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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF CHEMICAL ENGINEERING

BIOCHEMICAL ENGINEERING

UNIT – 1 INTRODUCTION – SCHA 1601

UNIT I INTRODUCTION

What Is Biochemical Engineering?

The biochemical engineer is the field that would use biology, chemistry and engineering to produce biopharmaceuticals, enzymes, biopolymers, biofuels and other products. If you are the biochemical student, you would need to have extensive knowledge of the bioreactors that would help for the growth of cells and various other concepts. The course will help you get acquainted with the knowledge that is required for you to become a proficient biochemical engineer. The person who holds this degree can also contribute to the environmental remediation, food processing, renewable energy and other areas.

There is a lot of dedication and commitment that is required for the student who is pursuing this subject to excel and complete the assignments immaculately. One can become a professional biochemical engineer when he/she has knowledge of various concepts of biochemical engineering. They also must have the practical knowledge on the topic.

The biochemical engineer would develop new chemical products that can be used by people and companies. The products that are used in day to day lives are developed by the biomedical engineer. The products we used every day are agricultural chemicals, petroleum-based products, fibrous products and cleaning products. There is a huge demand for these engineers in the market. The students can get a job with ease when their academic scores are impressive.

Biochemical should be well-acquainted with various topics related to this engineering.

- **Fluid Mechanics** - It allows the students to learn the mechanism and behaviour of fluids when they are in rest and motion.
- **Heat transfer** - The head transfer would let you transfer the energy from one body to another or from one location in the body to another location.
- **Chemical Kinetics** - This is also known as reaction kinetics that will let you learn about the rate of chemical reactions.
- **Process Dynamics** - The process is a dynamical system that keeps on changing its behaviour over a period.
- **Bio-separations** - The bio separation is the critical topic wherein the students would learn the processes to purify the biological products on a large scale with the help of basic engineering aspects.
- **Catalysis** - This is a process that helps you to modify the chemical reaction with the help of a catalyst.

MICROBIAL CELL STRUCTURE AND FUNCTION

The Prokaryotic Cell, Size, Shape and Arrangement of Bacterial Cells

THE PROKARYOTIC CELL

The members of the prokaryotic world make up a vast heterogeneous group of very small unicellular organisms. This group includes eubacteria, or true bacteria, and archaebacteria. Bacteria are one of the most important groups of the microbial world.

The chief distinguishing characteristics of prokaryotic cells are:

- Their genetic material (DNA) is not enclosed within a membrane.
- They lack other membrane-bounded organelles
- Their DNA is not associated with histone proteins (special chromosomal proteins found in eukaryotes).
- Their cell walls most of the time contains the complex polysaccharide peptidoglycan.
- They usually divide by binary fission. During this process, the DNA is copied and the cell splits into two cells.

Size, shape and arrangement of bacterial cells

Size:

Prokaryotes are among the smallest of all organisms (0.5 to 2.0 m m). Because of their small size, bacteria have a large surface-to-volume ratio. The smallest member of the genus is about 0.3µm in diameter. Even smaller cells have been reported like the nanobacteria or ultramicrobacteria appear to range from around 0.2µm to not less than 0.05µm. *E. coli* , a bacillusof about average size is 1.1 to 1.5 µm wide by 2.0 to 6.0 µm long. Spirochaetes occasionally reach 500 µm in length and the cyanobacterium *Oscillatoria* is about 7 µm in diameter. The bacterium, *Epulosiscium fishelsoni* , can be seen with the naked eye (600 m m long by 80 m m in diameter). Thus a few bacteria are much larger than the average eukaryotic cell (typical plant and animal cells are around 10 to 50 µm in diameter).

Shape and arrangement:

Typically bacteria have three basic shapes – spherical, rod like and spiral.

Spherical bacteria :

Coccus (pluralcocci) in pairs– **diplococcic;** in chains– **Streptococci** (*Streptococcus*); in cube like groups of eight– **Sarcinae** (Sarcina); in grape like structures– **Staphylococci** (*Staphylococcus*) (Fig.1)

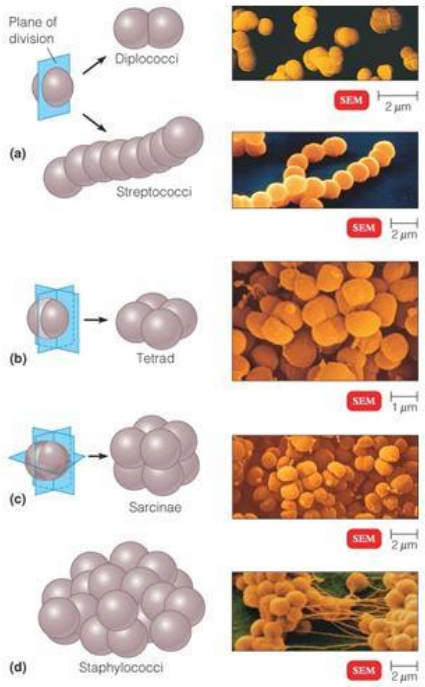


Fig. 1. Arrangement of spherical cells

Rod like bacteria - Bacillus (plural bacilli).

In pairs **– diplococci;** In chains – **streptococci.** Still others look like cocci and are called**coccobacilli** (Fig. 2).

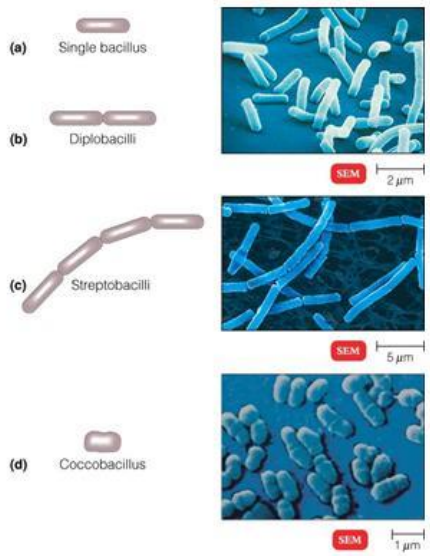


Fig. 2. Arrangement of rod like cells

Spiral bacteria

Comma shaped – Vibrio (*Vibrio*); Helical, long and curved – Spirilla (*Rhodospirillium*); Helical and flexible – Sprirochetes (*Treponema* and *Borrelia*) movement by axial filaments (Fig. 3).

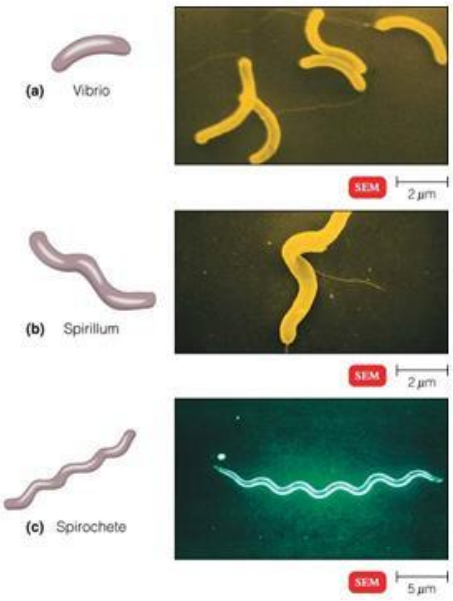


Fig. 3. Sprial bacteria

In addition there are: Star shaped cells (genus *Stella*); Square flat cells (halophilic archaebacteria); Triangular cells (*Haloarcula*); Branching mycelium – like filaments (*Streptomyces*) (Fig. 4)

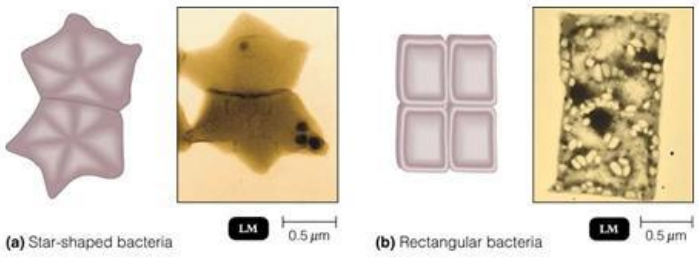


Fig. 4. Star shaped and rectangular shaped bacteria

Genetically most bacteria are monomorphic (single shape), but some have many shapes and are called pleomorphic (*Rhizobium* and *Corynebacterium*)

Structure:

A variety of structures are found I prokaryotic cells. The cells are almost always are bounded by a chemically complex cell wall. In between the cell wall and the periplasmic space lies the plasma membrane (Fig. 5). Internal membrane bound organelles are absent in prokaryotes and hence the internal is morphologically simple. The genetic material is localized in a discrete region called nucleoid and not separated from the surrounding cytoplasm by membrane. Ribososmes and inclusions are found scattered in the cytoplasmic matrix. Bacteria use flagella for locomotion, and in addition, many cells are surrounded by a capsule or slime layer external to the cell wall.

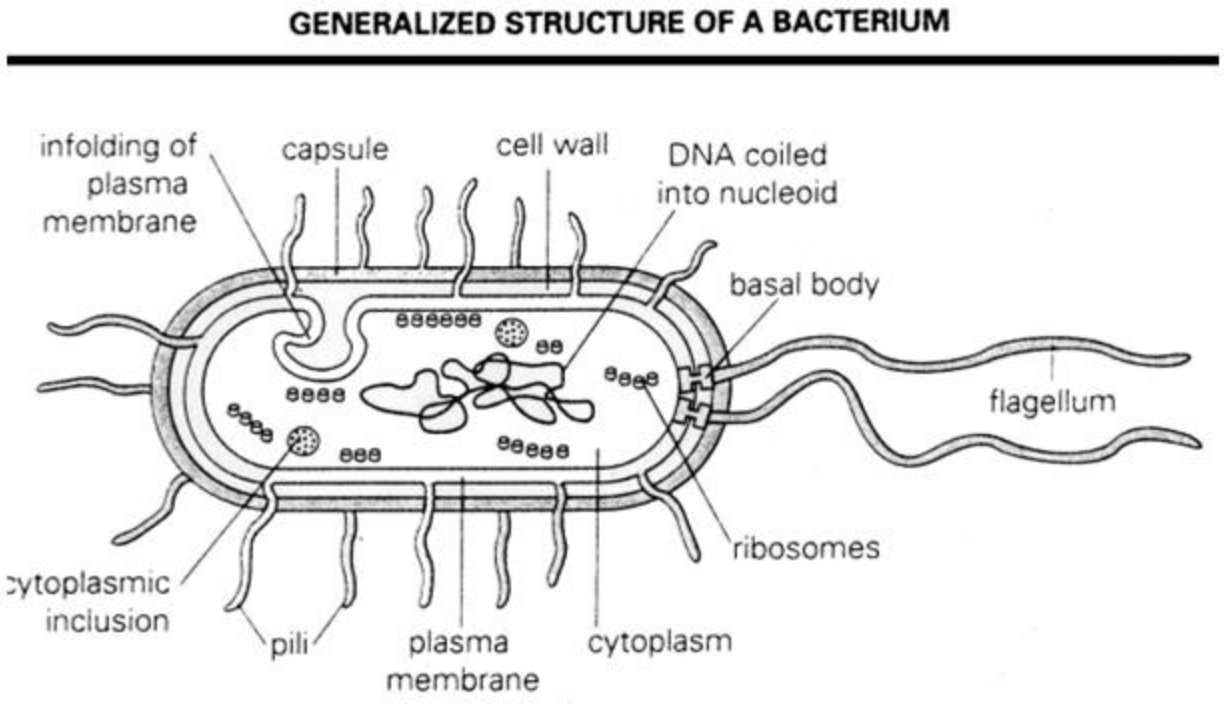


Fig.5. Generalized structure of a bacterium

The bacterial cell wall

Is a complex, semi-rigid structure that surrounds the underlying plasma membrane and protects the cell and the internal parts from adverse surrounding environment. Except for Mycoplasmas and some Achaea most bacteria have strong walls that give them shape and protect them from osmotic lysis; wall shape and strength is primarily due to peptidoglycan. The cell walls of pathogenic bacteria have components that contribute to their pathogenicity and protect a cell from toxic substances and are the site of action of several antibiotics. Major functions – prevent cells from rupturing, maintains shape and serves as a point of anchorage for flagella. Christian Gram developed the Gram stain in 1884, and it became evident that bacteria could be divided into two major groups based on their response to staining. Gram positive bacteria stained purple and the gram negative ones colored pink or red by the technique. The gram positive cell wall consists of a single thick layer of 20 to 80 nm of peptidoglycan or murein layer lying outside the cell membrane. In gram negative cells, the peptidoglycan layer is thin of about 2 to 7 nm surrounded by a 7 to 8 nm thick outer membrane. The gram positive cells are stronger than gram negative ones because of the peptidoglycan layer.

A space is seen between the plasma membrane and the outer membrane in electron micrographs of gram negative bacteria and a similar one but smaller gap may be observed between the plasma membrane and wall in gram positive bacteria. This space is called **periplasmic space.** The substance that occupies the space is the periplasm. The periplasmic space in gram negative bacteria ranges from 1nm to as great as 71 nm. It contains many proteins and participates in nutrient acquisition, hydrolytic enzymes attacking nucleic acids and phosphorylated molecules. The periplasmic space contains enzymes involved in peptidoglycan synthesis and modification

of toxic compounds that could harm the cell. Gram positive bacteria may not have a visible periplasmic space but appear to have as many periplasmic proteins and several enzymes that are secreted are often called **exozymes.**

The Archaea differs from other prokaryotes. Thewalls lack peptidoglycan and are composed of proteins, glycoprotein or polysaccharides.

Structure of Peptidoglycan

The cell wall of bacteria is composed of a macromolecular net

Gram positive cell walls

The cell wall consists of many layers of peptidoglycan, forming a thick, rigid structure, which contains a peptide interbridge. Gram positive cell walls also contain **teichoic acids,** polymers of glycerol and ribitol joined by phosphate groups. To the glycerol and ribotol groups, the amino acids such as D-alanine or sugars like glucose are attached (Fig. 7). There are two classes to teichoic acids; lipoteichoic acid (if they are attached to the lipid of the plasma membrane) and wall teichoic acid (which extend to the surface of the peptidoglycan and are negatively charged). Teichoic acids are not found in gram-negative bacteria.

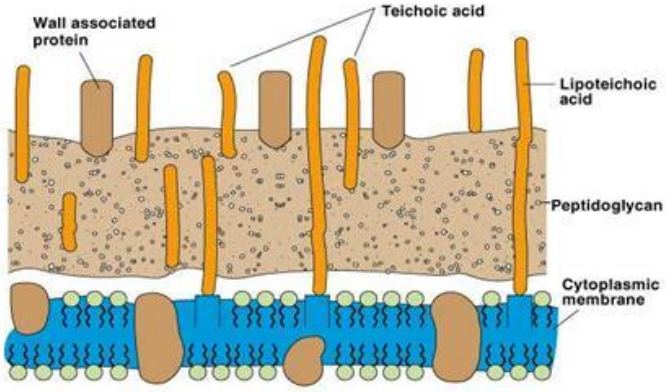


Fig. 7. Cell wall of Gram positive bacteria

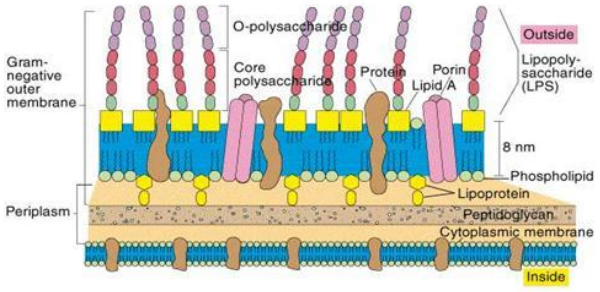
Some bacteria like *Staphylococci* and most other gram-positive bacteria have a layer of proteins on the surface of their cell wall peptidoglycan. Some are noncovalently attached by binding to the peptidoglycan, teichoic acids and other receptors and these proteins are involved in the interactions of the cell with its environment. Ex. S-layer proteins. Other surface proteins are covalently attached to the peptidoglycan and in gram positive pathogens these have roles such as aiding in adhesion to host tissues, preventing opsonization and blocking phagocytosis.

Gram negative cell walls

The cell wall contains only a thin layer of peptidoglycan. In *E. coli,* it is about 2-3nmthick and contains only one or two layers of sheets of peptidoglycan. The peptidoglycan is bounded to lipoproteins in the outer membrane and is embedded in a soft material, the **periplasmic gel.** Gram negative cell walls do not contain teichoic acids. They are more susceptible to mechanical breakage because of small amount of peptidoglycan. **Outer membrane -** found primarily in gram-negative cell consists of lipoproteins, liposaccharides and phospholipids and lies outside the thin peptidoglycan layer. The most abundant membrane protein is Braun's lipoprotein, a small lipoprotein covalently joined to the underlying peptidoglycan and embedded in the outer membrane by its hydrophobic end. The outer membrane e and plasma membrane appear to be in direct contact at many locations in the cell wall. The adhesion sites may be regions of direct contact or possibly true membrane fusions. It is proposed that substances can move into the cell through these adhesions sites rather than travelling through the periplasm.

The most unusual constituents of the outermembrane are its lipopolysaccharides. (LPSs). These contain both lipid and carbohydrate and consist of three parts: 1. Lipid A, 2. the core polysaccharide, and 3. the O side chain. The lipid A region contains two glucosamine sugar derivatives, each with three fatty acids and phosphate or pyrophosphate attached. This region is buried in the outer membrane and the remaining portion projects from the surface. The second region or the core polysaccharide is joined to lipid A and the third region or the O side chain which is a polysaccharide chain extends outward from the core. The O side chain is readily recognized by host antibodies, gram negative bacteria may thwart host defenses by readily changing the nature of their O side chains to avoid detection. The core polysaccharide contains charged sugars and phosphate, LPS contributes to the negative charge on the bacterial surface (Fig. 8). Lipid A is a major constituent of the outer membrane, and the LPS helps stabilize the membrane structure. Lipid A is often toxic and as a result LPS can act as an endotoxin and cause some symptoms that arise in gram-negative bacterial infections. The function of the outer membrane is to serve as a protective barrier. It prevents or slows the entry of bile salts, antibiotics, and other toxic substances that might kill or injure the bacterium. Provides a barrier to certain antibiotics (like penicillin), lysozyme detergents, heavy metals, bile salts and digestive.

Porins are proteins that permit small molecules to pass through the outer membrane (about 800 MW). S **pecific channel proteins –** allow specific substances like vitamin B12, iron, nucleotides and maltose.



- **Fig.8.** Cell wall of gram negative bacteria

Atypical cell walls:

• *Mycoplasma* – is a bacterial genus that naturally lacks cell walls. Their plasma membranes have lipids called sterols, which protect them from osmotic lysis.

• Archaeabacteria – have pseudomurein (N-acetylalosaminuronic acid) but no peptidoglycan.

• L forms - are mutant bacteria with defective cell walls .

Damage to the cell wall

The pres ence of cell wall is essential to protect bacteria against destruction by osmotic pressure. The bacterial cytoplasm is much more concentrated with solutes than in most microbial habitats which are hypotonic. During osmosis, water moves across selectively permeable membranes such as the plasma membrane from dilute solutions (higher water concentration) to more concentrated solutions (lower water concentration). Usually water generally enters the bacterial cells and the osmotic pressure may reach 20 atmospheres. Plasma membrane cannot resist such high pressures and the cell will swell and be physically disrupted and destroyed, a process called lysis. In hypertonic habitats, the water flows outward, and the cytoplasm shrivels up and pulls away from the cell wall. This phenomenon is called plamolysis and is useful in food preservation because many microorganisms cannot grow in dried foods and jellies as they cannot avoid plasmolysis. The importance of the cell wall in protecting bacteria can be demonstrated by treatment with lysozyme (naturally occurs in eukaryotic cells and is a constituent of tears, mucus and saliva), which attacks the peptidoglycan by hydrolyzing the bond that connects NAM and NAG units. Penicillin inhibits peptidoglycan synthesis. Gram positive cell walls are destroyed and the remaining cellular contents are referred to as**protoplast** .Gram negative cells are not completely destroyed and the remaining cellular contents are referred to as **spheroplast**. Protoplast and spheroplast are subject to osmotic lysis. Antibiotics such as penicillin destroy bacteria by interfering with the formation of the peptide cross bridges of peptidoglycan and ultimately cell wall synthesis.

Structures internal to the cell wall

In this lecture we shall be dealing with the structures internal to the cell wall of a bacterial cell. They include plasma membrane, organelles in the cytoplasm like nuclear area, ribosomes, inclusion bodies and endospores.

Plasma (cytoplasmic) membrane

Membranes are absolute requirement of all living organisms. It is the chief point of contact with the cell's environment and thus is responsible for much of its relationship with the outside world. Plasma membrane – encloses the cytoplasm and consists of phospholipids and proteins (**fluid mosaic model**).Most membrane-associated lipids are structurally asymmetric with polar and nonpolar ends. The polar ends interact with water and are hydrophilic and the nonpolar hydrophobic ends are insoluble in water. The lipid composition of bacterial membranes varies with environmental temperature in such a way that the membrane remains fluid during growth. Bacterial membranes usually differ from eukaryotic membranes in lacking sterols such as cholesterol and they contain pentacyclic sterol-like molecules called hopanoids and these are said to stabilize the bacterial membranes. Cell membranes are very thin structures about 5 to 10 nm thick and can be seen only with electron microscope. Plasma membranes have a complex internal structure; the small globular particles seen in these membranes are thought to be membrane proteins that lie within the membrane lipid bilayer (Fig. 9).

The most widely accepted current model for membrane structure is the fluid mosaic model of S. Jonathan Singer and Garth Nicholson. Two types of membrane proteins are seen, **Peripheralproteins** - which are loosely connected to the membrane and can be easily removed and are soluble in aqueous solutions and make up about 20 to 30% of total membrane protein. About 70 to 80% of membrane proteins are **integral proteins**. These cannot be easily extracted from membranes and are insoluble in aqueous solutions when freed of lipids. Integral proteins, like membrane lipids are amphipathic; their hydrophobic regions are buried in the lipid while the hydrophilic portions project from the membrane surface. The plasma membrane retains the cytoplasm, particularly in cells without cell walls, and separates it from the surroundings. Plasma membranes serve as a selectively permeable barrier; it allows particular ions and molecules to pass, either into or out of the cell, while preventing the movement of others. Transport systems can be used for such tasks as nutrient uptake, waste excretion, and protein secretion. The plasma membrane also is the location of a variety of crucial metabolic processes; respiration, photosynthesis, the synthesis of lipids and cell wall constituents, and probably chromosome segregation.

The bacterial plasma membrane can be destroyed by alcohols and polymixins which cause leakage of intracellular contents and subsequent cell death of the organism.

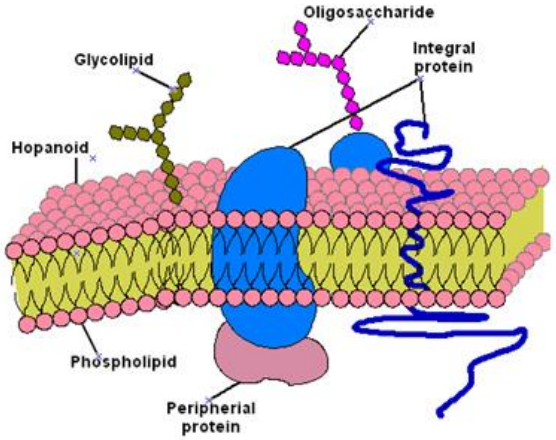


Fig. 9. Plasma membrane strcuture

Internal membrane systems:

Prokaryotes do not contain complex membrane systems as present in eukaryotes like chloroplast and mitochondria. They contain membranous structures like the one observed most is **mesosome**. Mesosomes – irregular infoldings or invaginations of the plasma membrane in the shape of vesicles, tubules, or lamellae. They can be seen in both gram positive and gram-negative bacteria. These are often found next to the septa or cross-walls in dividing bacteria and sometimes seems attached to the bacterial chromosome. Thus they seem to be involved in cell wall formation during division or play a role in chromosome replication and distribution to daughter cells.

Some bacteria have internal membrane systems quite different from the mesosomes. The infoldings of the plasma membrane can become extensive and complex in photosynthetic bacteria such as the cyanobacteria and purple bacteria or in bacteria with very high respiratory activity like the nitrifying bacteria. They may be aggregates of spherical vesicles, flattened vesicles, or tubular membranes. Their function may be to provide a larger membrane surface for greater metabolic activity.

Cytoplasm

Is the fluid component inside the plasma membrane. Cytoplasm is about 80% water and contains primarily proteins (enzymes), carbohydrates, lipids, inorganic ions and many low MW compounds. Major structures in the cytoplasm are DNA, ribosomes and inclusions.

Nuclear area

The striking difference between prokaryotic and eukaryotic systems is the way in which their genetic material is packaged. Prokaryotes lack a membrane-delimited nucleus. Contains a single long circular molecule of double-stranded DNA bacterial chromosomes do not include histones and are not surrounded by a nuclear envelope and located in an irregularly shaped region called **nucleiod** . The nuclear area can be spherical, elongated, or dumb-bell shaped. It is

attached to the plasma membrane and proteins of plasma membrane are believed to be responsible for replication of the DNA. It has been discovered recently that *Vibrio cholera* has more than one chromosome. Electron microscope studies have shown the nucleiod in contact with either the mesosome or the plasma membrane and hence evidence that the membranes may be involved in the separation of DNA into daughter cells during division. Chemical analysis reveals that they nucleoids are composed to about 60% DNA, 30% RNA and 10% protein. *E. coli*, which is about 2 to 6um long, the closed DNA circle measures approximately 1400um. Hence, it is evident that the DNA is efficiently packaged to fit within the nucleoid and the DA is looped and coiled extensively (Fig. 10).

Many bacteria possess extra chromosomal double stranded, circular DNA molecules called **plasmids**in addition to their chromosome. They replicate independently and are associated with plasma membrane proteins. Plasmids usually contain from five to 100 genes. Plasmids may carry genes for such activities as antibiotic resistance, tolerance to toxic metals, production of toxins, and synthesis of enzymes. Plasmid DNA is used for gene manipulation in biotechnology. Because plasmids move between different bacteria, drug resistance can spread throughout a population.

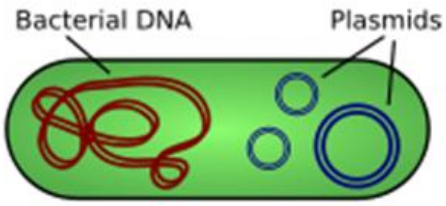


Fig. 10. Bacterial DNA and plasmids

Ribosomes:

The cytoplasmic matrix is also packaged with ribosomes, they also may be loosely attached to the plasma membrane. They look like small, featureless particles at low magnification electron microscope. They are made up of both protein and ribonucleic acid (RNA). Ribosome's function as sites of protein synthesis; matrix ribosomes synthesize proteins destined to remain within the cell, whereas the plasma membrane ribosomes make proteins for transport to the outside.The shape of each protein is determined by its amino acids sequence and the special proteins called molecular chaperones or chaperones aid the polypeptide in folding to its proper shape. Prokaryotic ribosomes are smaller than eukaryotic ribosomes (Fig. 11). Ribosomes are composed of two subunits, each subunit being composed of protein and a type of RNA called ribosomal RNA (rRNA).They are comm. Only 70S: 30S subunit (1 molecule of rRNA) and 50S subunit (2 molecules of rRNA) and have dimensions of about 14 to 15 nm, a molecular weight of approximately 2.7 million.The S in 70S stands for Svedberg value or sedimentation coefficient. It is the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater is its Svedberg value. The Sedimentation coefficient is a function of a particle's molecular weight, volume and shape. Several antibiotics, such as streptomycin, neomycin and tetracyclines, exert their antimicrobial effects by inhibiting protein synthesis on ribosomes.

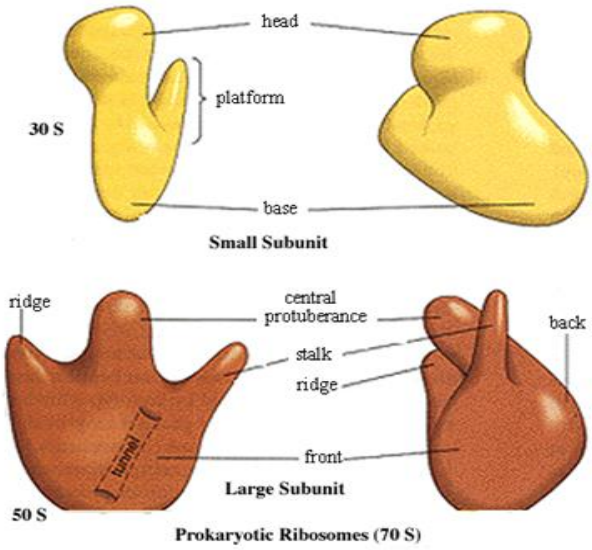


Fig. 11. Ribosmes in bacteria

Inclusions:

Inclusion bodies can be divided into two types:

Inclusion bodies not bounded by a membrane and lie free in the cytoplasm. Ex. Polyphosphate granules, cyanophycingranules and some glycogen granules.

Inclusion bodies enclosed by a membrane about 2-4nm thick. Ex.PolyB-hydroxybutyrate granules, some glycogen and sulfur granules, carboxysomes and gas vacuoles.

Organic inclusion bodies:

Glycogen:

Polymer of glucose units composed of long chains formed by alpha (1-4) glycosidic bonds and branching chains connected to themby alpha (1-6)glycosidic bonds. Ex. glycogen and starch, and their presence can be demonstrated when iodine is applied to the cells (glycogen granules appear reddish brown and starch granules appear blue).

Poly B- hydroxybutyrate:

Contains beta-hydroxybutyrate molecules joined by ester bonds between the carboxyl and hydroxyl groups of adjacent molecules. Appear in various species of *Mycobacterium, Bacillus, Azotobacter ,Spirillum* and other genera. Lipid inclusions are revealed by use of fat-soluble dyes, such as Sudan dyes.

Glycogen and PHB are carbon storage reservoirs providing material for energy and biosynthesis (Fig. 12).

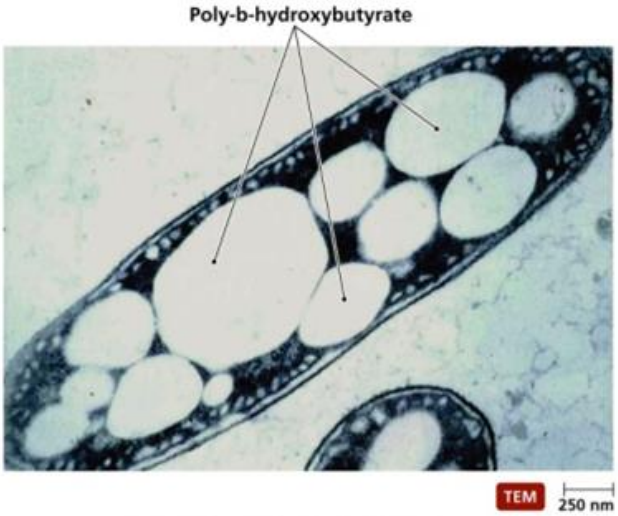


Fig. 12 . Poly B- hydroxybutyrate inclusions

Cyanophycin granules:

Cyanobacteria are composed of large amino acids containing approximately equal amounts of amino acids arginine and aspartic acid. These are used to store extra nitrogen for the bacteria.

Carboxysomes:

These are polyhedral and hexagonal inclusions that contain the enzyme ribulose 1,5-diphosphate carboxylase. ·Bacteria that use carbon dioxide as their sole source of carbon require this enzyme for carbon dioxide fixation during photosynthesis (Ex.nitrifying bacteria, cyanobacteria, and *Thiobacilli*).

Gas vacuoles:

These are hollow cavities found in many aquatic prokaryotes, including cyanobacteria, anoxygenic photosynthetic bacteria and halobacteria. Each vacuole consists of rows of several individual gas vesicles, which are hollow cylinders covered by protein. Their function is to maintain buoyancy so that the cells can remain at the depth in the water appropriate for them to receive sufficient amounts of oxygen, light and nutrients. They are impermeable to water and permeable to atmospheric gases.

Inorganic inclusion bodies:

Polyphosphate granules orMetachromatic granules:

Linear polymer of organo phosphates joined by ester bonds. Reservoirs for phosphate, an important component of cell nucleic acids and also energy reserves. Represents a reserve of

inorganic phosphate (polyphosphate) that can be used in the synthesis of ATP. Stain red with certain blue dyes, such as methylene blue, and are collectively known as volutin. Found in algae, fungi and protozoans, as well as bacteria. These granules are quite large and are characteristic of*Corynebacterium diphtheriae*, the causative agent of diphtheria, thus they have diagnostic significance.

Sulphur granules:

Sulphur bacteria, which belong to the genus *Thiobacillus*, derive energy by oxidizing sulfur and sulfur containing compounds. These bacteria may deposit sulfur granules in the cell, where they serve as an energy reserve. Purple photosynthetic bacteria use H_2S as electron donor and accumulate resulting sulfurin either the periplasmic space or in special cytoplasmic globules.

Magnetosomes:

Not for storage, but these are used by some bacteria to orient in the earth's magnetic field. These inclusion bodies contain iron in the form of magnetite (greigite or pyrite) (Fig. 13). Ex. *Aquaspirillum magnetotacticum*. Also present in heads of birds, dolphins, and turtles etc which aid in navigation.

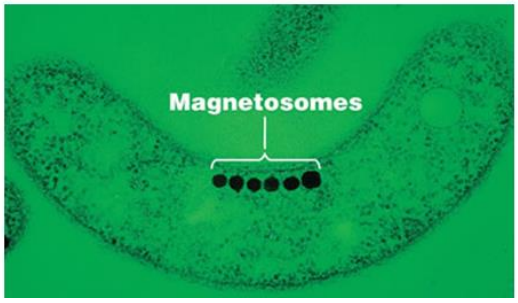


Fig. 13. Magnetosomes

Endospores "An escape pod for DNA"

Endospores are a survival mechanism:they are triggered to form during adverse environmental conditions. They are NOT reproductive structures as only one cell gives rise to one spore and endospores can be identified with special stains and differentiated from the vegetative cell (Fig. 14). Endospores are resistant to: heat: withstand boiling for over one hour; desiccation, UV radiation and chemical disinfectants. The resistance of these spores has serious consequence and some very pathogenic bacteria have the ability of produce such spores

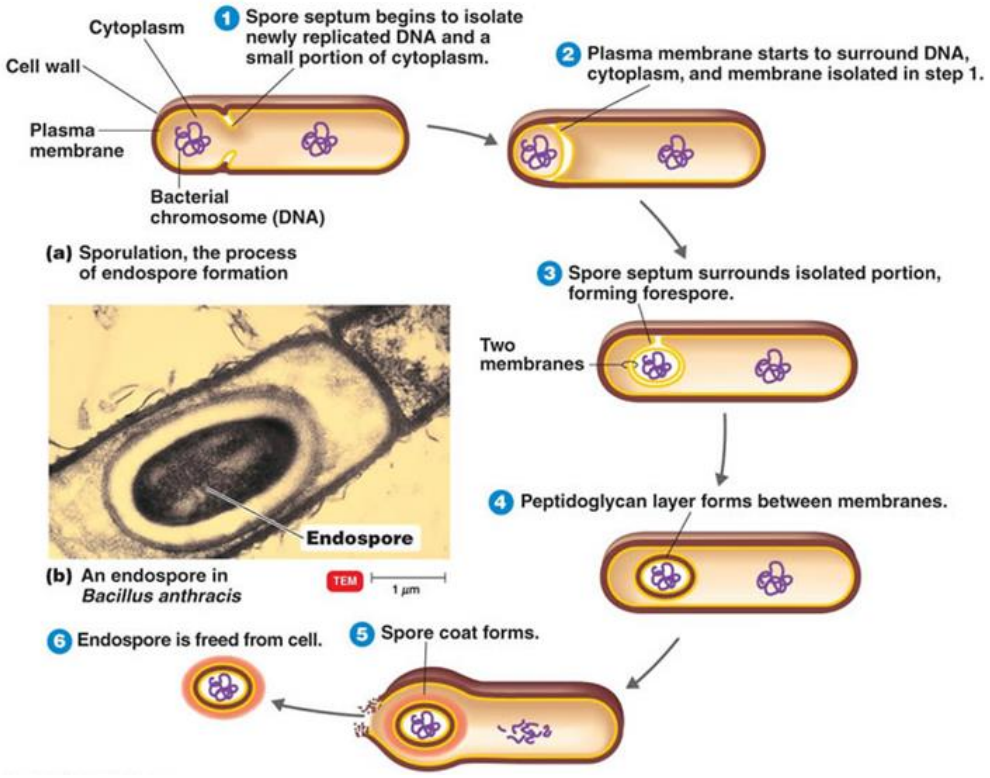


Fig. 14. Endospore formation

Structures External to the Cell Wall

In this lecture we shall look into the structures external to the cell wall of bacterial cells. This includes glycocalyx, fimbriae, pili, flagella, axial filaments

Glycocalyx (Capsules, Slime layers and S-layers)

It is a viscous (sticky), gelatinous polymer composed of polysaccharide, polypeptide or both. If the substance is organized and is firmly attached to the cell wall, the glycocalyx is described as a **capsule**(negative staining). If the substance is unorganized and only loosely attached to the cell wall, the glycocalyx is described as a **slime layer**. Capsules protect pathogenic bacteria from phagocytosis (process by which certain white blood cells engulf and destroy microbes) and contribute to virulence. Unencapsulated *Streptomyces pneumoniae* and *Bacillus anthracis* does not cause disease because the cells are readily phagocytosized. This allows the bacteria to attach to various surfaces, such as rocks in fast-moving streams, plant roots, human tooth and tissues and even other bacteria. Capsules also contain water which prevents them from desiccation. Other examples are *Streptococcus mutans*(dental caries), *Klebsiella pneumoniae* (respiratory tract). These can protect a cell against dehydration. Capsules and slime layers usually are made up of polysaccharides, but they may be constructed of othermaterial, like *Bacillus anthracis* has a capsule of poly D-glutamic acid. Capsules are clearly visible in the light microscope by using stains or special capsule stains.

A regularly structured layer called S-layer is usually seen in many gram positive and gram negative bacteria. It consists of proteins or glycoproteins and resembles a pattern something similar to floor tiles. The S-layer adheres directly to the outer membrane in case of gram negative bacteria and with the peptidoglycan surface in gram positive bacteria. These protect the bacteria against ion and pH fluctuations, osmotic stress, enzymes, or the predacious bacterium *Bdellovibrio* . The S layer also helps maintain the shape and envelope rigidity of at least bacterial cells and also promotes cell adhesion to surfaces. Sometimes, the layer also seems to protect some pathogens against complement attack and phagocytosis, thus contributing to their virulence.

Fimbriae and Pili:

Many gram negative bacteria have hairlike appendages that are shorter, straighter and thinner than flagella and are used for attachment rather than for motility. They are usually called fimbriae. These structures contain a protein called pilin. **Fimbriae** - occur at the poles of the bacterial cell, or they can be evenly distributed over the entire surface of the cell. Fimbriae of *Neisseria gonorrhoeae* the causative agent of gonorrhea help the microbe to colonize mucous membranes to cause the disease. At least some types of fimbriae attach bacteria to solid surfaces such as rocks in streams and host tissues.

Pilior sex pili or pilus - usually longer than fimbriae and number only one to ten per cell. Pili function to join bacterial cells prior to the transfer to DNA from one cell to another (sometimes called sex pili). They are genetically determined by sex factors or conjugative plasmids and are required for bacterial mating. Some bacterial viruses attach specifically to receptors on sex pili at the start of their reproductive cycle.

Flagella:

Motile bacteria move by use of flagella, threadlike locomotor appendages extending outward from the plasma membrane and cell wall. They are slender, rigid structures, about 20 nm across and up to 15 or 20 µm long. Bacterial species often differ distinctively in their patterns of flagella distribution (Fig. 15).

Monotrichous- single polar flagellum located at one end

Amphitrochous- With two flagella, one at each end

Lophotrichous - With two or more flagella at one or both ends

Peritrichous - flagella all over the surface

Atrichous - Bacteria without flagella (Cocci rarely have flagella)

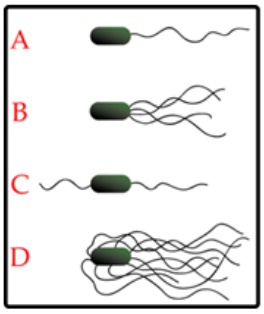


Fig. 15 . Flagellar arrangement. A. Monotrichous B. Lophotrichous C. Amphitrichous D. Peritrichous

Structure:

Transmission electron microscopic studies have shown that the bacterial flagellum is composed of three parts. 1) **Filament** – outermost region and contain the globular protein flagellin2) **Hook** – the filament is attached to hook, which consists of a different protein 3) **Basal body** - which anchors the flagellum to the cell wall and plasma membrane. It consists of a small central rod inserted into it are a series of rings (Fig. 16). The filament is a hollow, rigid cylinder constructed of a single protein called**flagellin** (MW from 30,000 to 60,000). Some bacteria have sheaths surrounding their flagella. For example *Bdellovibrio* has a membranous structure surrounding the filament. *Vibrio cholerae* has a lipopolysaccharide sheath.

The hook and basal body are quite different from the filament. Slightly wider than the filament, the hook is made of different protein subunits. The basal body is the most complex structure of the flagellum. In *E.coli* and Gram negative bacteria, the body has four rings connected to central rod. The outer L and P rings associate with the lipopolysaccharide and peptidoglycan layers. The inner M ring connects the plasma membrane.Gram positive have only twp basal body rings, an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan.

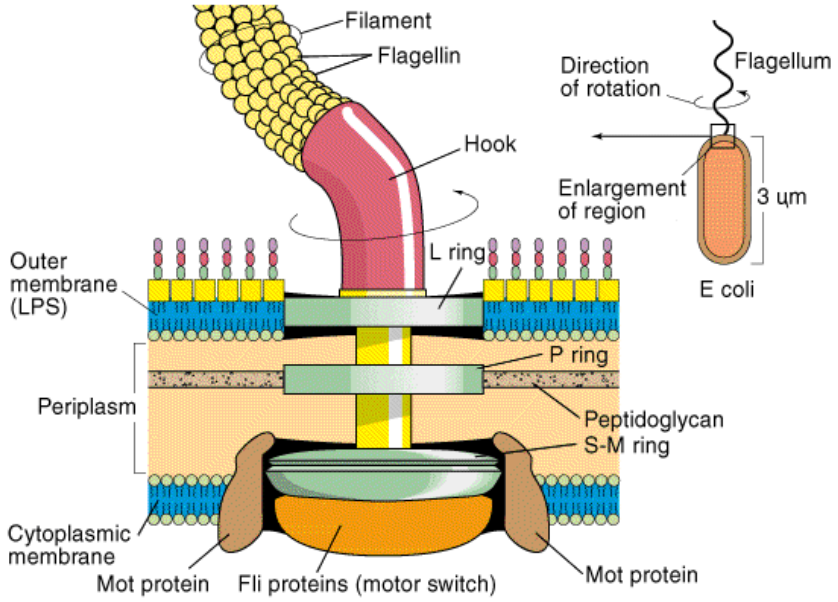


Fig. 16. Structure of bacterial flagella (Gram negative)

The synthesis of flagella is a complex process involving atleast 20 to 30 genes. Flagellin subunits are transported through the filament's hollow internal core. When they reach the tip, the subunits spontaneously aggregate under the direction of a special filament cap so that the filament grows at its tip rather than at the base. Filament synthesis is an excellent example of self-assembly.

Flagellar movement:

The mechanism of flagellar movement in prokaryotes is different from eukaryotic flagella. The bacterium moves when the helix rotates as the filament is in the shape of rigid helix. The flagella act just like propellers on a boat. The direction of flagellar rotation determines the nature of bacterial movement. The movement in monotrichous bacteria stop and tumble randomly by reversing the flagellar rotation. The polar flagella, rotate counter clockwise during normal forward movement, whereas the cell itself rotates slowly clockwise. Peritrichous bacteria also operate in a similar way. To move forward, the flagella rotate counter clockwise. As they do so, they bend at their hooks to for a rotating bundle that propels them forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles (Fig. 17).

