all nutrients are added at the beginning
- **Process:** Inoculum is added, fermentation proceeds without further additions, product is harvested at the end
- **Growth Phases:** Lag phase → Log phase → Stationary phase → Death phase
- **Advantages:** Simple operation, less contamination risk, easy to control
- **Disadvantages:** Lower productivity, downtime between batches, product concentration varies
- **Examples:** Penicillin, most antibiotics, enzymes

2. Fed-Batch Fermentation:

- **Definition:** Nutrients are added continuously or intermittently during fermentation
- **Process:** Initial medium is loaded, additional nutrients fed as needed, no product removal until end
- **Advantages:** Higher cell density, avoids substrate inhibition, controls catabolite repression
- **Disadvantages:** More complex control, volume increases over time
- **Examples:** Baker's yeast, recombinant proteins, high cell density cultures

3. Continuous Fermentation:

- **Definition:** Fresh medium is continuously added and product is continuously removed
- **Process:** Steady state is maintained, constant volume, cells in exponential growth
- **Types:** Chemostat (nutrient-limited), Turbidostat (cell density-controlled)
- **Advantages:** High productivity, consistent product quality, no downtime, easier automation
- **Disadvantages:** High contamination risk, genetic instability, complex control
- **Examples:** Beer production, single cell protein, wastewater treatment



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B. Based on Oxygen Requirement:

- **Aerobic Fermentation:** Requires oxygen (antibiotics, citric acid, vitamins)
- **Anaerobic Fermentation:** No oxygen required (ethanol, lactic acid, biogas)
- **Facultative:** Can grow with or without oxygen

General Requirements for Fermentation

- **Microorganism:** High productivity, genetic stability, non-pathogenic, easy to maintain
- **Substrate/Medium:** Inexpensive, readily available, supports good growth
- **Sterility:** Pure culture, contamination-free environment
- **Temperature Control:** Optimal temperature for organism (usually 25-37°C)
- **pH Control:** Maintain optimal pH (5-8 for most organisms)
- **Aeration:** Adequate oxygen supply for aerobic processes
- **Agitation:** Proper mixing for uniform distribution
- **Foam Control:** Antifoam agents to prevent excessive foaming
- **Monitoring:** Regular sampling and analysis
- **Downstream Processing:** Efficient product recovery and purification

Fermentation Media

Definition:

Fermentation medium is the nutrient solution that provides all essential components for microbial growth and product formation.

Components of Fermentation Medium:

1. Carbon Source (Energy Source):

- Sugars: Glucose, sucrose, lactose, molasses
- Starch: Corn starch, potato starch
- Oils and fats: Vegetable oils, animal fats
- Organic acids: Acetic acid, citric acid
- Usually 1-5% of medium

2. Nitrogen Source:

- **Organic:** Peptone, yeast extract, meat extract, soybean meal, corn steep liquor
- **Inorganic:** Ammonium salts (NH₄Cl, NH₄NO₃), urea, ammonia gas
- Concentration: 0.5-2% of medium



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3. Minerals (Macro and Micro elements):

- **Macronutrients:** Phosphorus (phosphates), Sulfur (sulfates), Potassium, Magnesium, Calcium
- **Micronutrients (Trace elements):** Iron, Zinc, Copper, Manganese, Cobalt, Molybdenum
- Required in small amounts but essential for enzyme function

4. Vitamins and Growth Factors:

- B-complex vitamins (thiamine, biotin, riboflavin)
- Amino acids (for auxotrophic strains)
- Nucleotides (purines, pyrimidines)

5. Water:

- Major component (80-90% of medium)
- Must be pure, free from contaminants

6. Additional Components:

- **Buffers:** Phosphate, carbonate systems to maintain pH
- **Antifoam agents:** Silicone oils, vegetable oils
- **Precursors:** Specific chemicals to direct product formation
- **Inducers:** Trigger gene expression (IPTG for recombinant proteins)

Types of Media:

1. **Defined/Synthetic Medium:** Exact chemical composition known, used for research
2. **Complex/Undefined Medium:** Exact composition unknown, contains natural ingredients (yeast extract), used industrially
3. **Semi-defined Medium:** Mixture of defined and complex components

Desirable Characteristics of Industrial Media:

- Low cost and readily available
- Support high productivity
- Minimal waste generation
- Consistent quality and composition
- Easy product recovery
- Long shelf life

Fermentation Equipment (Fermenters/Bioreactors)

Definition:

A fermenter (bioreactor) is a vessel in which microbial growth and product formation occur under controlled conditions.