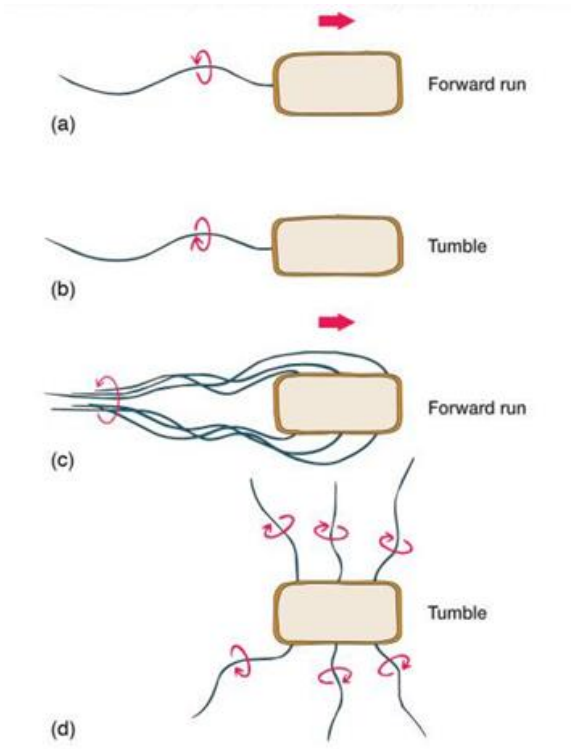


Fig. 17. Bacterial flagellar movement

Motility enables the bacterium to move toward a favorable environment or away from a particular stimulus called taxis . Chemotaxis (include chemicals) and phototaxis (include light). Bacteria do not always swim aimlessly but are attracted by such nutrients as sugars and amino acids, and are repelled by many harmful substances and bacterial waste products. Movement toward chemical attractants and away from repellents is known as **chemotaxis**. The mechanism ofchemotaxis in *E.coli* has been studied most. Forward swimming is due to counterclockwise rotation of the flagellum, whereas tumbling results from clockwise rotation. The bacteria must be able to avoid toxic substances and collect in nutrient-rich regions and at the proper oxygen levels. *E.coli* has four different chemoreceptors that recognize serine, aspartate and maltose, ribose and galactose and dipeptides respectively. These chemoreceptors often are called methyl-accepting chemotaxis proteins (MCPs)

Some bacteria can move bymechanisms other than flagellar rotation. Spirochetes are a group of bacteria that have unique structure and motility (*Treponemapallidum*, the causative agent of syphilis and *Borrelia burgdorgeri* , the causative agent of Lyme disease).Spirochetes travel through viscous substances such as mucus or mud by flexing and spinning movement caused by special a xial filaments - bundles of fibrils that arise at the ends of the cell beneath the outer sheath and spiral around the cell (fig. 18).The rotation of the filaments produces an opposing movement of the outer sheath that propels the spirochetes by causing them to move like corkscrews.

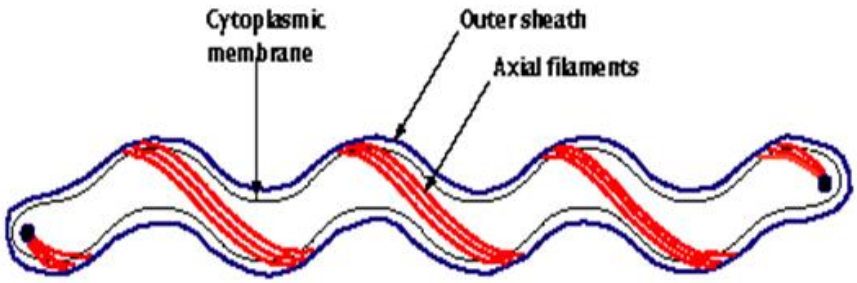


Fig. 18 . Axial filaments seen in spricohetes

A very different type of motility, gliding motility, is employed by many bacteria; cyanobacteria, myxobacteria and cytophagas and some mycoplasmas.

The Cell - Structure and Function

According the cell theory, the cell is the basic structural and functional unit of the organism. Although cells are differentiated or specialized in carrying out specific functions, all cells have similar structures and functions thus we can consider general cell characteristics.

Cell Components

Cells are highly organized. They are composed of structures that are responsible for specific cell functions, called organelles and inclusions. The organelles are permanent structures in the cell. The inclusions are temporary structures in the cell in the form of granules or droplets.

The cell (protoplasm) has three broad divisions.

1. The plasma membrane, the outer surface of the cell, separates the cell's internal environment from the environment outside of the cell. 2. The nucleus, the control center of the cell, is essential for the cell's survival and its ability to divide. 3. The cytoplasm (cytosol) surrounds the nucleus. The cytosol is the intracellular fluid portion of the cytoplasm; it has all the characteristics of a mixture. It consists of water and dissolved solute molecules and ion, true solution. It has suspended molecules, colloidal solution. The cell organelles and inclusions are suspended in the cytosol; each has specific structure and function, suspension.

The cell or plasma membrane (plasmalemma) is flexible but sturdy barrier that surrounds the cytoplasm and forms the limiting boundary of the cell. Membranes also surround many organelles to form the cytomembrane system of the cell. The cell membrane and the membranes of the organelles are semi-permeable to maintain the appropriate intracellular environment.

I. Cell Membrane

The basic organization of all cell membranes is that of the cell membrane.

A. Structure - Fluid Mosaic Model of Membrane Structure

The cell membrane is composed of a bimolecular layer of lipid (phospholipid and cholesterol) and various proteins embedded in the lipid in an asymmetrical pattern. The bimolecular layered arrangement is due to the lipids, which are amphipathic molecules; i.e. they have polar and nonpolar ends. The phospholipid polar hydrophilic ends are arranged toward aqueous regions. The nonpolar, hydrophobic ends arranged toward the interior of the membrane. The lipid of the membrane is semi-fluid in which the proteins float in no specific pattern to form a mosaic. The lipid determines basic structure of the membrane, while protein determines basic function. Carbohydrates are attached to the outer surface of the membrane by the way of protein (glycoprotein) and lipid (glycolipid). These surface sugar molecules behave as receptor or marker molecules. Example, these molecules as glycoproteins, serves as cell recognition site on the red blood cell identifying the person's blood type.

Some proteins extend through the membrane forming a channel to connect the interior of the cell with the external environment. These protein molecules are mainly carrier molecules, they are known as integral protein. Other proteins are on either surface; these are known as peripheral proteins. They mainly function for structural support.

B. Functions

The primary function of the cell membrane is control of movement of material into and out of the cell. All materials that enter or leave the cell must pass through the cell membrane. The properties of the membrane, molecular size, electric charge and solubility of the materials determine the ease with which materials pass through the cell membrane. The membrane is semi-permeable - some materials pass and other materials cannot pass. The cell membrane regulates and maintains the cell's intracellular environment and its homeostasis.

1. Simple diffusion through the lipid (hydrophobic) portion of the membrane**.** In general, small uncharged, non-polar lipid substances and nonlipid substances such as water, oxygen, and carbon dioxide pass the lipid part of the membrane by simple diffusion. Water-soluble substances, ions and larger molecules diffuse with difficulty.

2. Movement through the integral proteins of the membrane - the integral proteins form channels either for simple diffusion or for mediated transport (facilitated diffusion or active transport) for water, large neutral molecules, small polar molecules and ions.

3. Bulk (Vesicular) Transport - Endocytosis and Exocytosis

Endocytosis and exocytosis are active transport processes across the cell membrane involving very large molecules (macromolecules) and particulate material, such as whole cells by an engulfment process that requires energy. In the paragraphs above discussed movement of molecules and ions.

a. Endocytosis - in this process the cell surrounds large extracellular material either particulate or liquid with a part of the cell membrane to form a vesicle or vacuole that encloses the material. The vesicle separates from the membrane by pinching off.

1. Phagocytosis (cell eating) - the contents of the vesicle are large suspended particles. Example – Macrophages (a type of WBC) phagocytose microorganisms (bacteria) and viruses.

2. Pinocytosis (cell drinking) or Bulk-Phase Endocytosis - the contents of the vesicle are dissolved solutes (tiny droplets of extracelluar fluid) in solution.

3. Receptor Mediated Endocytosis – a selective type of endocytosis by which cells take up specific substances (ligands) by binding to specific receptors on the extracellular surface of the cell membrane. The substances are then transported into the cell with a minimal amount of fluid. The receptors are concentrated in regions of the cell membrane called clathrin-coated pits. The clathrin protein attaches to the cytoplasm side of the cell membrane. Many clathrin molecules come together forming an invagination of the cell membrane around the receptor-substance complex. The invaginated region of the cell membrane fuses and pinches off as a clathrin coated vesicle containing the receptor-substance complex. Immediately after invagination the vesicle loses its clathrin to become an uncoated vesicle. The clathrin molecules return to the inner surface of the cell membrane. The uncoated vesicle fuses with a vesicle known as an endosome. Within the endosome the substance separates from the receptors. The receptors accumulate in elongated protrusions of the endosome. These pinch off to form transport vesicles containing the receptors to return the receptors to the cell membrane. Other transport vesicles containing the endocytosed substance bud off the endosome and fuse with a lysosome. The enzymes within the lysosome digest the substance to simpler substances. Examples of substances taken in by receptor-mediated endocytosis are low-density lipoprotein (LDL), transferrin (an iron-transporting protein in the blood), some vitamins, antibodies and some hormones.

b. Exocytosis - in this process substances that the cell produces are secreted out of the cell. The substances are in membrane bound vesicles within the cell. The cell vesicles move through the cytosol to the cell membrane, fuse with the cell membrane. The contents of the vesicles are then released from the cell.

c. Transcytosis – vesicles undergo endocytosis on one side of the cell membrane, move across the cell, then
undergo exocytosis on the opposite side. Transcytosis occurs across the endothelial cells of the blood capillaries.

Endocytosis and exocytosis require energy. During both these processes, the cell membrane changes size.

4. Mediated Transport

a. Faciliated Diffusion - a type of mediated transport where the protein carrier molecule, permease, move molecules across the cell membrane along a concentration gradient, from high

concentration to low concentration. This is a passive process, which does not require expenditure of energy.

b. Active Transport - a type of mediated transport where the protein carrier molecule moves molecules across the cell membrane against a concentration gradient with the expenditure of energy (ATP).

Organelles

II. Nucleus

The nucleus is the cell's largest organelle usually found in the center of the cell.
Structure – The shape of the nucleus is spherical to oval. The nucleus is surrounded a double-layered membrane or envelope containing pores. It is through the pores that RNA leaves and other molecules can enter the nucleus by an active transport process.
Function -The nucleus contains information in the form of large aggregates of DNA – the genes to control cell structures and cell activities.

A. Chromatin (Chromosomes) Chromatin Vs Chromosomes

Chromatin
Structure - Suspended in the nuclear matrix (nucleoplasm) is the genetic material. The DNA of the cell is in the form of chromatin threads. Chemically the chromatin consists of DNA+protein (histone)+small amount of RNA. Euchromatin – extended chromatin active DNA, used by cells in making protein. Heterochromatin – condensed chromatin inactive DNA.
Function - DNA contains the information that controls cell functioning and activities in terms of protein synthesis and transmission of information from one generation to the next.
Chromosomes
The chromosomes are the condensed forms of chromatin in the form of short rods. The human chromosome complement is 46 chromosomes, 22 pairs of autosomes and two sex chromosome Female - XX, Male - XY.

B. Nuclear Membrane or Envelope
Structure – The nuclear membrane is a double membrane structure with pores. The pores form by fusion of
the inner and outer membranes.
Function - The pores allow proteins and RNA macromolecules to pass by an active transport process.
The nuclear membrane is semi-permeable.

C. Nucleolus
Structure - The nucleolus is a spherical, non-membrane bound structure, which appears during interphase,
and disappears during mitosis. The segment of the DNA that carries instructions for organizing the nucleolus
and synthesizing the r-RNA is called the nucleolar organizer region.

Function - The nucleolus is a nuclear structure that produces the components of the ribosomes and some
forms of RNA.

D. Nucleoplasm

It is colloid substance that has the same content as the cytosol. The chromatin threads and nucleolus are
suspended within the nucleoplasm.

III. Cytoplasm Organelles

A. Ribosomes

Structure - non-membrane bound small spherical solid structures composed of two subunits.
Chemically ribosomes consist of RNA+protein.
Function - Ribosomes are sites of protein synthesis.

B. Endoplasmic Reticulum (ER)

Structure - a network of membrane bound fluid filled channels throughout the cytoplasm (cisterns). ER is continuous with the cell membrane and nuclear membrane.

Function - the tubules of the ER serve as temporary storage area for substances and as a transport system for molecules synthesized by the cell. ER with attached ribosomes is called “rough" ER. ER without ribosomes is called "smooth" ER. Lipid and steroid (cholesterol) synthesis occurs in the membranes of "smooth" ER.

Ca^{++} ions are stored in the "smooth" ER tubules in muscle cells.

Proteins synthesized by ribosomes of the "rough" ER travel in the channels of the ER to the Golgi apparatus for secretion out of the cell. Ribosomes free in the cytoplasm make proteins for the cell's own use.

C. Golgi Apparatus or Complex and Secretory Vesicles

Structure - a stack of 4-6 parallel-flattened membranous sacs (cisternae) located near the nucleus from which secretory vesicles filled with secretory material get pinched off. The proteins once in the Golgi apparatus are modified and accumulate at the ends of the sacs. The ends of the sacs pinch off as secretory vesicles filled with the protein. The vesicles are called secretory granules. The granules then migrate to the periphery of the cell fuse with the cell membrane. The contents are released from the cell by exocytosis.

Function – The Golgi apparatus functions for secretion. It is most highly developed in secretory cells, such as gland cells and nerve cells.

D. Lysosomes

Structure - small membrane bound vesicles formed by the Golgi apparatus. They contain hydrolytic digestive enzymes at an acidic pH (5). The lysosomes are pinched off from the Golgi apparatus.

Function - the lysosomes function for digestion of phagocytosed substances. The lysosomes fuse with the phagocytic vesicles formed during endocytosis and pour their digestive enzymes into the vesicles. The enzymes then digest the material. The lysosomes are abundant in phagocytic WBC.

E. Peroxisomes

Structure - membrane bound organelles produced by division of preexisting peroxisomes.

Function - the peroxisomes function to inactivate toxic substances in the cell.

The peroxisomes contain enzymes that use O_2 molecules to oxidize or remove H atoms from organic substances to form H_2O_2.

If these substances were not oxidized and allowed to accumulate; they would be toxic to the cell - examples of toxic substances phenol, formaldehyde, formic acid, alcohol. The generated H_2O_2 is itself toxic and is decomposed by an enzyme in the peroxisome, catalase.

$$H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

F. Mitochondria

Structure - elongated (rod shaped) to spherical shaped structures bounded by a double membrane. The outer membrane is smooth and surrounds the mitochondrion. The inner membrane is extensively folded into projections called cristae on which enzymes are present. In the center of the mitochondrion is a semisolid matrix which enzymes are also present.

Function - mitochondria (powerhouses of cell) are involved in ATP production. Mitochondria contain enzymes, which are involved in aerobic, cell respiration, if O_2 is present.

Mitochondria are self-replicating as they contain mitochondrial DNA and ribosomes to produce proteins.

G. Cytoskeleton - The cell has an internal skeleton of tiny tubules and filaments which support the cell, hold the nucleus and organelles in place. It is also responsible for cell movement, changes in cell shape and movement of organelles.

Types of Cytoskeletal Structures

1. Microtubules - slender, hollow, cylindrical unbranched tubules consist of the protein tubulin. The microtubules provide spatial organization (support) to the organelles of the cell, form parts of cilia, flagella, centrioles and spindle fibers and provide for cell movement and distribution of material.

2. Microfilaments - small, solid, rods or cylinders made of the proteins actin and myosin that provide for the contractile activities of the cells. Also the microfilaments function to maintain support and shape of the cell.

3. Intermediate Filaments - they are in size between microtubules and microfilaments. They support cells,especially long thin cells like nerve cells.

H. Cilia, Flagella, Basal Body

Cilia and flagella are thin, cylindrical structures that are motile cytoplasmic projections from the cells surfaces. These structures move substances over the cell surface (cilia) or move the cell (flagella).

1. Cilia (um)

Structure - small, short, and numerous hair-like projections.

Function - wavelike rhythmic motion of the cilia creates a current that transport(move)material over a surface. Example - found in the respiratory system to move mucus over the surface, in the oviduct of the reproductive system moves the ova along.

2. Flagella (um)

Structure - long, whiplike and fewer in number/cell, 1-2/cell. They have more random motion than cilia.

Function – The flagellum's whip-like movement propels the sperm cell through an aqueous environment.

Example- in humans the flagella are only found in sperm.

Both cilia and flagella have the same organizational pattern and originate from a structure in the cytoplasm, the basal body. The internal structure of cilia and flagella consist of a core of cytoplasm surrounded by the cell membrane. Within the center of these cytoplasmic projections there are microtubules.The microtubules are arranged in a 9+2 pattern, Nine (9) pairs in a circle and one (1) pair in the center.

3. Basal Body

Structure - at the base of cilia and flagella. The basal body anchor the cilia and flagella to the cell, it consists of 9 groups of microtubules (3 tubules/group) and no central tubules. This same structure is found is the centrioles.

Function - organize microtubules of cilia and flagella.

I. Centrioles

Structure - Centrosome near the nucleus is a dense region of cytoplasm; within the centrosome are 2 pairs of cylindrical structures, the centrioles.

Function - the centrioles are involved in organizing the microtubules of the mitotic spindle. The spindle is involved in the separation of the chromosomes during cell division. Each centriole pair occurs as a group of microtubules at right angles to each other.

J. Microvilli

Structure - Very short cylindrical shaped extension of cytoplasm covered by the cell membrane.

Function - increase cell surface area for absorption. Microvilli do not beat. Within the microvilli are microfilaments which support the microvilli. Actin which is a contractile protein causing the microvilli to contract and expand. This creates movement that brings fresh material in contact with the microvilli for absorption.

IV. Cellular Inclusions

Temporary chemical substances in a cell in the form of granules or droplets produced by the cell or taken in by the cell. Examples - melanin pigment, glycogen granules, lipid (triglycerides) droplets, minerals, mucus droplets, dust and lipochrome, a pigment lipid. The inclusions may or may not be surrounded by a membrane. These inclusions are not permanent components of cells, they are constantly being used and replaced.

V. Life Cycle of the Cell - Interphase and Cell Division

Highly specialized cells do not undergo cell division after they have fully developed, such as muscle and nerve cells. Some cells of epithelial tissue and connective tissue that are less specialized are capable of reproduction throughout the life of the organism.

The life cycle of a cell has three basic events.

A. Interphase - Replication of the genetic material (DNA) or the chromatin threads (chromosomes) in the nucleus of the cell.

Cell Division

B. Karyokinesis (Mitosis or Nuclear Division) - Mitosis is the redistribution of genetic material (DNA) into two new nuclei, so that each nucleus contains the same genetic material (same number and kind of chromosomes) as the original nucleus.

C. Cytokinesis (Cytoplasmic Division) - the division of the cell's cytoplasm to produce two new daughter cells, each cell with its own nucleus and about 1/2 the cytoplasm of the mother (original) cell. The latter stages of mitosis and cytokinesis occurs simultaneously.

Somatic Cell Division - Division of body cells except for the gametes, the eggs and sperms. During somatic cell division there is exact replication and transmission of the chromosomes to each daughter cell.

The Life Cycle of the Cell

A. Replication of the DNA occurs during interphase, a period between cell divisions.

The interphase is divided into 3 periods:
G_1 - growth, metabolism, synthesis
S - DNA synthesis, centrioles replicated
G_2 - growth, metabolism, synthesis

During interphase the genetic material of the cell appears as indistinct chromatin threads within the nucleus. The DNA macromolecule replicates at this time.

Semi-Conservative Method of DNA Replication

The two linked chains of DNA macromolecule separate and each chain serves as a guide, template or pattern that specifies the order of nucleotides to be incorporated into a new DNA chain. The enzyme, DNA polymerase, moves along the DNA chains links the nucleotides into the new DNA chain according to complementary base pairing - A-T, C-G. As a result, a new DNA chain is formed complementary to the original template DNA chain. The newly replicated DNA chain remains attached to the original DNA template. This forms two complete DNA macromolecules exactly like the original DNA macromolecule. Thus each DNA macromolecule consists of one original DNA chain, which acted as a template and one newly synthesized chain. This is called the semi-conservative method of DNA replication.

Also during interphase centrioles, and mitochondria replicate, RNA and protein are synthesized. The cell grows in size. When interphase is complete cell division begins.
Microscopic view of interphase - nuclear membrane and nucleolus present, DNA in the uncoiled condition as chromatin threads.

Cell Division

B. Karyokinesis (mitosis, nuclear division) C. Cytokinesis (cytoplasmic division)

Several phases of mitosis are recognized but mitosis is a continuous event not a series of discrete steps.
1.Prophase - during prophase the paired centrioles separate, each pair moving to opposite poles of the cell. The microtubules or spindle fibers are organized by the centrioles to form the mitotic spindle**.** The mitotic spindle is responsible for the separation of the chromosomes to the opposite poles of the cell. There are three types of mitotic spindle fibers (microtubules).

a. Non-kinetochore microtubules – The lengthening of the non-kinetochore microtubules between the centrioles push the centrosomes, with the centrioles, to opposite poles of the cell so the spindle extends from pole to pole. These microtubules are linked near the center of the cell, push against each other forcing the centrosomes apart.

b. Kinetochore microtubules –The chromosomes attach to the kinetochore microtubules by the centromere.

c. Aster microtubules – The aster microtubules attach the centrioles to the cell membrane.

Also during prophase, the nucleolus disappears and the nuclear membrane disintegrates and disappears. The chromatin threads of DNA, condense to become tightly coiled and visible as short rod-like bodies, the chromosomes. The shortening of the chromatin threads into rod shape chromosomes prevents entangling of the chromatin threads as they move during mitosis, so the DNA material is not broken and lost. Since each chromosome has replicated, it consists of two separate structures, the chromatids. The chromatids are held together by a spherical body, the centromere that is surrounded by protein, the kinetochore. Each chromatid consists of an original DNA chain and a replicated DNA chain.

By late prophase, the centrioles have reached the opposite poles and completed the spindle. The chromosomes move onto the spindle and attach to the spindle by their centromeres and kinetochore. The chromosomes then move to the middle or equator of the cell halfway between the centrioles, the equatorial or metaphase plate.

The kinetochore microtubules after attaching to the chromosomes by the centromere pull the chromosomes to the middle of the cell.

2. Metaphase - in metaphase the chromosomes are attached by their centromeres to the spindle fibers along the equatorial or metaphase plate of the spindle i.e., the exact center of the mitotic spindle.

3. Anaphase - the chromatids of each chromosome separate as the centromere splits and each chromatid ecomes an independent chromosome, each consist of two chains of DNA. The chromosomes are attached to the kinetochore end of the microtubules and are dragged or pulled

by the centromere toward opposite poles of the cell as the kinetochore microtubules breakdown and shorten. The chromosomes appear V-shaped as the centromeres lead the way dragging the rest of the chromosomes toward the poles.

During late anaphase or early telophase the beginning of cytokinesis is seen as an inward pinching (cleavage furrow) of the cell membrane in the middle of the cell.

The process of moving and separating the chromosomes as short, compact bodies helps in the parceling out of the genetic material to the two new daughter cells. Diffuse chromatin threads would tangle and break that would result in imprecise separation of the genetic material to the daughter cells.

4. Telophase - during telophase the nuclear membrane and the nucleolus reform. The mitotic spindle disappears and the double stranded chromosomes uncoil to become chromatin threads again.

By the end of telophase the cytokinesis is complete as the inward pinching of cell membrane separate the dividing mother cell into two daughter cells. At the end of telophase the two daughter cells assume the interphase condition and the division is complete.

C. Cytokinesis

Division of the cell's cytoplasm and organelles. The process begins in late anaphase or early telophase with indentation of the plasma membrane at the center of the cell. This is the cleavage furrow. The cleavage furrow is produced by the contraction of the actin microfilaments just below the cell membrane. The actin contracting forms a contractile ring that pulls the cell membrane progressively inward constricting the center of the cell until the cell is pinched into two cells. The plane of the cleavage furrow is always perpendicular to the mitotic spindle so the two sets of chromosomes will segregate into each daughter cell. When cytokinesisis complete the two daughter cells are in interphase. Then daughter cells begin the cell life cycle again.

VI. Protein Synthesis

DNA directs protein synthesis by two processes.

A. Transcription

Transcription occurs in the nucleus. It is a process by which genetic information stored in the sequence of N-bases of the DNA nucleotides is rewritten in the N-base sequence of the RNA. Transcription means transcribing words which are in the same language, i.e. transcribing a segment of DNA into a specific RNA molecule.

There are 3 kinds of RNA molecules.
1. Messenger RNA (m-RNA)
2. Ribosomal RNA (r-RNA) and Ribosomes
3. Transfer RNA (t-RNA)

B. Translation

Translation occurs in the cytoplasm. Translation is a process by which information in the N-base sequence of m-RNA specifies the amino acid sequence of the protein. Translation means translating from one language to another, i.e., translating the information of the N-bases of the nucleotides in the RNA the specific order of amino acids of the protein molecule.

Genetic Code

In translation the ribosomes "read' in sequence 3 N-bases at a time. The 3N-bases or triplet constitutes a "word', called a codon. Each codon specifies an amino acid. The 4 N-bases (A,T(U),C,G) can be arranged to form 64 different 3 N-base codons. Since there are 20 amino acids, the system has more than enough capacity to specify each amino acid. Also several different codons can specify the same amino acid (redundancy) and some codons serve as starting and stopping for reading the message.

A gene is a group of nucleotides, on the DNA molecule that is specific for an amino acid sequence to form a specific protein. The specific protein then influences the character or the trait. The nucleotide sequence is the key to heredity.

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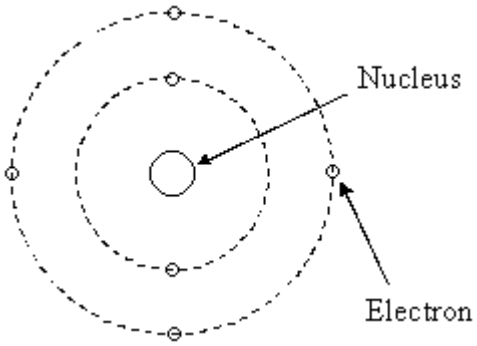
Biochemistry

Introduction

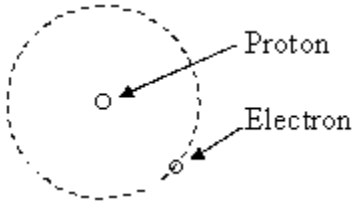
Organic molecules are molecules that contain ***carbon*** and ***hydrogen***. All living things contain these organic molecules: ***carbohydrates***, ***lipids***, ***proteins***, and ***nucleic acids***. These molecules are often called ***macromolecules*** because they may be very large, containing thousands of carbon and hydrogen atoms and because they are typically composed of many smaller molecules bonded together. These four macromolecules will be discussed in the second half of this chapter..

Carbon

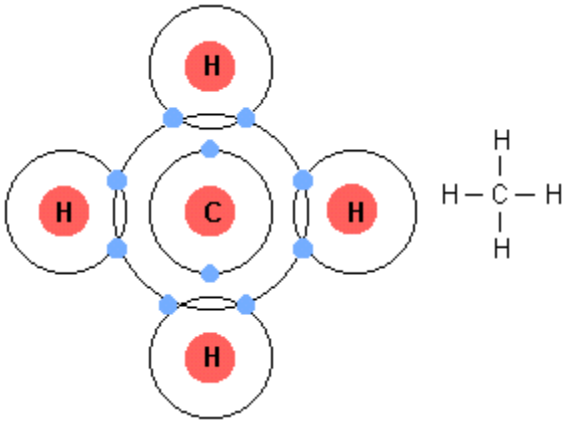
Carbon has four electrons in its outer shell:



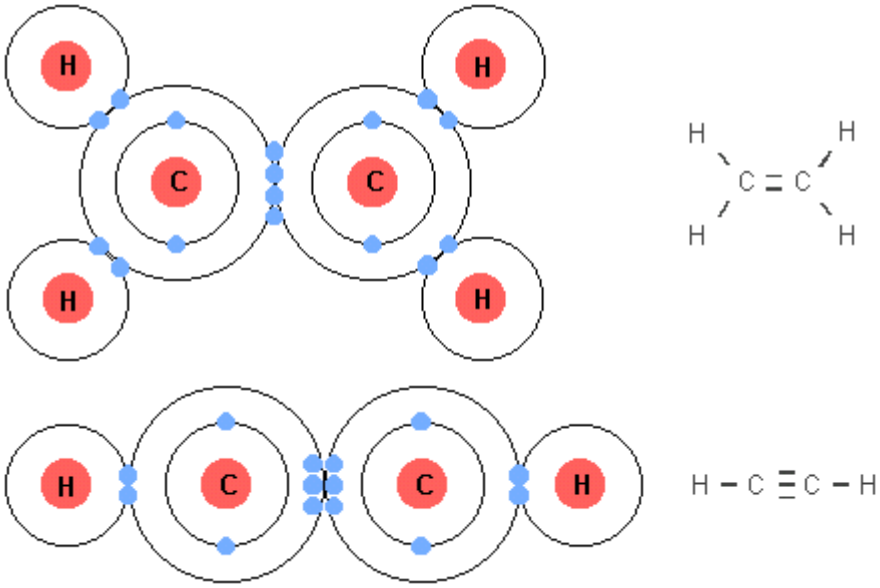
Hydrogen has one electron and one proton:



Carbon can bond by covalent bonds with as many as 4 other atoms. The diagram below shows a molecule of methane. Lines can be used to represent bonds in the shorthand formula seen in the right side of the diagram.



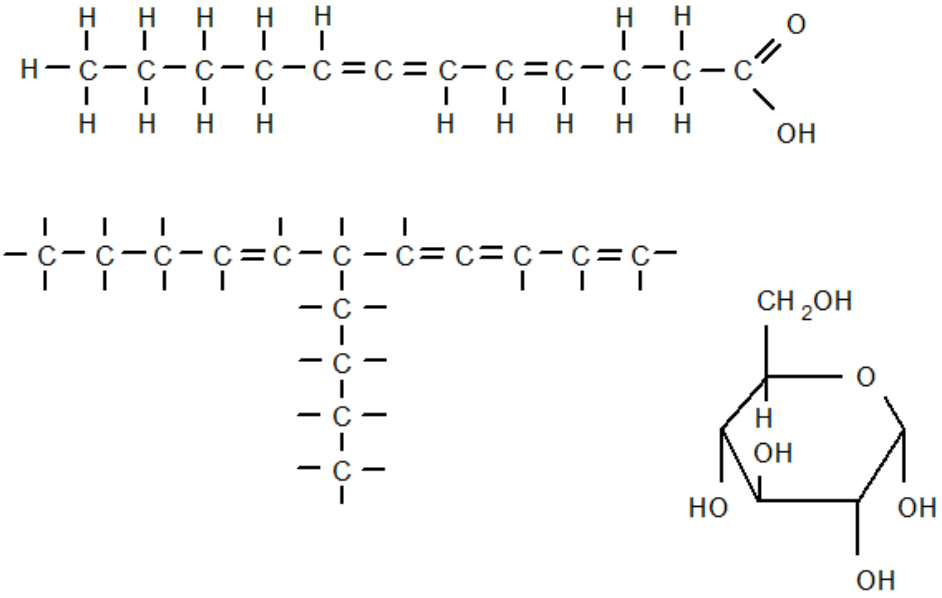
Carbon can also form double covalent (shares 2 pairs of electrons) or triple covalent bonds (shares 3 pairs) as shown below:



Carbon can form 4 covalent bonds because it has 4 electrons in its outer shell. It can form the following number of bonds. Notice that in each case below, there is a total of four bonds.

- 4 single bonds
- two double bonds
- one double bond and two single bonds
- one triple and one single bond

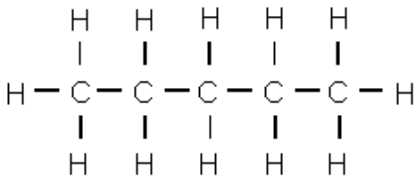
Long chains of carbon atoms are common. The chains may be branched or form rings as shown in the examples below:



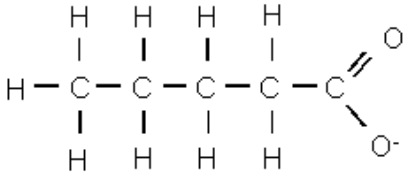
Hydrophilic and Hydrophobic

Polar and ***ionic*** molecules have positive and negative charges and are therefore attracted to water molecules because water molecules are also polar. They are said to be ***hydrophilic*** because they interact with (dissolve in) water by forming hydrogen bonds.

Nonpolar molecules are ***hydrophobic*** (means "water fearing"). They do not dissolve in water.



Nonpolar molecules are hydrophobic.

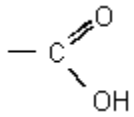


Polar and ionic molecules are hydrophilic.

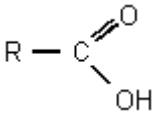
Portions of large molecules may be hydrophobic and other portions of the same molecule may be hydrophilic.

Functional Groups

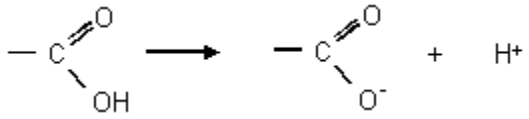
Organic molecules may have functional groups attached. A functional group is a group of atoms of a particular arrangement that gives the entire molecule certain characteristics. Functional groups are named according to the composition of the group. For example, COOH is a carboxyl group.



Organic chemists use the letter "R" to indicate an organic molecule. For example, the diagram below can represent a carboxylic acid. The "R" can be any organic molecule.

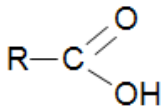
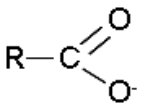
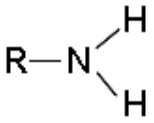
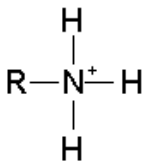
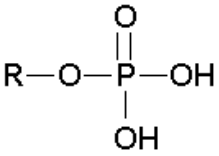
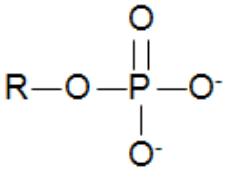
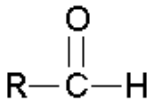
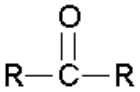


Some functional groups are polar and others can ionize. For example, if the hydrogen ion is removed from the COOH group, the oxygen will retain both of the electrons it shared with the hydrogen and will have a negative charge. The hydrogen that is removed leaves behind its electron and is now a hydrogen ion (proton).



If polar or ionizing functional groups are attached to hydrophobic molecules, the molecule may become hydrophilic due to the functional group. Some ionizing functional groups are: -COOH, -OH, -CO, and $-NH_2$.

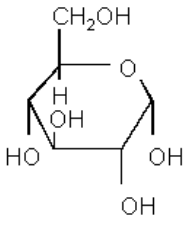
Some important functional groups are shown below.

Name	**Structure**	
	Non-ionized	**Ionized**
Hydroxyl	R—OH	
Carboxyl		
Amino		
Phosphate		
Sulfhydryl	R—SH	
Aldehyde		
Ketone		

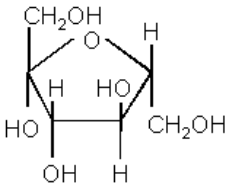
Isomers

Different molecules that are composed of the same number and kinds of atoms are called ***isomers***. Three kinds of isomers are described below.

Structural isomers differ in their overall construction as shown in glucose and fructose. Glucose and fructose are both $C_6H_{12}O_6$ but the atoms are arranged differently in each molecule.



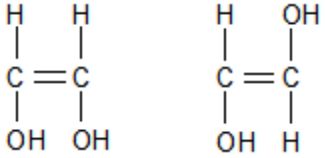
glucose



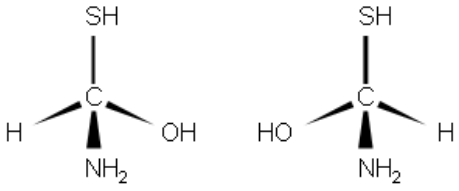
fructose

Geometric isomers maintain the same carbon skeleton but a double bond occurs between carbon atoms. The

location of atoms bonded to a double-bonded carbon atom differs. The two molecules shown are geometric isomers because the double bond cannot rotate. If the bond between the two carbon atoms below were a single bond, they would not be isomers because atoms attached by single bonds can rotate. The carbon atoms would be able to rotate from one orientation to another if the bond were a single bond.

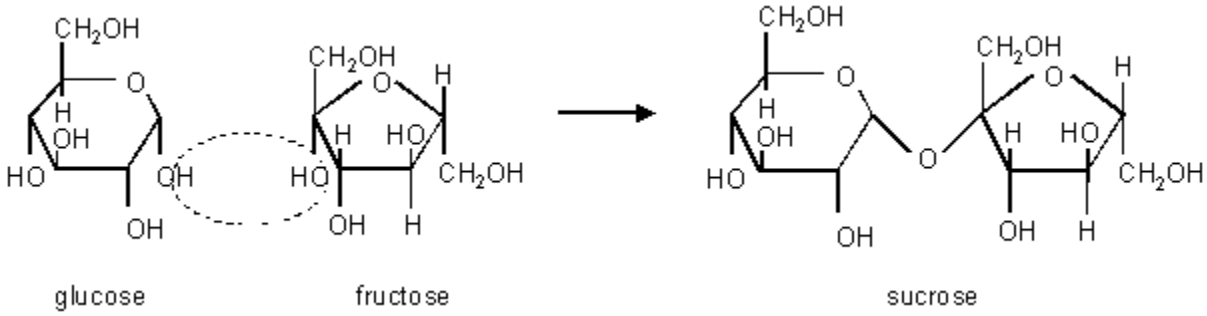


Enantiomers are molecules that are mirror images of each other.



Condensation

In biological systems, macromolecules are often formed by removing H from one atom and OH from the other (see the diagram below). The H and the OH combine to form water. Small molecules (monomers) are therefore joined to build macromolecules by the removal of water. The diagram below shows that sucrose (a sugar) can be produced by a condensation reaction of glucose and fructose.



This is called a ***condensation*** or ***dehydration*** reaction. Energy is required to form the bond.

Hydrolysis

This is a type of reaction in which a macromolecule is broken down into smaller molecules. It is the reverse of condensation (above).

Macromolecules and Monomers

Many of the common large biological molecules (macromolecules) are synthesized from simpler building blocks (monomers). Each of the types of molecules listed in the table are discussed below.

Example of a Macromolecule	**Monomer**
polysaccharide (complex carbohydrate)	monosaccharide (simple sugar)
fat (a lipid)	glycerol, fatty acid
protein	amino acid
nucleic acid	nucleotide

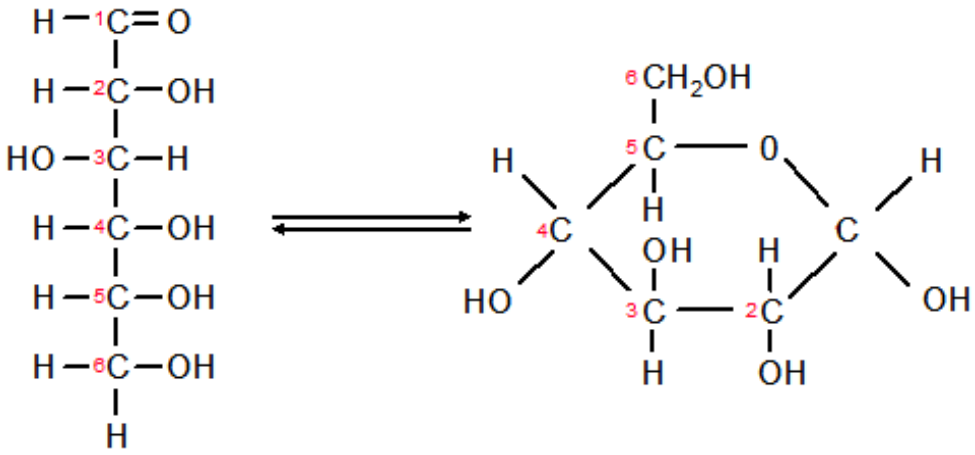
Carbohydrates

The general formula for carbohydrates is $(CH_2O)_n$.

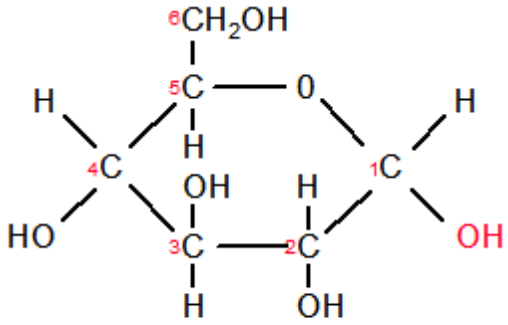
Monosaccharides:

Monosaccharides are simple sugars, having 3 to 7 carbon atoms. The names of most sugars end with the letters ***ose***. Example: Glucose, fructose and galactose are monosaccharides; their structural formula is $C_6H_{12}O_6$.

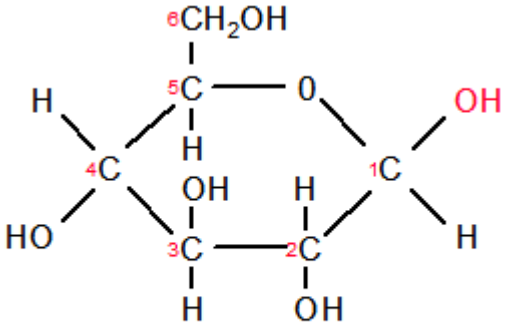
Glucose and other kinds of sugars may be linear molecules as shown below but in aqueous solution they become a ring form.



There are two isomers of the ring form of glucose. They differ in the location of the OH group on the number 1 carbon atom (in red below).



α-glucose



β-glucose

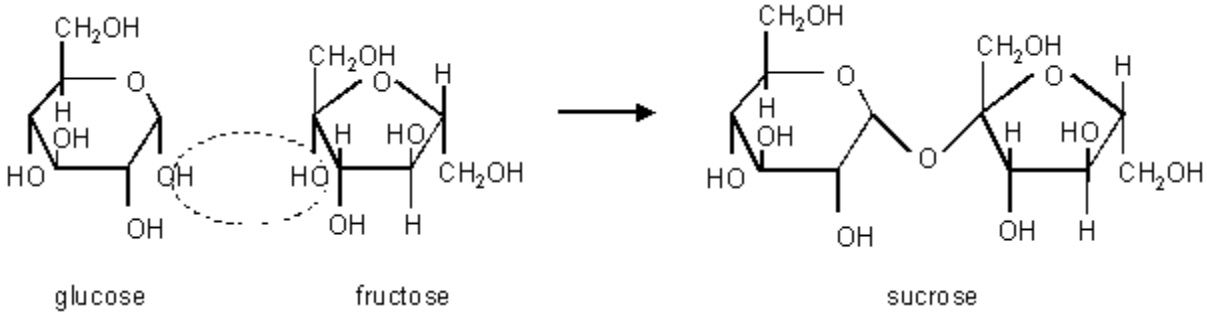
The number 1 carbon atom (numbered in red above) of the linear form of glucose is attached to the oxygen on the number 5 carbon atom.

Simple sugars store energy for cells. Cells also use simple sugars to construct other kinds of organic molecules.

Disaccharides:

Disaccharides are composed of 2 monosaccharides joined together by a condensation reaction.

Examples: Sucrose (table sugar) is composed of glucose and fructose.



glucose fructose sucrose

Like glucose, sucrose stores energy. Plants synthesize sucrose to transport to nonphotosynthetic parts of the plant.

Lactose is found in milk. It is formed when glucose bonds to galactose.

The digestion of carbohydrates typically involves hydrolysis reactions in which complex carbohydrates (polysaccharides) are broken down to ***maltose*** (a disaccharide). Maltose is then further broken down to produce two glucose molecules.

Polysaccharides:

Monosaccharides may be bonded together to form long chains called polysaccharides.

Starch and Glycogen

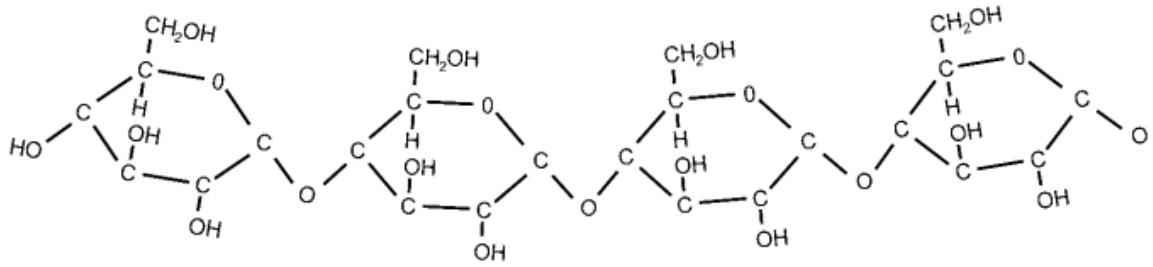
Starch and glycogen are polysaccharides that function to store energy. They are composed of glucose monomers bonded together producing long chains.

Animals store extra carbohydrates as glycogen in the liver and muscles. Between meals, the liver breaks down glycogen to glucose in order to keep the concentration of glucoses in the blood stable. After meals, as glucose levels in the blood rise, it is removed from and stored as glycogen.

Plants produce starch to store carbohydrates.

Amylopectin is a form of starch that is very similar to glycogen. It is branched but glycogen has more branches. Amylose is a form of starch that is unbranched.

Below: Glycogen or Starch

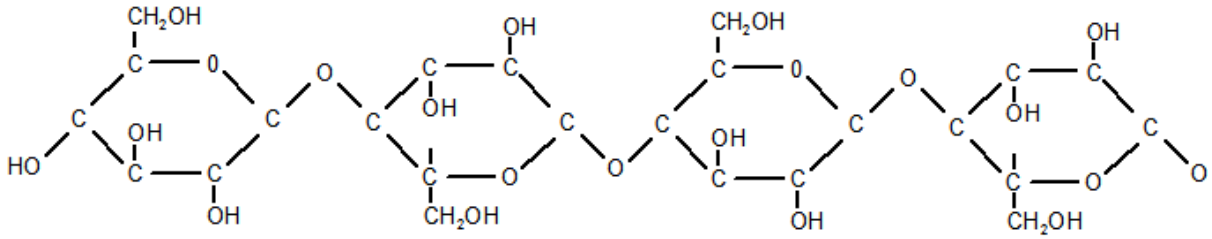


Cellulose and Chitin

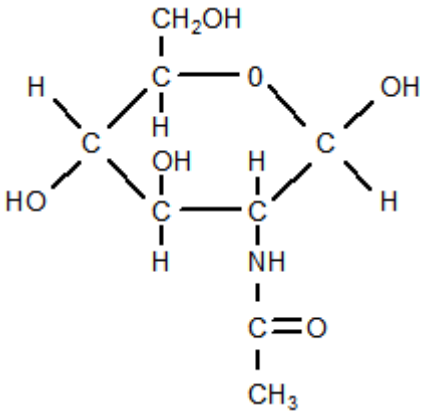
Cellulose and Chitin are polysaccharides that function to support and protect the organism. The cell walls of plants are composed of cellulose. The cell walls of fungi and the exoskeleton of arthropods are composed of chitin.

Cellulose is composed of beta-glucose monomers; starch and glycogen are composed of alpha-glucose. The bond orientation between the glucose subunits of starch and glycogen allows the polymers to form compact spirals. The monomers of cellulose and chitin are bonded together in such a way that the molecule is straight and unbranched. The molecule remains straight because every other glucose is twisted to an upside-down position compared to the two monomers on each side. Cellulose fibers are composed of long parallel chains of these molecules. The chains are attached to each other by hydrogen bonds between the hydroxyl groups of adjacent molecules.

Below: Cellulose



The glucose monomers of chitin (N-acetyl glucosamine) have a side chain containing nitrogen.



Cotton and wood are composed mostly of cellulose. They are the remains of plant cell walls.

Digestibility of Cellulose and Chitin

Humans and most animals do not have the necessary enzymes needed to break the linkages of cellulose or chitin.

Animals that can digest cellulose often have microorganisms in their gut that digest it for them.

Fiber is cellulose, an important component of the human diet.

Lipids

Lipids are compounds that are insoluble in water but soluble in nonpolar solvents.

Some lipids function in long-term energy storage. One gram of fat stores more than twice as much energy as one gram of carbohydrate.

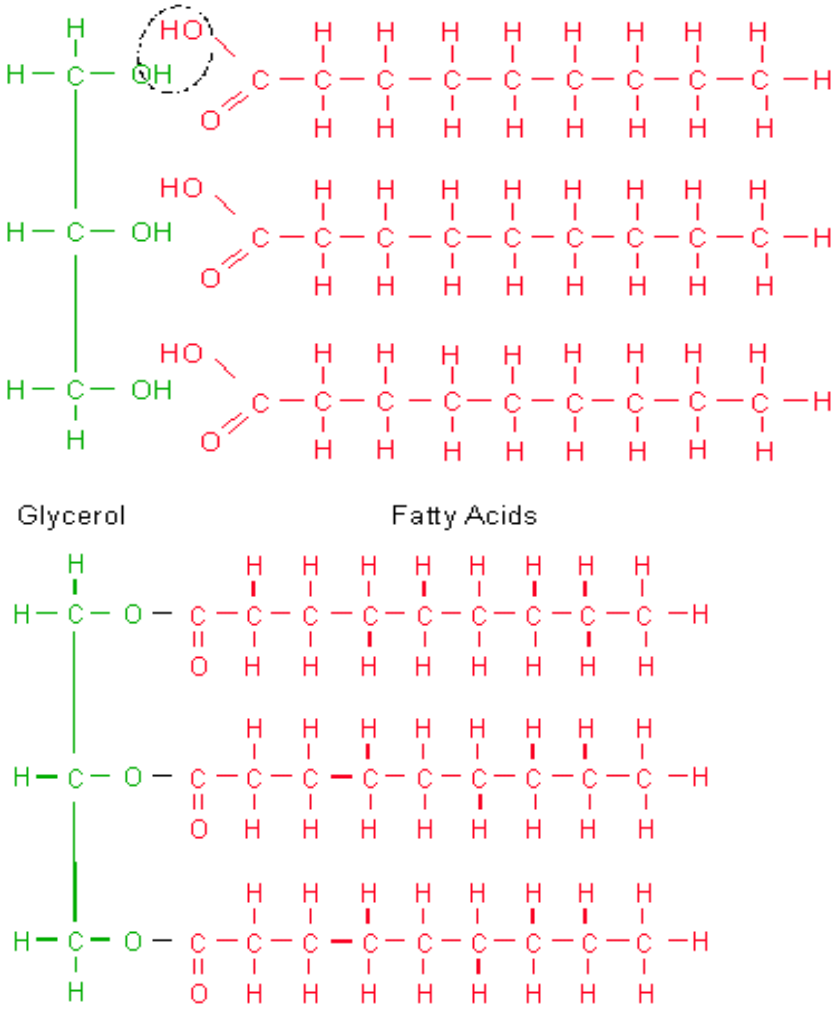
Lipids are also an important component of cell membranes.

Fats and Oils (Triglycerides)

Fats and oils are composed of fatty acids and glycerol.

Fatty acids have a long hydrocarbon (carbon and hydrogen) chain with a carboxyl (acid) group. The chains usually contain 16 to 18 carbons.

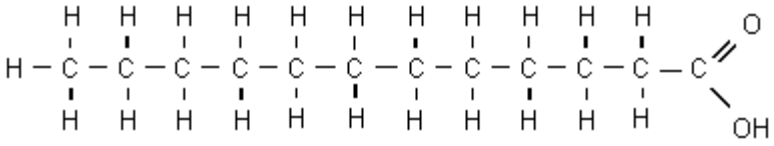
Glycerol contains 3 carbons and 3 hydroxyl groups. It reacts with 3 fatty acids to form a ***triglyceride*** or ***fat*** molecule.



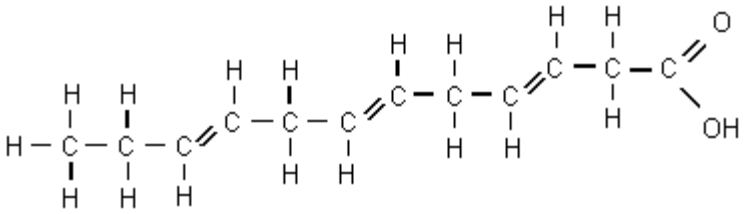
Fats are nonpolar and therefore they do not dissolve in water.

Saturated and Unsaturated Fat

Saturated fatty acids have no double bonds between carbons. ***Unsaturated*** fatty acids have at least one double bond. Each double bond produces a "bend" in the molecule (see diagram above). Molecules with many of these bends cannot be packed as closely together as straight molecules, so these fats are less dense. As a result, triglycerides composed of unsaturated fatty acids melt at lower temperatures than those with saturated fatty acids. For example, butter contains more saturated fat than corn oil, and is a solid at room temperature while corn oil is a liquid.



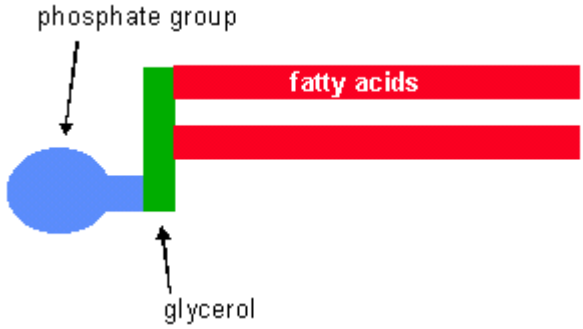
Saturated Fatty Acid



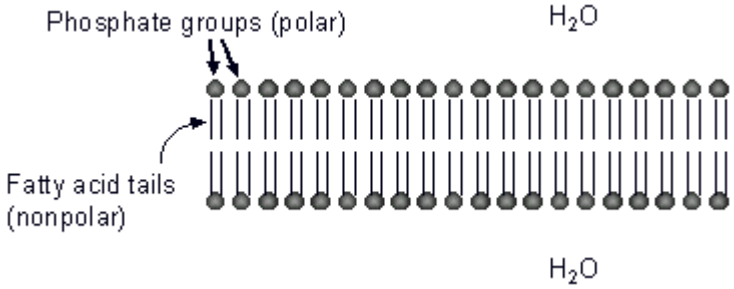
Unsaturated Fatty Acid

Phospholipids

Phospholipids have a structure like a triglyceride (see diagram above), but contain a phosphate group in place of the third fatty acid. The phosphate group is polar and therefore capable of interacting with water molecules.



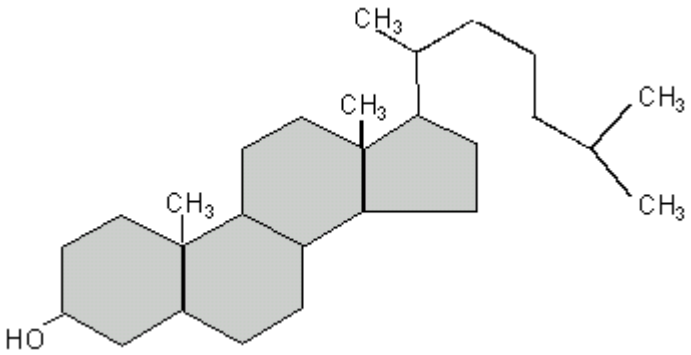
Phospholipids spontaneously form a bilayer in a watery environment. They arrange themselves so that the polar heads are oriented toward the water and the fatty acid tails are oriented toward the inside of the bilayer (see the diagram below). In general, nonpolar molecules do not interact with polar molecules. This can be seen when oil (nonpolar) is mixed with water (polar). Polar molecules interact with other polar molecules and ions. For example table salt (ionic) dissolves in water (polar). The bilayer arrangement shown below enables the nonpolar fatty acid tails to remain together, avoiding the water. The polar phosphate groups are oriented toward the water.



Membranes that surround cells and surround many of the structures within cells are primarily ***phospholipid bilayers***.

Steroids

Steroids have a backbone of 4 carbon rings.



Cholesterol (see diagram above) is the precursor of several other steroids, including several hormones. It is also an important component of cell membranes.

Saturated fats and cholesterol in the diet can lead to deposits of fatty materials on the linings of the blood vessels.

Waxes

Waxes are composed of a long-chain fatty acid bonded to a long-chain alcohol

They form protective coverings for plants and animals (plant surface, animal ears).

Proteins

Importance of proteins

Some important functions of proteins are enzymes (bio chemical reactions), hormones, storage (egg whites of birds, reptiles; seeds), transport (hemoglobin), contractile (muscle), protective (antibodies), membrane proteins (receptors, membrane transport, antigens), structural, and toxins (botulism, diphtheria)

Enzymes

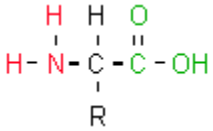
Enzymes are proteins that speed up the rate of biochemical reactions. Example: The presence of an enzyme in the chemical reaction causes hypothetical chemicals A and B to react, producing C:

$$A + B \xrightarrow{\text{enzyme}} C$$

Proteins are able to function as enzymes due to their shape. For example, enzyme molecules are shaped like the reactants, allowing the reactants to bind closely with the enzyme.

Amino Acids

Amino acids are the building blocks of proteins.

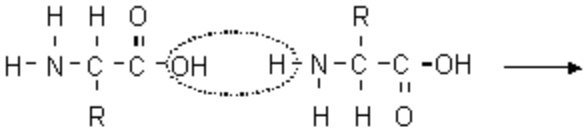


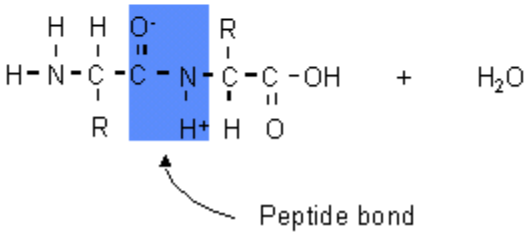
Twenty of the amino acids are used to make protein. Each has a carboxyl group (COOH) and an amino group (NH_2).

Each amino acid is different and therefore has its own unique properties.

Some amino acids are hydrophobic, others hydrophilic. The carboxyl or amino group may ionize (forming NH_3^+ or COO^-). The "R" group of some amino acids is nonpolar and the "R" group of some others is polar or it ionizes.

Amino acids are joined together by a ***peptide bond***. It is formed as a result of a condensation reaction between the amino group of one amino acid and the carboxyl group of another.





Polypeptides

Two or more amino acids bonded together are called a ***peptide***. A chain of many amino acids is referred to as a ***polypeptide***. The complete product, either one or more chains of amino acids, is called a ***protein***.

There is unequal sharing of electrons in a peptide bond. The O and the N are negative and the H is positive.

Levels of structure

The large number of charged atoms in a polypeptide chain facilitates hydrogen bonding within the molecule, causing it to fold into a specific 3-dimensional shape.

The 3-dimensional shape is important in the activity of a protein.

Primary Structure: Primary structure refers to the sequence of amino acids found in a protein.

Secondary structure: The oxygen or nitrogen atoms of the peptide bond are capable of hydrogen-bonding with hydrogen atoms elsewhere on the molecule. This bonding produces two common kinds of shapes seen in protein molecules, coils (helices) and pleated sheets. The helices and pleated sheets are referred to as a proteins secondary structure.

Tertiary structure: Tertiary structure refers to the overall 3-dimensional shape of the polypeptide chain.

Hydrophobic interactions with water molecules are important in creating and stabilizing the structure of proteins. Hydrophobic (nonpolar) amino acids aggregate to produce areas of the protein that are out of contact with water molecules.

Hydrophilic (polar and ionized) amino acids form hydrogen bonds with water molecules due to the polar nature of the water molecule.

Hydrogen bonds and ***ionic bonds*** form between R groups to help shape the polypeptide chain.

Disulfide bonds are covalent bonds between sulfur atoms in the R groups of two different amino acids. These bonds are very important in maintaining the tertiary structure of some proteins.

The shape of a protein is typically described as being globular or fibrous. Globular proteins contain both coils and sheets.

Quaternary structure: Some proteins contain two or more polypeptide chains that associate to form a single protein. These proteins have quaternary structure. For example, hemoglobin contains four polypeptide chains.

Denaturation

Denaturation occurs when the normal bonding patterns are disturbed causing the shape of the protein to change. This can be caused by changes in temperature, pH, or salt concentration. For example, acid causes milk to curdle and heat (cooking) causes egg whites to coagulate because the proteins within them denature.

If the protein is not severely denatured, it may regain its normal structure.

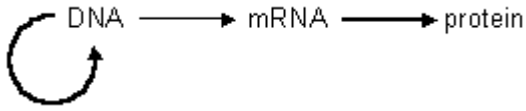
Other Kinds of Proteins

Simple proteins contain only amino acids. Conjugated proteins contain other kinds of molecules. For example, ***glycoproteins*** contain carbohydrates, nucleoproteins contain nucleic acids, and ***lipoproteins*** contain lipids.

Nucleic acids

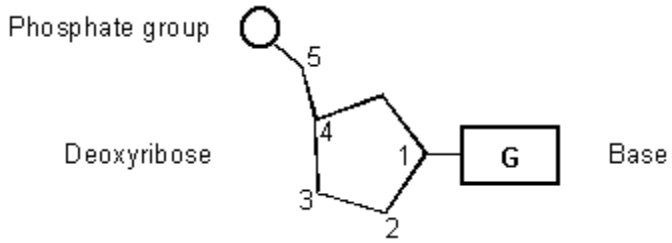
DNA

DNA (deoxyribonucleic acid) is the genetic material. An important function of DNA is top store information regarding the sequence of amino acids in each of the body's proteins. This "list" of amino acid sequences is needed when proteins are synthesized. Before protein can be synthesized, the instructions in DNA must first be copied to another type of nucleic acid called messenger RNA.

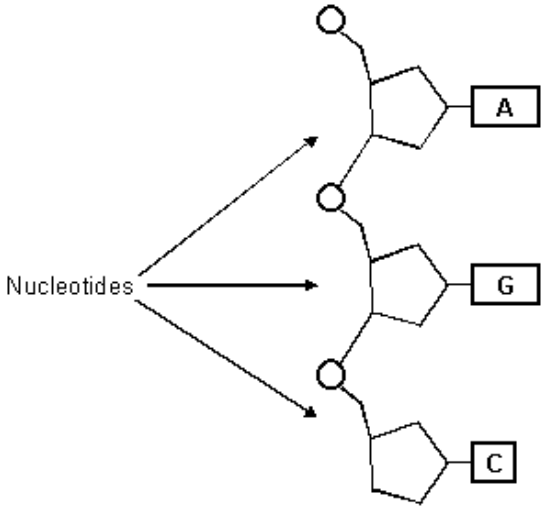


Structure of DNA

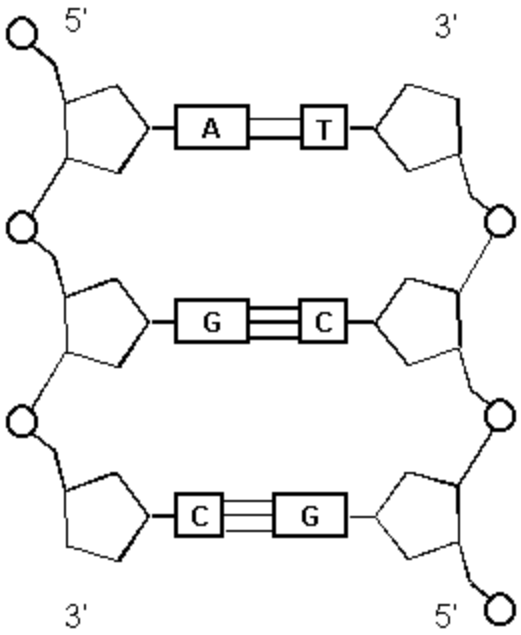
Nucleic acids are composed of units called nucleotides, which are linked together to form a larger molecule. Each nucleotide contains a base, a sugar, and a phosphate group. The sugar is deoxyribose (DNA) or ribose (RNA). The bases of DNA are adenine, guanine, cytosine, and thymine. Notice that the carbon atoms in one of the nucleotides diagrammed below have been numbered.



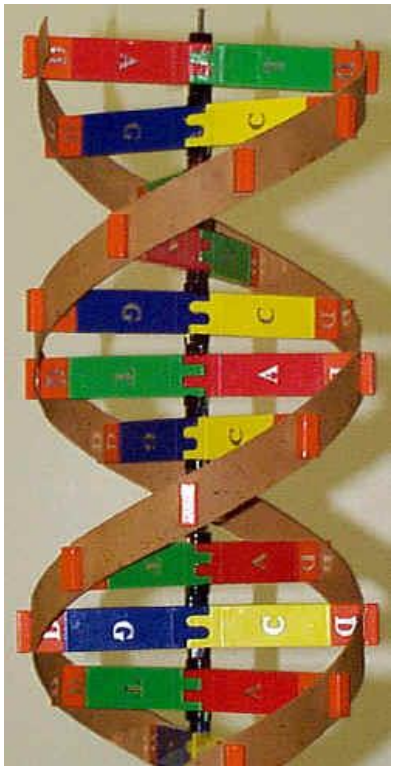
The diagram below shows how nucleotides are joined together to form a "chain" of nucleotides.



DNA is composed of two strands in which the bases of one strand are hydrogen-bonded to the bases of the other. The sugar-phosphate groups form the outer part of the molecule while the bases are oriented to the center.



The strands are twisted forming a configuration that is often referred to as a ***double helix***. The photograph below is of a model of DNA.



Complimentary base pairing

The adenine of one strand is always hydrogen-bonded to a thymine on the other. Similarly, Guanine is always paired with Cytosine.

A-T

G-C

Antiparallel

The end of a single strand that has the phosphate group is called the 5' end. The other end is the 3' end.

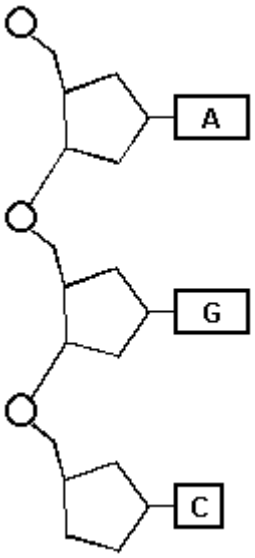
The two strands of a DNA molecule run in opposite directions. Note the 5' and 3' ends of each strand in the diagram.

RNA

RNA (ribonucleic acid) is similar to DNA and is involved in the synthesis of polypeptides and proteins as discussed above. The table below lists differences between DNA and RNA.

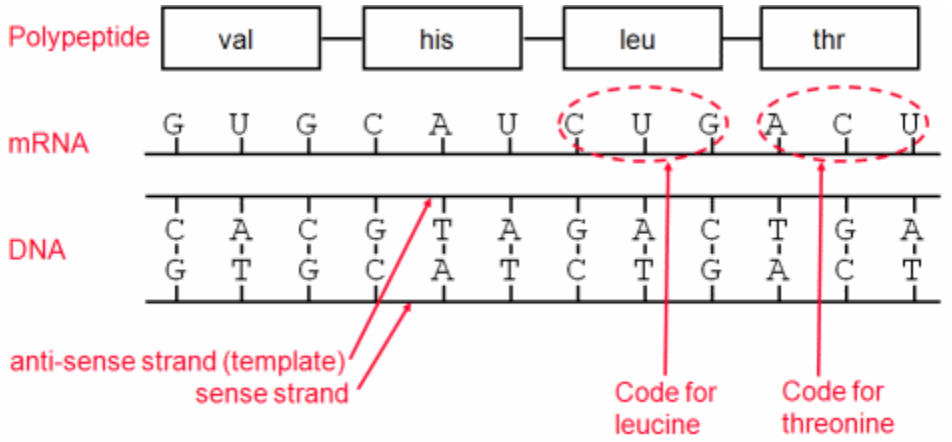
	DNA	**RNA**
# Strands	2	1 (see diagram below)
Sugar	deoxyribose	ribose
Bases	A, T, G, C	A, U, G, C

RNA is single-stranded as shown below.



Codons

One strand of DNA (the anti-sense strand) is used as a template to produce a single strand of mRNA. The bases in the mRNA strand are opposite (complimentary) to the bases in the DNA template strand; it resembles the sense strand of DNA except that the base thymine is replaced by uracil. The mRNA contains three-letter (three-base) codes used to determine the sequence of amino acids in the polypeptide that it codes for. For example, in the diagram below, GUG is the code for valine. The sequence of codes in DNA therefore determines the sequence of amino acids in the protein.

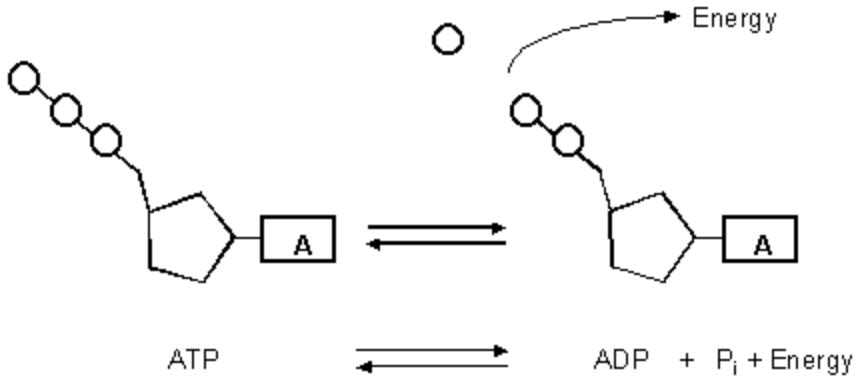


Each three-letter code in mRNA is a ***codon***. It is the code for one amino acid.

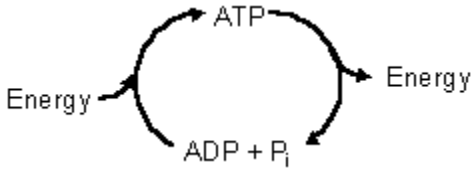
ATP

ATP (adenosine triphosphate) is a nucleotide that is used in energetic reactions for temporary energy storage.

Energy is stored in the phosphate bonds of ATP. When ATP breaks down to form ADP and P_i, energy is released. Normally, cells use the energy stored in ATP by breaking one of the phosphate bonds, producing ADP. Energy is required to convert ADP + P_i back to ATP.



ATP is continually produced and consumed as illustrated below.



ENZYMES

What are Enzymes?

What are Enzymes? Enzymes are substances that function as organic catalysts, in other words, they either start chemical reactions or make them run faster. They accomplish this while remaining unchanged themselves. Enzymes are composed of two parts, a protein portion called the apoenzyme and a nonprotein portion, either a coenzyme (organic) or cofactor (inorganic). Enzymes are present in every cell in both plants and animals; and are responsible for regulating the biochemical reactions necessary to sustain life.

Enzymes are highly specific, both in the substrate they affect, and in the reactions they catalyze. They can exist both in active and in inactive forms, and many enzymes occur naturally in both active and inactive forms in cells. They can, however, be permanently inactivated by altering their environmental conditions, such as pH or temperature.

There are six main groups of enzymes - hydrolases, isomerases, ligases, lyases, oxidoreductases, and transferases. The enzymes involved in food decomposition and in the digestive process are hydrolases. They break down proteins (proteases), carbohydrates (carbohydrases or amylases), and fats (lipases). Plants also contain enzymes to break down fiber (cellulases).

The enzymes most often utilized in dietary supplements function in the same way as the enzymes found naturally in food and as digestive enzymes in the human body. There are supplemental enzymes available that are capable of breaking down almost any food. For example, there are several different carbohydrate hydrolyzing enzymes, such as amylase (for polysaccharides), lactase (for milk sugar), invertase (for sucrose), and cellulase (for plant fibers).

What are the different types of enzymes?

Because enzymes have so many applications, scientists have found it helpful to classify them based on what they do, what substances they act upon (substrates), and the reaction they start or accelerate. There are six main groups of enzymes, each having fundamentally different activities.

- Hydrolases break down proteins, carbohydrates, and fats such as during the process of digestion. They do this by adding a water molecule, thus the name hydrolases.
- Isomerases catalyze the rearrangement of chemical groups within the same molecule.
- The ligases catalyze the formation of a bond between two substrate molecules through the use of an energy source.

- Lyases catalyze the formation of double bonds between atoms by adding or subtracting chemical groups.
- Oxidoreductases make oxidation-reduction (the process by which an atom loses an electron to another atom) possible.
- Transferases transfer chemical groups from one molecule to another. Your body contains many enzymes from each group.

- **Examples of Industrial Enzymes:**

Enzymes	**Source or Type**	**Applications**
Carbohydrases		Laundry and dishwashing detergents, industrial pipe/tank cleaners, textiles, pulp and paper, fermentation ethanol
Alpha-amylase	Bacterial a-amylase (e.g., *Bacillus subtilis*), Fungal a-amylase (e.g., *Aspergillus niger*), Alkaline a-amylase	Textiles, starch syrups, laundry and dishwashing detergents, paper desizing, fermentation ethanol, animal feed
ß-amylase	From a strain of Bacillus	Brewing, maltose syrup
Cellulase		Dishwashing detergents, animal feed, textiles, bioenergy production
ß-Glucanase	exo-ß-1,4-glucanase, endo-ß-1,4-glucanase	Brewing industry
ß-Glucosidase		Transforms isoflavone phytoestrogens in soymilk
Dextranase	Made by various microorganisms (e.g., *Leuconostoc mesenteriodes*)	Hydrolyzes the polysaccharide dextran
Dextrinase (almost identical to a-glucosidase)		Cleaves dextrin into two molecules of glucose
a-Galactosidase (melibiase)		Could increase yield of sucrose; potential use in the beet sugar industry
Glucoamylase	*Aspergillus niger*, Rhizopus, Endomyces	Manufacture of dextrose syrup and high-fructose syrup
Hemmicellulase/Pentosanase/Xylanase	*Thermomyces lanuginosus, Penicillium simplicissimum*	Baking, fruit juice manufacture, wood pulp processing
Invertase		Manufacture of invert syrup from cane or beet sugar (use is minor)
Lactase	*Kluyveromyces lactis*,	Eliminates lactose from dairy foods

	Asperigillus oryzae, Bacillus	
Naringinase		Debitter citrus peel
Pectinase		Fruit processing
Pullulanase	*Klebsiella aerogenes, Bacillus acidipullulyticus, Bacillus subtilis*	Antistaling agent in baked goods
Proteases		Brewing, baking goods, protein processing, distilled spirits, laundry and dishwashing detergents, lens cleaners, leather and fur, chemicals
Acid proteinase	*Endothia parasitica,* Rhizopus, *Aspergillus niger, A. oryzae*	Baking, improves dough handling
Alkaline protease	*Bacillus subtilis, Bacillus licheniformis*	Detergents, leather and fur
Bromelain	Pineapple stem	Food industry
Pepsin	Porcine or bovine stomach	Cheese production
Aminopeptidase	*Lactococcus lactis*	Food and animal feed
Subtilisin	*Bacillus subtilis* var. Carlsberg, *Bacillus lichenformis*	Chiral resolution of chemical compounds or pharmaceuticals
Lipases and Esterases	Phospholidases, pregastric esterases, phosphatases	Cleaners, leather and fur, dairy, chemicals
Aminoacylase	Porcine kidney, *Aspergillus melleus*	Optical resolution of amino acids
Glutaminase	Bacillus, Aspergillus	Conversion of glutamine to glutamate
Lysozyme	Chicken egg white, *Saccharomyces cerevisiae, Pichia pastoris*	Antibacterial (germicidal in dairy industry)
Penicillin acylase	*Bacillus megaterium, Escherichia coli*	Chemical synthesis
Isomerase		Converts glucose syrup to high-fructose syrup in food industry
Oxireductases		Chemicals, detergent bleaches, pulp bleaching
Alcohol dehydrogenase	*Saccharomyces cerevisiae, Thermoanarobium brockii*	Chiral synthesis of chemicals

Amino acid oxidase	Porcine kidney, snake venom	Chiral resolution of racemic amino acid mixtures
Catalase	*Aspergillus niger*	desugaring of eggs
Chloroperoxidase	Algae, bacteria, fungi, mammalian tissues	Steroid synthesis
Peroxidase	Horseradish	Laundry and wood pulp bleaches
Acetolactate decarboxylase		Brewing industry
Aspartic ß-decarboxylase		Manufacture of L-alanine from L-aspartic acid
Histidase	Achromobacter liquidum	Cosmetics
Cyclodextrin glycosyltransferase		Manufacture of cyclodextrins from starch

- *Source:* Diversa & Novo Nordisk

For the purpose of this discussion, enzymes can be divided into three groups.

- Metabolic enzymes (enzymes which your body produces that work in blood, tissues, and organs)
- Digestive enzymes (enzymes that break down food into usable material)
- Food enzymes (enzymes that are contained in raw food)

When raw food is ingested, enzymes present within the food are released, thereby assisting the body's digestive processes in breaking down the food into its simplest components for utilization within the body. However cooking and processing destroys those enzymes, forcing the body to supply the additional enzymes needed to adequately digest the food.

There are several categories of food enzymes:

- Lipase, breaks down fats that are found in most dairy products, nuts, oils, and meat.
- Lactase, breaks down lactose (milk sugars).
- Protease, breaks down proteins that are found in meats, nuts, eggs, and cheese.
- Amylase, breaks down carbohydrates, starches, and sugars, prevalent in potatoes, fruits, vegetables, and many snack foods.
- Cellulase, breaks down cellulose, the fibrous structure that makes up most plant cell walls

To provide enough enzymes to relieve the burden on the body, the choices are to eat more raw food or the take a supplemental enzyme-based product.

How do Enzymes aid in digestion?

Enzymes are an integral part of the digestive process. From the time food enters the mouth, enzymes are at work breaking the food down into smaller and smaller units until it can be absorbed through the intestinal wall. These enzymes come from two sources, those found in the food itself, and those produced in the body.

All raw food naturally contains the proper types and proportions of enzymes necessary to assist in the process of decomposition. In addition, when raw food is eaten, chewing ruptures the cell membranes and releases these indigenous food enzymes, many of which survive and contribute to the digestive process. These enzymes include protease, which breaks long protein chains (polypeptides) into smaller amino acid chains and eventually into single amino acids, amylase that reduces large carbohydrates (starches and other polysaccharides) to disaccharides including sucrose, lactose, and maltose, lipase that digests fats (triglycerides) into free fatty acids and glycerol, and cellulase. Cellulase, which is not found in the human system, breaks the bonds found in fiber.

Ideally, the human body is capable of producing these same enzymes, with the exception of cellulase, necessary to digest food and allow for the absorption of nutrients. However, with estimates of as many as twenty million Americans suffering from various digestive disorders, optimal conditions are not the case.

Most food enzymes are essentially destroyed under the conditions used to cook and process food, leaving foods devoid of enzyme activity. Placing the full digestive burden on the body, the body's digestive process can become over-stressed. Digestive problems can result, causing improper digestion and malabsorption of nutrients that can have far reaching effects. Consequences of malabsorption can include impaired immunity, allergic reaction, poor wound healing, skin problems and mood swings. Supplemental enzymes can improve the level of digestion and help assure that the maximum level of nutrient absorption is attained.

Supplemental enzymes of microbial and plant origin work at the pH found in the upper stomach. Food sits in the upper portion of the stomach for as long as an hour before gastric secretions begin their action. Several studies have shown that the enzymes in saliva continue their digestive activity in the upper stomach and can digest up to 30% of the ingested protein, 60% of ingested starch and 10% of ingested fat during the 30 to 60 minutes after consumption. Although salivary enzymes accomplish a significant amount of digestion, their activity is limited to a pH level above 5.0. Supplemental microbial enzymes, and some plant enzymes, are active in the pH range of 3.0 to 9.0 and can facilitate the hydrolysis of a much larger amount of protein, carbohydrates and fat before Hydrochloric Acid is secreted in sufficient amounts to neutralize their activity. Obviously, these enzymes can contribute significantly in improving food nutrient utilization.

Why are food enzymes missing in cooked and processed foods?

Modern food processing techniques and all types of cooking destroy nearly 100% of the enzymes naturally occurring in food. Enzymes are completely denatured when exposed to temperatures over 118 deg. for any length of time. The modern diet consisting of cooked and processed food is essentially devoid of active enzymes.

What is the difference between pancreatic enzymes, plant enzymes, and microbial enzymes?

Pancreatic enzymes:
- derived from animal tissues
- activity limited to a narrow pH range
- very specific in action
- activated by body’s enzymes

• easily destroyed by acidity of the stomach
• delayed effect
• does not break down fibers/certain carbohydrates
• no sucrase, maltase, or lactase activity

Plant enzymes:
• derived from certain plants such as pineapple or papaya
• effective within a broad pH range
• predominantly proteolytic activity

Microbial (fungal) enzymes:
• derived from selected microorganisms by the process of fermentation
• broad pH range (approximately 3.0 - 9.0)
• activated in upper stomach
• begin working immediately
• broad action on a variety of foods

Supplemental pancreatic, plant, and microbial enzymes are all designed to enhance digestion. However, plant and microbial enzymes use a "proactive" approach and begin working on foods sooner after ingestion. Pancreatic enzymes usually begin working approximately 30 minutes after food reaches the stomach. Because of their stability in the acidic environment of the upper stomach, plant and microbial enzymes can begin their digestive action immediately after the food reaches this region. With the increased exposure to digestive enzyme activity, food has a better chance of being broken down into small, more readily absorbed particles.

Choosing a quality enzyme supplement is more difficult then just looking for a plant or fungal-based product. Enzyme potency and activity level is not evaluated by weight, because weight does not necessarily correlate with digestive capability. The activity level of a digestive enzyme is measured by assaying the quantity of digestion (hydrolysis) that occurs under specific conditions. This activity depends upon concentration, quantity, pH, temperature, and substrate.

Are there physiological effects from consuming an enzyme-free diet?

According to Dr. Howell, "cooked, enzyme-free diets contribute to a pathological over-enlargement of the pituitary gland [which in turn] regulates the other glands". Likewise, the human pancreas is grossly hypertrophied in relation to all other members of the animal kingdom. Howell concluded that the massive size of the human pancreas is the result of a pathological adaptation to a cooked food diet. Research conducted at the University of Minnesota showed significant changes in the organ weights of rats fed heat-treated food. Both the pancreas and submaxillary glands increased in weight by 20 to 30 percent. Research by Grossman further substantiates the adaptive role that diet plays in pancreatic secretion.

How many are aware of the function of enzymes?

Very few are aware of the function of enzymes in the body. Enzymes are substances, which make life possible. They are needed for every chemical reaction that occurs in our body. Without enzymes, no activity at all would take place. Neither vitamin, mineral, nor hormones could do any work - without enzymes.

Can the importance of pre-digestion be substantiated?

Research shows that nearly all creatures including rodents, whales, canines and birds have distinct organs that enable the exogenous enzymes of food the necessary time to act, before initiating the body's own digestive process. For example, seeds and grains lie in a birds crop for eight to twelve hours, during which proteolytic and amylolytic enzymes in the seed begin hydrolyzing proteins and starch. The food enzyme stomach concept in humans is supported by research on the extended activity of salivary amylase. The amylolytic activity of ptyalin alone can digest as much as 45% of the starch in a meal, before gastric secretions inhibit its action. Further studies in the 1940's showed that as much as 60% starch, 30% protein and 10% fat were digested before pepsin was activated.

Why take supplemental enzymes?

Supplemental enzymes replace the enzymes once present in raw food. Most enzymes are lost in cooking and processing. Only raw or uncooked food contains enzymes. Nature put these enzymes in food to aid in digesting the food you eat so your body's enzymes would not have to handle all the work. By taking a supplemental enzyme you free up your body's enzymes. When enzymes are missing from your food, the full burden of digestion falls on your own digestive system. Food sits in your stomach for nearly an hour before your body's digestive enzymes are secreted. It is during this time that food enzymes do their best work breaking down complex food molecules. These supplemental enzymes are temporarily inactivated in the stomach, but not before they have already accomplished much of their mission of breaking down the food molecules. During the first 30 to 60 minutes after eating, enzymes are hard at work, predigesting food long before the stomach acids render some of them inactive. Enzymes not destroyed in the stomach are re-activated in the small intestine.

Today's typical diet of cooked, canned, and convenience foods make it very important to take supplemental enzymes to relieve some of your body's digestive stress. Since your body will put a higher priority on digestion than on maintaining health, it will steal enzymes from the immune system to finish digestion. Taking a supplemental enzyme can help take stress off not only your digestive organs, but also your immune system.

How Enzymes Work?

- Active sites on enzymes = places to which a specific substrate binds
- Enzyme-substrate complexes form (when substrates attach to active sites on the enzymes) to break apart or put together substances at a fast rate
  E+ S → ES → E + P

There are two models of enzyme action

1. *lock & key model* → substrate & the enzyme fit together perfectly
2. *induced-fit model* →enzymes change shape slightly to accommodate the substrate
   Draw figure 4-15

Factors affecting enzyme action

1. *Temperature* – enzymes work best at certain temperatures, – *37^{o}C is best for human enzymes in the* body

2. *pH* – enzymes work best at certain pH; basic, neutral, and/or acidic environments

ex.) Amylase in saliva at pH 7, Pepsin in the stomach at pH 2-3, & Trypsin in the intestines at pH 9

3. S*ubstrate & enzyme concentrations* – how fast reactions take place depends on how much of the substrate & enzyme is available.

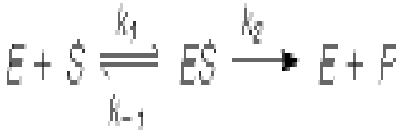
4. *Coenzymes* – helpers such as vitamins & minerals

Why we learn enzyme kinetics?

Enzyme kinetics is important to understand

1. the mechanism of enzyme action
2. the role of an enzyme in the cell
3. how the activity of enzyme can be controlled

When we talk about enzyme kinetics → it relates to
the velocity of PRODUCT being formed or
the velocity of SUBSTRATE being changed to product



The reaction rate (same as velocity or rate of formation of products) can be written as

V = k2[ES]

[ES] is usually not a measurable concentration. Easily measurable items are the substrate (or product) and the total concentration of enzyme, which is the sum of the free enzyme and complexed enzyme.

That is **[E]t = [E] + [ES],**

where [E]t is total enzyme, [E] is free enzyme, and [ES] is complexed enzyme.

Briggs and Haldane proposed a model that avoided the equilibrium assumption. It assumes that the more ES that is present, the faster ES will dissociated either to products or back to reactants. Therefore, when the reaction is started by mixing enzymes and substrates, the ES concentration builds up at first, but quickly reaches a steady state, in which it remains almost constant. The steady state will persist until almost all of the substrate has been consumed

Single-enzyme kinetics

Kinetics of enzyme-catalysed reactions

Under conditions of constant temperature and pressure, the change in the Gibbs free energy ΔG determines the direction and the extent in which a reaction may occur, and life processes are no exception to this rule. For a simple process that converts S into P,

$$\Delta G = \Delta G^{o} + RT \ln \frac{[P]}{[S]} \qquad \text{(Eq. 1.1)}$$

When ΔG < 0, this process may proceed in the forward direction (S is converted into P), when ΔG > 0 the reaction proceeds backward (P is converted into S), and when ΔG = 0, there is no net progress and the reaction is in equilibrium. In other words, when [S] and [P] have their equilibrium values, ΔG = 0, and the equilibrium constant K_{eq} is:

$$K_{eq} = \frac{[P]_{eq}}{[S]_{eq}} = e^{-\frac{\Delta G^{\circ\prime}}{RT}} \qquad \text{(Eq. 1.2)}$$

Although ΔG determines the direction of the reaction, the rate is a different matter. In living cells the rate of a reaction largely is determined by the enzyme that catalyses the reaction. According to biochemistry, the conversion of S to P, catalysed by enzyme E, under initial conditions (no P present) could proceed as:

E + S <—> ES —> E + P

The rate of this reaction is given by the well-known Michaelis-Menten expression:

$$v = \frac{V_{max}[S]}{[S] + K_M} \qquad \text{(Eq. 1.3)}$$

In this expression V_{max} is the maximal rate of the reaction when [S] is increased, and the Michaelis constant K_M is the concentration of S where the rate is exactly half of V_{max}.

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derivation of Michaelis-Menten equation

The reaction scheme is:

$$E + S \underset{k_2}{\overset{k_1}{<\!\!-\!\!>}} ES \underset{k_4}{\overset{k_3}{<\!\!-\!\!>}} E + P$$

Odd-numbered kinetic constants (k_1 and k_3) apply to the forward direction (left to right), even-numbered kinetic constants (k_2 and k_4) to the backward reaction.

First assumption: steady state for ES $\frac{d[ES]}{dt} = 0$

$$k_1 [E] [S] + k_4 [E] [P] = k_2 [ES] + k_3 [ES]$$

$$(k_1 [S] + k_4 [P]) [E] = (k_2 + k_3) [ES]$$

With total enzyme concentration $e = [E] + [ES]$

$$\frac{[ES]}{e} = \frac{(k_1[S] + k_4[P])}{(k_1[S] + k_4[P]) + (k_2 + k_3)}$$

and

$$\frac{[E]}{e} = \frac{(k_2 + k_3)}{(k_1[S] + k_4[P]) + (k_2 + k_3)}$$

The rate of the reaction is:

$$v = \frac{d[P]}{dt} = -\frac{d[S]}{dt} = k_1 [E] [S] - k_2 [ES] = k_3 [ES] - k_4 [E] [P]$$

(so S —> P is the forward direction). Putting in the expressions for [E] and [ES]:

$$v = \frac{(k_1 k_3 [S] - k_2 k_4 [P])e}{(k_1[S] + k_4[P]) + (k_2 + k_3)} \qquad \text{(Eq. 1.4)}$$

In this expression the maximal forward rate is achieved when [S] —> ∞. The rate then is

$$V_{max}(\text{forward}) = V_S = k_3 e$$

The maximal backward rate is approached when [P] —> ∞. The rate expression predicts that v —> – k_2 e. However, V_P is defined as positive number:

$$V_{max}(\text{backward}) = V_P = k_2 e$$

Michaelis constants (K_M's) can be defined both for S (K_S) and for P (K_P):

$$K_S = \frac{k_2 + k_3}{k_1} \qquad K_P = \frac{k_2 + k_3}{k_4} \qquad \text{(Eq. 1.5)}$$

Introducing V_S, K_S, V_P and K_P into Eq. 1.4, this turns into:

$$v = \frac{V_S \frac{[S]}{K_S} - V_P \frac{[P]}{K_P}}{1 + \frac{[S]}{K_S} + \frac{[P]}{K_P}} \qquad \text{(Eq. 1.6)}$$

This expression relates the rate v to the concentrations of S and P. The relation between these three variables contains four parameters: K_S, V_S, K_P and V_P.