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Essential Components of a Fermenter:

1. **Vessel Body:**
 - a. Cylindrical tank with hemispherical or ellipsoidal top and bottom
 - b. Material: Stainless steel (SS 316) - corrosion resistant, non-toxic
 - c. Height to diameter ratio: Usually 2:1 to 3:1
 - d. Capacity: From few liters (lab) to 500,000 liters (industrial)
2. **Agitation System:**
 - a. Impeller/stirrer mounted on central shaft
 - b. Motor drives the shaft (top or bottom driven)
 - c. Types of impellers: Rushton turbine, marine propeller, paddle
 - d. Baffles (vertical plates) prevent vortex formation
3. **Aeration System:**
 - a. Air inlet with sparger (perforated ring/pipe at bottom)
 - b. Air filter (HEPA) for sterile air
 - c. Air flow controller and pressure gauge
 - d. Exhaust gas outlet with filter
4. **Temperature Control System:**
 - a. Cooling jacket or internal cooling coils
 - b. Heating elements (for sterilization)
 - c. Temperature sensors (thermocouples, RTD)
 - d. Automatic control via computer/PLC
5. **pH Control System:**
 - a. pH electrode/probe
 - b. Acid and alkali addition ports
 - c. Peristaltic pumps for controlled addition
 - d. Automatic pH controller
6. **Foam Control System:**
 - a. Foam sensor/detector
 - b. Antifoam addition system
 - c. Mechanical foam breakers
7. **Ports and Openings:**
 - a. Inoculation port
 - b. Sampling port
 - c. Harvest outlet
 - d. Nutrient addition ports
 - e. Pressure relief valve
 - f. Sight glass for visual inspection
8. **Monitoring and Control Systems:**
 - a. Dissolved oxygen (DO) sensor



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- b. Pressure gauge
- c. Level sensors
- d. Computer control system (SCADA)
- e. Data logging and recording

Sterilization Methods

Definition:

Sterilization is the complete elimination or destruction of all forms of microbial life including bacteria, viruses, fungi, and spores.

Importance:

- Ensures pure culture fermentation
- Prevents contamination and product spoilage
- Maintains product quality and consistency
- Reduces risk of pathogenic organisms

Methods of Sterilization:

A. Heat Sterilization:

1. Moist Heat (Steam Sterilization):

- **Autoclaving:** Most common method, uses steam under pressure
- **Conditions:** 121°C at 15 psi for 15-20 minutes
- **Applications:** Media, glassware, instruments, fermenter sterilization
- **In-situ sterilization:** Fermenter is sterilized in place before use
- **Mechanism:** Coagulation and denaturation of proteins
- **Advantages:** Effective, economical, no toxic residues
- **Disadvantages:** May destroy heat-sensitive nutrients

2. Dry Heat Sterilization:

- **Hot Air Oven:** 160-180°C for 2-4 hours
- **Applications:** Glassware, metal instruments, powders, oils
- **Mechanism:** Oxidation of cell components
- Less effective than moist heat, requires higher temperature and longer time

B. Filtration Sterilization:

- **Principle:** Physical removal of microorganisms by passing through membrane
- **Filter types:** Membrane filters (0.2-0.45 μm pore size), depth filters, HEPA filters
- **Materials:** Cellulose acetate, cellulose nitrate, polysulfone, PTFE
- **Applications:** Heat-sensitive solutions (vitamins, antibiotics), air sterilization



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- **Air filtration:** HEPA filters remove 99.97% of particles $\geq 0.3 \mu\text{m}$
- **Advantages:** No heat damage, suitable for heat-labile substances
- **Disadvantages:** Cannot remove viruses completely, filters may clog