It can be verified easily that at [S] = K_S and [P] = 0, the half maximal forward rate is obtained, and at [S] = 0 and [P] = K_P, the half maximal backward rate. Special forms of Eq. 1.6 (such as Eq. 1.3 when [P] = 0) can be used under different conditions.

The Haldane relationship

When the net rate is zero, equilibrium is reached. This occurs when the numerator of the rate expression (Eq. 1.6) is zero:

$$V_S \frac{[S]_{eq}}{K_S} - V_P \frac{[P]_{eq}}{K_P} = 0$$

From this follows the Haldane relation:

$$K_{eq} = \frac{[P]_{eq}}{[S]_{eq}} = \frac{V_S K_P}{V_P K_S} \qquad \text{(Eq. 1.7)}$$

The four parameters in the kinetic expression are mutually dependent. Sometimes it is useful to use this interdependence to eliminate e.g. V_P from the rate expression:

$$v = \frac{\frac{V_S}{K_S}\left([S] - \frac{[P]}{K_{eq}}\right)}{1 + \frac{[S]}{K_S} + \frac{[P]}{K_P}} \qquad \text{(Eq. 1.8)}$$

This form shows that of the four parameters in the kinetic expression, one (K_{eq}) is determined by the reaction (independent of the enzyme), while the three others (K_S, K_P and $V_{max} = V_S$) depend on the enzyme.

Special conditions

When studying an isolated enzyme with the aim to determine the values of K_M's and V_{max}'s, one can choose experimental conditions such that this is a relatively easy task. Some examples:

(a) *Initial conditions (forward reaction):* the second assumption is [P] = 0. The rate expression to be used is:

$$v = \frac{V_S \frac{[S]}{K_S}}{1+\frac{[S]}{K_S}} \quad \text{or} \quad v = \frac{V_S[S]}{[S]+K_S} \quad \left(= \frac{V_{max}[S]}{[S]+K_M} \right) \qquad \text{(Eq. 1.9)}$$

From this familiar expression V_S and K_S can be estimated by a non-linear fitting procedure, or by a linear fit to e.g. a Lineweaver-Burk graph (see Fig. 1.1).

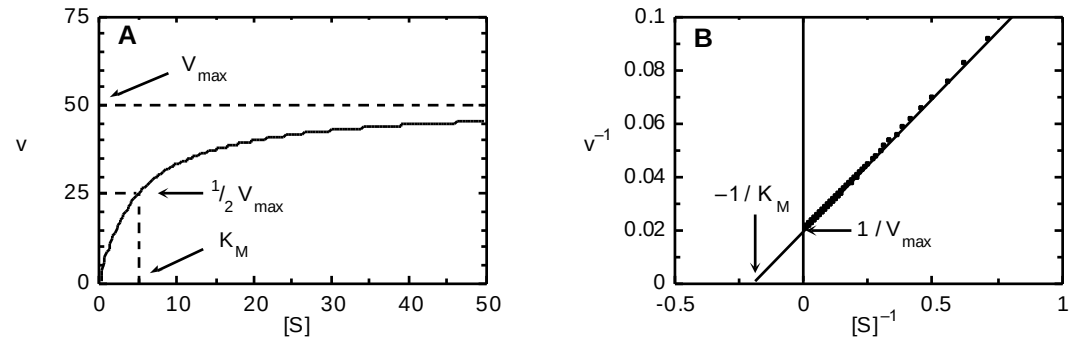


Fig. 1.1 Michaelis-Menten kinetics with initial conditions assumption.
Parameter values: V_S (V_{max}) = 50, K_S (K_M) = 5, [P] = 0. A. Rate plotted against substrate concentration. B. Lineweaver-Burk graph.

(b) *Initial conditions (backward reaction):* the second assumption is [S] = 0. The rate expression to be used is:

$$v = \frac{-V_P \frac{[P]}{K_P}}{1+\frac{[P]}{K_P}} \text{ or } \quad v^* = \frac{V_P[P]}{[P]+K_P} \quad \left(= \frac{V_{max}[P]}{[P]+K_M} \right) \qquad \text{(Eq. 1.10)}$$

(with $v^* = -v$).
Using initial conditions, the third assumption generally is made that the substrate (either S or P) is in large excess of the total amount of enzyme e. Then [ES] ([ES] ≤ e) can be neglected and [S] = s or [P] = p (the total concentrations added).

c) *Moiety-conserved conditions.* In this case the second assumption is [S] + [ES] + [P] = q is constant. Again using the third assumption that substrates are in large excess above enzyme (q >> e), [ES] drops out of the conservation relation, and this turns into: [S] + [P] = q. This can be used to e.g. eliminate [P] from the rate expression, yielding a hyperbolic relation between the

two remaining variables v and [S] (see § 3.4). Although it seems that introduction of q is adding an extra parameter, scrutiny of the rate equation (while keeping in mind that K_{eq} has a given value) reveals that only three parameters may be chosen independently. A few possible relations between v and [S] are shown in Fig. 1.2.

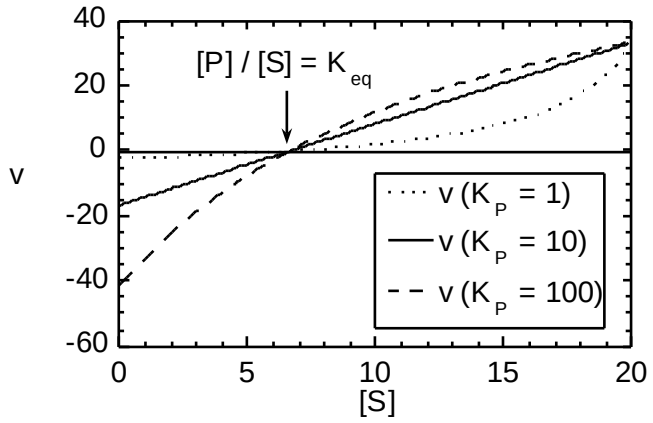


Fig. 1.2 Michaelis-Menten kinetics with moiety conservation ([S] + [P] = constant).
Parameter values: V_S = 50, K_S = 10, K_{eq} = 2, [S] + [P] = 20, K_P as indicated. Note that all three curves intersect the [S]-axis (v = 0) at the equilibrium point. The curve is concave when $K_S > K_P$, linear when $K_S = K_P$ and convex when $K_S < K_P$.

One expects that initial conditions are extremely rare in cells (generally substrates and products are present); however, moiety conservation very often is a useful assumption in studying enzyme activity in the cell.

Irreversibilty and product sensitivity

It is often thought that assuming initial conditions in the forward direction is a good approach to study irreversible reactions in the cell. "Irreversible" implies that K_{eq} is very large. Making K_{eq} very large turns Eq. 1.8 into:

$$v = \frac{\frac{V_S}{K_S}[S]}{1+\frac{[S]}{K_S}+\frac{[P]}{K_P}} = \frac{V_S[S]}{[S]+K_S+[P]\frac{K_S}{K_P}} \qquad \text{(Eq. 1.11)}$$

This expression differs from that valid under initial (forward) conditions (Eq. 1.9) in the presence of the term in the denominator containing [P]. This makes the rate *product sensitive*, an important feature of the kinetics, as we will see. To use initial conditions kinetics the reaction need not only be irreversible, but also product insensitive. For this K_P needs to be very large,

which by itself helps irreversibility by increasing K_{eq} (see Eq. 7). One way of using "initial conditions" is to define an effective [P]-dependent K_M for S:

$$K_S' = K_S\left(1+\frac{[P]}{K_P}\right)$$

Which turns Eq. 1.11 into Eq. 1.9. However, to use this expression, [P] should be constant under experimental conditions.

---

reversible inhibition

Again, the reaction scheme is:

$$E + S \underset{k_2}{\overset{k_1}{<\!\!-\!\!>}} ES \underset{k_4}{\overset{k_3}{<\!\!-\!\!>}} E + P$$

with the addition that there is reversible binding of an inhibitor I to the enzyme:

E + I <—> EI with (dissociation) inhibition constant $K_i^E = \frac{[E][I]}{[EI]}$

And

ES + I <—> ESI with (dissociation) inhibition constant $K_i^{ES} = \frac{[ES][I]}{[ESI]}$

EI and ESI are inactive forms: they cannot bind or release S or P.
In the derivation of kinetic expressions, the fourth assumption is made that these reactions are at equilibrium. An extension of the third assumption is that also the inhibitor is present in large excess with respect to the enzyme.
Using these assumptions it can be derived that the total enzyme concentration now is:

$$e = [E] + [EI] + [ES] + [ESI] = [E]\left(1+\frac{[I]}{K_i^E}\right) + [ES]\left(1+\frac{[I]}{K_i^{ES}}\right) \quad \text{(Eq. 1.12)}$$

With this modification, a rate expression similar to Eq.1.6 can be derived, with (primed) parameters that depend on the inhibitor in the following manner:

$$V_S' = \frac{V_S}{\left(1+\frac{[I]}{K_i^{ES}}\right)} \qquad V_P' = \frac{V_P}{\left(1+\frac{[I]}{K_i^{ES}}\right)} \quad \text{(Eq. 1.13)}$$

$$K_S' = K_S \frac{\left(1+\frac{[I]}{K_i^E}\right)}{\left(1+\frac{[I]}{K_i^{ES}}\right)} \qquad K_P' = K_P \frac{\left(1+\frac{[I]}{K_i^E}\right)}{\left(1+\frac{[I]}{K_i^{ES}}\right)} \qquad \text{(Eq. 1.14)}$$

When the inhibitor doesn't bind to E or to ES, the corresponding K_i is infinite.

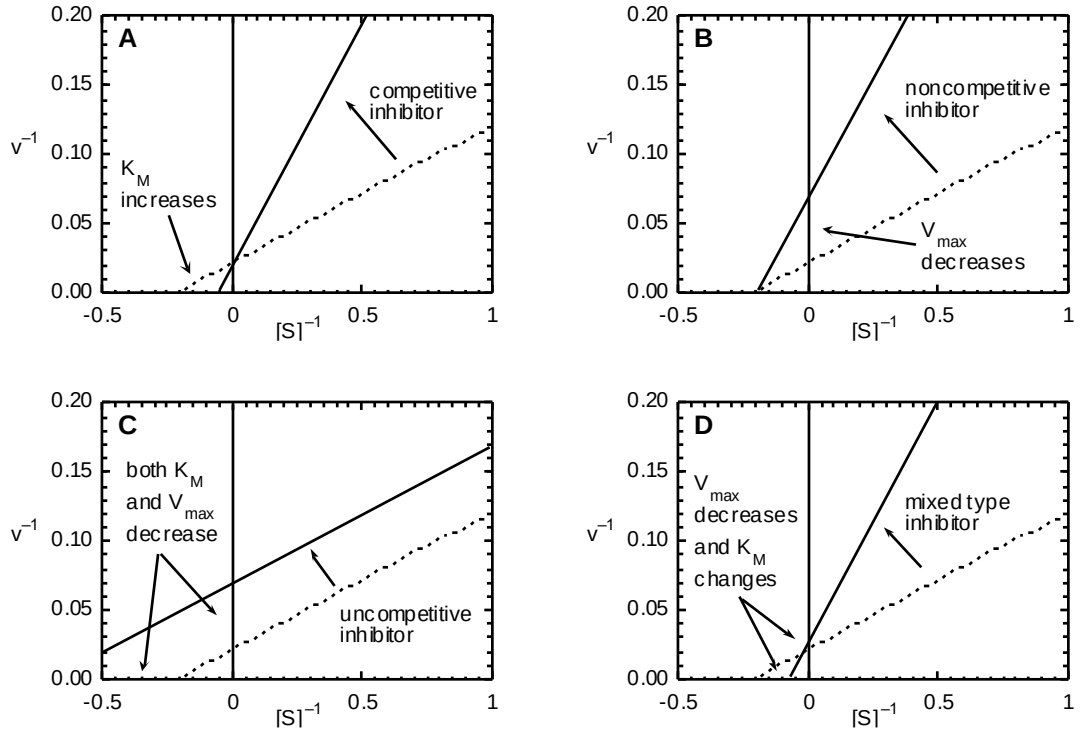


Fig. 1.3 The Lineweaver-Burk graph as a tool to distinguish types of reversible inhibition. Initial conditions, with parameter values: $V_S = 50$, $K_S = 5$, $K_{eq} = 2$, [P] = 0, [I] = 0.5. A. Competitive inhibition: $K_i^E = 0.2$, K_i^{ES} infinite. B. Noncompetitive inhibition: $K_i^E = K_i^{ES} = 0.2$.C. Uncompetitive inhibition: K_i^E infinite, $K_i^{ES} = 0.2$ D. Mixed-type inhibition: $K_i^E = 0.2$, $K_i^{ES} = 1$.

Special cases are:
(a) Exclusive binding of the inhibitor to E (*competitive* inhibition). Only K_S' and K_P' depend on [I] (they increase with [I]). This occurs when inhibitor I competes with substrate (S, or P) for the same site on the enzyme.
(b) Binding of the inhibitor to E and ES with the same K_i (pure *non-competitive* inhibition). Only V_S' and V_P' depend on [I] (they decrease with [I]).

(c) The inhibitor exclusively binds to ES (*uncompetitive* inhibition). V_S', K_S', V_P' and K_P' depend on [I] (they all decrease with [I]).
(d) A fourth possibility is that the inhibitor binds to E and ES with different K_i (*mixed type* inhibition). All four parameters change.
Under initial conditions (e.g. [P] = 0) the four types of reversible inhibition are most easily distinguished in e.g. a Lineweaver-Burk graph (see Fig. 1.3).
In a completely analogous way one can think of different forms of (reversible) *activation* of an enzyme.

Enzyme kinetics and the living cell

We have seen in what way one can describe the kinetics of the simplest enzyme-catalysed reaction. We will not discuss enzymes with more than a single substrate here, but in principle similar treatments of their kinetics have been developed. One should be aware that kinetic data about enzymes often have been determined under "initial conditions" of one kind or other, and kinetic behaviour of these enzymes inside the cell generally has to be understood under quite different conditions. Important considerations here are reversibility of the catalysed reaction (depending on K_{eq}), product sensitivity (depending on K_P) and moiety conservation.

Enzyme: increase the rate of a reaction without itself being used up, a biological catalyst
Substrate: the substance being acted on

Reaction rates are dictated by:
1. state of matter of reactants
2. concentration of reactants
3. temperature
4. presence of a catalyst

Why are they important?
To digest food in a beaker or test tube in an attempt to attain the same products as your body at body temperature you would be waiting for years.
To break the peptide bonds in proteins your body uses peptase. To break the glycosidic bonds of amylose(starch) your body uses amylase. Peptase and amylase are two of the body's thousands of enzymes. Without them you would starve.

Enzymes have:
- Ability to increase rate - rate is increase by orders of magnitude
- High specificity - enzymes can catalyze a specific molecule, their function can be as specific to catalyze a specific stereo orientation of a molecule or as general as to catalyze the reactions of an organic functional group.

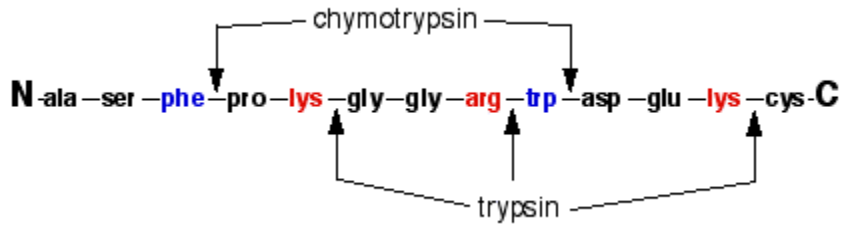
- Ability to be regulated - cells can activate or deactivate the enzyme when the environment and situation are right.

Nomenclature:
The nomenclature for enzymes have undergone a change. Initially an enzyme was named with the suffix –in. Today the –ase suffix has replaced the –in ending. Trypsin and chymotrysin are examples of old nomenclature and sucrase is and example of the new nomenclature.

Specific Enzymes:

Trypsin, the enzyme which selectively cleaves a substrates peptide bonds following the basic amino amino acids arginine or lysine, is an example of the old nomenclature.

Chymotrypsin is a variation of trypsin and cleaves peptide bonds on the C-terminus of the tryptophan, tyrosine, phenylalanine, and methionine amino acids.



Sucrase, the enzyme used to break the substrate sucrose's glycosidic bond, is an example of the new nomenclature.

Classification:
1. Reductase
    a. redox reactions
    b. reduce ethanol to ethanal
2. Transferase
    a. moves functional groups from one molecule to another, intermolecular
    b. kinase transfers phosphate groups
3. Hydrolase
    a. hydrolysis reactions
    b. lipase breaks a triglyceride into glycerol and fatty acids
    c. trypsin breaks peptide bond following lysine and arginine
4. Lyase
    a. used to add or remove a group to or from a double bond, but not by redox or hydrolysis
    b. pyruvate decarboylase – turns pyruvic acid into ethanal and CO_2
5. isomerase
    a. rearrange a molecule into a different isomer, structural or stereo
    b. rearranges galactose into glucose
6. ligase
    a. join to molecules
    b. DNA ligase creates DNA strands

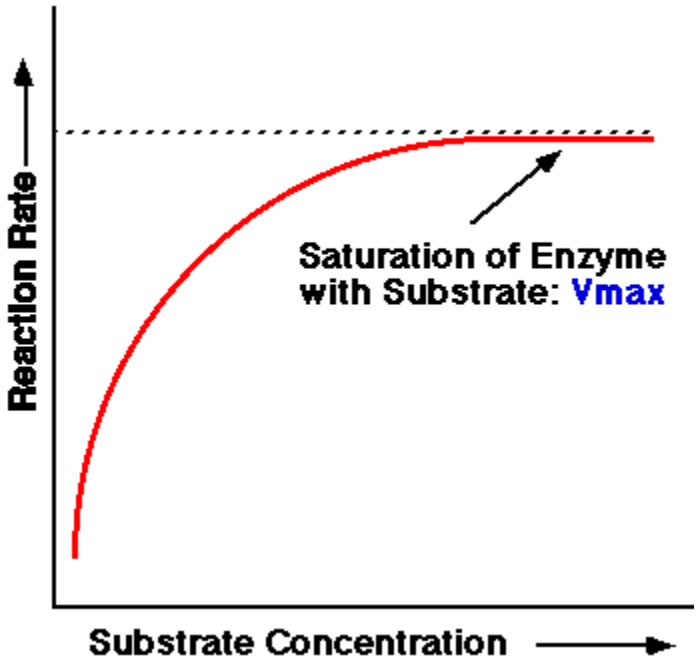
Mechanism Theories:
The **Lock & Key** theory describe a situation where the substrate fits perfectly into the enzyme's activity site. The activity site is simply the location of the enzyme where the substrate docks. The **Induced Fit** model begins with an enzyme activity site which does not exactly fit the substrate, but is stretched to accommodate the substrate. This second theory gives a more flexible image of an enzyme than the first.

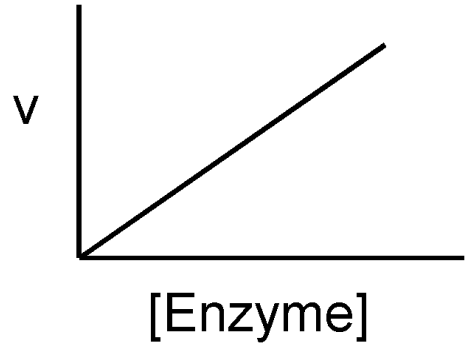
The ability for an enzyme to dock and undock molecules is essential in increasing the rate of the reactions. This turnover number the number of substrate molecules the enzyme is able to act on in one minute. This turnover number has been standardized by the chemical community into the enzyme international number. Which is the number of catalytic conversions per minute of 1 micromole of substrate, under specific conditions of temperature and pH.

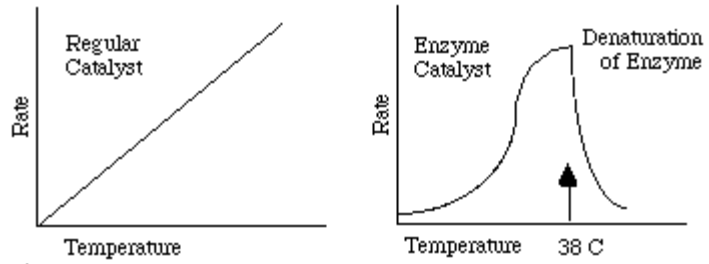
Enzyme Activity Factors:
An enzyme catalyzed reaction may be written as:

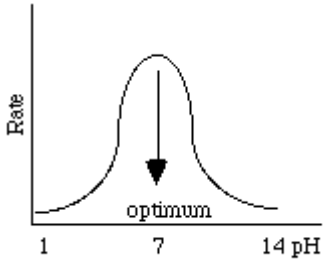
$$E + S \longleftrightarrow ES \longleftrightarrow E + P$$

E is the enzyme
S is the substrate (reactant)
ES is the enzyme-substrate complex
P is the product









The Equation which describes rate of reaction (r) as a function of substrate concentration (S) is the Michaelis-Menton Equation.

$$r = \frac{V_{max}[S]}{K_m + [S]}$$

Vmax is the function of the concentration of the enzyme [E] and the Turnover Number of the enzyme.

K_m is the Michaelis-Menton constant and is determined experimentally, just as the rate constant is for a rate law equation.

Remember, the Turnover Number refers to the efficiency of the enzyme and is expressed as the number of molecules of substrate converted to product per second.

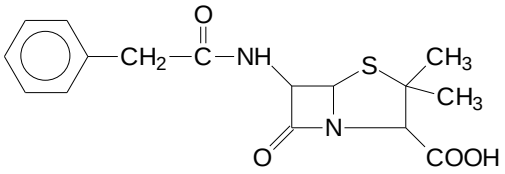
The Turnover Number of enzymes can range from 10 to 100,000 molecules per second, demonstrating the effective catalytic nature of some enzymes.

Enzyme Inhibition:

1. Chemicals other than substrates and products may interact with an enzyme influencing the reaction rate.

2. Chemicals which bind to the active site but do not react will compete for formation of the ES complex and are known as **competitive Inhibitors**. Raising substrate concentrations will overcome this type of inhibition.
3. Chemicals which bind somewhere else than the active site but decrease the turnover constant for the enzyme are known as **non-competitive inhibitors**. Raising the substrate concentration will not overcome this type of inhibition.
4. Some agents simply denature or otherwise destroy the enzyme causing irreversible Inhibition. This type of inhibition is see often with chemicals which form covalent bonds with the enzyme. CN^-, cyanide ion is an example of an irreversable inhibitor. It binds to the cytochrome oxidase, a cofactored enzyme, and prevents it from allowing cell respiration. If $Na_2S_sO_3$ is administered quickly the CN^- can be removed from the cofactored enzyme. Most heavy metals, Pb, Hg denature enzymes in a similar manner.

Not all inhibitors are bad for you. Penicillins act as an inhibitors for the transpeptidase enzyme which builds the protein portion of bacteria cell walls.



penicillin G structure

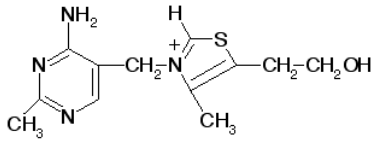
Cofactors:
All enzymes are proteins. But sometimes, for a protein to function it requires the assistance of another molecule or ion, a **cofactor**. Cofactors are normally thought of as metal ions, but when this cofactor is a small organic molecule it is named a **coenzyme**. The magnesium ion is required for the body to use glucose-phosphate compounds. Coenzymes are also called vitamins. Further yet, some coenzymes themselves must by accompanied by another organic molecule to function properly. If an enzyme requires a cofactor the protein is named **apoenzyme** until the cofactor has been added.

VITAMIN	FUNCTION	DEFICIENCY SYMPTOM
WATER SOLUBLE		
Ascorbic Acid (C)	Hydroxylases	Scurvy - connective tissue
Thiamine (B1)	reductases	Beriberi-fatigue
Riboflavin (B2)	reductases (Flavine)	Skin disease
Pyridoxine (B6)	Aminotransferases	Anemia
Niacin	reductases (NAD)	Pellagra -skin and nerve
Folic Acid (M)	Methyltransferases	Anemia
Vitamin B12	Isomerase	Anemia
Pantothenic Acid	Acyltransferase (CoA)	Weight Loss
Biotin (H)	Carboxylases	Dermatitis
FAT SOLUBLE		

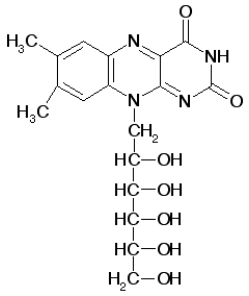
Vitamin A	Vision and Cell Differentiation	Night Blindness
Vitamin D	Calcium Metabolism	Rickets - bone problems
Vitamin E	Antioxidant	Fragile cell membranes
Vitamin K	Blood Clotting	Delayed blood clotting

VITAMIN C:

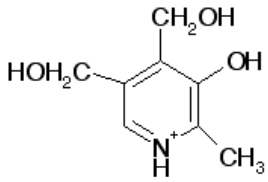
THIAMIN (B_1)



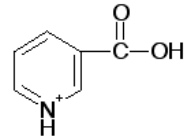
RIBOFLAVIN (B2)



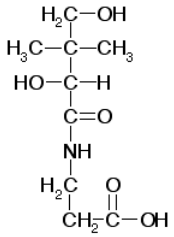
PYRIDOXINE (B6)



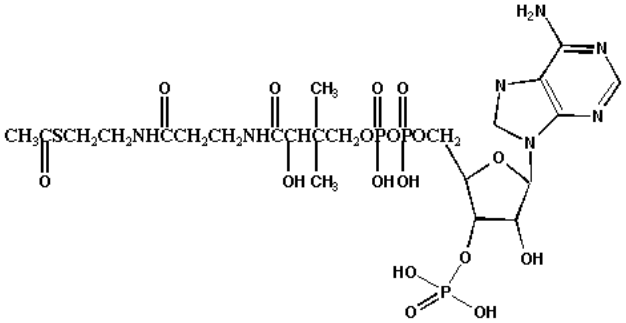
NIACIN



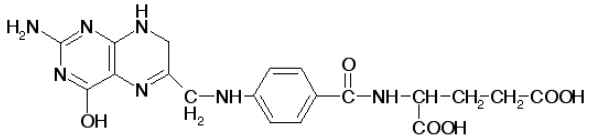
PANTOTHENIC ACID



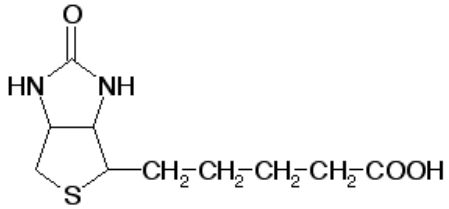
Acetyl-CoA Contains Pantothenic Acid



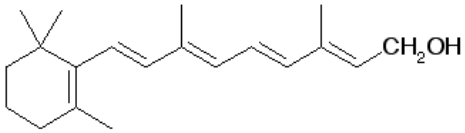
FOLIC ACID (M)



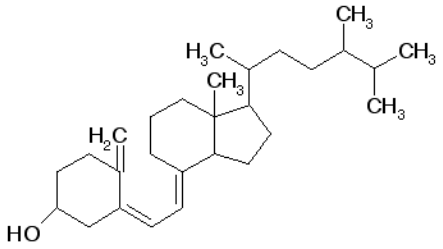
BIOTIN



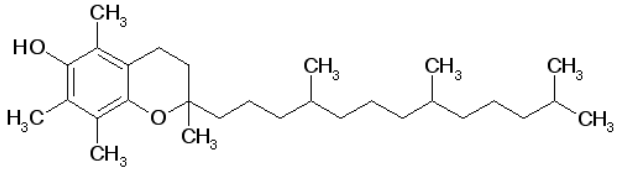
VITAMIN A (alcohol)



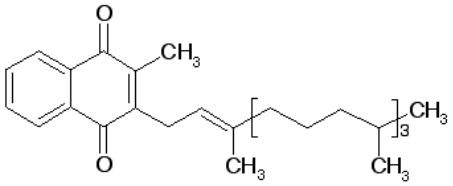
VITAMIN D



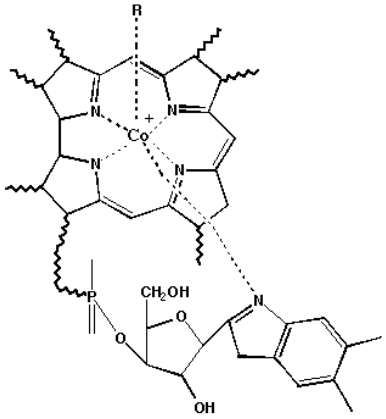
VITAMIN E



VITAMIN K



VITAMIN B_{12}



Enzyme Deficient Diseases:

Galactosemia:

A genetic disease in which the body has an inability to metabolize galactose. Elevated levels of galactose in blood and urine along with vomiting, diarrhea, liver enlargement are signs of this disease. Can cause death in days is lactose is not removed from their diet

albinism:

Lack of the enzyme tyrosinase which increases the rate of melanin production of the skin. UV light is the main regulator of this enzyme. Increased exposure to UV causes activity of tyrosinase to begin. This escalates the rate of melanin production resulting in a tan.

Lactose Intolerance:
Lactose intolerance develops when the body has difficulty digesting whole and skim milk and other dairy products. Lactose is a milk sugar and like most sugars, it is broken down by enzymes in the intestinal tract so it can be absorbed as an energy source. The enzyme that breaks down lactose is called lactase. When the intestine does not contain lactase, then lactose intolerance can occur. It is a troublesome and annoying problem, but it is never a serious one.

As commonly expected, infants and small children have the enzyme lactase so they can digest mothers' milk. However, during childhood, lactase begins to disappear in many people. Some ethnic groups are more likely to develop lactose intolerance. By adolescence, it is gone in about 75% of African-Americans, Jews, Native Americans, Mexicans, and in 90% of Asians. So the condition is very common.

When undigested lactose reaches the colon (large intestine), it is broken apart by bacteria. Lactic acid and other acidic chemicals result. It is these products that create the symptoms of lactose intolerance. These symptoms include nausea, abdominal cramps and rumbling, bloating, rectal gas (flatus), and diarrhea. They usually occur 30 minutes to two hours after ingesting lactose-containing foods. The severity of symptoms usually depends on the amount of lactose ingested and how much of the enzyme, lactase, remains in the intestinal tract.

Tay-Sachs Disease:
This disorder is named after a physician, Dr Bernard Sachs, who noted in 1887 that a number of children of Central and Eastern Europe (Ashkenazic) Jewish ancestry, who were born with no apparent problems, degenerated physically and mentally and died by the age of about four. The affected children were found to have a cherry-red spot at the back of the eye by an ophthalmologist, Dr Warren Tay, and thus the condition became known as Tay-Sachs disease.

The symptoms first appear at about the age of 6 months when an apparently happy healthy baby stops smiling, crawling or turning over, loses its ability to grasp or reach out, and gradually becomes blind, paralyzed and unaware of his/her surroundings. Death usually occurs by the age of 3 or 4 years.

Babies born with this condition lack a particular enzyme called hexosaminidase A or Hex A. Hex A normally breaks down a fatty substance found in the brain called GN12 ganglioside. Small amounts of this substance are essential for proper brain function. In a child with Tay-Sachs disease, the Hex A enzyme is missing and the fatty substance accumulates in the brain cells, irreversibly damaging the cells.

The mutation causing Tay-Sachs is in a gene on a chromosome and its effects are "recessive" or hidden by the presence of the correct copy of the gene. The pattern of inheritance in families who have this mutation is thus described as autosomal recessive inheritance There are four possibilities for the combinations of genes passed from the parents.

If a couple who are both carriers of a mutation in their Hex A gene have a baby, there is a 25% chance, in every pregnancy, that they will have a baby who is affected with Tay-Sachs disease.

If only one of the parents is a carrier of the mutation, there is no chance that the baby will inherit the mutation causing Tay-Sachs disease but there is a 50% chance that their child will be a healthy carrier of the mutation.

Introduction

What do you mean by "Immobilization"?

Word "immobilize" means to make anything unable to move at its own. We can entrap or fix something on certain supportive material for their immobilization. However, during this process it is very important to maintain their physiological conditions.

In 1950, for the first time intentionally enzyme was immobilized on solid material.

Immobilization of Enzyme defined, as „the imprisonment of an enzyme in a phase that allows interaction with substrate effectors or inhibitor molecules but it is separate from them".

What should be immobilized - cell or enzyme?

The selection of immobilization of cell or enzyme depends on so many criteria like number of step in the process requirement of coenzyme importance of contaminating reactions, cost, stability, and catalytic specificity.

When can we use enzyme for immobilization?

- When, the reaction is a single step
- When, co-enzyme is not involved
- When, there is use of single enzyme

What are the benefits of enzyme immobilization?

- It is Cost effective
- It requires smaller reactor
- It needs shorter process time

Benefits of Cell Immobilization

- Do not require coenzyme
- Enzyme remains stable in the cell

For reactions, those are more complex, immobilized cells are used, rather than immobilized enzyme

Immobilization achieved by any of the following methods

1. Adsorption
2. Physical entrapment
3. Covalent cross-linking
4. Micro-encapsulation

Adsorption

In this method, enzymes adsorbed onto supporting matrix. A range of specific or non-specific bond force may use like electrostatic force, hydrophobic interactions, or affinity bonding to specific ligand.

Advantages of Adsorption

- It is Easy

- It is Simple
- It gives High yield

Limitations of Adsorption

- Enzyme will leach with change in pH, or ionic strength
- Substrate with same charge as polymer may not gain access to enzyme except at high concentration, which in turn causes loss of enzyme

Physical Entrapment

Enzyme entrapped in Polymer matrix. The biocatalyst dissolved in a solution of the polymer‟s precursors, and polymerization initiated. Two types of polymers are used Polyacrylamide type gel and naturally occurring type gel materials such as, cellulose triacetate, agar, gelatin, carrageenan and alginate.

Advantages of Entrapment

- It is Simple
- It provides variability of pore size for the immobilization of cells or enzyme
- Mild conditions are used in the preparation of immobilized cells or enzymes

Limitations of Entrapment

- Viable cells bursts from gel material
- Free radicals formed during polymerization are toxic

Covalent cross-linking

Enzyme or cell bounded covalently with matrix. Enzyme can bind directly with reactive group of polymer or enzyme and polymer bridged by the use of bi-functional reagent. The principle groups on the enzyme through which it coupled are hydroxyl and amino group and to a lesser extent sulfhydryl groups.

Many commercially available activated polymers are hydrogels such as, celluloses or, polyacrylamide, onto which some reactive group like, diazo, carbodiimide or azide attached.

Bi-functional group used are of two types

1. Gluteraldehyde
2. Cyanuric chloride or Metal like Titanium Four

Gluteraldehyde is simple, and it bound to polymer and enzyme. Cyanuric chloride is multifunctional group, which react with cellulose and hydroxy substituted compound or enzyme.

Titanium four is multivalent group, which react with glass and used for the immobilization of cells or enzyme.

Advantages of covalent cross-linking

- This technique is Easy
- It gives High yield
- There is no Leaching of Enzyme

Limitations of covalent cross-linking

- Enzymes frequently inactivated
- Toxic reagents are used
- Preparations are complex

Micro-Encapsulation

Enzymes enclosed in a semipermeable membrane capsule. Materials used for the preparation of capsule are nylon or biodegradable polylactate or liposomes.

Advantages of Micro-encapsulation

- Surface area to volume ratio is high
- Replacement of enzyme is easy
- Highly viscosity substrate may be use

Limitations of Micro-encapsulation

- Membrane bound enzyme can denature
- This technique used for low molecular weight compound

Applications of Immobilization

1) Immobilized Enzyme-aminoacylase used for the first time by the immobilization method for the production of L-amino acids

2) In food industry, fructose syrup is produce from glucose by use of immobilized enzyme glucose-isomerase

3) Immobilized enzyme used in biosensor

4) Immobilized enzymes used in various analytical techniques where one can diagnose clinical problems 5) Accurate analysis of sample done with the help of specific immobilized enzyme and sensitive chemical analytical techniques uses immobilized enzyme

6) Immobilized Enzyme or Cells used in industry for the production of various industrial products Conclusion

Enzyme immobilization is one of the most hopeful approaches for deploying enzyme-based processes in biotransformation, diagnostics, pharmaceutical and food industries.

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1. Bioprocess Engineering (Basic Concepts) by M. L.Shuler& F.Kargi,Prentice Hall of India, 2003
2. Biochemical Engineering Fundamentals by J.E.Bailey & D. F. Ollis, McGraw Hill Book Company, 1986.
3. Chemical, Biochemical, and Engineering Thermodynamics by Stanley I. Sandler
4. Bioprocess Engineering Principles by Pauline M. Doran

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF CHEMICAL ENGINEERING

BIOCHEMICAL ENGINEERING

UNIT – 3 KINETICS OF MICROBIAL GROWTH – SCHA 1601

Cell Growth Kinetics

- Introduction
- Growth patterns and kinetics in batch culture
  - growth phases
  - effect of factors: oxygen supply
  - heat generation
- Growth kinetics (Monod Equation)
- Growth in continuous culture (ideal chemostat)

Growth Kinetics

Introduction

- Autocatalytic reaction: The rate of growth is directly related to cell concentration

substrates + cells → extracellular products + more cells

$$\sum S + X \rightarrow \sum P + nX$$

S: substrate concentration (g/L); X: cell mass concentration (g/L);

P: product concentration (g/L); n: increased number of biomass.

Net specific growth rate (1/time):

$$\mu_{\text{net}} \equiv \frac{1}{X}\frac{dX}{dt}$$

t: the time

Growth Kinetics

Introduction

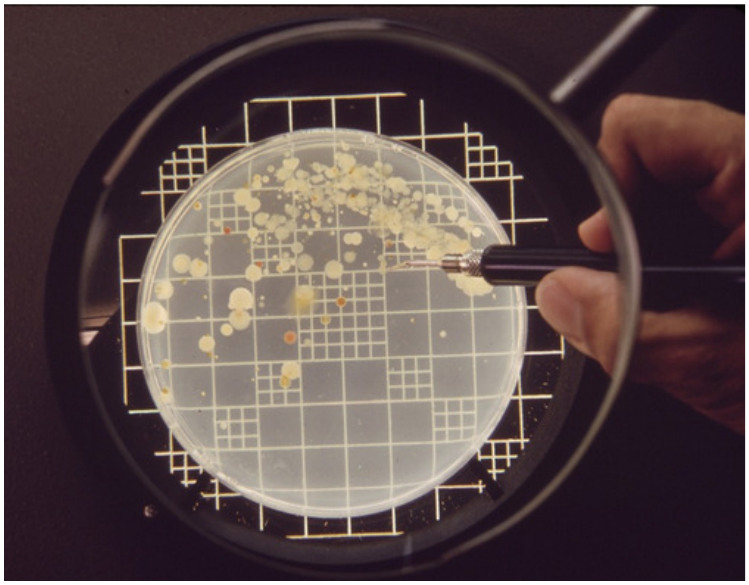
Net specific growth rate (1/time):

$$\mu_{\text{net}} = \mu_g - k_d$$

μ_g : Gross specific growth rate (1/time)

k_d : The rate of loss of cell mass due to cell death or endogenous metabolism

Endogenous metabolism: during the stationary phase, the cell catabolizes cellular reserves for new building blocks and for energy-producing monomers.

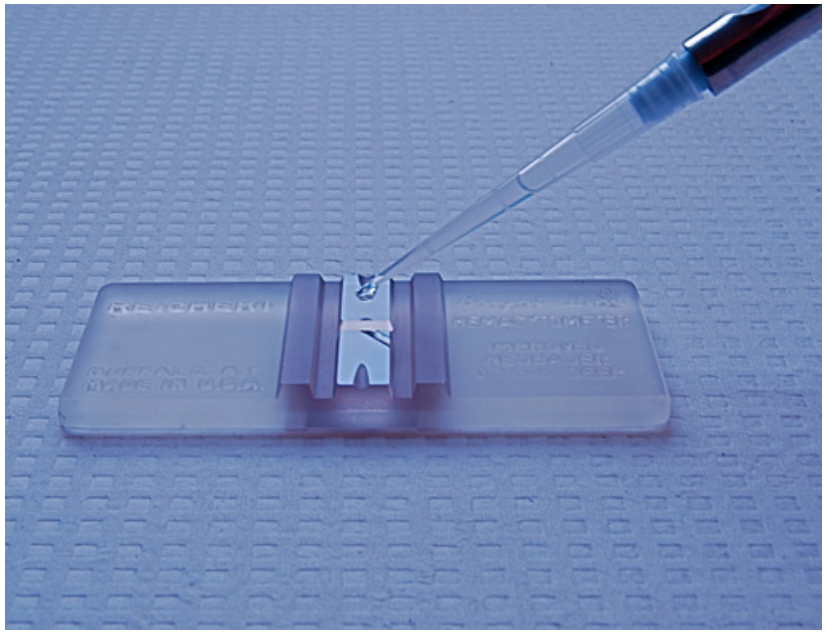


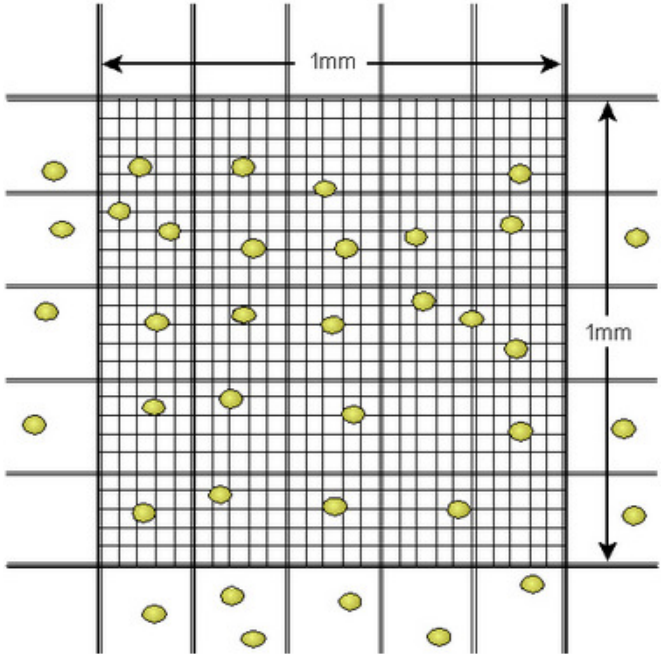
Introduction

- Quantifying cell concentration:

  <u>cell number density</u>:

  Petroff-Hausser slide (hemocytometer), plate counts, etc.





Growth Kinetics

Introduction

- Quantifying cell concentration:

  - direct: no suspended solid and interference compounds.

  <u>cell mass concentration</u> – preferred

  dry weight, optical density (turbidity) (600-700nm Wave Length)

Growth Kinetics

- Quantifying cell concentration:

  - indirect: direct method is inapplicable. (mold solid state fermentation)

    Cell mass can be determined by measurement of protein, DNA or ATP. e.g. 1mg ATP/g dry weight bacterial cell.

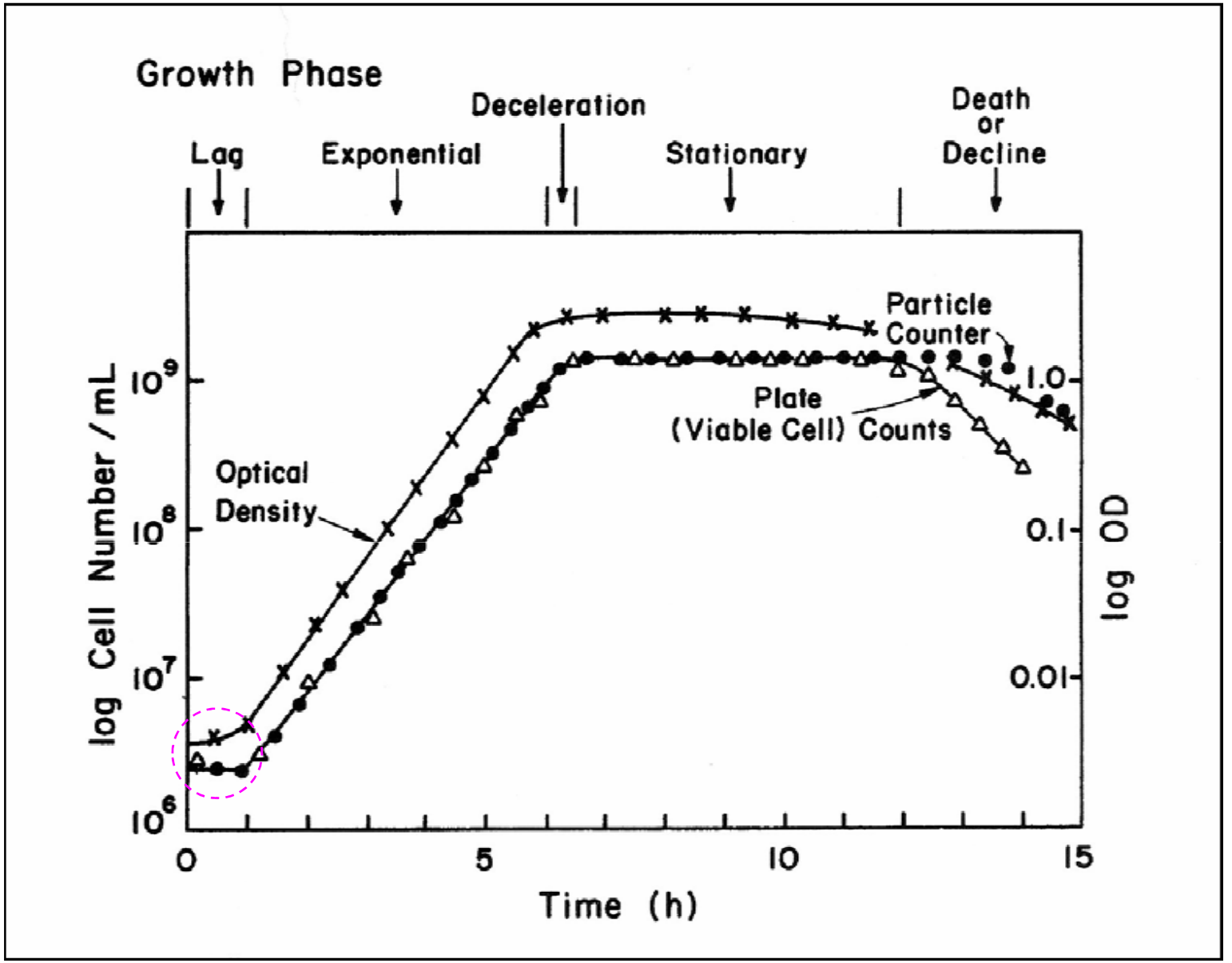
If 100 mg ATP/L is measured, then the cell mass:

100 mg (ATP/L)/1 mg ATP/g dry cells=100 (g dry weight cells/L)

Growth Kinetics

- Growth patterns and kinetics in batch culture
  - growth phases

  In batch culture:
    - lag phase
    - logrithmic or exponential growth phase
    - deceleration phase
    - stationary phase
    - death phase



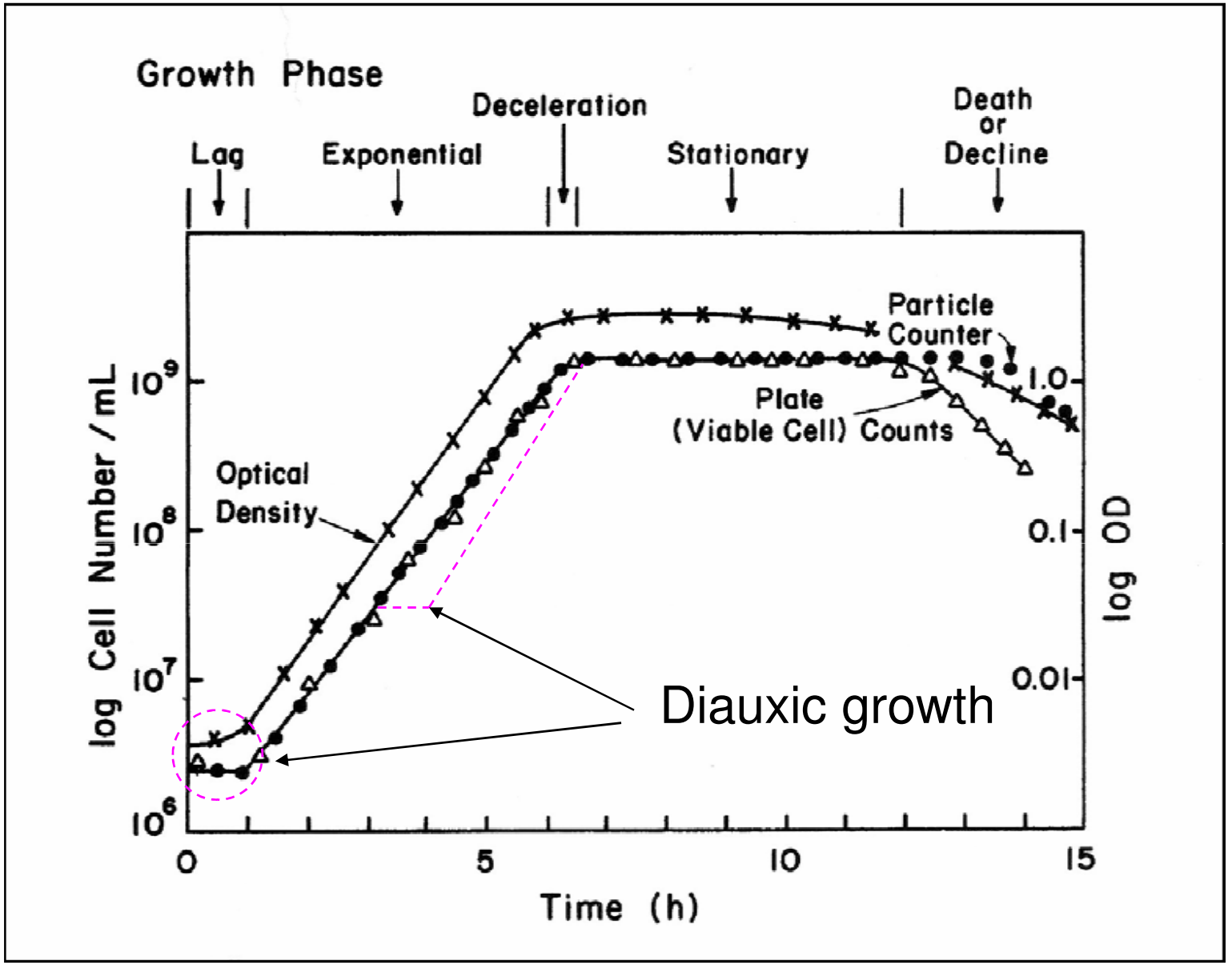
Typical growth curve for a bacterial population

Batch Growth Kinetics

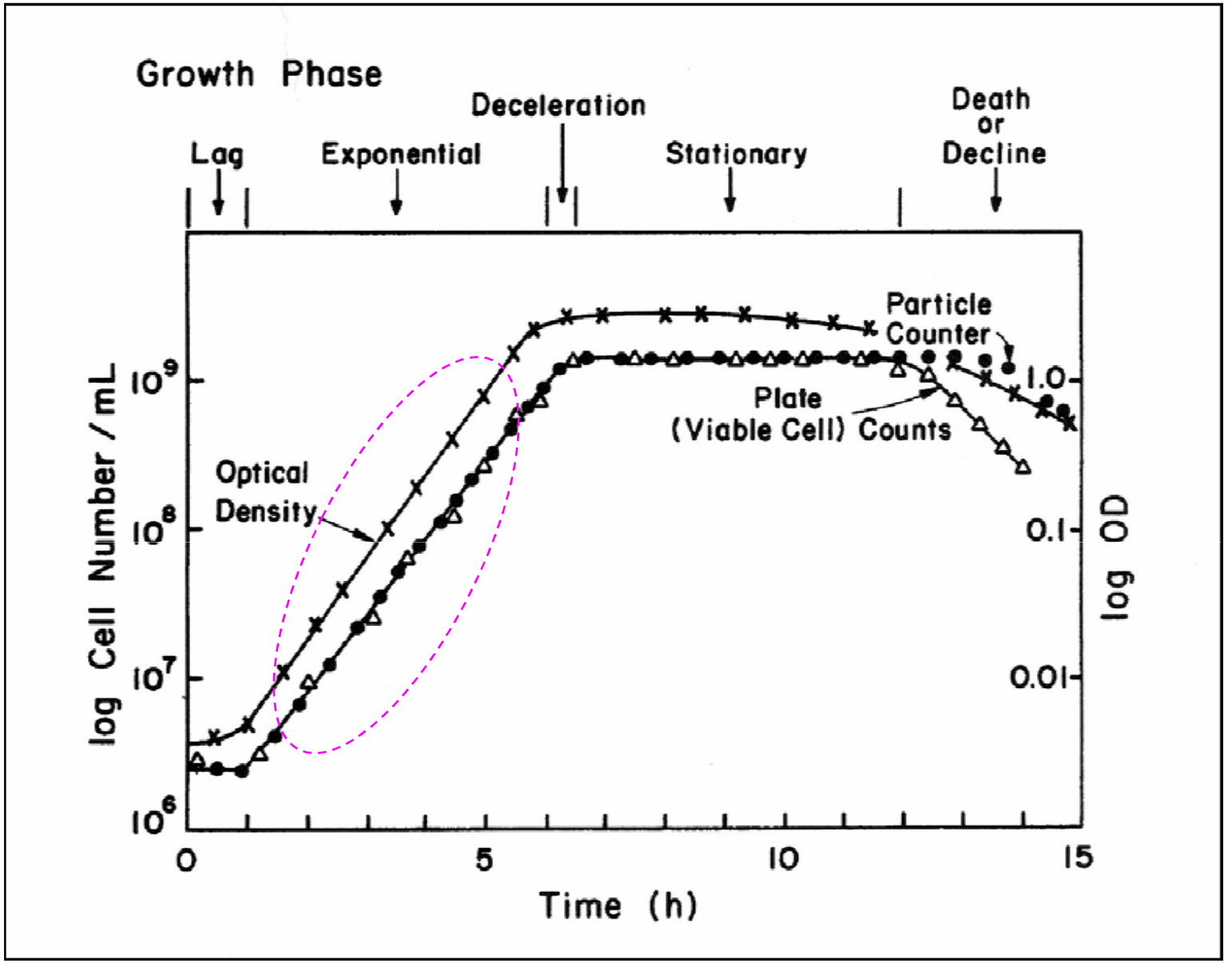
Lag phase

A period of adaptation for the cells to their new environment

- New enzymes are synthesized.
- A slight increase in cell mass and volume, but no increase in cell number
- Prolonged by low inoculum volume, poor inoculum condition (high % of dead cells), age of inoculum, nutrient-poor medium
- Multiple lag phases: (diauxic growth) medium contains more than one carbon source



Typical growth curve for a bacterial population



Typical growth curve for a bacterial population

Batch Growth Kinetics

Exponential growth phase

In this phase, the cells have adjusted to their new environment and multiply rapidly (exponentially)

- Balanced growth –all components of a cell grow at the same rate.
- Growth rate is independent of nutrient concentration, as nutrients are in excess.

Batch Growth Kinetics

Exponential growth phase

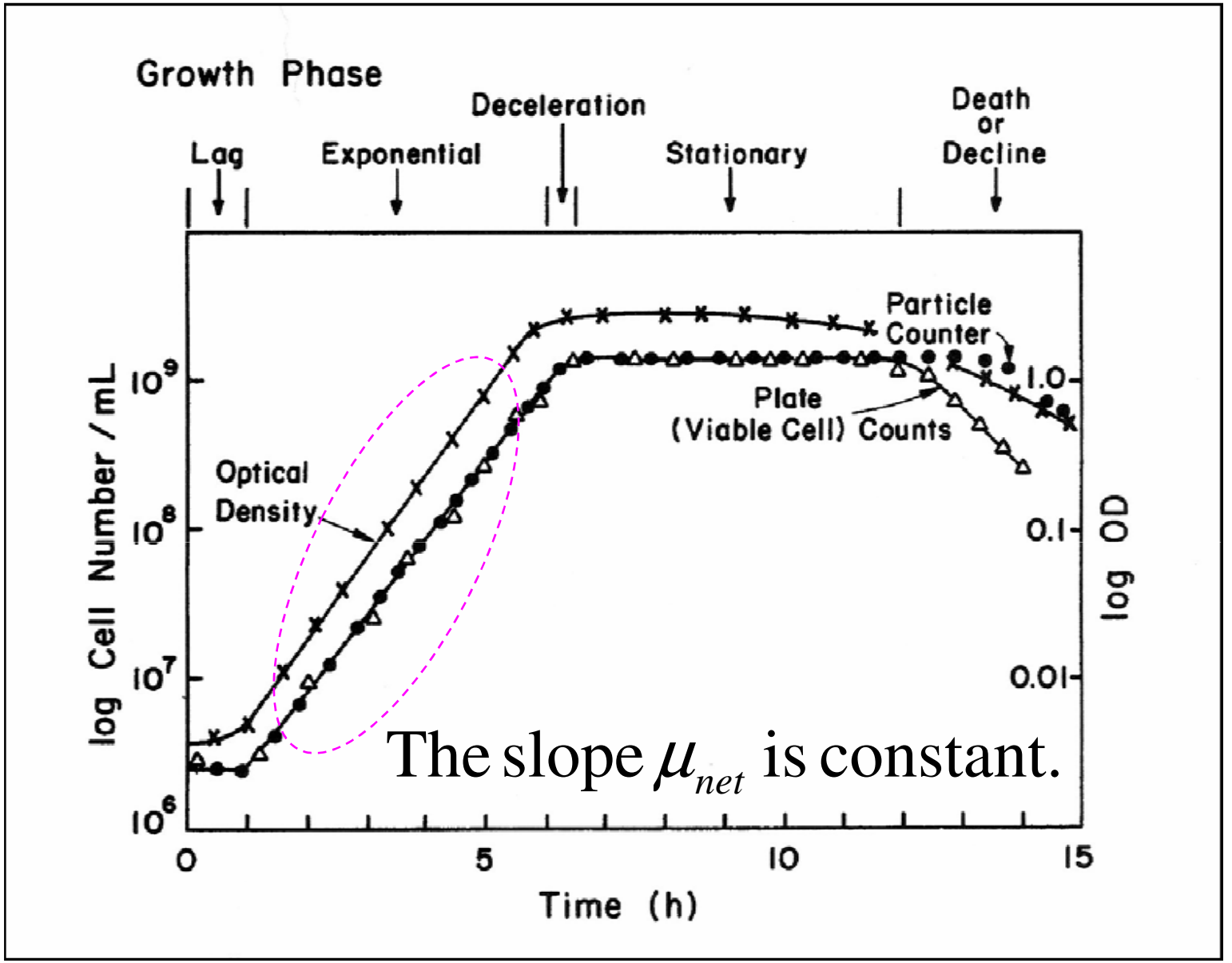
The balance of cell mass in a batch culture gives:

$$\frac{dX}{dt} = \mu_{net} X, \; X = X_0 \;\; at \;\; t = 0$$

Integration of the above equation yields:

$$\ln \frac{X}{X_0} = \mu_{net} t \text{ , or } X = X_0 e^{\mu_{net} t}$$

X and X_0 are cell concentrations at time t and $t = 0$



Typical growth curve for a bacterial population

Batch Growth Kinetics

Exponential growth phase

$$\mu_{net} = \mu_{R} = \mu_{\max}$$

$\mu_{\max}$ is the maximum specific growth rate (1/time)

TABLE 2.1. *Some representative values of μ_{max} (obtained under the conditions specified in the original reference) for a range of organisms*

Organism	μ_{max} (h^{-1})	Reference
Vibrio natriegens	4.24	Eagon (1961)
Methylomonas methanolytica	0.53	Dostalek *et al.* (1972)
Aspergillus nidulans	0.36	Trinci (1969)
Penicillium chrysogenum	0.12	Trinci (1969)
Fusarium graminearum Schwabe	0.28	Trinci (1992)
Plant cells in suspension culture	0.01–0.046	Petersen and Alfermann (1993)
Animal cells	0.01–0.05	Lavery (1990)

Batch Growth Kinetics

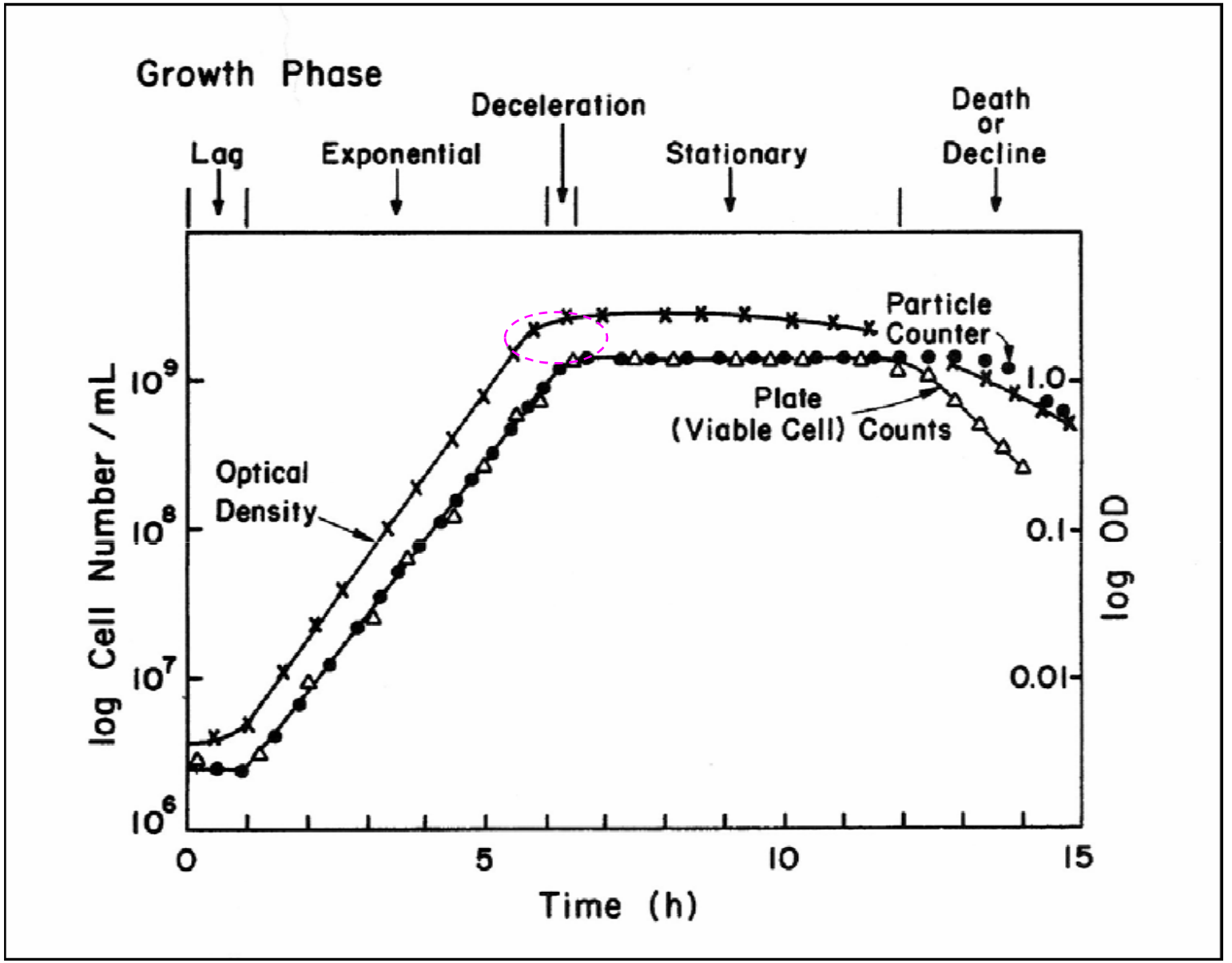
Exponential growth phase

$$\mu_{net} = \mu_{R} = \mu_{\max}$$

$\mu_{\max}$ is the maximum specific growth rate (1/time)

Doubling time of cell mass: the time required to double the microbial mass:

$$\tau_d = \frac{\ln X / X_0}{\mu_{\text{net}}} = \frac{\ln 2}{\mu_{\text{net}}} = \frac{0.693}{\mu_{\text{net}}}$$



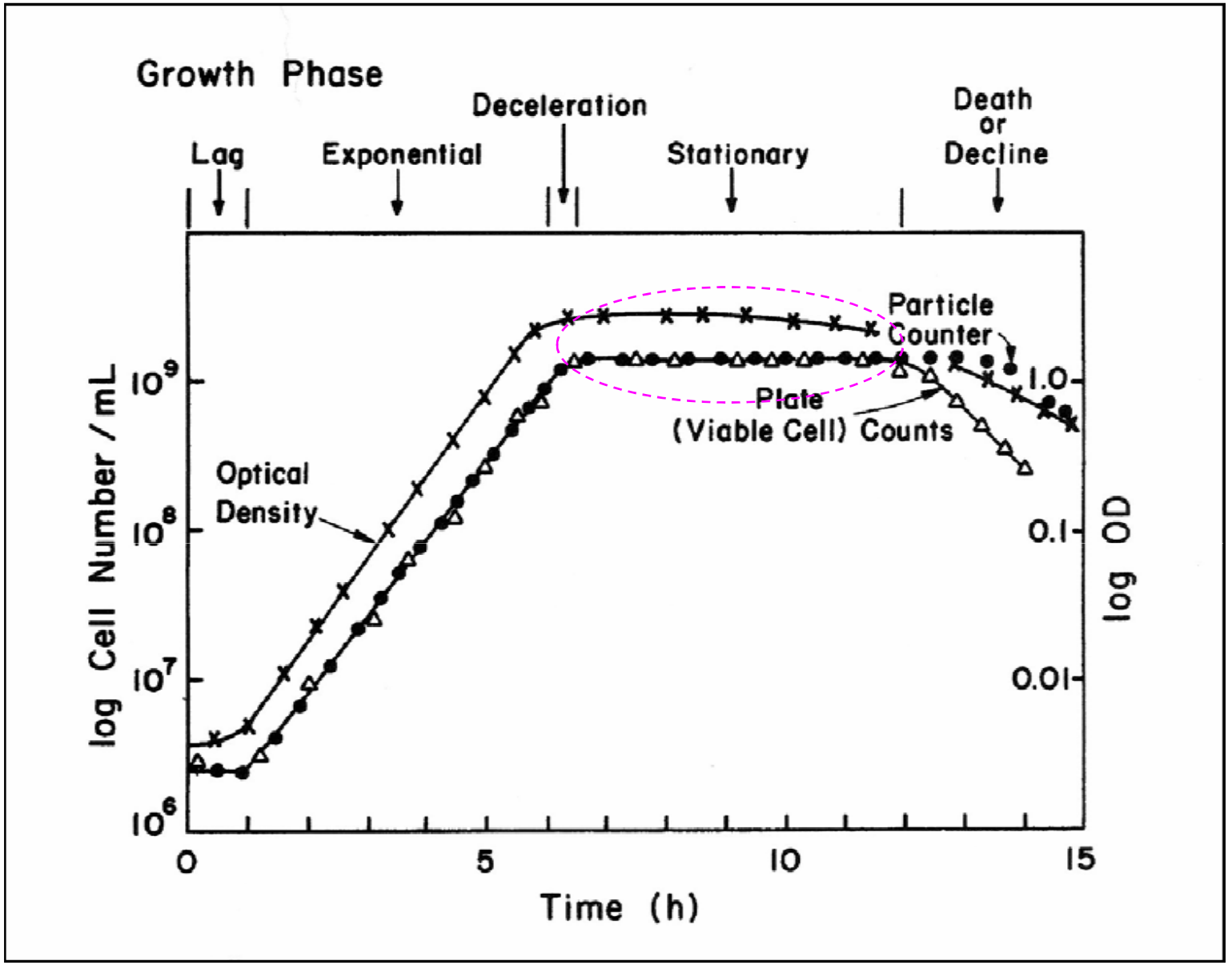
Typical growth curve for a bacterial population

Batch Growth Kinetics

Deceleration growth phase

Very short phase, during which growth decelerates due to either:

- Depletion of one or more essential nutrients
- The accumulation of toxic by-products of growth (e.g. Ethanol in yeast fermentations)
- Period of unbalanced growth: Cells undergo internal restructuring to increase their chances of survival



Typical growth curve for a bacterial population

Batch Growth Kinetics

Stationary Phase:

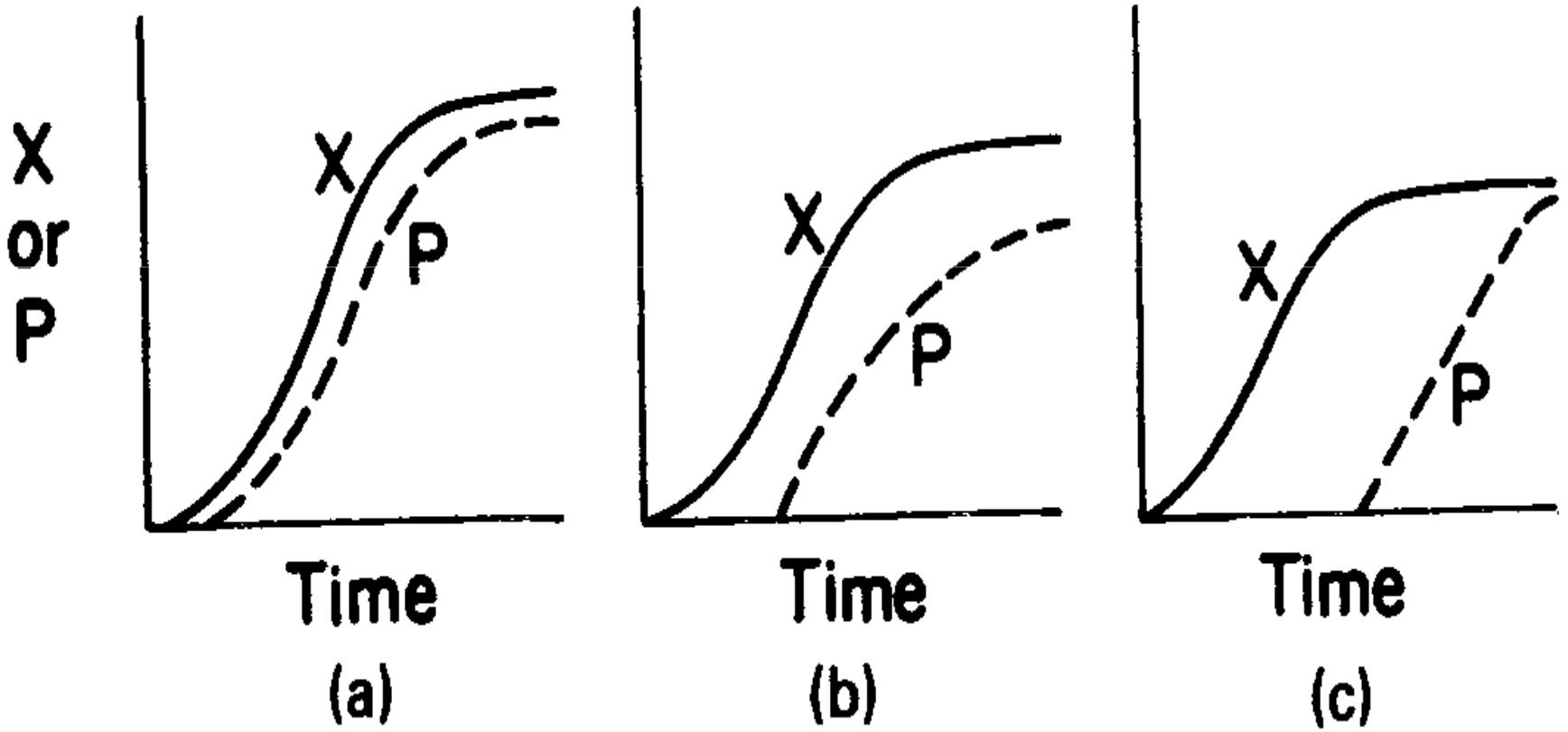
With the exhaustion of nutrients (S≈0) and build-up of waste and secondary metabolic products

- The growth rate equals the death rate.
- There is no net growth in the organism population.
- Cells may have active metabolism to produce secondary metabolites.

  Primary metabolites are growth-related: ethanol by *S. cerevisae.*

  Secondary metabolites are non-growth-related: antibiotics, pigments.

Kinetic Pattern of Growth and Product Formation



Growth-associated Mixed-growth-associated Non growth-associated

Batch Growth Kinetics

Stationary phase

- Cell lysis may occur and viable cell mass may drop.

  A second growth phase may occur and cells may grow on lysis products of lysed cells (cryptic growth)

- Endogenous metabolism occurs by catabolizing cellular reserves for new building blocks and energy-producing monomer (maintenance energy).

  The rate describing the conversion of cell mass into maintenance energy or the loss of cell mass due to cell lysis:

$$\frac{dX}{dt} = -k_d X$$

k_d is the rate constant for endogenous metabolism.

Batch Growth Kinetics

<u>Death Phase</u>:

The living organism population decreases with time, due to a lack of nutrients and toxic metabolic by-products.

The rate of death usually follows:

$$\frac{dN}{dt} = -k_d' N$$

k_d' is the first-order death rate constant.

Batch Growth Kinetics

Yield coefficients: defined based on the amount of consumption of another material.

$$\text{Growth yield}: \quad Y_{X/S} \equiv -\frac{\Delta X}{\Delta S}$$

$$\text{Product yield}: \quad Y_{P/S} \equiv -\frac{\Delta P}{\Delta S}$$

$$\text{Growth yield based on consumption of oxygen}:$$

$$Y_{X/O_2} \equiv -\frac{\Delta X}{\Delta O_2}$$

$$\Delta S = \Delta S_{\substack{\text{assimilation} \\ \text{into biomass}}} + \Delta S_{\substack{\text{assimilation} \\ \text{into an} \\ \text{extracellular} \\ \text{product}}} + \Delta S_{\text{growth energy}} + \Delta S_{\substack{\text{maintenance} \\ \text{energy}}}$$

Batch Growth Kinetics

Yield coefficients: defined based on the amount of consumption of another material.

For most bacteria and yeast:

$$Yx/s = 0.4\text{-}0.6 \text{ g/g glucose}$$

$$Yx/_{O_2} = 0.9 - 1.4 \text{ g/g } O_2$$

At the end of the batch growth period, the measured yields are apparent as of endogenous metabolism occurring, Kd > 0, which changes the metabolic pathways of the substrate.

e.g.

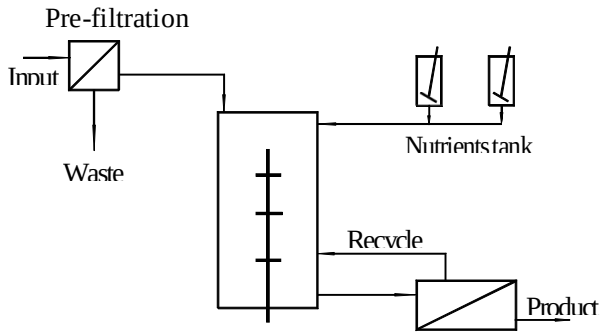
$$Y^{M}_{X/S} > Y^{App}_{X/S}$$

CONTENTS

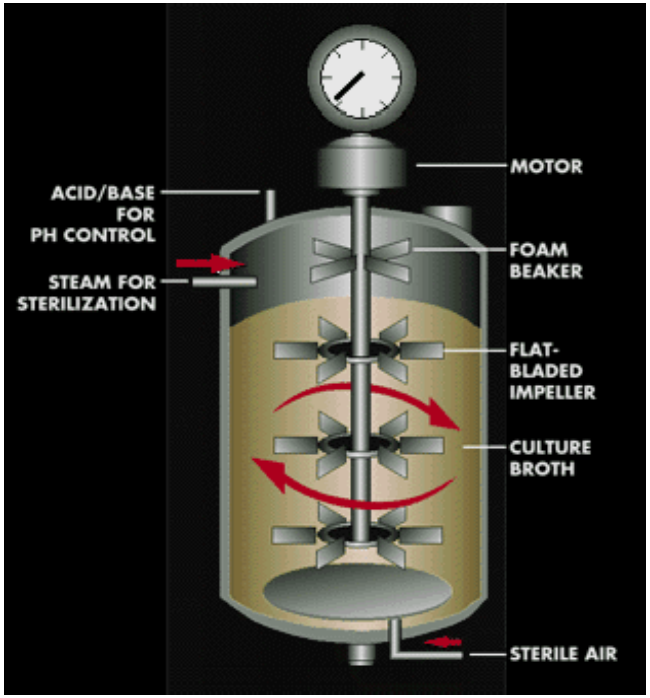
- Batch and Continuous types
- Immobilized whole cell and enzyme reactors
- High performance bioreactors
- Sterile and non-sterile operations
- Reactors in series
- Design of reactors and scale up

BIOREACTORS

- Bioreactor: device, usually a vessel, used to direct the activity of a biological catalyst to achieve a desired chemical transformation
- Fermenter: type of bioreactor in which the biocatalyst is a living cell



Product separation&purification



Bioreactor

- Microbial fermenters:
  - cell concentration increases with the expense of the substrate
  - mixed flow reactor is suitable
- Enzymatic reactors:
  - enzyme concentration does not change
  - non- autocatalytic type
  - plug flow reactor is suitable

- The main requirements any bioreactor should satisfy are:
  - high quality
  - easy to use
  - perfectly sterile
  - precise measurement
  - control and recording of all culture parameters
  - inexpensive

OPERATION MODES OF BIOREACTORS

Operation mode

- Batch
- Continuous
- Modification of the above eg. Fed-batch

Oxygen Supply

ONLY FOR MICROBIAL CELL BIOREACTORS

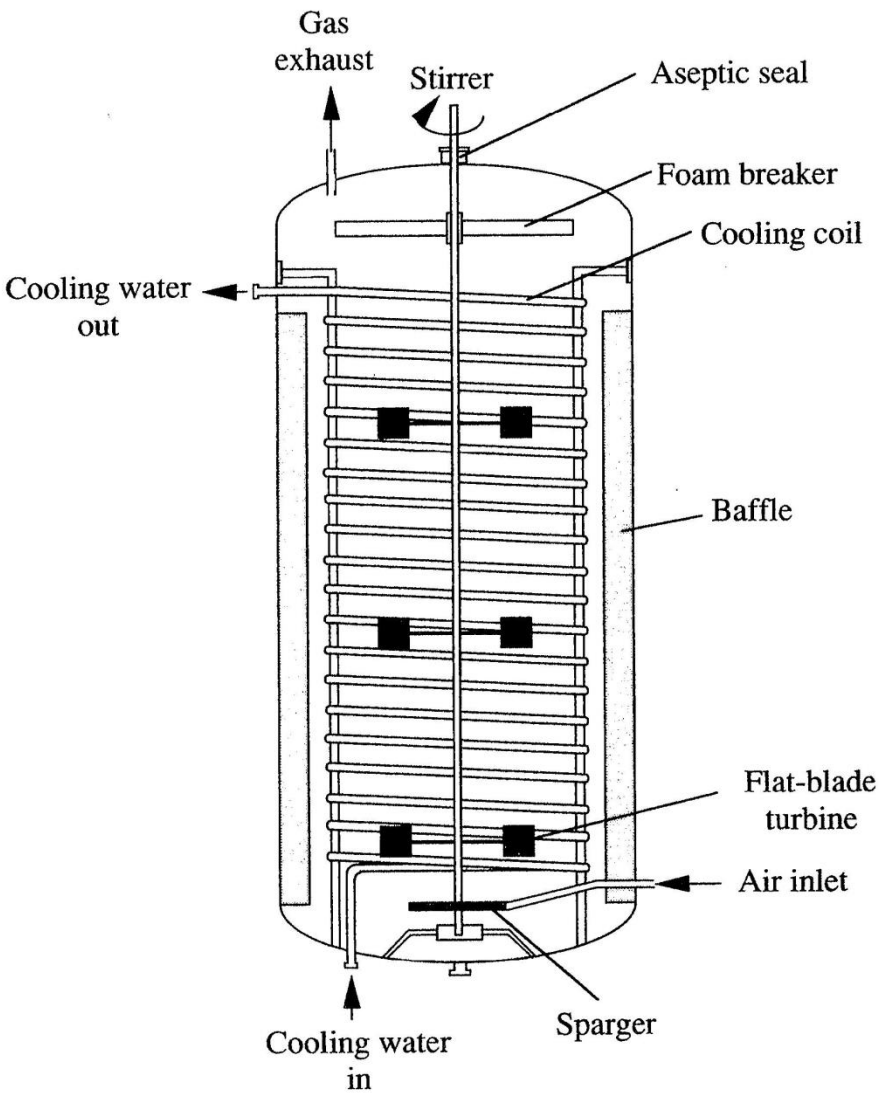
- Aerobic bioreactor: Need adequate mixing and aeration
- Anaerobic bioreactor: no need for sparging or agitation

Form of biocatalyst

- Free or immobilized enzyme
- Free or immobilized microbial cell

BIOREACTORS - CONFIGURATIONS

STIRRED TANK



Mixing method: Mechanical agitation

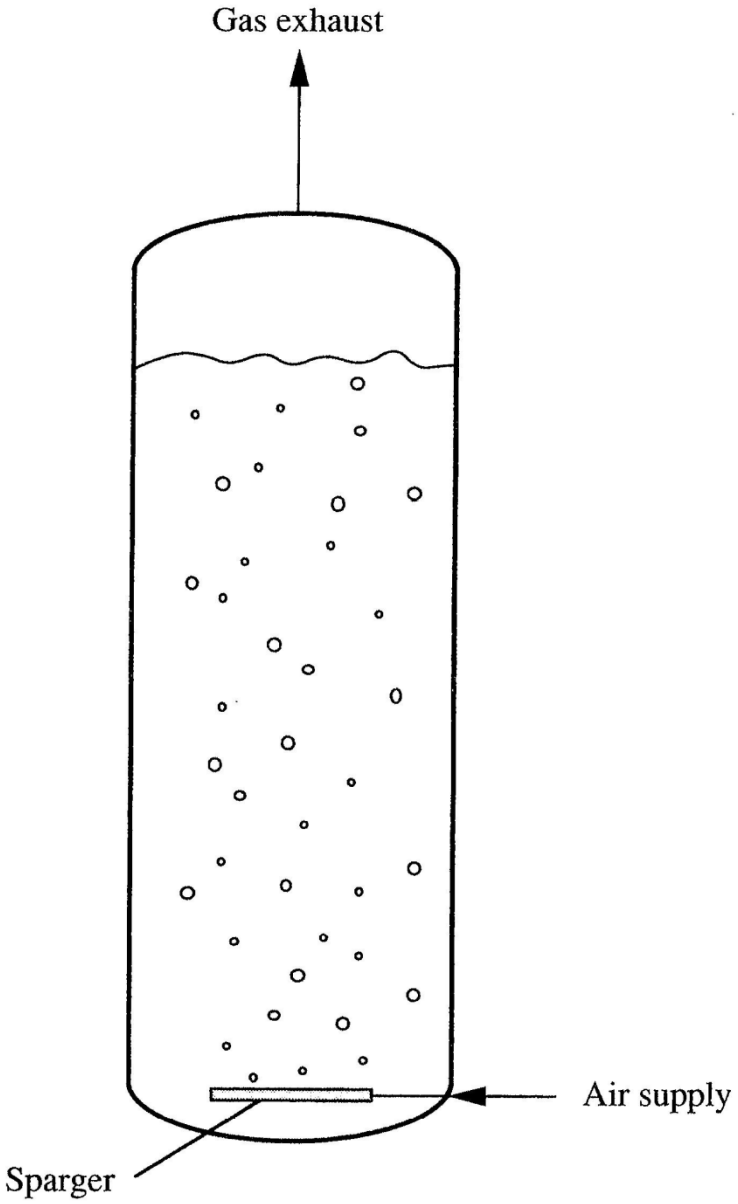
•Baffles are usually used to reduce vortexing

• Applications: free and immobilized enzyme reactions

•High shear forces may damage cells

•Require high energy input

BUBBLE COLUMN

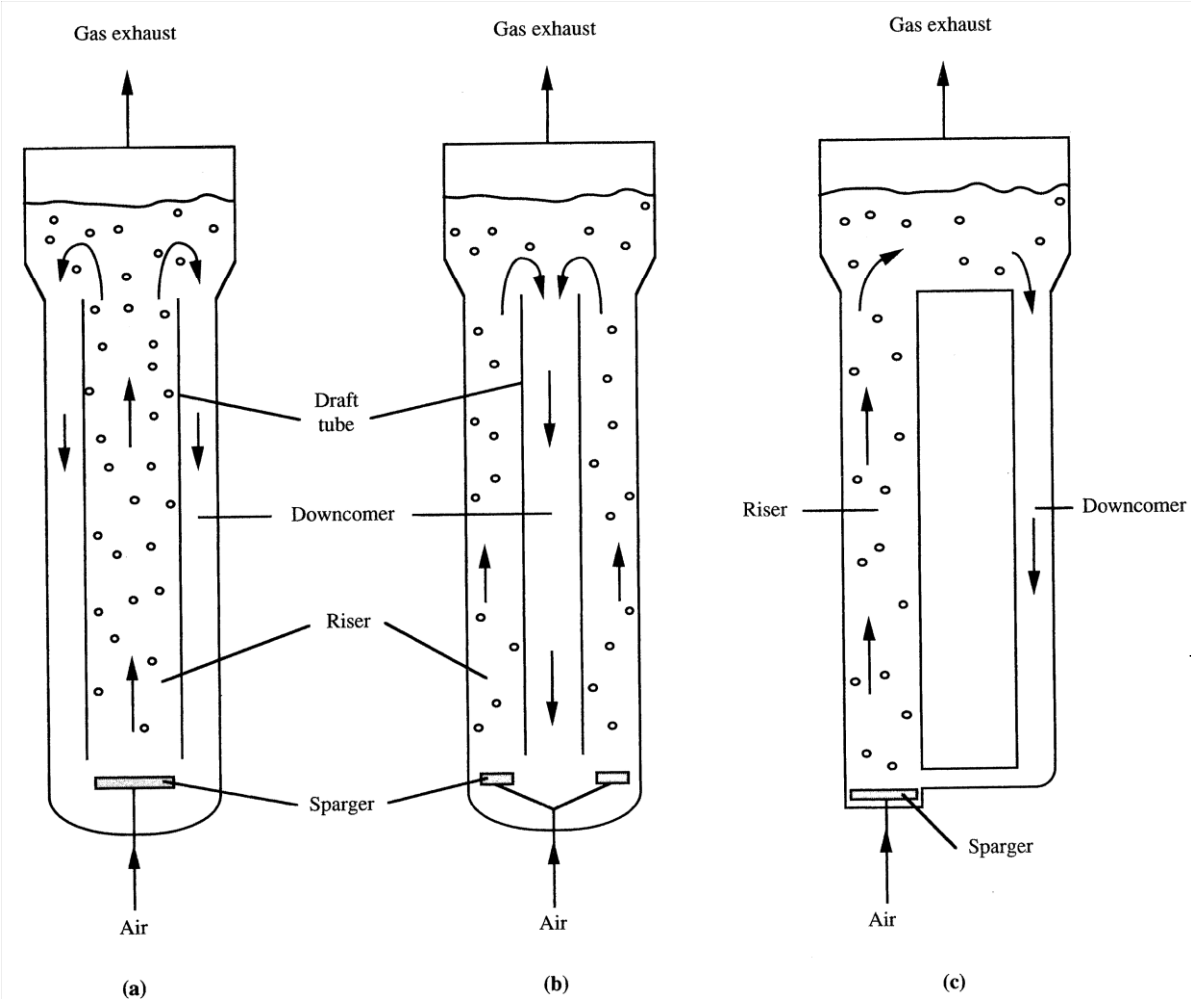


Mixing method: Gas sparging

- Simple design
- Good heat and mass transfer
- Low energy input

Gas-liquid mass transfer coefficients depend largely on bubble diameter and gas hold-up.