C. Radiation Sterilization:

- **UV Radiation:** 260-280 nm wavelength, damages DNA
- Used for surface sterilization, laminar flow hoods, water treatment
- Limited penetration, not for liquids or solids
- **Ionizing Radiation:** Gamma rays, X-rays (high penetration)
- Used for disposable medical devices, pharmaceuticals

D. Chemical Sterilization:

- **Ethylene Oxide (ETO):** Gas sterilization for heat-sensitive equipment
- **Formaldehyde:** Fumigation of rooms and equipment
- **Hydrogen Peroxide:** Vapor phase sterilization
- **Disinfectants:** Alcohol, phenolics, halogens (surface sterilization only)

Aeration Process

Definition:

Aeration is the process of supplying oxygen to the fermentation medium to meet the respiratory requirements of aerobic microorganisms.

Importance of Aeration:

- Provides oxygen for aerobic metabolism
- Removes carbon dioxide and volatile metabolites
- Maintains dissolved oxygen levels
- Enhances mixing and mass transfer
- Prevents anaerobic conditions and byproduct formation

Methods of Aeration:

1. Surface Aeration:

- Oxygen diffuses from air-liquid interface
- Suitable for shallow vessels
- Limited application in industrial fermentation
- Used in: Tray fermentation, solid-state fermentation

2. Submerged Aeration (Sparging):

- Most common method in industrial fermentation
- Sterile air is bubbled through medium via sparger
- Sparger designs: Perforated pipe, ring sparger, porous sparger
- Creates small bubbles for better oxygen transfer
- Combined with mechanical agitation for efficiency



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Factors Affecting Oxygen Transfer:

4. **Air Flow Rate:** Higher flow increases oxygen supply (typical: 0.5-1.5 vvm - volumes per volume per minute)
5. **Agitation Speed:** Breaks bubbles into smaller size, increases surface area
6. **Bubble Size:** Smaller bubbles provide larger surface area for transfer
7. **Medium Viscosity:** High viscosity reduces oxygen transfer
8. **Temperature:** Higher temperature decreases oxygen solubility
9. **Pressure:** Higher pressure increases oxygen solubility
10. **Cell Density:** Higher density increases oxygen demand

Oxygen Transfer Rate (OTR):

- Measure of how quickly oxygen is transferred from air to liquid
- Must exceed Oxygen Uptake Rate (OUR) of microorganisms
- Critical parameter for fermenter design and operation
- Monitored using dissolved oxygen (DO) probes

Problems Associated with Aeration:

- Foam formation (controlled by antifoam agents)
- Shear stress on cells (mycelial fungi are sensitive)
- Heat generation (requires cooling)
- Power consumption (major operational cost)
- Contamination risk if air not properly sterilized

Stirring (Agitation)

Definition:

Stirring or agitation is the mechanical mixing of fermentation medium using impellers to create uniform conditions throughout the vessel.

Purposes of Agitation:

- **Mixing:** Uniform distribution of nutrients, cells, and oxygen
- **Oxygen Transfer:** Breaks air bubbles into smaller sizes, increases surface area
- **Heat Transfer:** Uniform temperature distribution, prevents hot spots
- **Dispersion:** Keeps cells in suspension, prevents settling
- **Mass Transfer:** Facilitates nutrient uptake and waste removal
- **Homogenization:** Ensures uniform conditions (pH, temperature, DO)

Types of Impellers:

1. Rushton Turbine (Disc Turbine):

- Most common impeller in fermentation
- Flat disc with 6 vertical blades
- Creates radial flow pattern



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- Excellent for gas dispersion
- High shear, suitable for bacterial fermentation
- Power consumption: High

2. Marine Propeller:

- 3 angled blades like a ship propeller
- Creates axial flow (top to bottom)
- Good for bulk mixing
- Low shear stress
- Suitable for shear-sensitive organisms (fungi)
- Power consumption: Low to medium

3. Paddle Impeller:

- Flat blades attached to central shaft
- Simple design, low cost
- Creates tangential flow
- Used in low viscosity media
- Gentle mixing

4. Pitched Blade Turbine:

- Blades at 45° angle
- Combines radial and axial flow
- Good mixing efficiency
- Moderate shear stress

Baffles:

- Vertical plates attached to fermenter wall
- Usually 4 baffles, width = 1/10 to 1/12 of tank diameter
- Prevent vortex formation (swirling)
- Improve mixing efficiency
- Increase turbulence and oxygen transfer
- Reduce power consumption

Factors Affecting Agitation:

- **Impeller speed:** Higher speed = better mixing but more shear and power
- **Impeller size:** Larger diameter = wider zone of influence
- **Number of impellers:** Multiple impellers for tall fermenters
- **Medium viscosity:** High viscosity requires more power
- **Tank geometry:** Height/diameter ratio affects flow patterns
- **Cell sensitivity:** Shear-sensitive cells need gentle agitation

Problems with Excessive Agitation:

- Cell damage due to shear stress
- Mycelial breakage in fungal cultures



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- Increased foam formation
- Higher power consumption and operational costs
- Heat generation

Large-Scale Production Fermenter Design

Industrial fermenters range from 10,000 to 500,000 liters. Design considerations are critical for efficient and economical production.

Features:

1. Vessel Construction:

- Material: Stainless steel (SS 316L) for corrosion resistance
- Shape: Cylindrical with dished ends
- H:D ratio: 2:1 to 3:1 for good mixing
- Working volume: 70-80% of total volume (headspace for foam)
- Smooth internal surface (electropolished) for easy cleaning
- Pressure rating: Designed for steam sterilization (2-3 bar)

2. Agitation System:

- Multiple impellers (2-4) on single shaft for tall vessels
- Top or bottom-driven motor
- Variable speed drive for control
- Mechanical seal to prevent contamination
- Power input: 2-10 kW/m³ for aerobic fermentation

3. Aeration System:

- Air compressor and air filters
- Multiple filter stages (pre-filter, HEPA filter)
- Ring sparger at bottom of vessel
- Air flow rate: 0.5-1.5 vvm
- Exhaust gas treatment (condenser, filter)

4. Temperature Control:

- Cooling jacket (annular space around vessel)
- Internal cooling coils for large fermenters
- Chilled water circulation system
- Steam supply for heating and sterilization
- RTD or thermocouple sensors
- Automatic temperature controller ($\pm 0.5^{\circ}\text{C}$ accuracy)

5. Instrumentation and Control:

- pH probe and controller
- Dissolved oxygen (DO) probe
- Pressure gauge and relief valve



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- Level sensors
- Foam sensors
- Load cells (for mass measurement)
- SCADA system for automation and data logging

Various Control Systems:

- **Temperature Control:** Automatic regulation via cooling water/steam valves based on sensor input
- **pH Control:** Automatic addition of acid or alkali via peristaltic pumps to maintain setpoint
- **Dissolved Oxygen Control:** Adjusted by varying agitation speed and/or air flow rate
- **Foam Control:** Automatic antifoam addition when foam level exceeds threshold
- **Pressure Control:** Pressure relief valves and backpressure regulators
- **Feed Control (Fed-Batch):** Controlled substrate feeding based on DO, pH, or time
- **Cascade Control:** DO controlled by sequential adjustment of agitation → air flow → oxygen enrichment
- **Computer Control (SCADA):** Centralized monitoring, data logging, recipe management, alarm systems

Scale-Up Considerations:

- Maintain same power per unit volume (P/V)
- Keep oxygen transfer rate constant
- Preserve mixing time and shear rate
- Validate sterilization effectiveness
- Optimize heat transfer capacity
- Consider geometric similarity

Production of Specific Products

Production of Penicillins

Introduction:

- Penicillin is a beta-lactam antibiotic discovered by Alexander Fleming in 1928
- First mass-produced antibiotic during World War II
- Produced by fungus *Penicillium chrysogenum* (previously *P. notatum*)
- Secondary metabolite (produced during stationary phase)

Microorganism:



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- **Organism:** *Penicillium chrysogenum* (filamentous fungus)
- Strain improvement: Original wild strain produced ~2 mg/L, modern strains produce >50 g/L
- Methods: Mutation and selection, genetic engineering
- Strain maintenance: Stored as spores on agar slants or in liquid nitrogen

Fermentation Medium:

- **Carbon source:** Lactose (preferred for penicillin production), glucose, molasses
- **Nitrogen source:** Corn steep liquor (rich in nutrients), ammonia, ammonium salts
- **Precursor:** Phenylacetic acid or phenoxyacetic acid (for Penicillin G production)
- **Minerals:** Phosphates, sulfates, trace elements
- **pH:** 6.5-7.0 (controlled throughout fermentation)
- **Temperature:** 25-27°C

Process:

1. Inoculum Preparation:

- Spore suspension → Shake flask (48-72 hours) → Seed fermenter (24-48 hours)
- Inoculum size: 5-10% of production fermenter volume

2. Production Fermentation:

- **Method:** Fed-batch fermentation
- **Duration:** 120-200 hours
- **Growth Phase (0-40 hours):** Rapid mycelial growth, high aeration
- **Production Phase (40-200 hours):** Penicillin synthesis, controlled nutrient feeding
- Continuous feeding of lactose and precursor to maintain optimal concentration
- High aeration (0.5-1.0 vvm) and agitation for oxygen supply
- Foam control using silicone-based antifoam
- **Final concentration:** 40-50 g/L
- **Dissolved oxygen:** Maintained at 20-40% saturation

3. Recovery and Purification:

- **Filtration:** Remove mycelium using rotary vacuum filter
- **Extraction:** pH adjustment and solvent extraction (butyl acetate or amyl acetate)
- **Re-extraction:** Transfer penicillin to aqueous phase
- **Crystallization:** Penicillin G potassium or sodium salt
- **Drying:** Vacuum drying or freeze-drying



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- **Purity:** >95% (pharmaceutical grade)

Types of Penicillin:

- **Penicillin G (Benzylpenicillin):** Most common, requires phenylacetic acid precursor
- **Penicillin V (Phenoxymethylpenicillin):** Requires phenoxyacetic acid, acid-stable, oral administration
- **6-APA:** Precursor for semi-synthetic penicillins (ampicillin, amoxicillin)

Production of Citric Acid

Introduction:

- Citric acid is an organic acid (tricarboxylic acid)
- Used in food, beverages, pharmaceuticals, cosmetics
- World production: Over 2 million tons per year
- Primary metabolite (part of Krebs cycle), but accumulates under specific conditions

Microorganism:

- **Organism:** *Aspergillus niger* (filamentous fungus)
- Selected for high citric acid yield and low oxalic acid production
- Non-pathogenic, GRAS (Generally Recognized As Safe) status

Fermentation Medium:

- **Carbon source:** Molasses (beet or cane), glucose, sucrose (15-25%)
- **Nitrogen source:** Ammonium nitrate, ammonium sulfate (low concentration 0.2-0.5%)
- **Minerals:** Phosphates, magnesium, potassium
- **Critical:** Low iron and manganese (inhibit citric acid production, promote oxalic acid)
- **pH:** Initial 5-7, drops to 1.5-2.0 during production
- **Temperature:** 28-30°C

Process:

1. Surface Culture Method (Traditional):

- Medium in shallow aluminum or stainless steel trays
- Spores sprayed on surface
- Mycelial mat forms on surface
- Duration: 7-10 days
- Yield: 70-80% (w/w of sugar)
- Disadvantages: Labor-intensive, large space requirement, contamination risk

2. Submerged Culture Method (Modern):



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- Stirred tank fermenter
- High aeration (1-2 vvm) and agitation
- Morphology control: Pellet formation desired (small, compact pellets)
- Duration: 5-7 days
- Batch or fed-batch process
- Yield: 80-90% (w/w of sugar)
- Final concentration: 100-150 g/L
- Advantages: Automated, less space, better control, higher productivity

3. Recovery and Purification:

- **Filtration:** Remove mycelium
- **Precipitation:** Add calcium hydroxide to form calcium citrate (insoluble)
- **Acidification:** Add sulfuric acid to regenerate citric acid and precipitate calcium sulfate
- **Crystallization:** Concentrate and cool to crystallize citric acid monohydrate
- **Drying:** Produce anhydrous citric acid
- **Purity:** >99.5% (food grade)

Factors Favoring Citric Acid Production:

- High sugar concentration (excess carbon)
- Low nitrogen concentration (limits growth, promotes production)
- Low pH (prevents oxalic acid formation)
- Deficiency of metal ions (Fe, Mn, Zn)
- High dissolved oxygen
- Optimal temperature (28-30°C)

Production of Vitamin B12 (Cyanocobalamin)

Introduction:

- Vitamin B12 (cobalamin) is essential for human health
- Required for DNA synthesis, red blood cell formation, neurological function
- Only synthesized by certain bacteria and archaea
- Most complex vitamin structure (contains cobalt)
- Used as supplement, in animal feed, and for treating pernicious anemia

Microorganisms:

- **Primary organisms:** Propionibacterium freudenreichii, Pseudomonas denitrificans
- **Others:** Streptomyces species, certain Bacillus species
- Often dual fermentation process using both organisms

Fermentation Medium:



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- **Carbon source:** Glucose, molasses, corn steep liquor
- **Nitrogen source:** Yeast extract, peptone, ammonium salts
- **Essential:** Cobalt salts (CoCl_2 or CoSO_4) - 1-10 mg/L
- **Precursors:** 5,6-dimethylbenzimidazole, betaine
- **pH:** 6.5-7.5
- **Temperature:** 25-30°C

Process:

1. Two-Stage Fermentation (Most Common):

- **Stage 1 (Anaerobic):** Propionibacterium produces intermediate (60-100 hours)
- **Stage 2 (Aerobic):** Pseudomonas converts to final B12 (40-60 hours)
- Total duration: 100-160 hours
- Final concentration: 50-150 mg/L

2. Single-Stage Fermentation:

- Uses Pseudomonas denitrificans only
- Aerobic fermentation
- Duration: 80-120 hours
- Simpler but lower yield

3. Recovery and Purification:

- **Cell lysis:** Heat treatment or autolysis to release B12 from cells
- **Extraction:** pH adjustment and solvent extraction
- **Chromatography:** Ion exchange or adsorption chromatography
- **Crystallization:** Final purification step
- **Conversion:** Convert to cyanocobalamin (stable form) by adding cyanide
- **Purity:** >98% (pharmaceutical grade)

Production of Glutamic Acid

Introduction:

- Glutamic acid is an amino acid
- Mainly used as monosodium glutamate (MSG) - flavor enhancer
- World production: Over 3 million tons per year
- Also used in pharmaceuticals and cosmetics

Microorganism:

- **Organism:** Corynebacterium glutamicum (Gram-positive bacterium)
- Also: Brevibacterium species
- Non-pathogenic, safe for food production

Fermentation Medium:

- **Carbon source:** Glucose, molasses, starch hydrolysate (10-15%)



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- **Nitrogen source:** Ammonium sulfate, urea, corn steep liquor
- **Essential:** Biotin (vitamin) - limited amount for glutamate excretion
- **Minerals:** Phosphates, magnesium, potassium, iron
- **pH:** 7.0-8.0 (controlled with ammonia addition)
- **Temperature:** 30-37°C

Process:

- **Method:** Batch or fed-batch fermentation
- **Duration:** 24-48 hours
- **Key factor:** Biotin limitation - causes cell membrane permeability changes
- Alternative triggers: Penicillin addition, surfactants, fatty acid limitation
- **Aeration:** High (1-1.5 vvm)
- **Dissolved oxygen:** >20% saturation
- **Final concentration:** 100-130 g/L
- **Yield:** 50-60% (w/w of sugar)

Recovery:

- **Cell removal:** Filtration or centrifugation
- **Isoelectric precipitation:** Adjust pH to 3.2 (isoelectric point)
- **Crystallization:** Produce L-glutamic acid crystals
- **MSG production:** Neutralize with sodium hydroxide to form monosodium glutamate
- **Drying:** Spray or vacuum drying
- **Purity:** >99% (food grade MSG)

Production of Griseofulvin

Introduction:

- Griseofulvin is an antifungal antibiotic
- Used for treatment of fungal skin infections (ringworm, athlete's foot)
- Acts by disrupting fungal cell division (mitotic spindle inhibitor)
- Administered orally for systemic infections

Microorganism:

- **Organism:** *Penicillium griseofulvum* (filamentous fungus)
- Also produced by: *Penicillium patulum*, *Penicillium janczewskii*
- Strain improvement through mutation and selection

Fermentation Medium:

- **Carbon source:** Glucose, sucrose, lactose (3-5%)
- **Nitrogen source:** Corn steep liquor, yeast extract, peptone
- **Minerals:** Phosphates, sulfates, trace metals
- **pH:** 5.5-7.0



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- **Temperature:** 24-26°C

Process:

- **Method:** Submerged fermentation (batch)
- **Duration:** 120-200 hours (5-8 days)
- **Growth phase:** 0-72 hours, rapid mycelial growth
- **Production phase:** 72-200 hours, secondary metabolite synthesis
- **Aeration:** Moderate (0.5-1.0 vvm)
- **Agitation:** Gentle to avoid mycelial damage
- **Final concentration:** 0.5-2.0 g/L

Recovery and Purification:

- **Filtration:** Separate mycelium from broth
- **Extraction:** Solvent extraction with chloroform or acetone
- **Crystallization:** Concentrate and cool to crystallize griseofulvin
- **Purification:** Recrystallization from organic solvents
- **Drying:** Vacuum drying
- **Purity:** >97% (pharmaceutical grade)

Blood Products

Collection, Processing and Storage of Whole Human Blood

A. Collection of Whole Blood:

1. Donor Selection:

- **Age:** 18-65 years (can vary by country)
- **Weight:** Minimum 50 kg
- **Hemoglobin:** Male ≥ 13 g/dL, Female ≥ 12.5 g/dL
- Must be healthy, no infections, not on certain medications
- Screening for HIV, Hepatitis B, Hepatitis C, Syphilis, Malaria
- Interval between donations: Minimum 3 months

2. Collection Method:

- **Volume:** 450 mL \pm 45 mL (one unit)
- **Container:** Sterile plastic blood bag with anticoagulant
- **Anticoagulant:** CPD (Citrate-Phosphate-Dextrose) or CPDA-1 (CPD-Adenine)
- **Ratio:** 63 mL anticoagulant for 450 mL blood
- **Procedure:** Aseptic venipuncture (antecubital vein), 5-10 minutes collection time
- Gentle mixing during collection to ensure anticoagulant distribution
- Samples taken for blood typing and screening tests



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B. Processing of Whole Blood:

1. Separation into Components:

- **Centrifugation:** Blood is centrifuged to separate components
- **Red Blood Cells (RBCs):** Bottom layer (45% of blood volume)
- **Buffy Coat:** Thin middle layer containing WBCs and platelets (<1%)
- **Plasma:** Top layer (55% of blood volume)
- Components separated using sterile connecting devices and transfer bags

2. Blood Typing and Screening:

- ABO blood group determination
- Rh factor (D antigen) testing
- Antibody screening (for irregular antibodies)
- Infectious disease testing: HIV, HBV, HCV, Syphilis, Malaria (NAT testing)
- Proper labeling with donor number, blood group, expiry date

C. Storage of Whole Blood:

- **Temperature:** 2-6°C (refrigerator with continuous temperature monitoring)
- **Storage duration:**
 - CPD: 21 days
 - CPDA-1: 35 days
- **Storage conditions:** Upright position, gentle handling
- **Quality control:** Regular checks for hemolysis, bacterial contamination
- **Before transfusion:** Visual inspection (no clots, discoloration, hemolysis)
- Compatibility testing (cross-matching) before transfusion

Dried Human Plasma

Introduction:

- Dried plasma (lyophilized plasma) is dehydrated blood plasma
- Developed during World War II for battlefield use
- Long shelf life, easy to transport and store
- Reconstituted with sterile water before use

Preparation Process:

1. Plasma Separation:

- Blood collected in anticoagulant (citrate-based)
- Centrifugation to separate plasma from cells
- Plasma transferred to separate container under sterile conditions
- Pooled from multiple donors (after screening)



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2. Freeze-Drying (Lyophilization):