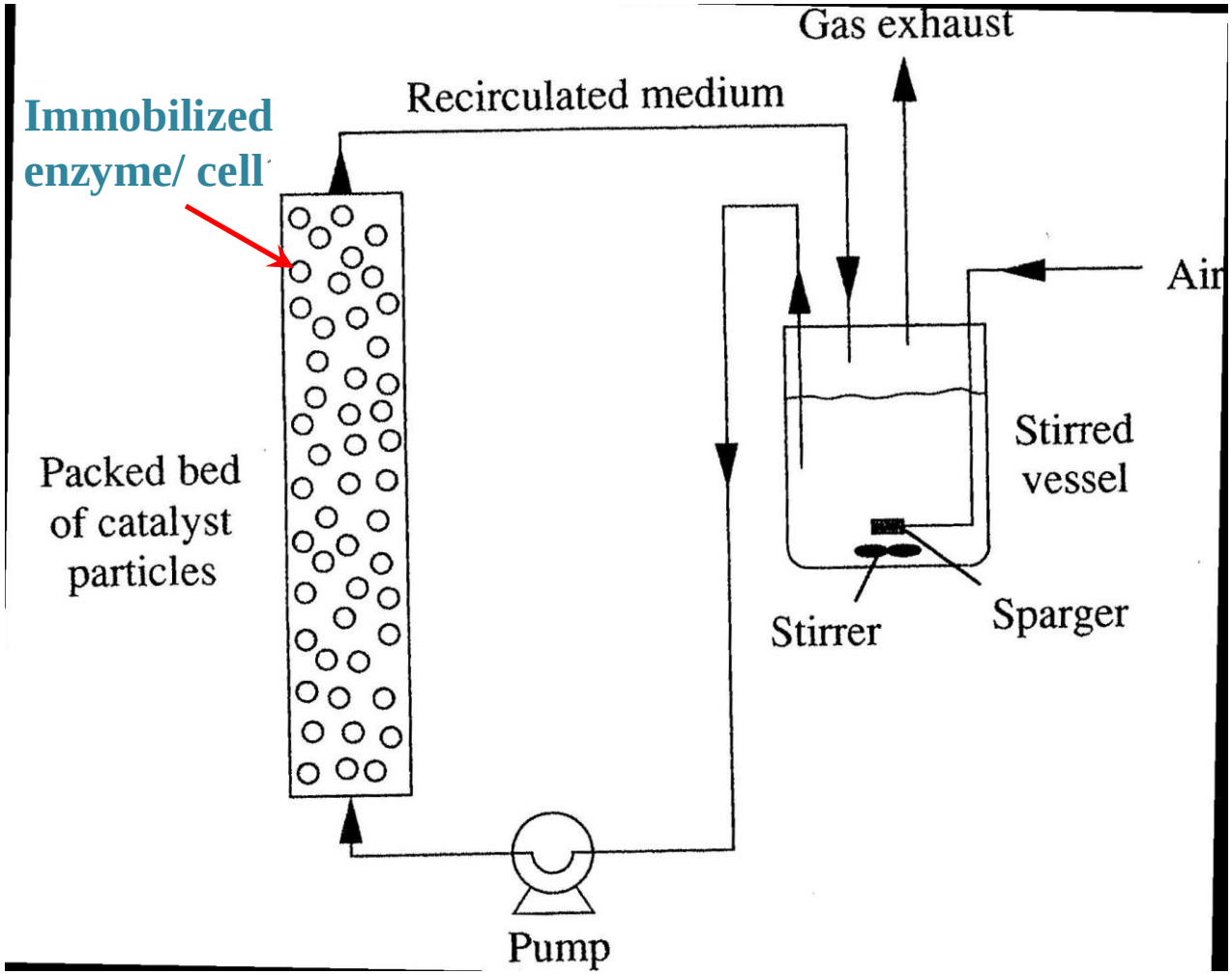
AIRLIFT REACTOR



Mixing method: airlift

- Compared to bubble column reactors, in an airlift reactors, there are two liquid steams
- up-flowing
- down-flowing steams
- Liquid circulates in an airlift reactor as a result of density difference between riser and down comer

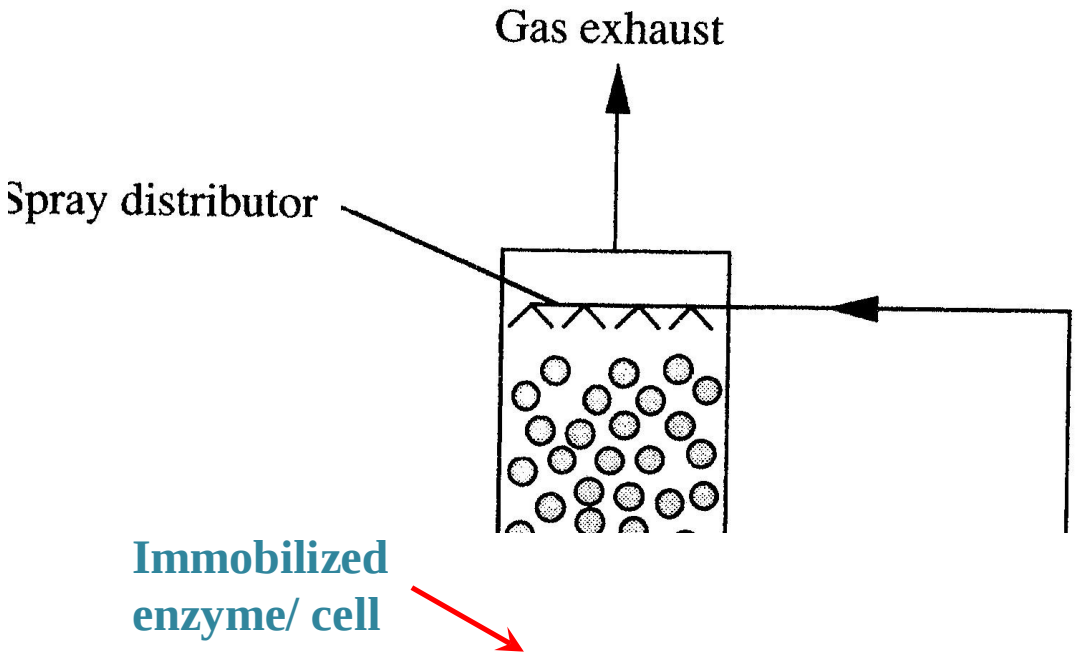
PACKED-BED REACTOR



- Packed-bed reactors are used with immobilized cells or enzyme biocatalysts

- Medium can be fed either at the top or bottom and forms a continuous liquid phase

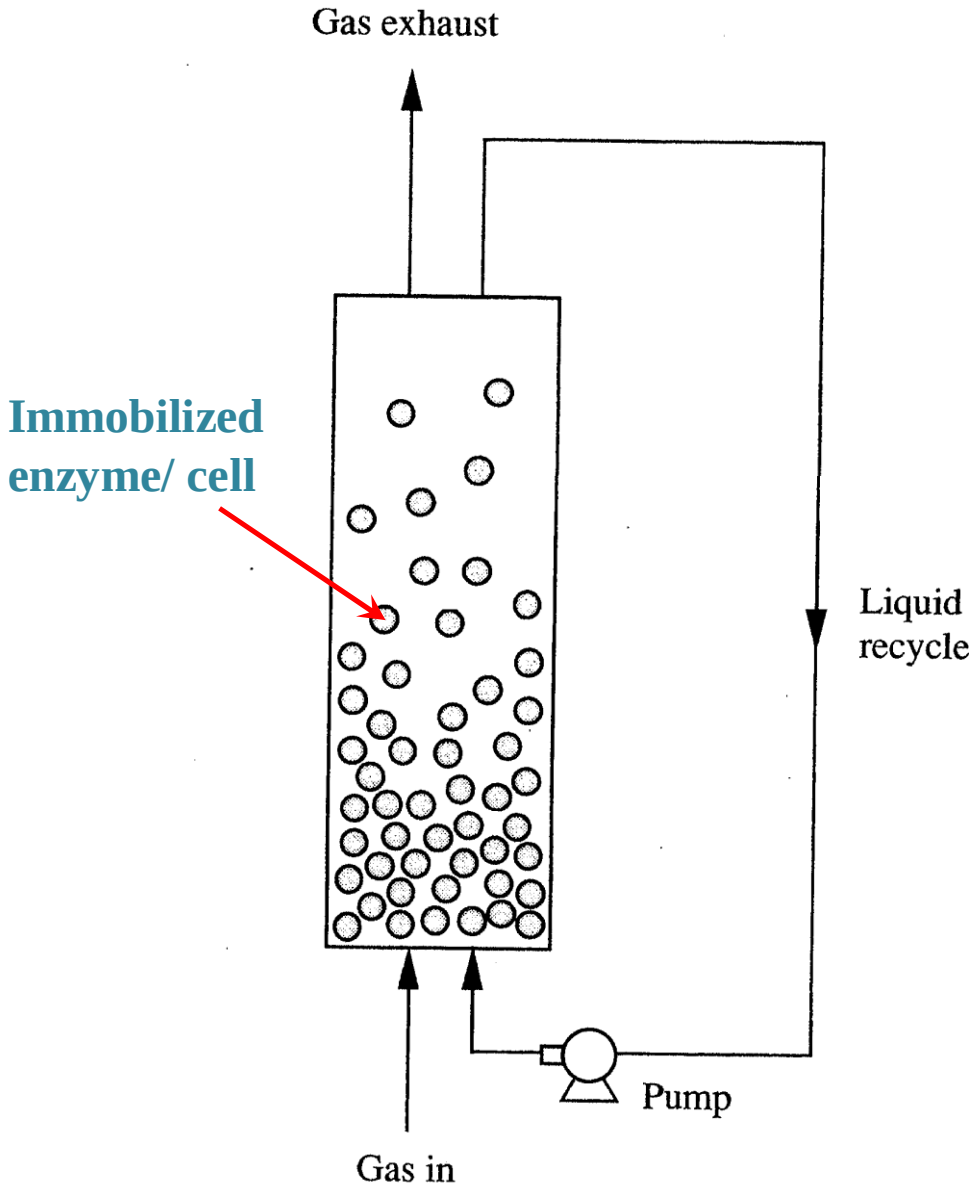
TRICKLE-BED REACTOR



- The trickle-bed reactor is another variation of the packed bed reactors

- Liquid is sprayed onto the top of the packing and trickles down through the bed in small rivulets

FLUIDIZED BED REACTOR

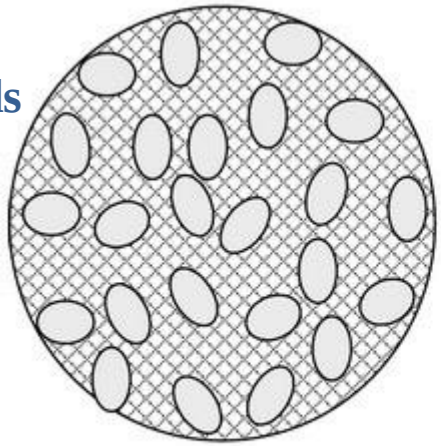


- In up flow mode
- Bed expands at high liquid flow rates
- Upward motion of the immobilized enzymes or cells

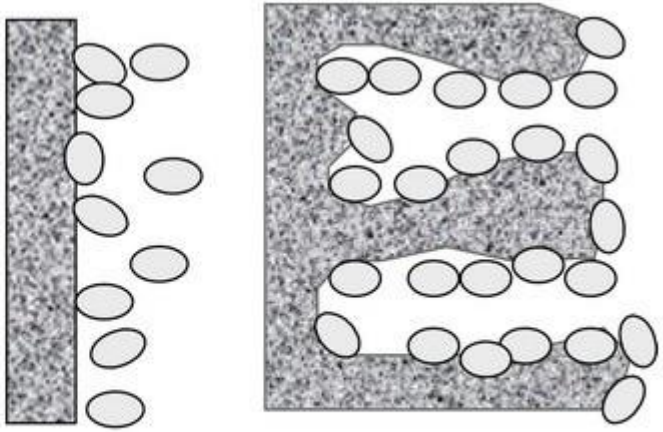
CELL IMMOBILIZATION TECHNIQUES

[Adsorption or covalent bonding]

Entrapment of cells within spheres of Ca^{2+} alginate



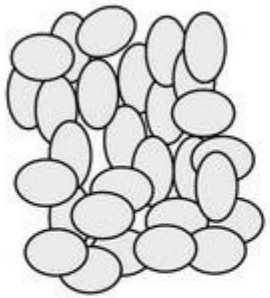
(I) Entrapment within a porous matrix



(a) (b)

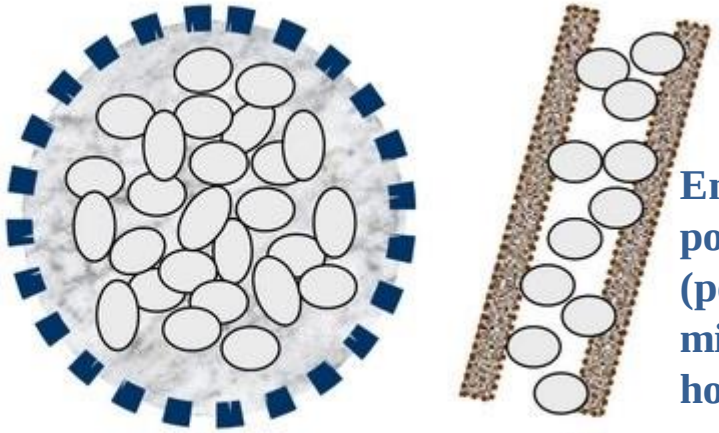
(II) Attachment or adsorption on solid carrier

Support materials:
Porous glass, cotton fiber, gelatin, titanium oxide, diethylaminoethyl cellulose



Aggregation: Cells clump together and form aggregates or cross linking is induced by glutaraldehyde.

(III) Self-aggregation



(a) (b)

(IV) Mechanical containment behind a barrier

Encapsulation in polymer membranes (polyester) or microspheres (PVA) or hollow fibers (PVC)

CELL IMMOBILIZATION TECHNIQUES

Method	Support Material	Cells	Reaction
Adsorption	Gelatin	*Lactobacilli*	Lactose ⟹ lactic acid
	Porous glass	*Saccharomyces*	Glucose ⟹ ethanol
	Cotton fibers	*Zymomonas*	Glucose ⟹ ethanol
	DEAE Cellulose	*Nocardia*	Steroid conversion
Covalent bonding	Cellulose + cyanuric chloride	*S. cerevisiae*	Glucose ⟹ ethanol
	Titanium oxide	*Acetobacter*	Vinegar
Cross linking	Glutaraldehyde	*E. coli*	Fumaric acid
Entrapment	Aluminium alginate	*Candida tropicalis*	Phenol degradation
	Calcium alginate	*S. cervisiae*	Glucose ⟹ ethanol
Encapsulation	Polyester	*Streptomyces sps.*	Glucose ⟹ fructose
	Alginate polylysine	Hybridoma cells	Monoclonal antibodies

Ideal bioreactor

- Low mixing time
- High gas-liquid mass transfer
- Equipped with in-situ sensors and direct digital control (DDC) system

High performance bioreactor :

- distinguish between the dynamics of the cell reaction and the dynamics of the environment
- minimize the influence of the bioreactor dynamics on the overall performance of the process by control of the environmental conditions

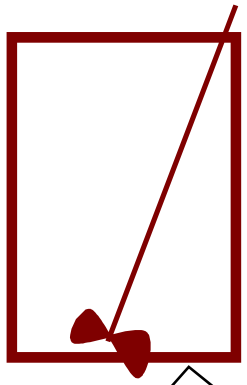
BIOREACTOR OPERATION MODES

IMPORTANT

Reactor type/mode of operation	Advantages	Disadvantages
Stirred tank reactors		
1. Steady state (CSTR)	(a) Large-scale production of cheap products STR, *especially CSTR*, is by far the best due to low capital and labor costs (b) CSTR (or fed-batch) needed when production of the desired product is catabolite repressed (c) Due to autocatalytic nature of microbial reactions, *productivity* is high (d) Product quality is constant	(a) Infection is a risk, e.g., caused by a short stop of the continuous feed sterilization by steam (b) The strain may mutate to a nonproducing strain after long production time (c) Downstream equipment can be difficult to operate in the continuous mode (d) Very inflexible
2. Batch operation	(a) Easily switched between different production duties with low retrofitting costs (b) Can be properly sterilized. Small risk of infection and mutation (short production time)	(a) High labor costs (b) Much idle time for sterilization, outgrowth of inoculum, and cleaning (c) Safety problems when filling and emptying reactor
3. Fed-batch operation	(a) Same advantages as CSTR (a and b) (b) The production time is limited with smaller risk of mutations	(a) More labor cost than CSTR (b) Large volume to be downstream processed between runs. Holding tanks used
Plug flow reactor		
1. Steady state	(a) Very high conversion of the substrate can be obtained (b) Fixed-bed operation (immobilized enzymes or cells). Film reactors (c) High conversion of gas-phase substrates (loop-reactors)	(a) Requires cells in feed and it can only be used *after* another reactor (b) The large difference in holding time between gas and liquid prevents the use of a single-pass PFR

BATCH OPERATION - ENZYME

- A batch bioreactor is normally equipped with an agitator to mix the reactant
- pH of the reactant is maintained by employing either buffer solution or a pH controller



$$r = \frac{dC_s}{dt} = \frac{r_{\max} C_S}{K_m + C_S}$$

Batch operation with stirring

Change of Cs with time, t

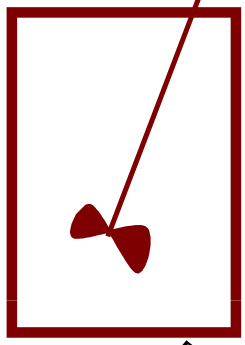
$$K_m \ln \frac{C_{s0}}{C_s} + \left(C_{s0} - C_s \right) = r_{\max} t$$

BATCH OPERATION - ENZYME

- A batch bioreactor is normally equipped with an agitator to mix the reactant

- pH of the reactant is maintained by employing either buffer solution or a pH controller

- A foam breaker may be installed to disperse foam



$$r_s = \frac{dC_s}{dt} = \frac{r_{max} C_s}{K_m + C_S}$$

Batch operation with stirring

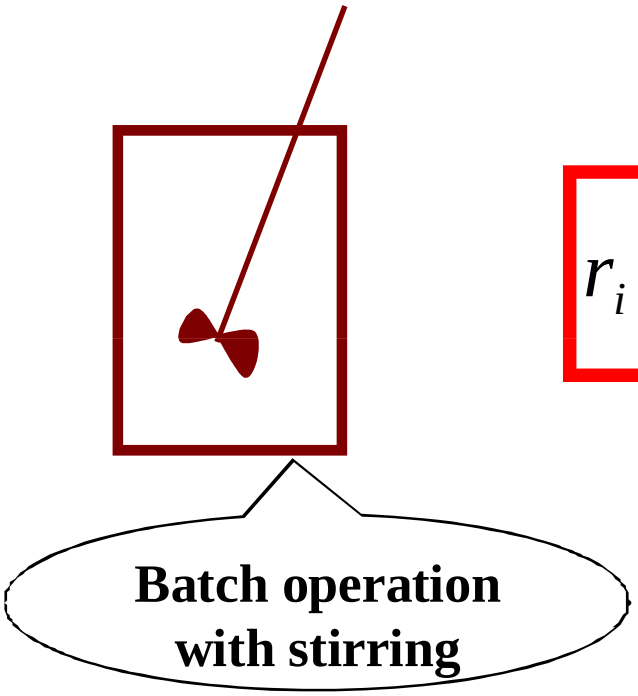
$$K_m \ln \frac{C_{s0}}{C_s} + (C_{s0} - C_s) = r_{max} t$$

Change of C_s with time, t

BATCH OPERATION - FERMENTER

- A batch bioreactor is normally equipped with an agitator to mix the reactant
- pH of the reactant is maintained by employing either buffer solution or a pH controller

- A foam breaker may be installed to disperse foam

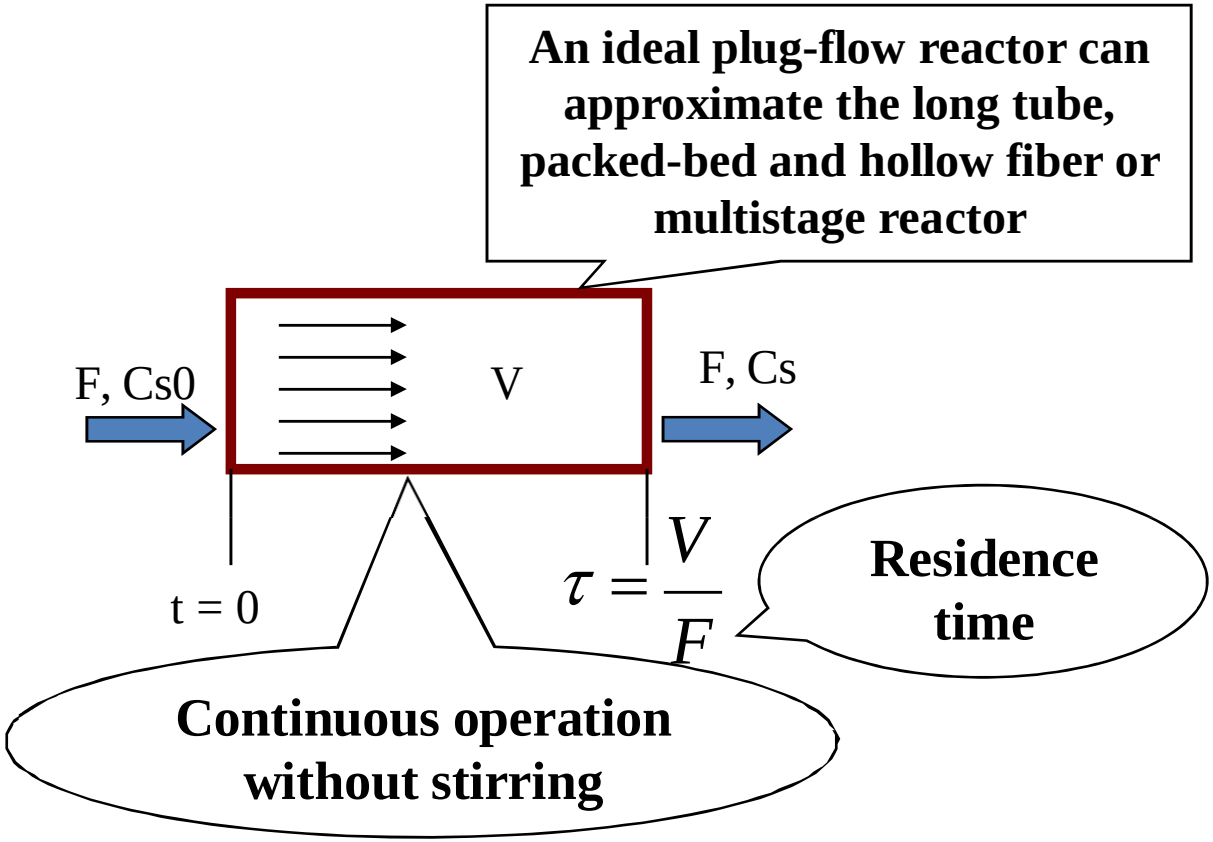


$$r_i = \frac{dC_i}{dt}$$

r_i depends on what?

PLUG-FLOW MODE

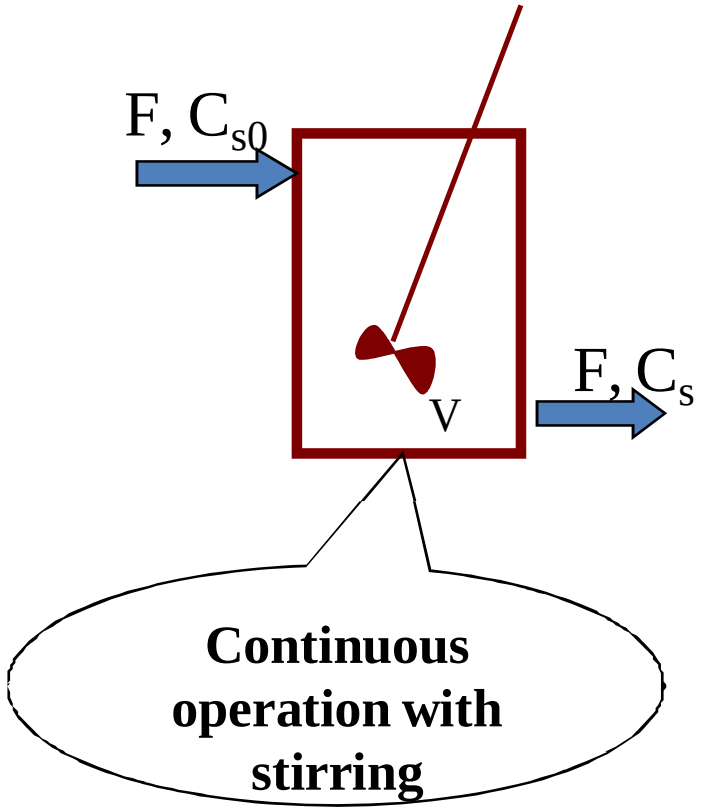
- Substrate enters one end of a cylindrical tube
- Packed with immobilized enzyme
- Product stream leaves at the other end



$$K_m \ln \frac{C_{s0}}{C_s} + \left(C_{s0} - C_s\right) = r_{\max} t$$

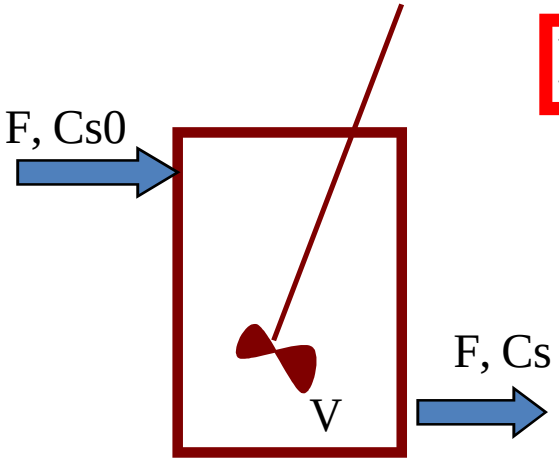
CONTINUOUS STIRRED-TANK - CHEMOSTAT

A continuous stirred-tank reactor (CSTR) is an ideal reactor which is based on the assumption that the reactants are well mixed



CSTR- Enzyme bioreactor

Mass balance of substrate:



Input - Output – Consumption = Accumulation

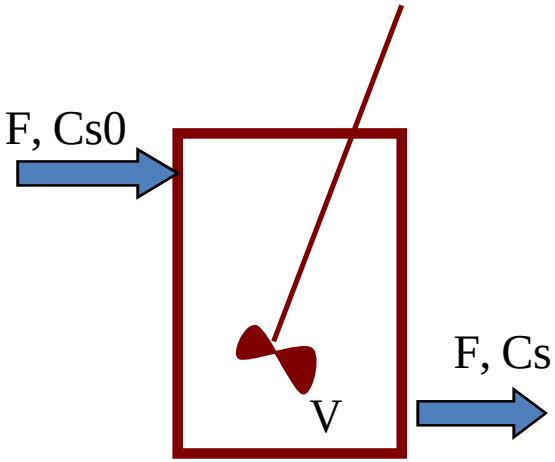
$$FC_{s0} - FC_s - rV = V\frac{dC_s}{dt}$$

Steady state: $\frac{dC_s}{dt} = 0$

Michaelis-Menten rate: $r = \frac{r_{\max} C_S}{K_m + C_S}$

$$FC_{s0} - FC_s - V\frac{r_{\max} C_s}{K_m + C_s} = 0$$

Mass balance of substrate:



$$FC_{s0} - FC_s + V\frac{r_{\max}C_s}{K_m + C_s} = 0$$

$$\frac{F}{V} = \frac{r_{\max}C_s}{(C_{s0} - C_s)(K_m + C_s)}$$

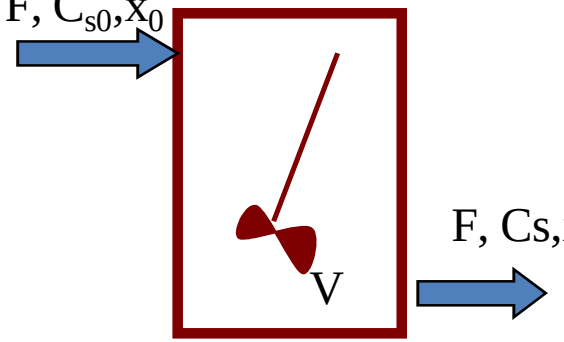
$$\frac{F}{V} = \frac{1}{\tau}$$

$$C_s = -K_m + \frac{r_{\max}C_s\tau}{C_{s0} - C_s}$$

CSTR- Microbial cell bioreactor/ Fermenter

Mass balance of biomass (X):

Input - Output +Formation – Consumption = Accumulation



$$FX_0 - FX + r_X V = V\frac{dX}{dt}$$

Steady state: $\frac{dX}{dt} = 0$

Feed is sterile: $X_0 = 0$

From definition of specific growth rate $r_X = \mu_{net} X$

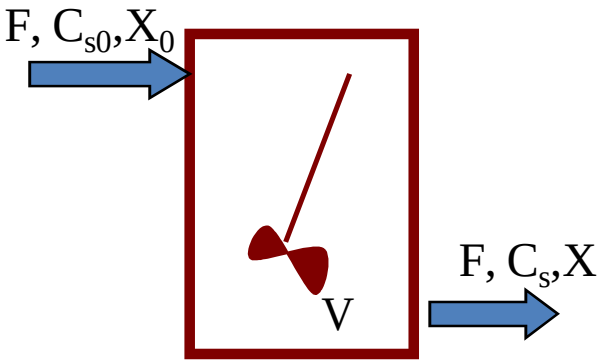
since, $\frac{F}{V} = \frac{1}{\tau} = D$

$$-\frac{F}{V}X + \mu_{net}X = 0$$

$$\Rightarrow \mu_{net} = D$$

CSTR- Microbial cell bioreactor/ Fermenter

Substrate concentration (C_S)



At steady state:

$$\mu_{net} = D$$

According to Monod model, in exponential growth phase

$$\mu_{net} = \mu_g = \frac{\mu_{\max} C_s}{K_s + C_s}$$

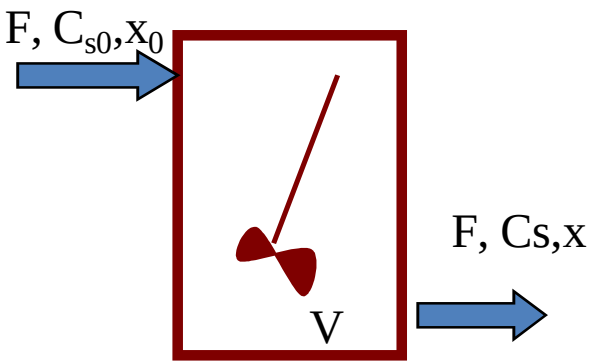
$$\frac{\mu_{\max} C_s}{K_s + C_s} = D$$

$$\Rightarrow C_s = \frac{DK_s}{\mu_{\max} - D}$$

since, $\frac{F}{V} = \frac{1}{\tau} = D$

CSTR- Microbial cell bioreactor/ Fermenter

Biomass concentration (X)



From the definition of $Y_{X/S}$:

$$Y_{X/S} = \frac{\Delta X}{\Delta C_s} = \frac{X - X_0}{C_{s0} - C_s}$$

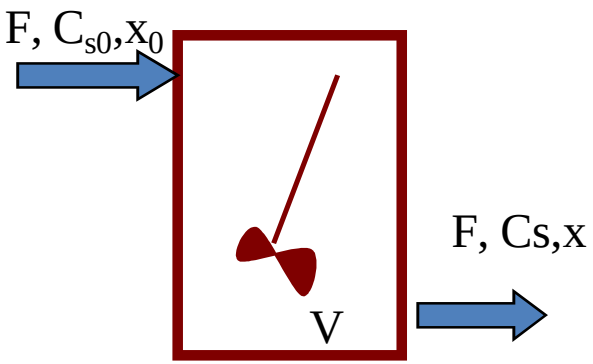
since, $C_s = \dfrac{DK_s}{\mu_{max} - D}$

& $X_0 = 0$ (sterile feed)

$$X = Y_{X/S}\left(C_{s0} - \frac{DK_s}{\mu_{max} - D}\right)$$

CSTR- Microbial cell bioreactor/ Fermenter

Biomass concentration (X)



From the definition of $Y_{X/S}$:

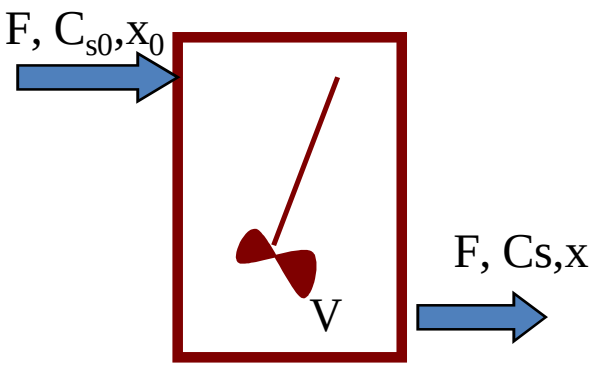
$$Y_{X/S} = \frac{\Delta X}{\Delta C_s} = \frac{X - X_0}{C_{s0} - C_s}$$

since, $C_s = \dfrac{DK_s}{\mu_{max} - D}$

& $X_0 = 0$ (sterile feed)

$$X = Y_{X/S}\left(C_{s0} - \frac{DK_s}{\mu_{max} - D}\right)$$

CSTR- Microbial cell bioreactor/ Fermenter



Steady state biomass concentration :

$$X = Y_{X/S}\left(C_{s0} - \frac{DK_s}{\mu_{\max} - D}\right)$$

Case 1: *Washout*

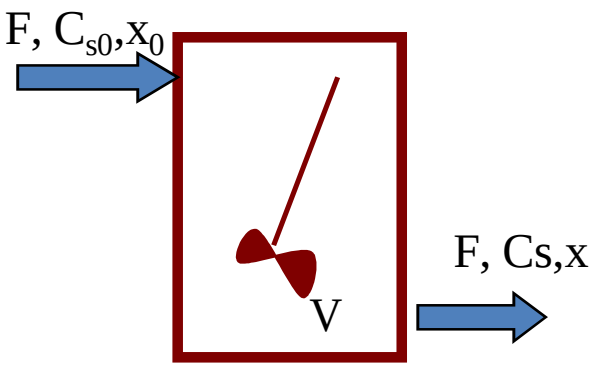
Flow rate (F) at which X=0,

i.e. all the biomass leaves the reactor,

$$\Rightarrow \left(C_{s0} - \frac{DK_s}{\mu_{\max} - D}\right) = 0$$

$$D_{Washout} = \frac{F_{Washout}}{V} = \frac{\mu_{\max} C_{s0}}{C_{s0} + K_s}$$

CSTR- Microbial cell bioreactor/ Fermenter



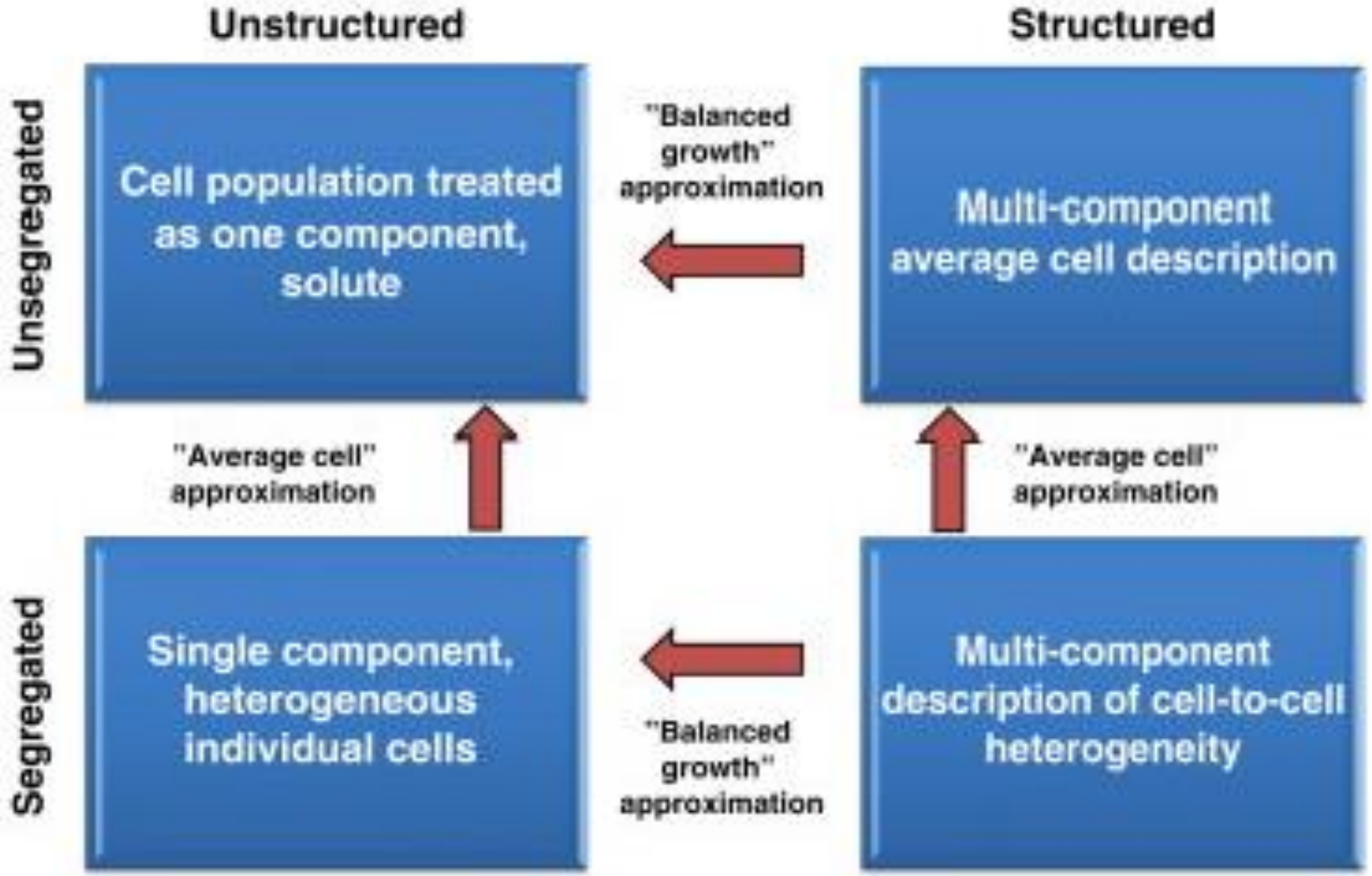
Steady state biomass concentration :

$$X = Y_{X/S}\left(C_{s0} - \frac{DK_s}{\mu_{\max} - D}\right)$$

Case 2 : Optimal flow rate for max. *biomass production*

Flow rate at which $\frac{d(DX)}{dD} = 0,$

$$D_{opt} = \frac{F_{opt}}{V} = \mu_{\max}\left(1 - \sqrt{\frac{K_s}{K_s + C_{s0}}}\right)$$


Unstructured
Structured
Unsegregated
Segregated
Cell population treated as one component, solute
Multi-component average cell description
Single component, heterogeneous individual cells
Multi-component description of cell-to-cell heterogeneity
"Balanced growth" approximation
"Balanced growth" approximation
"Average cell" approximation
"Average cell" approximation

References:

1. Bioprocess Engineering (Basic Concepts) by M. L.Shuler& F.Kargi,Prentice Hall of India, 2003
2. Biochemical Engineering Fundamentals by J.E.Bailey & D. F. Ollis, McGraw Hill Book Company, 1986.
3. Chemical, Biochemical, and Engineering Thermodynamics by Stanley I. Sandler
4. Bioprocess Engineering Principles by Pauline M. Doran

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DEPARTMENT OF CHEMICAL ENGINEERING

BIOCHEMICAL ENGINEERING

UNIT – 4 MIXING AND MASS TRANSFER – SCHA 1601

Unit 4

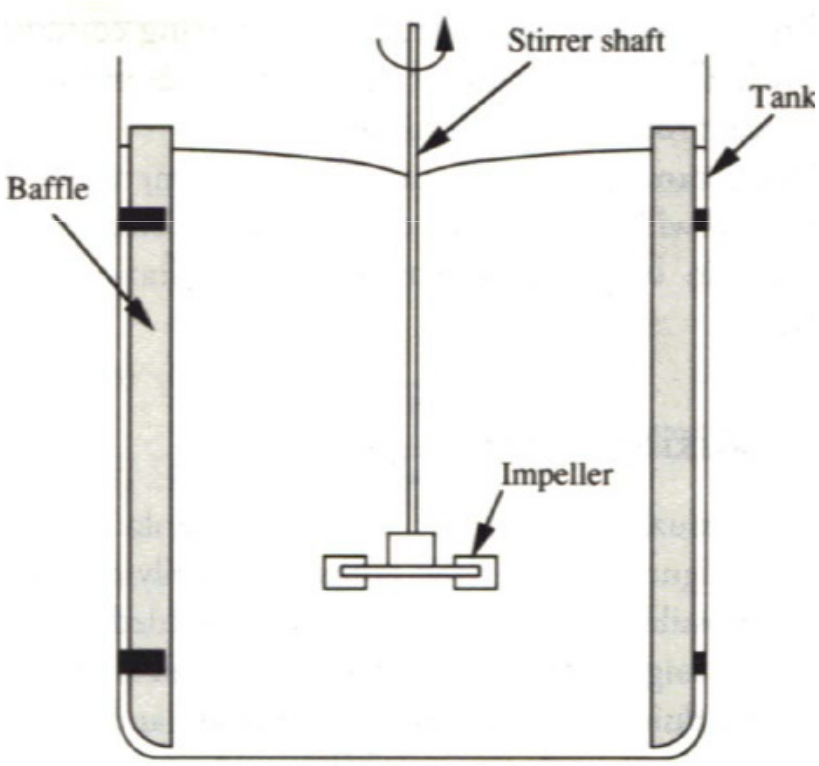
Transportation in microbial systems

Agitation

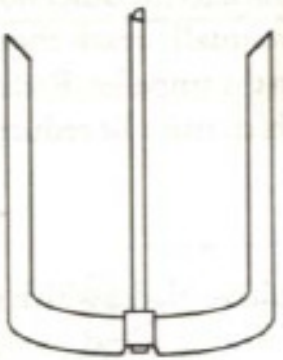
- ***Mixing, a physical process which aims at reducing non-uniformities in fluids by eliminating gradients of concentration and temperature in a bioreactor.***

- ***In a very large extend, it decides the performance of a bioreactor.***

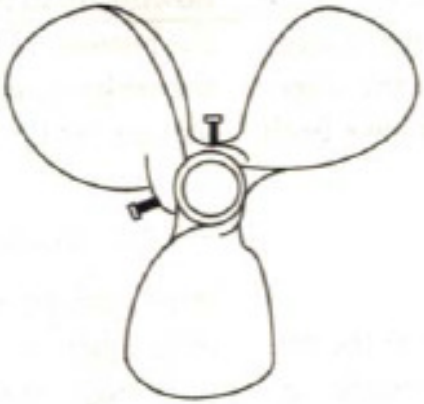
- ***Biochemical reactions are multiphasic involving gas (oxygen)-liquid (nutrient medium) –solid(microbial cells) and efficient mixing improves the contact of substrate, oxygen and other nutrients to cells during cell culture.***



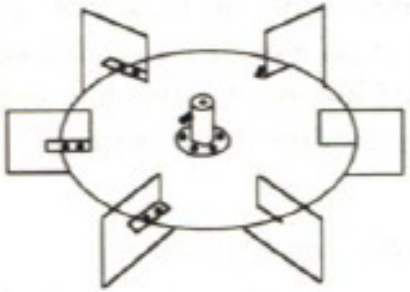
Agitation - Impellers



Anchor

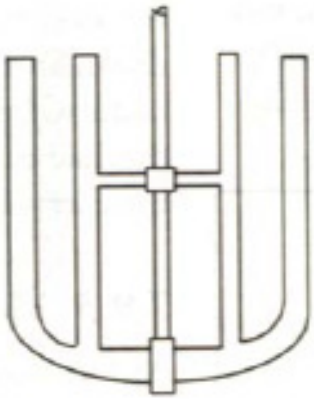


Propeller



6-flat-blade disc-turbine

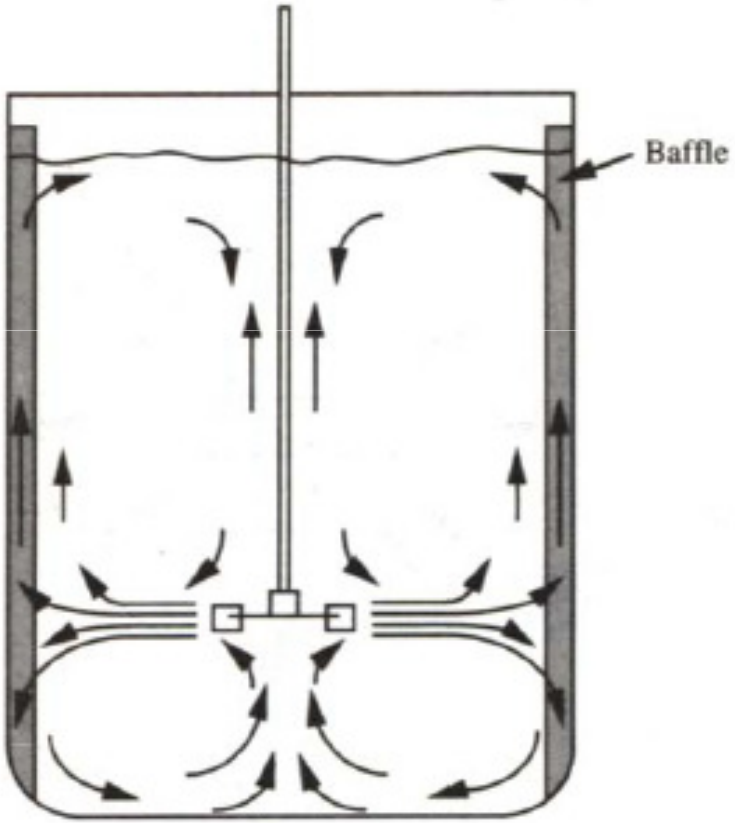
Paddle



Gate anchor

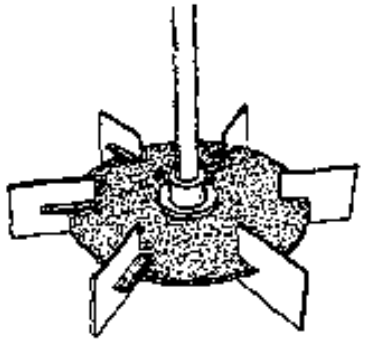
Helical screw

Agitation – Radial flow impellers

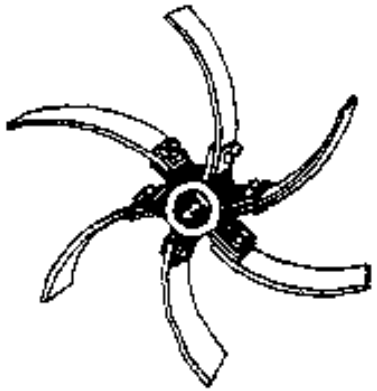


- **Radial flow impellers impose essentially shear stress to the fluid and are suitable for mixing of very viscous fluids.**

- **Radial flow impellers should be used in situations where high shear rates are needed, such as in dispersion processes**

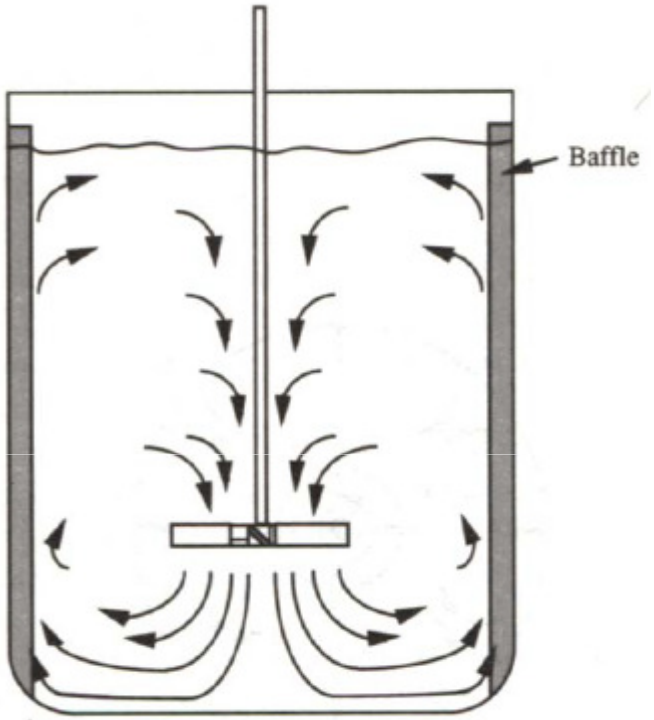


Disk Style Flat Blade Turbine
Commonly Referred to as
the Rushton Impeller

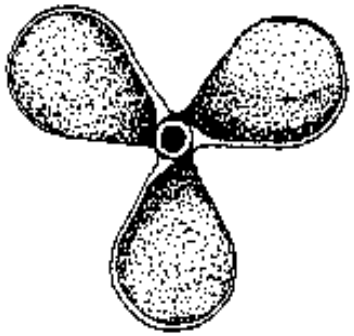


Sweptback or Curved Blade Turbine
(a Spiral Turbine)

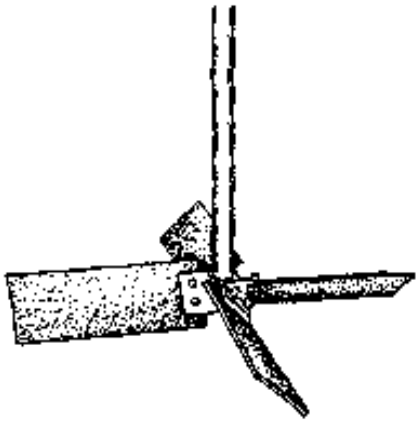
Agitation – Axial flow Impellers



Axial flow impellers are very useful in mixing solid-liquid suspensions because they prevent the solid particles from settling at the bottom of the tank



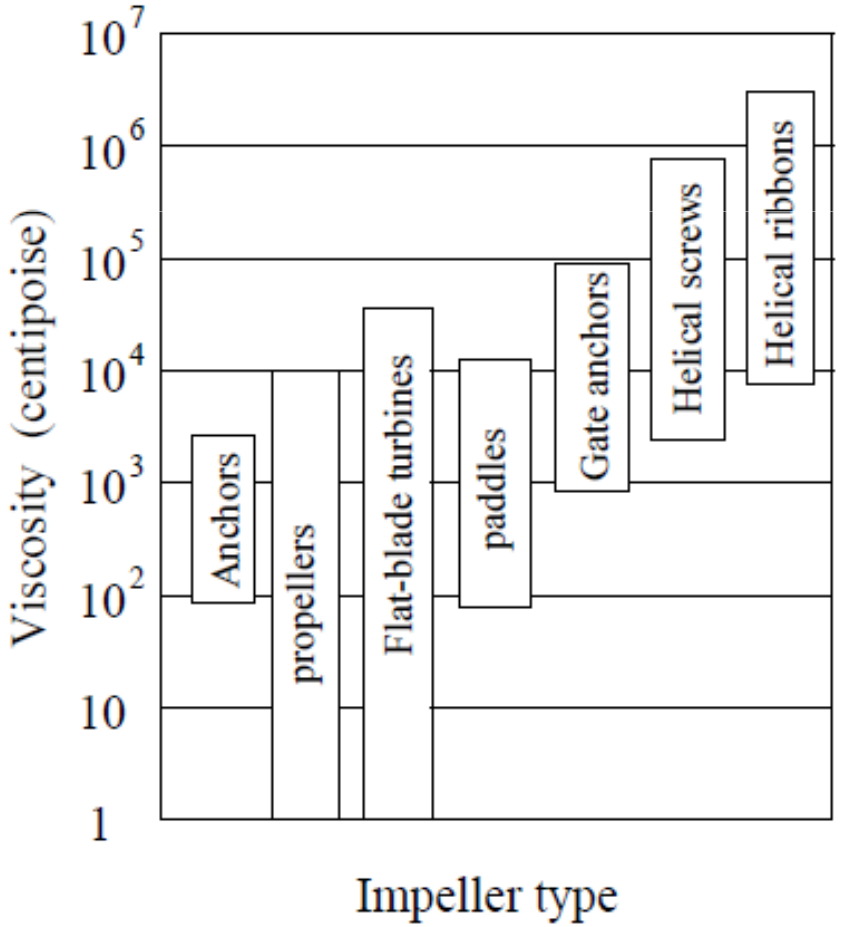
Propeller



45° Pitched Blade Turbine

Agitation – Choice of impeller

For situations involving very viscous fluids where laminar mixing is present, the diameter of the impeller approaches the diameter of the tank. The larger impellers such as anchors and helical ribbons are used.



Agitation – Mechanism of Mixing

For mixing to be effective,

- fluid circulated by the impeller must sweep the entire vessel in a reasonable time.
- the velocity of fluid leaving the impeller must be sufficient to carry material into the most remote parts of the tank.
- Turbulence must also be developed in the fluid.

These factors can be described as a combination of three physical processes:

- Distribution
- Dispersion
- Diffusion

- **Distribution: (Reactor scale)**

  The process whereby materials are transported to all regions of the vessel by bulk circulation currents is called distribution. In large tank, the size of the circulation paths is also large and the time taken to traverse them is long and distribution is slow.

- **Dispersion: (Length scales: about the size of the eddies)**

  The process of breaking up bulk flow into smaller and smaller eddies as a result of turbulence is called dispersion. If the rotational speed of the impeller is sufficiently high, superimposed on the distribution process is turbulence. Turbulence flow occurs when fluid no longer travels along streamlines but moves erratically in the form of cross-currents. Dispersion facilitates rapid transfer of material throughout the vessel.

- **Diffusion: (Molecular length scale)**

  Molecular diffusion becomes a prominent mechanism to achieve mixing at length scale smaller than the size of the eddies. Driven primarily by concentration gradients is generally regarded as a slow process, however, over small distances (typical of biochemical systems) it can be accomplished quite rapidly.

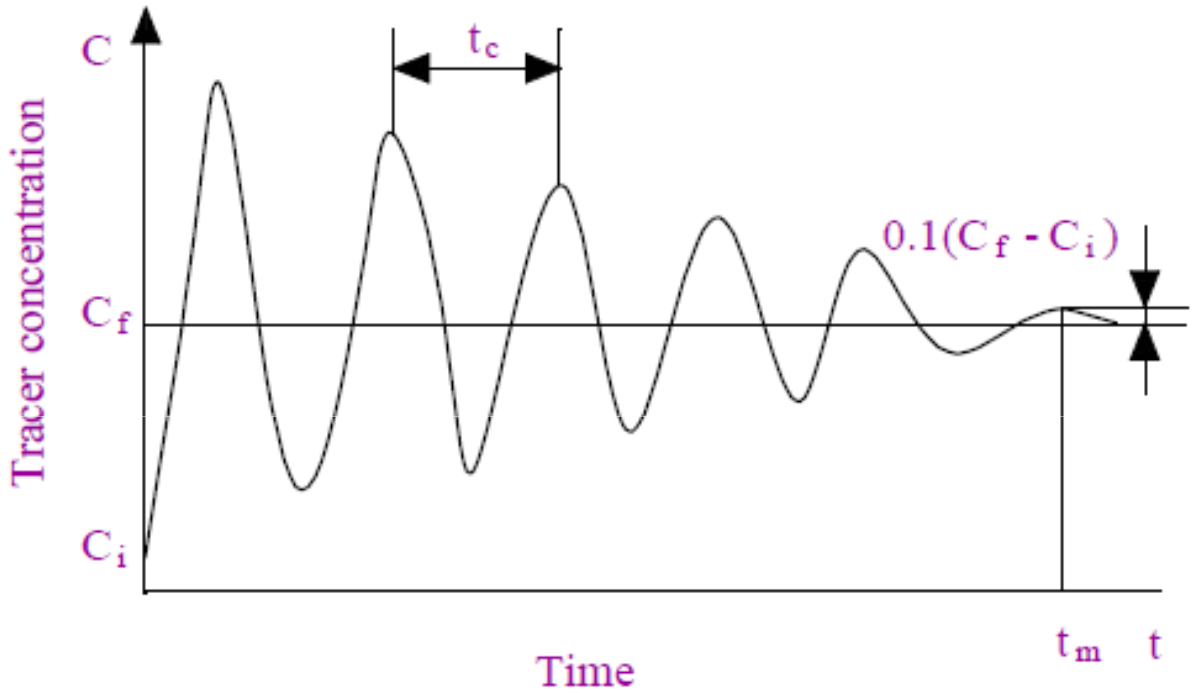
Agitation –Ideal & Non-ideal mixing

- **Ideal mixing:** Under ideal mixing conditions, the fluid in the reactor is perfectly mixed– that is, the contents are uniform throughout the reactor volume. In practice, an ideal mixing would be obtained if the mixing is sufficient and the liquid is not too viscous.

- **Non-ideal mixing** behaviour is commonly observed in stirred tank bioreactors. **Mixing time (t_m)** is a useful parameter for assessing mixing efficiency and is applied to characterize bulk flow in fermenters.

- The mixing time t_m is the time required to achieve a given degree of homogeneity starting from the completely segregated state. It can be measured by injecting a tracer into the vessel and following its concentration at a fixed point in the tank. Tracers in common use include acids, bases and concentrated salt solutions.

- Let us assume a small pulse of tracer is added to fluid in a stirred tank already containing tracer material at concentration C_i.
- *When* flow in the system is circulation, the tracer concentration measured at some fixed point in the tank will follow a pattern similar to that shown in figure.



- Before mixing is complete, a relatively high concentration will be detected every time the bulk flow brings tracer to the measurement point.
- The peaks in concentration will be separated by a period approximately equal to the average time taken for fluid to traverse one bulk circulation loop. In stirred tank this period is called the circulation time t_c.
- After several circulations the desired degree of homogeneity is reached.

Definition of the mixing time t_m depends on the degree of homogeneity required. Usually, mixing time is defined as the time after which the concentration of tracer differs from the finial concentration C_f by less than 10% of the total concentration difference ($C_f - C_i$). At t_m the tracer concentration is relatively steady and the fluid composition approaches uniformity. For a single-phase liquid in a stirred tank with several baffles and small impeller, there is an approximate relationship between mixing time and circulation time

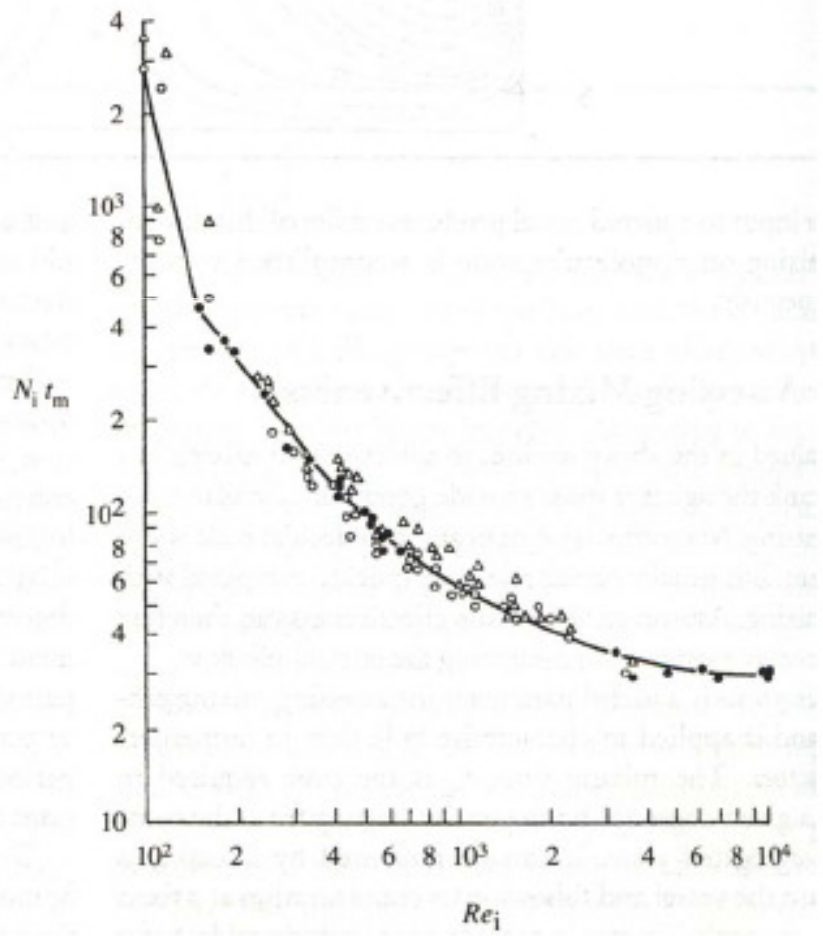
$$t_m = 4t_c \tag{6.2}$$

We can predict that mixing time in stirred tanks will depend on variables such as the size of the tank and impeller, fluid properties such as viscosity, and stirred speed. The relationship between mixing time and several of these variables has been determined experimentally for different impellers; results for a Rushton turbine in a baffled tank are shown in Fig 6.11. The dimensionless number $N_i t_m$ is plotted as a function of the impeller Reynolds number $(Re)_i$. t_m is the mixing time based on a 10% deviation from final conditions, and N_i is rotational speed of the stirrer. $N_i\, t_m$ represents the number of stirrer rotations required to homogenize the liquid.

At low Reynolds number, $N_i t_m$ increases significantly with decrease of $(Re)_i$. However, as Reynolds number is increased above about 5 $\times$ 10^3, $N_i t_m$ approaches a constant value which persists at high $(Re)_i$. For Rushton turbines, this constant value can be estimated using the following relationship.

$$N_i t_m = \frac{1.54V}{D_i^3}$$

$$(Re)_i = \frac{N_i D_i^2 \rho}{\mu}$$



Example 6.1 Estimation of mixing time

A fermentation broth with viscosity 10^{-2}Pa s and density 1000 kg m^{-3} is agitated in a 2.7 m^3 baffled tank using a Rushton turbine with diameter 0.5 m and stirred speed 1 s^{-1}. Estimate the mixing time.

Solution:

From Eq.(6.4):

$$(Re)_i = 2.5 \times 10^4$$

$(Re)_i > 5 \times 10^3$, therefore $N_i t_m$ is constant and can be calculated from Eq.(6.3):

$$N_i t_m = 33.3$$

Therefore:

$$t_m = 33.3/1 = 33.3 \text{ s}$$

6.5 Power Requirements for Mixing

Usually, electrical power is used to drive impellers in stirred tanks. For a given stirred speed, the power required depends on the resistance offered by the fluid to rotation of the impeller. Average power consumption per unit volume for industrial bioreactors ranges from 10 kW m^{-3} for small vessels to 1~2 kW m^{-3} for large vessels. Friction in the stirrer motor gearbox and seals reduces the energy transmitted to the fluid; therefore, the electrical power consumed by stirrer motors is always greater than the mixing power by an amount depending on the efficiency of the drive. Energy costs for operation of stirrers in bioreactors are an important consideration in process economics.

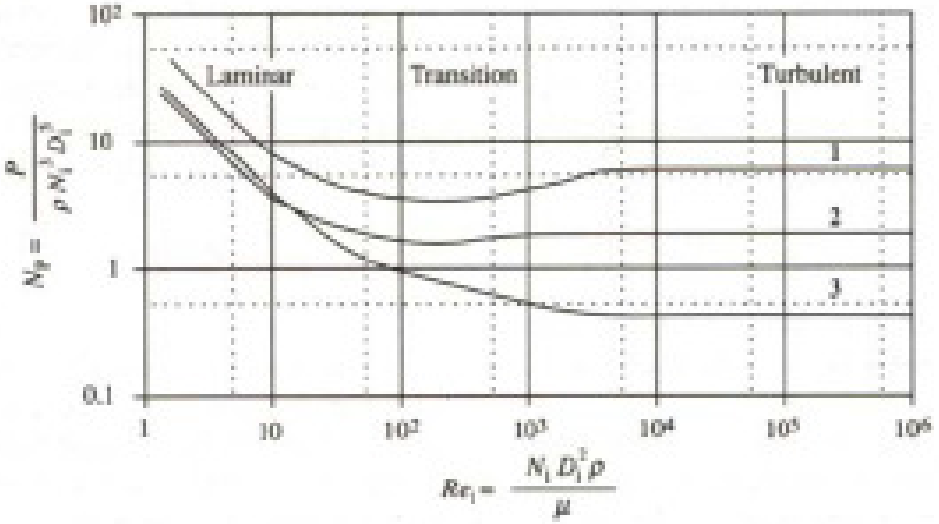
6.5.1 Ungassed Newtonian Fluids

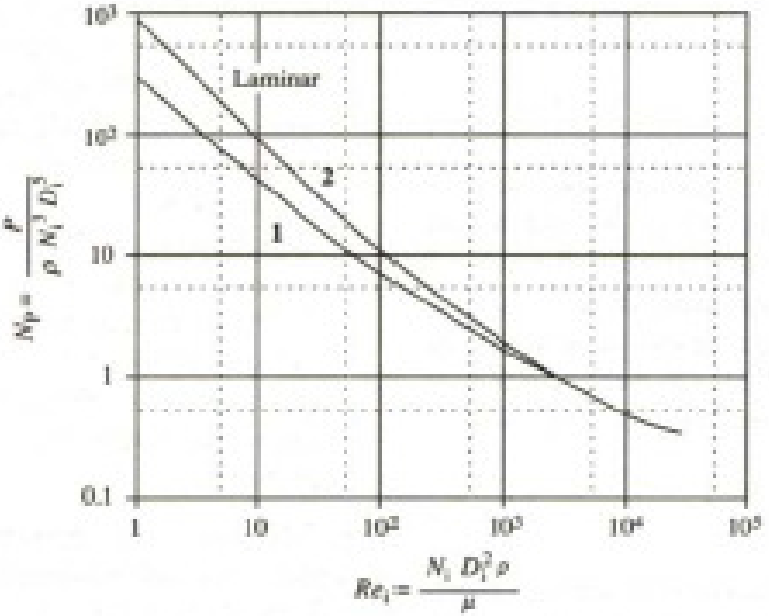
Mixing power for non-aerated fluids depends on the stirrer speed, the impeller diameter and geometry, and properties of the fluid such as density and viscosity. The relationship between these variables is usually expressed in terms of dimensionless numbers such as the impeller Reynolds number $(Re)_i$ and the power number N_p. N_p is defined as:

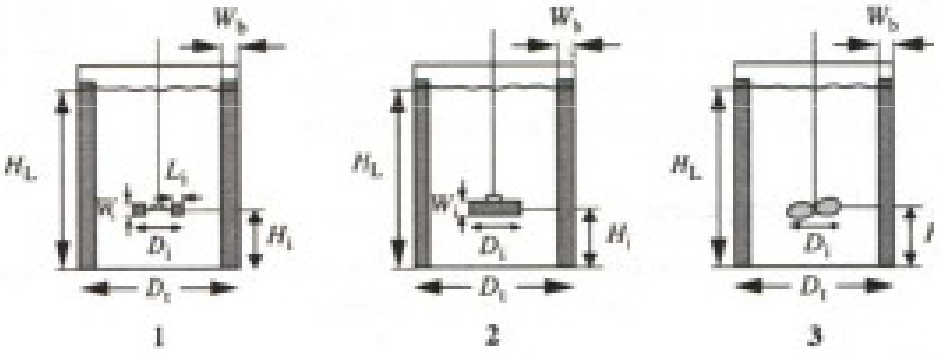
$$N_p = \frac{P}{\rho N_i^{\,3} D_i^{\,5}} \tag{6.5}$$

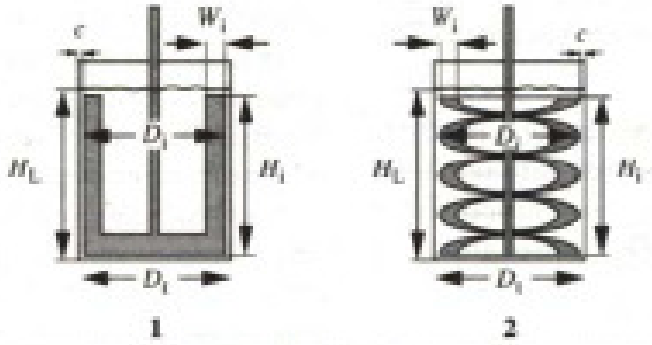
and

$$P = N_p \rho N_i^3 D_i^5 \tag{6.6}$$









Impeller	D_t/D_i	H_L/D_i	H_i/D_i	Baffles	
				W_b/D_i	Number
1. Rushton turbine $W_i/D_i = 0.2$, $L_i/D_i = 0.25$	3	3	1	0.1	4
2. Paddle $W_i/D_i = 0.25$	3	3	1	0.1	4
3. Marine propeller Pitch = D_i	3	3	1	0.1	4

Impeller	D_t/D_i	c/D_i	H_i/D_i	W_i/D_i
1. Anchor	1.02	0.01	1	0.1
2. Helical ribbon	1.02	0.01	1	0.1

- *Laminar region*: The laminar regime corresponds to $(Re)_i < 10$ for many impellers; for stirrers with very small wall-clearance such as the anchor and helical-ribbon mixer, laminar flow persists until $(Re)_i = 100$ or greater. In the laminar regime:

$$N_p \propto 1/(Re)_i$$

or

$$P = k_1 \mu N_i^2 D_i^3 \qquad (6.7)$$

- *Turbulent regime*: Power number is independent of Reynolds number in turbulent flow. Therefore:

$$P = N_p' \rho N_i^3 D_i^5$$

Table 6.1 Constants in Eq.(6.7) and (6.8)

Impeller type	k_1 , $(Re)_i = 1$	N_p', $(Re)_i = 10^5$
Rushton turbine	70	5~6
Paddle	35	2
Marine propeller	40	0.35
Anchor	420	0.35
Helical ribbon	1000	0.35

Example 6.2 Calculation of power requirements

A fermentation broth with viscosity 10^{-2} Pa s and density 1000 kg m^{-3} is agitated in a 50 m^3 baffled tank using a marine propeller 1.3 m in diameter. The tank geometry is as specified in Figure 6.13. Calculate the power required for a stirred speed of 4 s^{-1}.

Solution:

From Eq.(6.4):

$$(Re)_i = \frac{4 \times 1.3^2 \times 1000}{10^{-2}} = 6.76 \times 10^5$$

From Figure 6.13, flow at this $(Re)_i$ is fully turbulent and

$$N_p' = 0.35$$

Therefore:

$$P = 0.35 \times 1000 \times 4 \times 1.3^5 = 8.3 \times 10^4 \text{ kgm}^2 \text{ s}^{-3} = 83 \text{ kW}$$

6.5.2 Ungassed Non-Newtonian Fluids

Impeller Reynolds number based on the apparent viscosity μ_a:

$$(Re)_i = \frac{N_i D_i^{\,2} \rho}{\mu_a} \qquad (6.9)$$

For power-law fluids:

$$(Re)_i = \frac{N_i D_i^{\,2} \rho}{K \gamma^{n-1}} \qquad (6.10)$$

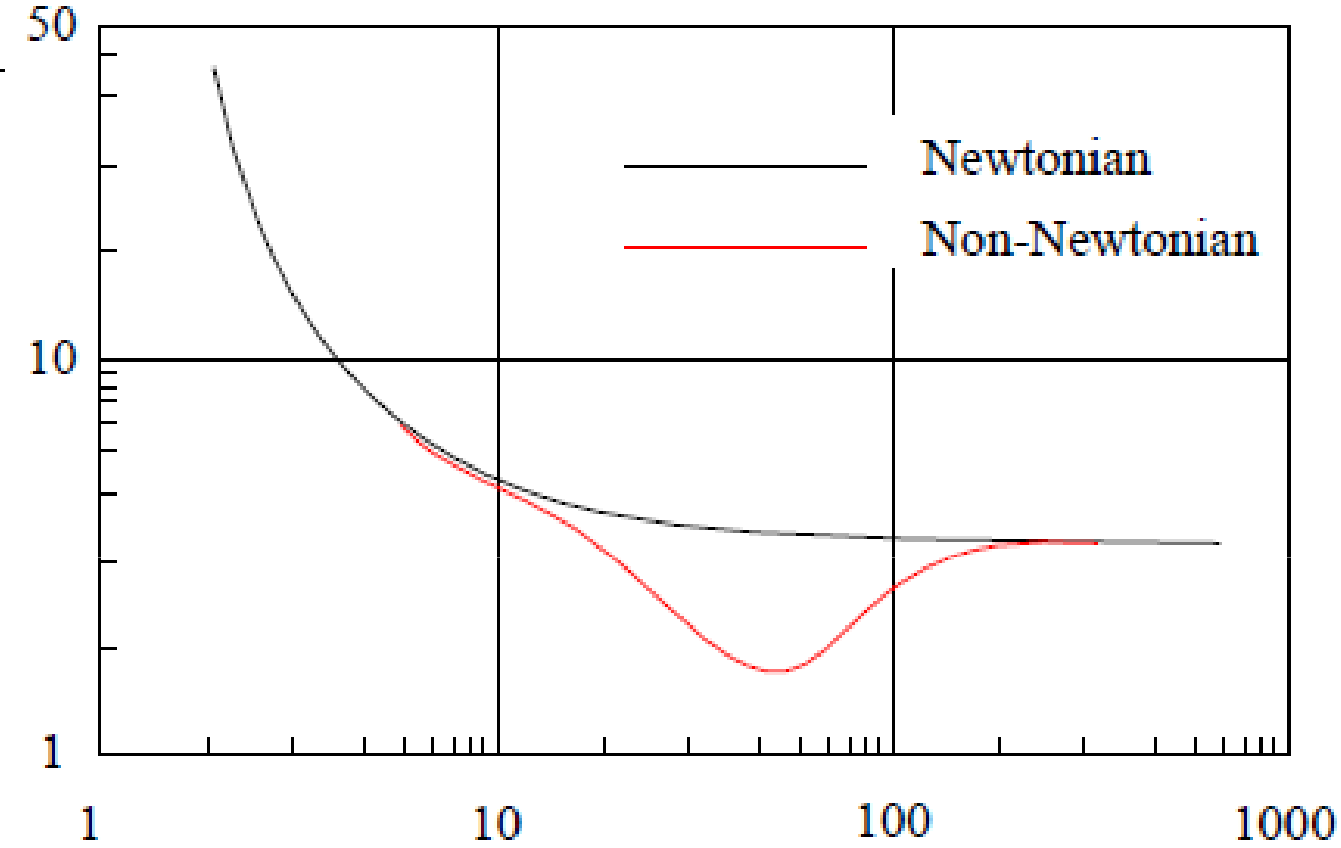
For stirred tanks, an approximate relation for pseudoplastic fluids is often used:

$$\gamma = k N_i \qquad (6.11)$$

Substituting Eq.(6.11) into (6.10) gives:

$$(Re)_i = \frac{N_i^{\,2-n} D_i^{\,2} \rho}{K k^{n-1}} \qquad (6.12)$$

$$N_P = \frac{P}{\rho N_i^{\,3} D_i^{\,5}}$$



$$(\mathrm{Re})_i = \frac{\rho N_i D_i^{\,2}}{\mu} \text{ or } \frac{\rho N_i D_i^{\,2}}{\mu_a}$$

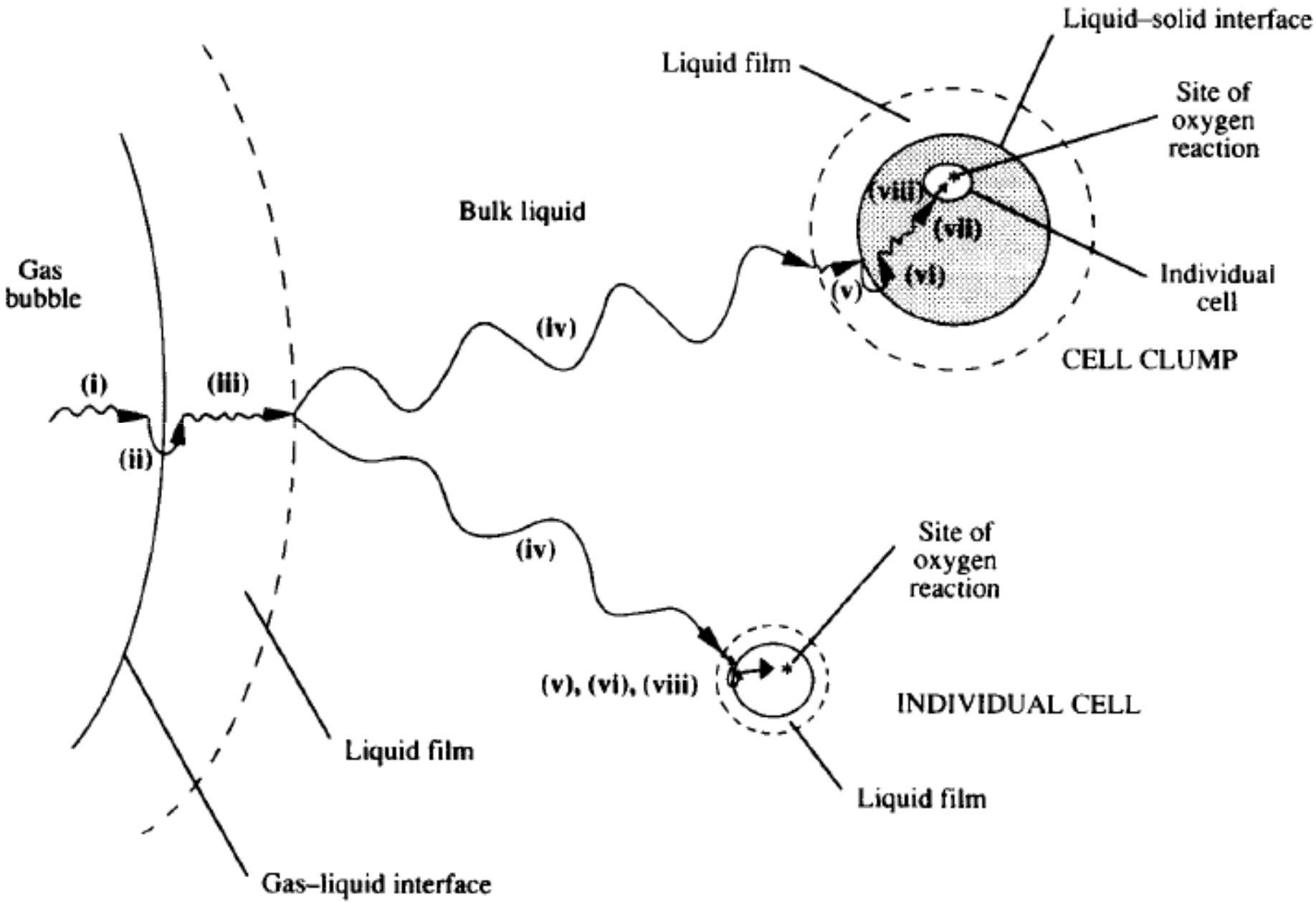
6.5.3 Gassed Fluids

All of the changes in hydrodynamic behavior duo to gassing are not completely understood. Power consumption is strong controlled by gas-cavities formation; because this process is discontinuous and appears somewhat randomly, reduction in power consumption is typically non-uniform. The random nature of gas dispersion in agitated tanks means that it is difficult to obtain an accurate prediction of power requirements. However, an expression for the ratio of gassed to ungassed power as a function of operating conditions has been obtained.

$$\frac{P_g}{P_0} = 0.10\left(\frac{F_g}{N_i V}\right)^{-0.25}\left(\frac{N_i^{\,2} D_i^{\,4}}{g W_i V^{2/3}}\right)^{-0.20}$$

Oxygen Transport in Bioreactors

Oxygen Transport in a Microbial Bioreactior



Oxygen Transport in a Microbial Bioreactor

$$k_L a\,(C^*_{AL} - C_{AL}) = q_O x$$

(i) Transfer through the bulk gas phase in the bubble is relatively fast.

(ii) The gas–liquid interface itself contributes negligible resistance.

(iii) The liquid film around the bubbles is a major resistance to oxygen transfer.

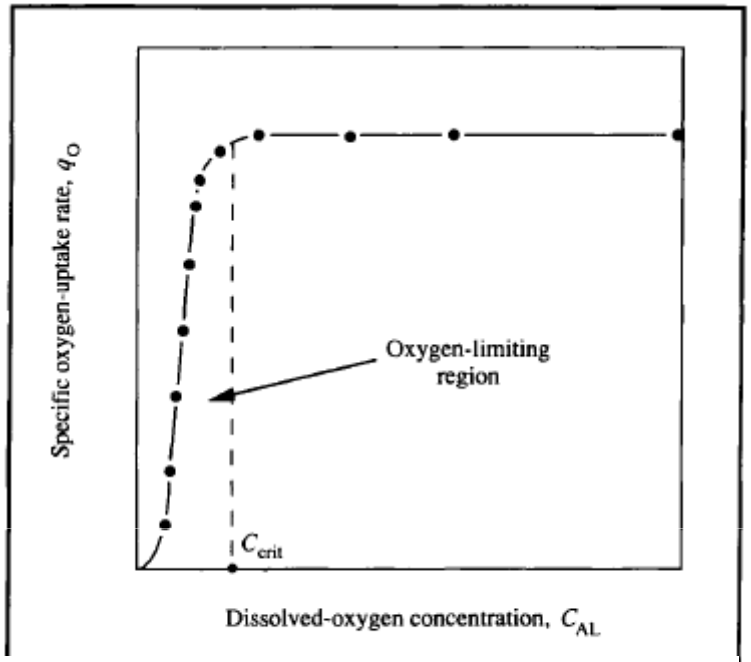
(iv) In a well-mixed fermenter, concentration gradients in the bulk liquid are minimised and mass-transfer resistance in this region is small. However, rapid mixing can be difficult to achieve in viscous fermentation broths; if this is the case, oxygen-transfer resistance in the bulk liquid may be important.

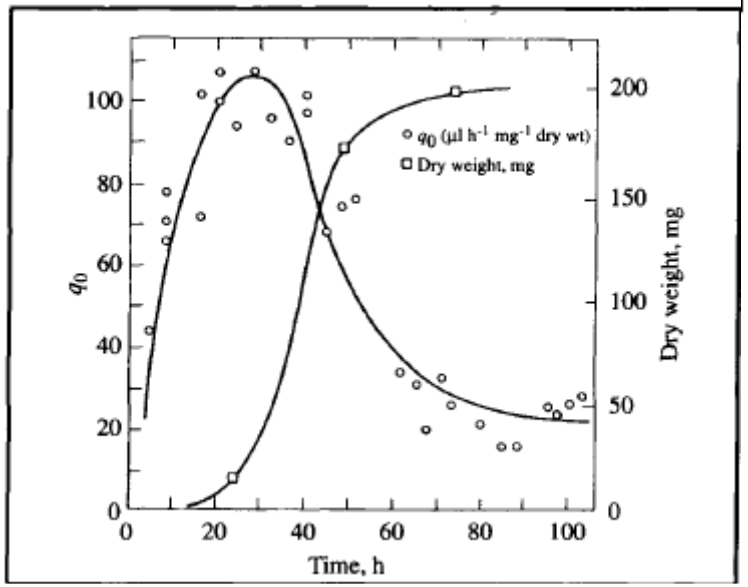
(v) Because single cells are much smaller than gas bubbles, the liquid film surrounding each cell is much thinner than that around the bubbles and its effect on mass transfer can generally be neglected. On the other hand, if the cells form large clumps, liquid-film resistance can be significant.

(vi) Resistance at the cell–liquid interface is generally neglected.

(vii) When the cells are in clumps, intraparticle resistance is likely to be significant as oxygen has to diffuse through the solid pellet to reach the interior cells. The magnitude of this resistance depends on the size of the clumps.

(viii) Intracellular oxygen-transfer resistance is negligible because of the small distances involved.





Measurement of $K_L a$

- **It is extremely difficult to measure both 'K_L' and *'a'* in a fermentation and, therefore, the two terms are generally combined in the term *$K_L a$, know as the volumetric mass-transfer coefficient.***
- **The units of *$K_L a$,* are reciprocal time (h^{-1}).**
- **The volumetric mass-transfer coefficient is used as a measure of the aeration capacity of a fermenter.**
- **The larger the *$K_L a$,* the higher the aeration capacity of the system.**
- **The *$K_L a$* value will depend upon the design and operating conditions of the fermenter and will be affected by the variables such as**
  - **aeration rate,**
  - **agitation rate and impeller design.**

Need for measurement of $K_L a$

- The determination of the $K_L a$ of a fermenter is essential in order
  - to establish its aeration efficiency and
  - to quantify the effects of operating variables on the provision of oxygen.
- The equations describing oxygen transfer are based on dissolved oxygen concentration.
- The solubility of oxygen is affected by dissolved solutes therefore pure water and a fermentation medium saturated with oxygen have different dissolved oxygen concentrations.

- Determination of $K_L a$ value is done by following method:
  - (i) Sulphite oxidation technique
  - (ii) Gassing-out techniques
    - Static gassing out method
    - Dynamic gassing out method
  - (iii) Oxygen-balance technique

(i) Sulphite oxidation technique

- The oxygen-transfer rates is determined by the oxidation of sodium sulphite solution.
- This technique does not require the measurement of dissolved oxygen concentrations.
- Based on the rate of conversion of a *0.5* M solution of sodium sulphite to sodium sulphate in the presence of a copper or cobalt catalyst:

$$Na_2SO_3 + 0.5O_2 \longrightarrow Na_2SO_4$$

- The volumes of the thiosulphate titrations are plotted against sample time and the oxygen transfer rate may be calculated from the slope of the graph.

- As oxygen enters solution it is immediately consumed in the oxidation of sulphite, so that the sulphite oxidation rate is equivalent to the oxygen-transfer rate.

- Since the dissolved oxygen concentration, is zero then the $K_L a$ may then be calculated from the equation:

$$\underbrace{\frac{1}{2}\frac{d[SO_4]}{dt}}_{\text{Experimentally determined}} = \text{OTR} = K_L a \,.\, C^* \qquad \text{(i)}$$

$$K_L a = \text{OTR} / C^*$$

where OTR is the oxygen transfer rate.

C* is the oxygen solubility, a constant depending on medium composition, temperature and pressure.

(ii) Gassing-out techniques

- The estimation of the $K_L a$ of a fermentation system by gassing-out techniques depends upon monitoring the increase in dissolved oxygen concentration of a solution during aeration and agitation.

- The oxygen transfer rate will decrease during the period of aeration as C_L approaches C* due to the decline in the driving force $(C^* - C_L)$.

- The oxygen transfer rate, at particular time, will be equal to the slope of the tangent to the curve of values of dissolved oxygen concentration against time of aeration, as shown in Fig.

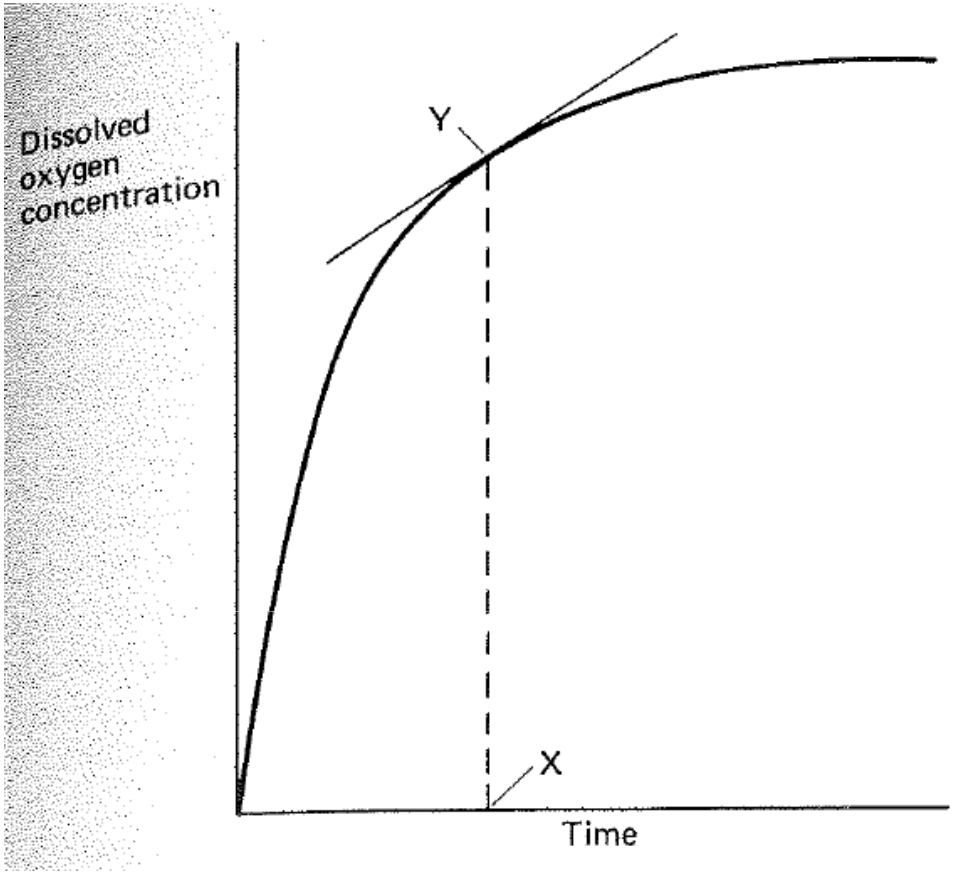


Fig. The increase in dissolved oxygen concentration of a solution over a period of aeration. The oxygen transfer rate at time X is equal to the slope of the tangent at point Y.

- To monitor the increase in dissolved oxygen over an adequate range it is necessary first to decrease the oxygen level to a low value.
- Two methods have been employed to achieve this lowering of the dissolved oxygen concentration –
  - the static method and
  - the dynamic method.