- **Freezing:** Plasma frozen rapidly to -40°C to -50°C
- **Primary drying:** Sublimation of ice under vacuum (removes ~95% water)
- **Secondary drying:** Desorption of bound water at slightly higher temperature
- **Final moisture content:** $<1\%$
- Process time: 24-48 hours
- Maintains protein structure and activity

3. Packaging and Storage:

- Packaged in sterile, sealed vials or bottles
- Nitrogen gas atmosphere to prevent oxidation
- **Storage temperature:** $2-8^{\circ}\text{C}$ (refrigerated) or room temperature
- **Shelf life:** 3-5 years (much longer than liquid plasma)
- Protected from light and moisture

Reconstitution:

- Add sterile water for injection (same volume as original plasma)
- Gentle swirling to dissolve (no shaking)
- Use within 4 hours after reconstitution
- Do not re-freeze reconstituted plasma

Uses:

- Volume replacement in shock and burns
- Coagulation factor replacement
- Emergency situations where fresh plasma not available
- Military and remote medical facilities

Plasma Substitutes

Definition:

Plasma substitutes (plasma expanders or volume expanders) are solutions used to replace blood volume in cases of severe blood loss, shock, or dehydration. They are synthetic or semi-synthetic alternatives to blood plasma.

Types of Plasma Substitutes:

A. Crystalloid Solutions:

1. Normal Saline (0.9% NaCl):

- Isotonic solution
- Simple, inexpensive
- Short duration in circulation (distributes to all body compartments)
- Used for initial fluid resuscitation



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- Can cause hyperchloremic acidosis in large volumes

2. Ringer's Lactate (Hartmann's Solution):

- Contains: Sodium, potassium, calcium, chloride, lactate
- More physiological than normal saline
- Lactate metabolized to bicarbonate (helps correct acidosis)
- Preferred for trauma and surgical patients

3. Dextrose Solutions (5% Dextrose in Water):

- Provides free water and calories
- Not for volume replacement (distributes to all compartments)
- Used for hydration and maintenance

B. Colloid Solutions:

1. Dextrans:

- **Description:** Polysaccharides produced by *Leuconostoc* bacteria
- **Types:** Dextran 40 (low molecular weight), Dextran 70 (high molecular weight)
- **Mechanism:** High osmotic pressure retains fluid in circulation
- **Duration:** 4-6 hours
- **Side effects:** Allergic reactions, bleeding tendency, renal impairment
- **Uses:** Shock, prophylaxis of thromboembolism
- **Maximum dose:** 20 mL/kg/day

2. Gelatin Solutions:

- **Description:** Degraded animal collagen (bovine or porcine)
- **Types:** Modified fluid gelatin (Gelofusine), Succinylated gelatin
- **Molecular weight:** 30,000-35,000 Da
- **Duration:** 2-4 hours
- **Advantages:** No effect on coagulation, less allergic reactions than dextrans
- **Uses:** Acute blood loss, hypovolemic shock

3. Hydroxyethyl Starch (HES):

- **Description:** Modified natural polysaccharide from corn or potato starch
- **Types:** Low, medium, high molecular weight (130,000-670,000 Da)
- **Duration:** 4-24 hours (depends on molecular weight)
- **Advantages:** Longer duration, good volume expansion
- **Side effects:** Coagulation defects, pruritus, renal impairment
- **Uses:** Surgery, trauma, sepsis
- **Restrictions:** Restricted use in critically ill patients due to safety concerns

4. Albumin:



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- **Description:** Human serum albumin (natural protein)
- **Concentrations:** 5% (iso-oncotic), 20-25% (hyperoncotic)
- **Source:** Pooled human plasma, heat-treated for safety
- **Duration:** 12-24 hours
- **Advantages:** Most physiological, no coagulation effects, viral inactivation
- **Disadvantages:** Expensive, derived from human source
- **Uses:** Severe hypoalbuminemia, cirrhosis with ascites, burns, nephrotic syndrome

Comparison: Crystalloids vs Colloids:

- **Crystalloids:** Cheap, readily available, short duration, require 3-4x volume
- **Colloids:** Expensive, longer duration, more effective volume expansion, risk of side effects
- **Choice:** Initial resuscitation with crystalloids, colloids for severe cases or when crystalloids insufficient



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