Static gassing out method

- **The oxygen concentration of the solution is lowered by gassing the liquid out with nitrogen gas, so that the solution is 'scrubbed' free of oxygen.**

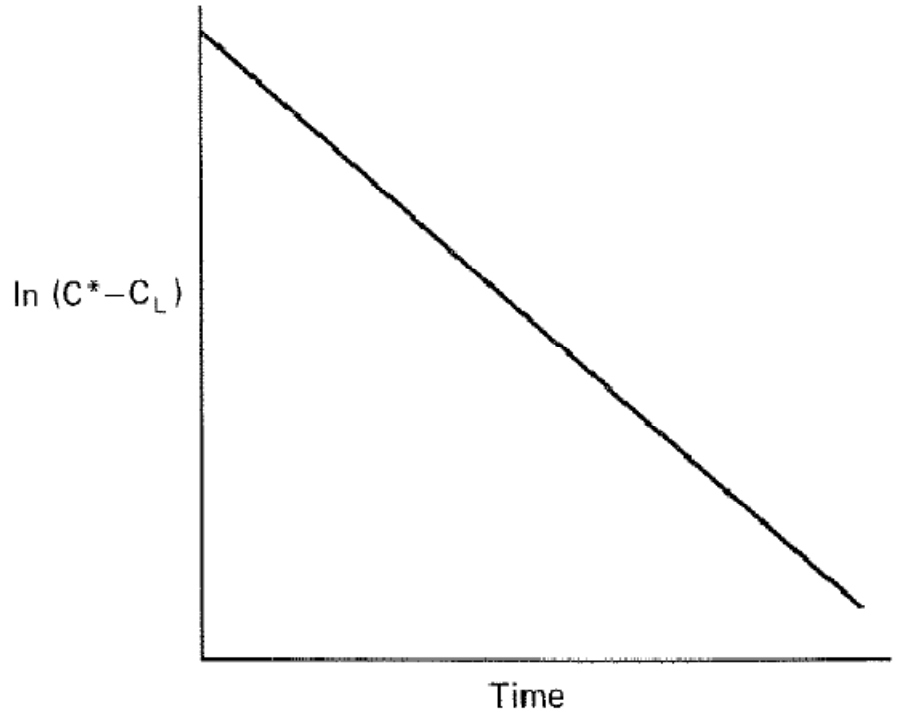
- **The deoxygenated liquid is then aerated and agitated and the increase in dissolved oxygen monitored using some form of dissolved oxygen probe.**

- **The increase in dissolved oxygen concentration is given by**

$$dC_L/dt = K_L a(C^* - C_L)$$

- **Initial condition: At t=0, C_L=0. Solving the first order ODE and taking logarithm,**

$$\ln(C^* - C_L) = -K_L a\, t$$



A plot of the ln(C* - C*L)* against time of aeration, the slope of which equals -*KLa.*

Dynamic gassing out method

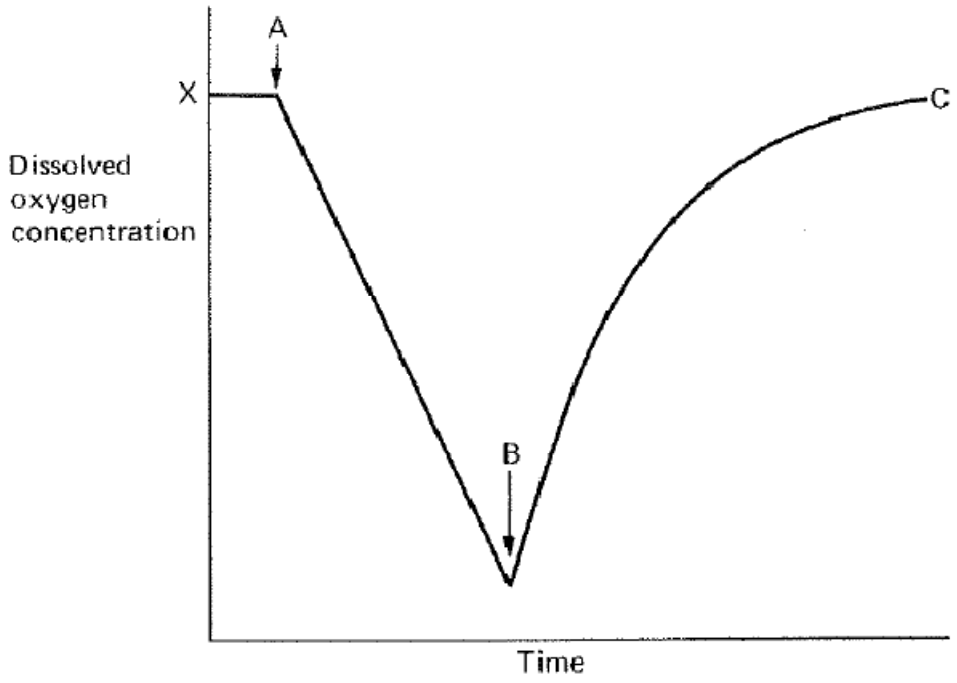
$$OTR = dC_L/dt = K_La(C^*-C_L) - Xq_{O2}$$

--------(iii)

Where,

- *X* is the concentration of biomass and
- q_{O2} is the specific respiration rate (mmoles of oxygen g-l biomass h- I).
- The term xq_{O2} is given by the slope of the line AB in Fig -1.

Fig.1. Dynamic gassing out for the determination of *KLa* values. Aeration was terminated at point A and recommenced at point B.



- Equation (iii) may be rearranged as:

$$C_L = -1/K_La\{(dC_L / dt)+ Xq_{O2}\}+C^* \qquad \text{----------(iv)}$$

- Now from equation (iv), a plot of C_L versus $dC_L/dt + Xq_{O2}$ will yield a straight line, the slope of which will equal $-1/K_La$, as shown in Fig. 2.

Fig. 2 . The dynamic method for determination of *KLa* values. The information is gleaned from Fig. 9.7. by taking tangents of the curve, Be, at various values of C*L'*

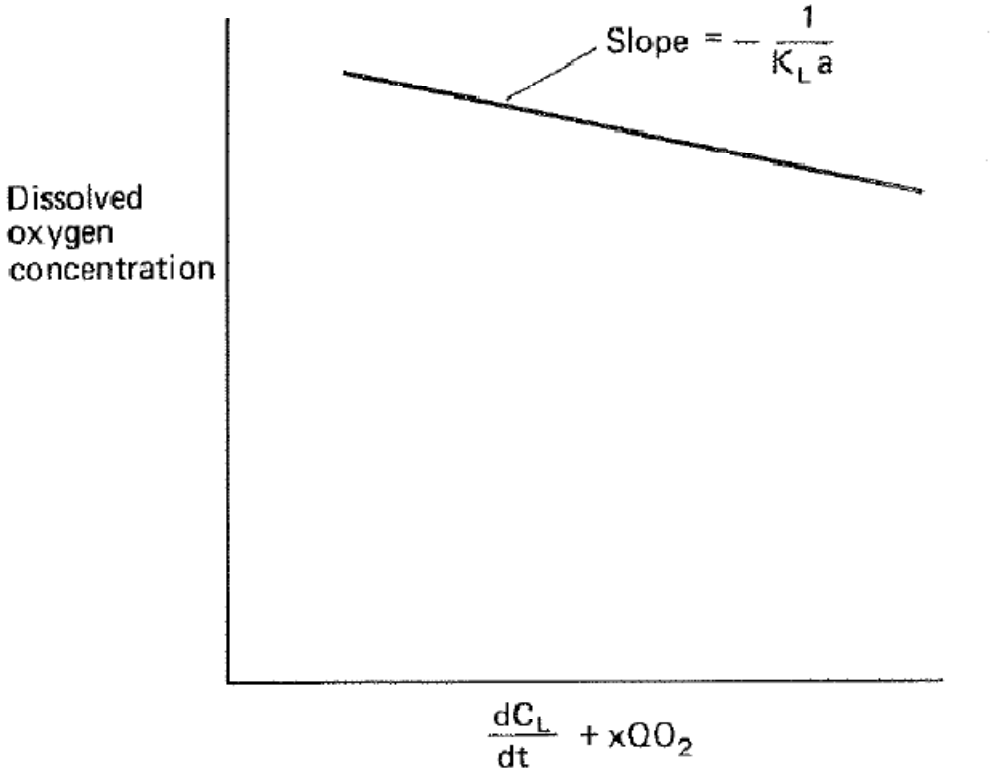
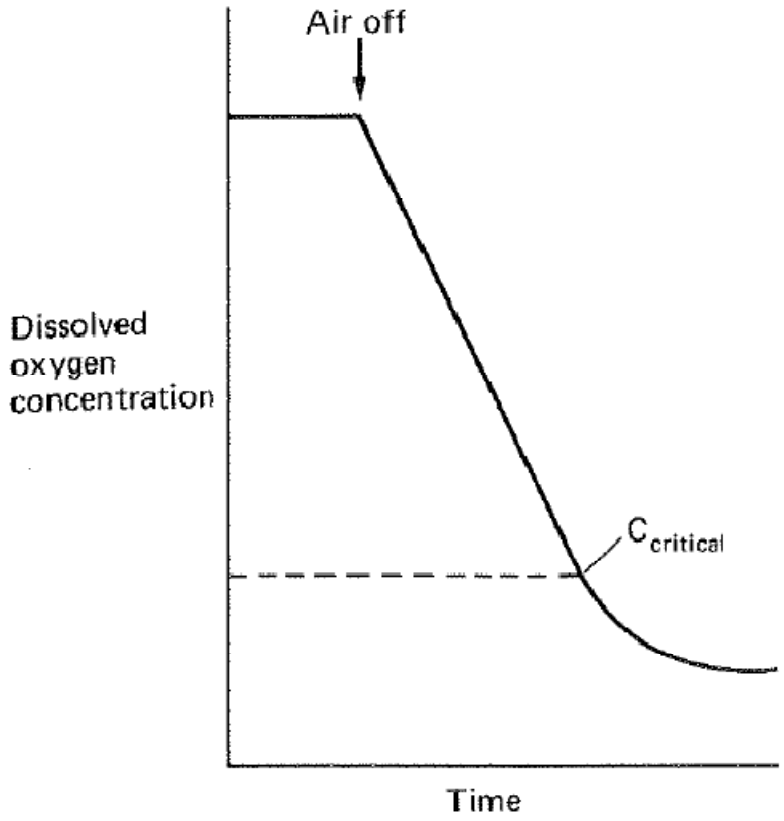


Fig. 3. The occurrence of oxygen limitation during the dynamic gassing out of a fermentation.



Advantages

- The dynamic gassing-out method has the advantage over the previous methods of determining the $K_L a$ during an actual fermentation and may be used to determine $K_L a$ values at different stages in the process.

- The technique is also rapid and only requires the use of a dissolved-oxygen probe, of the membrane type.

Limitations

- A major limitation in the operation of the technique is the range over which the increase in dissolved oxygen concentration may be measured.

- It may be difficult to apply the technique during a fermentation which has an oxygen demand close to the supply capacity of the fermenter.

- Both the dynamic and static methods are also unsuitable for measuring $K_L a$ values in viscous systems.

(iii) Oxygen-balance technique

- Use to measure $K_L a$ during fermentation process.
- The amount of oxygen transferred is determined, directly into solution in a set time interval.
- The procedure involves measuring the following parameters:

The procedure involves measuring the following parameters:

(i) The volume of the broth contained in the vessel, V_L (dm3).

(ij) The volumetric air flow rates measured at the air inlet and outlet, Q_i and Q_o' respectively (dm^3 $min^{\sim 1}$).

(iii) The total pressure measured at the fermenter air inlet and outlet, P_i and P_o, respectively (atm. absolute).

(iv) The temperature of the gases at the inlet and outlet, 1; and T_o, respectively (K).

(v) The mole fraction of oxygen measured at the inlet and outlet, Y_i and Y_o' respectively.

- The oxygen transfer rate may then be determined from the following equation (Wang *et al., 1979):*

$$OTR = (7.32 \times 10^5/V_L)(QiPiyi/Ti - QoPoyo/To)$$

----------*(v)*

- Where 7.32×10^5 is the conversion factor equaling (60min h ~l) [mole/22.4 dm3 (STP)] (273 K/l atm).
- These measurements require accurate flow meters, pressure gauges and temperature-sensing devices as well as gaseous oxygen analysers.
- The ideal gaseous oxygen analyser is a mass spectrometer analyser which is sufficiently accurate to detect changes of 1 to 2%.

- The *KLa* may be determined, provided that *CL* and C* are known, from equation(1) :

$$dC_L / dt = K_L a(C^*-C_L)$$

Or $$OTR = K_L a\,(C^*-C_L)$$

Or $$K_L a = OTR/(C^*-C_L)$$

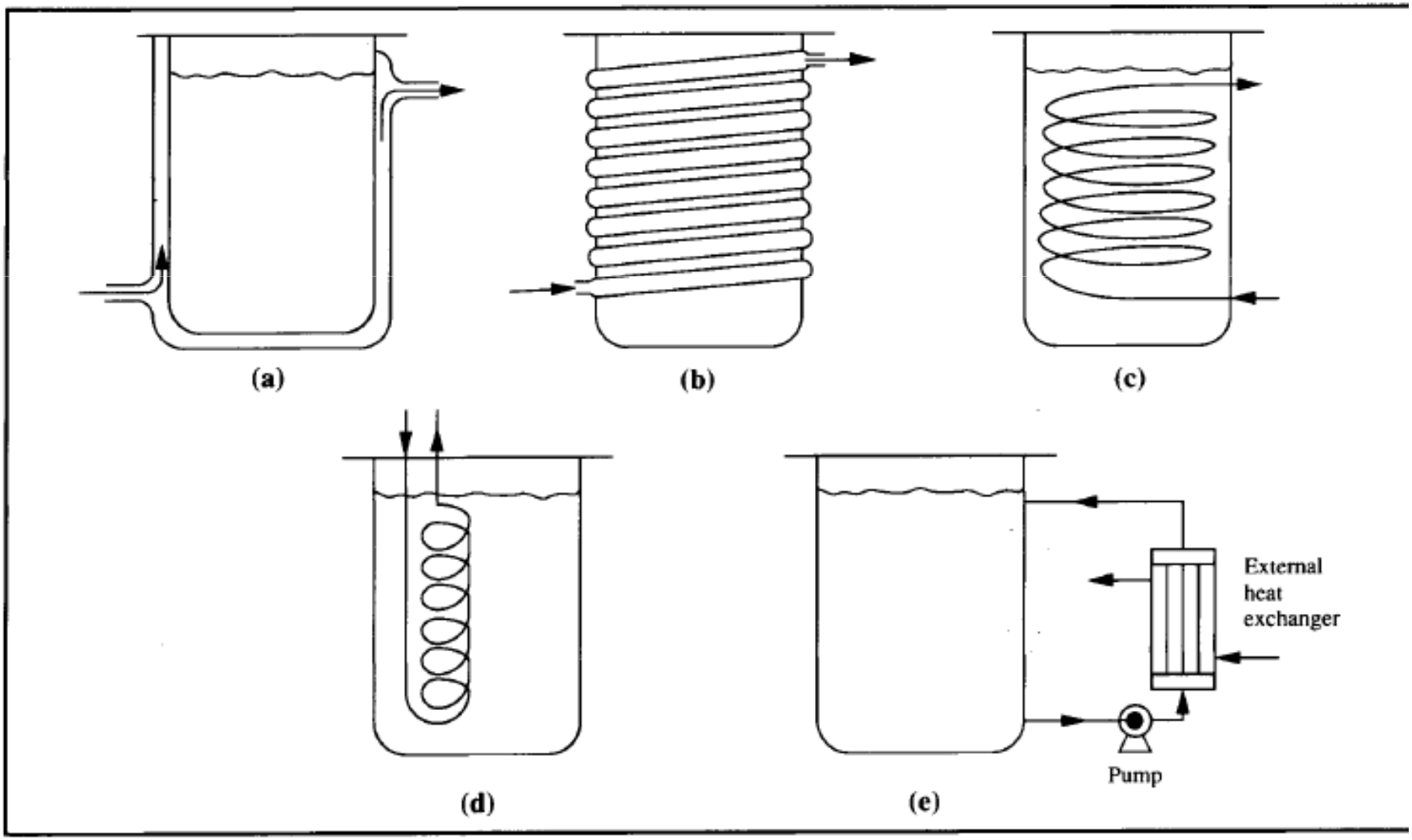
- The oxygen-balance technique appears to be the simplest method for the assessment of $K_L a$ and

- Has the advantage of measuring aeration efficiency during a fermentation.

FACTORS AFFECTING $K_L a$ VALUES IN FERMENTATION VESSELS

- A number of factors have been demonstrated to affect the *KLa* value. Such factors include
  - the air-flow rate employed in vessels,
  - the degree of agitation inside vessels ,
  - the rheological properties of the culture broth and
  - the presence of antifoam agents.

Figure 8.1 Heat-transfer configurations for bioreactors: (a) jacketed vessel; (b) external coil; (c) internal helical coil; (d) internal baffle-type coil; (e) external heat exchanger.



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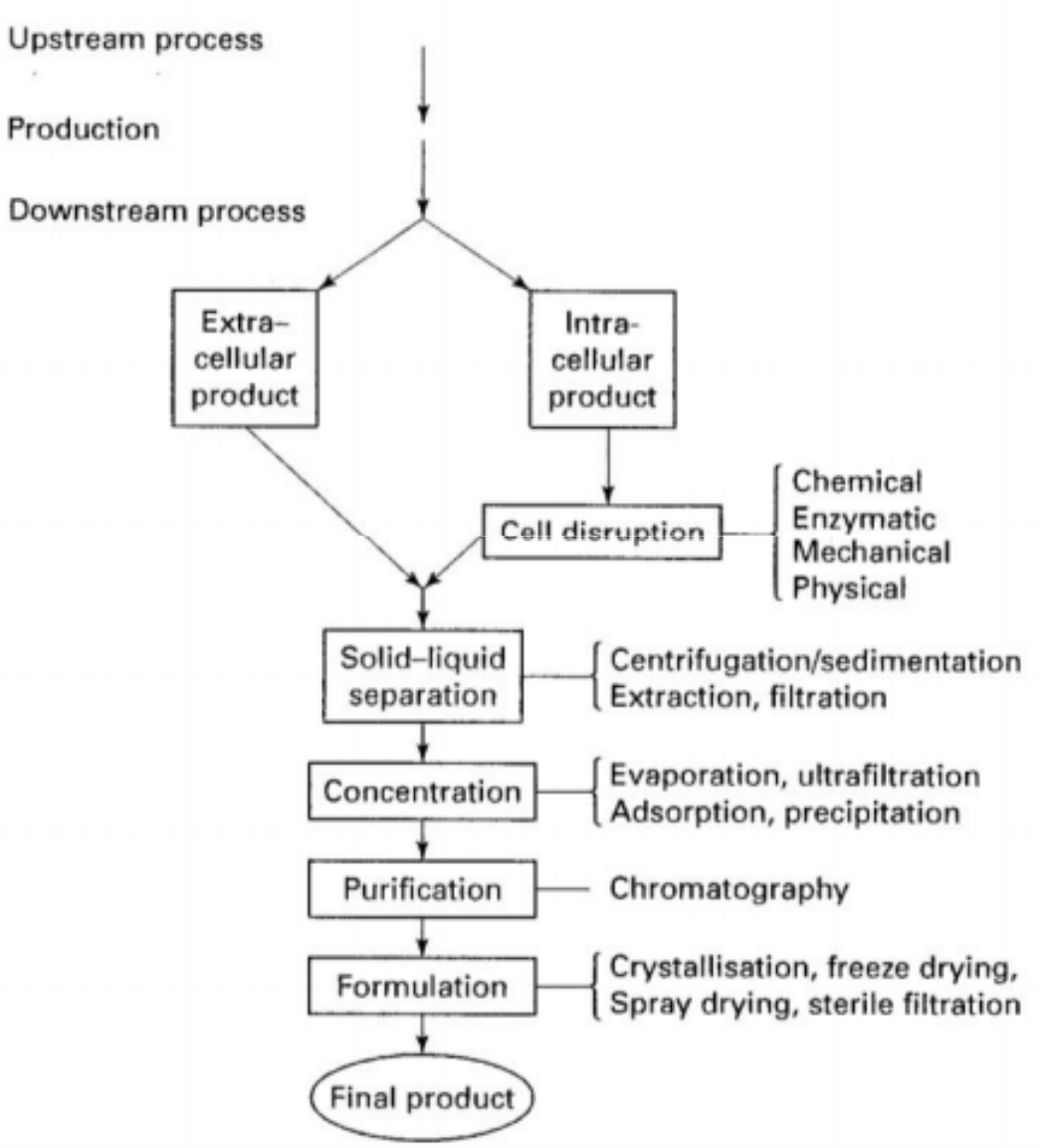
SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF CHEMICAL ENGINEERING

BIOCHEMICAL ENGINEERING

UNIT – 5 DOWNSTREAM PROCESSING – SCHA 1601

OVERVIEW OF BIOSEPARATIONS



- **Up to 90% of new product cost is in downstream processing.**
- **More than 60% of the cost of third and fourth generation antibiotics is in purification.**
- **For recombinant DNA fermentation products, downstream processing can account for 80 to 90% of the overall processing cost.**

- **Most bioseparations have four similar steps:**

  (1) Separation of insolubles

  * **Objectives: remove or collect cells, cell debris, or other particulates**
  * **Typical operations: filtration, centrifugation**
  * **Relatively little product concentration or improvement of product quality occurs.**

6.4.1 Removal of insolubles

In some processes, the product of interest is the biomass itself or the product can be extracellular in the broth; however, in both cases microbial cells must be separated. Typical sizes for cells can be anywhere between 0.5–1 μm for bacteria, 1–7 μm for yeast, 5–15 μm for molds, 20–40 μm for plant cells, and 10–0 μm for suspensions of animal cell (Dutta, 2008).

Removal of insolubles usually involves filtration or centrifugation. Filtration separates the solids from the liquid by forcing the fluid through a filter or solid support on which particles are deposited. This method is fairly straightforward for dilute suspension of large and rigid particles. However, small particles and deformability of the cells can make the process more complicated. There are many types of filters available but plate and frame pressure filters and rotary vacuum filters are most common. Plate and frame pressure filters are employed for small-scale separation of bacteria and fungi from fermentation broth. Rotary vacuum filters are preferred for large-scale commercial processes due to lower labor costs (Perry & Chilton, 1973). Even though filtration is straightforward, for some products pretreatments may be required. Heating for protein denaturation, addition of electrolytes to promote coagulation and flocculation, and addition of filter aids (earths and perlites) to increase porosity and decrease the compressibility of cakes are some pretreatment examples (Belter et al., 1988).

On the other hand, centrifugation utilizes the density difference between the solids and the surrounding fluid, when solid particles settle down and fluid stands as a clear phase. Even though centrifugation requires more expensive equipment and produces a wet cake compared to filtration, it is still an alternative for separation of small particles, between 100 and 0.1 μm (Shuler & Kargi, 2008). Two basic types of centrifuges are the tubular and the disk centrifuge (Figure 6.3). The tubular centrifuge consists of a cylindrical rotating element in a stationary case. Broth is fed through the bottom and clear liquid collected from top, leaving the solids on the wall of the bowl. The tubular centrifuge can be inexpensive, but is not suitable for large-scale and continuous processes. The disk centrifuge includes a short, wide bowl that turns on a vertical axis. Closely spaced cone-shaped disks decrease the distance of particles to be removed and increase efficiency. This type of centrifuge is utilized for continuous and large-scale processes but is more expensive than the tubular centrifuge (Belter et al., 1988).

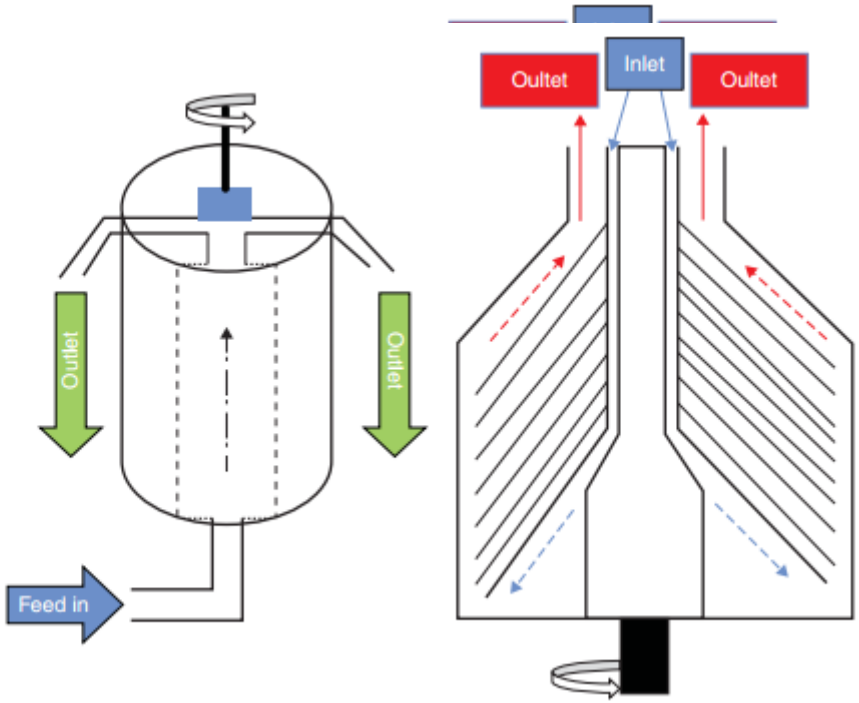
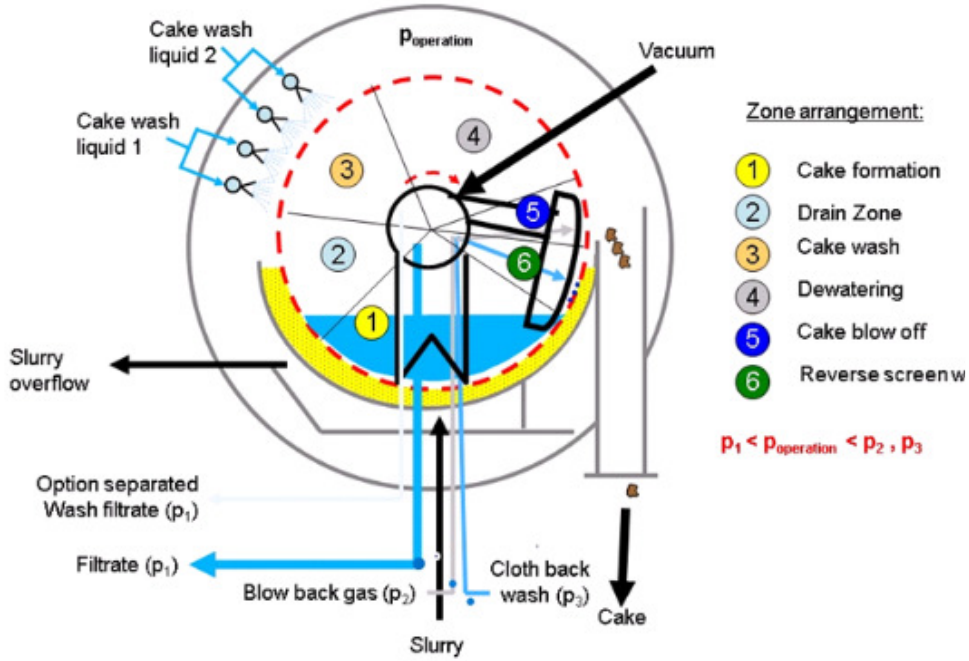


Figure 6.3 Schematics for tubular centrifuge (*left*) and disk centrifuge (*right*).

Rotary drum filter



- **Most bioseparations have four similar steps (2/3):**

  (1) Separation of insolubles

(2) Isolation of soluble products

* **Objectives: remove materials of widely divergent properties compared to the desired product**
* **Typical operations: extraction, adsorption, ultrafiltration, precipitation**
* **Appreciable concentration and product quality increases usually occur.**

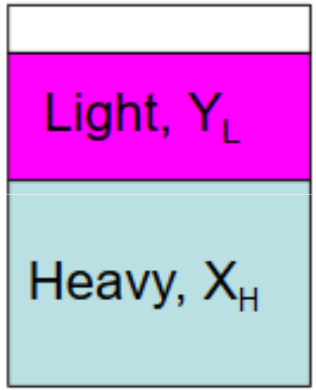
6.4.3 Removal of solubles

The next step in product recovery is separating intracellular (after cell disruption) and extracellular products from the broth and many methods are available for this purpose. Recovery and purification is a very wide topic, and only a few methods will be explained briefly in this section (Belter et al., 1988; Shuler & Kargi, 2008).

Extraction is the method of separating the solutes of a liquid solution by contact with another insoluble liquid, the solvent (Dutta, 2008). The desired product can be selectively extracted out of the solution into the solvent phase by selecting an appropriate solvent. A good solvent should be easily recovered, have large density difference between two phases, non-toxic, and cheap. The solvent-rich phase is called the extract, whereas the residue of the liquid is called the raffinate (Dutta, 2008).

Liquid-liquid extraction:

- Difference of solubility in two immiscible liquid
- Applicable: separate inhibitory fermentation products such as ethanol and acetone-butanol from fermentation broth.
  antibiotics (i.e. solvent amylacetate)
- Requirements of liquid extractants :



  nontoxic, selective, inexpensive, immiscible with fermentation broth and
  high distribution coefficient: $K_D=Y_L/X_H$

  Y_L and X_H are concentrations of the solute in light and heavy phases, respectively.

  The light phase is the organic solvent and the heavy phase is the fermentation broth.

Adsorption is a technique that relies on the fact that specific substances in solution can be adsorbed selectively by certain solids as a result of physical or chemical interactions between the molecules of the solid and substance adsorbed. Adsorption is a very effective method for separation of dilute substances, because it is very selective while the solute loading on the solid surface is limited (Dutta, 2008). Adsorption can be due to intermolecular forces (van der Waals forces between the molecules and substance), chemical interaction between a solute and ligand, or ionic forces. Adsorption can occur via stage-wise or continuous-contact methods.

Adsorption

Adsorb soluble product from fermentation broth onto solids.

Approaches: physical adsorption, ion exchange

Adsorption capacity: mass of solute adsorbed per unit mass of adsorbent

Affected by properties of adsorbents:

functional groups and their numbers, surface properties

by properties of solution: solutes, pH, ionic strength and temperature

- Difference of Affinity of product in the solid and liquid phase.
- Applicable: soluble products from dilute fermentation

Precipitation is a commonly used method for the recovery of proteins or antibiotics (Belter et al., 1988). It can be induced by addition of salts, organic solvents, or heat. The addition of salts precipitates proteins, since protein solubility is reduced by increase in salt concentration in the solution. This method is effective and cheap, but it causes protein denaturation (Belter et al., 1988). Ammonium sulfate is the most commonly used salt but has the disadvantage that it is hard to remove from precipitated protein. Organic solvents precipitate protein at a lower temperature by decreasing the dielectric constant of the solution. Acetone, ethanol, and methanol are the most commonly employed organic solvents. Heating is another way of promoting precipitation of proteins by denaturation. It is used to eliminate unwanted proteins; however, it is difficult to precipitate the unwanted proteins without damaging the desired product.

Precipitation

Reduce the product solubility in the fermentation broth by adding salts.

- Applicable: separate protein or antibiotics from fermentation broth
- Methods:
  - salting-out by adding inorganic salts such as ammonium sulfate, or sodium sulfate at high ionic strength (factors: pH, temperature)
    - salts interact more stronger with water
    - cause little denaturation
    - inexpensive

Chromatography employs a mobile phase and a stationary phase. The mobile phase is the solution containing solutes to be separated and eluent that carries the solution through the stationary phase. The stationary phase can be adsorbent, ion exchange resin, porous solid or gel and is mostly packed in a cylindrical column. A solution is injected at one end of the column and eluent carries the solution through the stationary phase to the other end of the column. Each solute in the original solution moves at a rate proportional to its relative affinity for the stationary phase and is separated at the end of the column (Ogez et al., 1989). Chromatography is employed to purify proteins and peptides from a complex solution at laboratory scale. For large-scale chromatography, a stationary phase must be selected which has the required strength and chemical stability (Dutta, 2008).

Gel Permeation Chromatography

Gel Permeation chromatography has been used extensively in the purification of proteins, nucleic acids, and other biological macromolecules since its inception (148,149). Gel chromatography (also called GPC, gel filtration chromatography or SEC) is a mild purification technique that separates molecules based on size, using an inert (noninteracting) matrix with a carefully controlled pore structure. The pore structure of the gel matrix is such that smaller molecules are able to diffuse into more of the matrix than larger molecules, and hence the larger molecules elute first from the column followed by the other solutes, in order of decreasing size. It is important to note that the solute "size" reported from a gel chromatography assay is dependent on the shape of the molecule. Two proteins of the same molecular weight can have different elution characteristics depending on their shape, e.g., one is a rod-like molecule and one is spherical in shape. Most commercially available "calibration" standard samples assume a spherical shape.

Gel permeation chromatography is a very mild purification technique that allows operation at conditions suited for the stability of the desired protein, and it can be operated in the presence of cofactors, at high or low pH, at 37°C or 4°C, with or without detergents, etc. It is also a suitable technique for both preparative practices and analytical techniques. Preparative gel permeation chromatography is frequently used as a polishing step where low capacities are acceptable and the feed stock is relatively clean (150). Dimers and degradation products are the most common impurities removed from the product in gel chromatography steps. Additionally, GPC is frequently used as a buffer-exchange step.

Membrane filtration is used to separate small particles using polymeric membranes under pressure. Based on the size of particles, membrane filtration is subcategorized as microfiltration, ultrafiltration, and reverse osmosis. Microfiltration is employed for colloids, fat globules, and cells with a pore size of 0.01–10 µm (Grandison & Finnigan, 1996). Ultrafiltration employs the same principle but is used for smaller particles with a pore size of 10–200 µm. In reverse osmosis, a macromolecule and a solvent are separated by a semi-permeable membrane under pressure (Belter et al., 1988). The pore size (1–10 µm) of the membrane for reverse osmosis enables all suspended and dissolved material to be separated (Dutta, 2008). First-generation membranes were made of cellulose acetate. Nowadays, inorganic and organic membranes are available (Lewis, 1996). Detailed description of membrane filtration can be found in the study of Grandison and Lewis (1996).

Membrane separation:

- Microfiltration: 0.1 - 10 μm, bacterial and yeast cells.
- Ultrafiltration: macromolecules (2000 <MW< 500,000)
- Dialysis: removal of low-MW solutes: organic acids (100<MW<500) and inorganic ions (10<MW<100).
- Reverse osmosis: a pressure is applied onto a salt-containing phase, which drives water from a low to a high concentration region. MW < 300.

The common features of the above methods:

- Use membrane
- Driving forces: pressure

- **Most bioseparations have four similar steps (3/3):**

  (1) Separation of insolubles

  (2) Isolation of products

(3) Purification

* **Objectives: remove impurities of similar chemical functionality and physical properties**

* **Typical operations: chromatography, affinity methods, fractional precipitation, electrophoresis**

(4) Polishing

* **Objectives: remove liquids**

* **Typical operations: drying, crystallization (not always possible)**

6.4.4 Purification

Especially for pharmaceuticals, finely purified final product is required. Therefore, crystallization, ultrafiltration, and various chromatographic methods are employed for final purification. Crystallization operates at low temperature and minimizes the thermal degradation of heat-sensitive materials (Shuler & Kargi, 2008). Drying is the removal of solvent from purified wet product. The physical properties of the product, its heat sensitivity, and desirable final moisture content have to be considered to conduct a drying process. Vacuum-tray driers, freeze driers, rotary drum driers, spray drum driers, and pneumatic conveyor driers are the major types used for purification (Shuler & Kargi, 2008).

Cells are smaller in size (hence centrifugation) and the product is inside the cell (hence cell disruption)

Purification of a soluble intracellular bacterial protein

Cell harvesting
e.g. centrifugation
→ supernatant (spent broth)

Disruption of recovered cells
e.g. liquid shear homogenization

Cellular debris separation
e.g. centrifugation
→ cell debris

Protein precipitation of the cell-free extract
e.g. ammonium sulphate

Recovery of protein by centrifugation

Desalting of resuspended protein pellet
gel filtration

Ion-exchange chromatography
e.g. DEAE cellulose

Dialysis

Lyophilization

Purification of an organic acid from a fungal culture

Broth clarification
e.g. rotary vacuum filtration
→ mycelium

Clarified broth decolorization
e.g. activated charcoal treatment

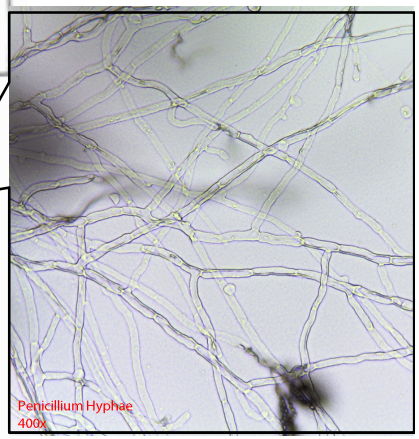
Evaporation

Crystallization

Recovery of crystals
e.g. filtration

Crystal cleaning

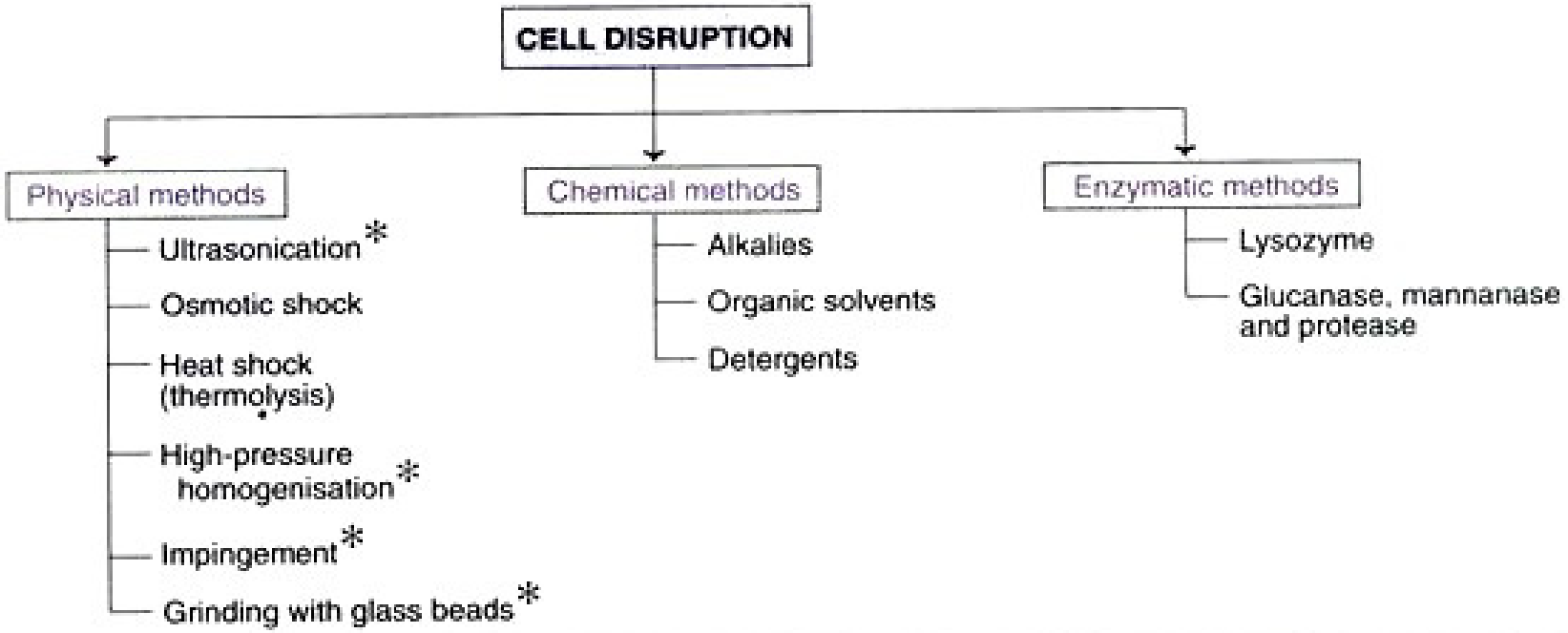
Crystal drying



Cells are larger in size and hence easy to separate by filtration and the product is outside the cell i.e. extracellular (hence the filtered cells are discarded and only the supernatant is processed)

There are several biotechnological products (vitamins, enzymes) which are located within the cells. Such compounds have to be first released (maximally and in an active form) for their further processing and final isolation. The microorganisms or other cells can be disintegrated or disrupted by physical, chemical or enzymatic methods.

If the product is intracellular (example. Insulin) we need to rupture the cell using one of these technique to get the product out of the cell.



Impingement:
In this procedure, a stream of suspended cells at high velocity and pressure are forced to hit either a stationary surface or a second stream of suspended cells (impinge literally means to strike or hit). The cells are disrupted by the forces created at the point of contact. Micro fluidizer is a device developed based on the principle of impingement. It has been successfully used for breaking E. coli cells. The advantage with impingement technique is that it can be effectively used for disrupting cells even at a low concentration.

Grinding with glass beads:
The cells mixed with glass beads are subjected to a very high speed in a reaction vessel. The cells break as they are forced against the wall of the vessel by the beads. Several factors influence the cell breakage-size and quantity of the glass beads, concentration and age of cells, temperature and agitator speed. Under optimal conditions, one can expect a maximal breakage of about 80% of the cells.

Ultra sonication:
Ultrasonic disintegration is widely employed in the laboratory. However, due to high cost, it is not suitable for large-scale use in industries.

Osmotic shock:
This method involves the suspension of cells (free from growth medium) in 20% buffered sucrose. The cells are then transferred to water at about 4°C. Osmotic shock is used for the release of hydrolytic enzymes and binding proteins from Gram-negative bacteria.

Heat shock (thermolysis):
Breakage of cells by subjecting them to heat is relatively easy and cheap. But this technique can be used only for a very few heat-stable intracellular products.

High pressure homogenization:
This technique involves forcing of cell suspension at high pressure through a very narrow orifice to come out to atmospheric pressure. This sudden release of high pressure creates a liquid shear that can break the cells

Chemical methods of cell disruption:

Treatment with alkalies, organic solvents and detergents can lyse the cells to release the contents.

Alkalies:

Alkali treatment has been used for the extraction of some bacterial proteins. However, the alkali stability of the desired product is very crucial for the success of this method e.g., recombinant growth hormone can be efficiently released from E. coli by treatment with sodium hydroxide at pH 11.

Organic solvents:

Several water miscible organic solvents can be used to disrupt the cells e.g., methanol, ethanol, isopropanol, butanol. These compounds are inflammable; hence require specialised equipment for fire safety. The organic solvent toluene is frequently used. It is believed that toluene dissolves membrane phospholipids and creates membrane pores for release of intracellular contents.

Detergents:

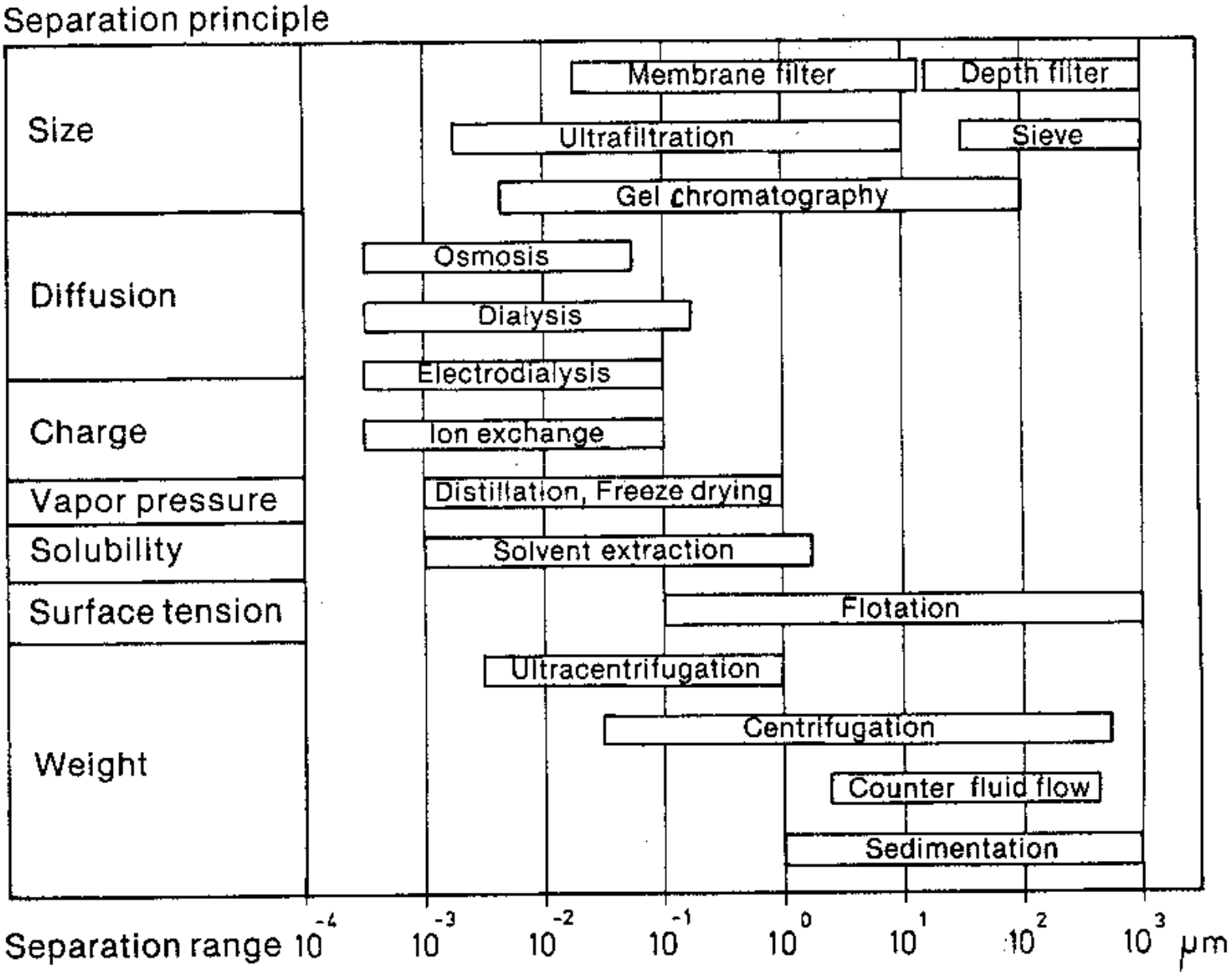
Detergents that are ionic in nature, cationic-cetyl trimethyl ammonium bromide or anionic-sodium lauryl sulfate can denature membrane proteins and lyse the cells. Non-ionic detergents (although less reactive than ionic ones) are also used to some extent e.g., Triton X-100 or Tween. The problem with the use of detergents is that they affect purification steps, particularly the salt precipitation. This limitation can be overcome by using ultrafiltration or ion-exchange chromato-graphy for purification.

Enzymatic methods of cell disruption:

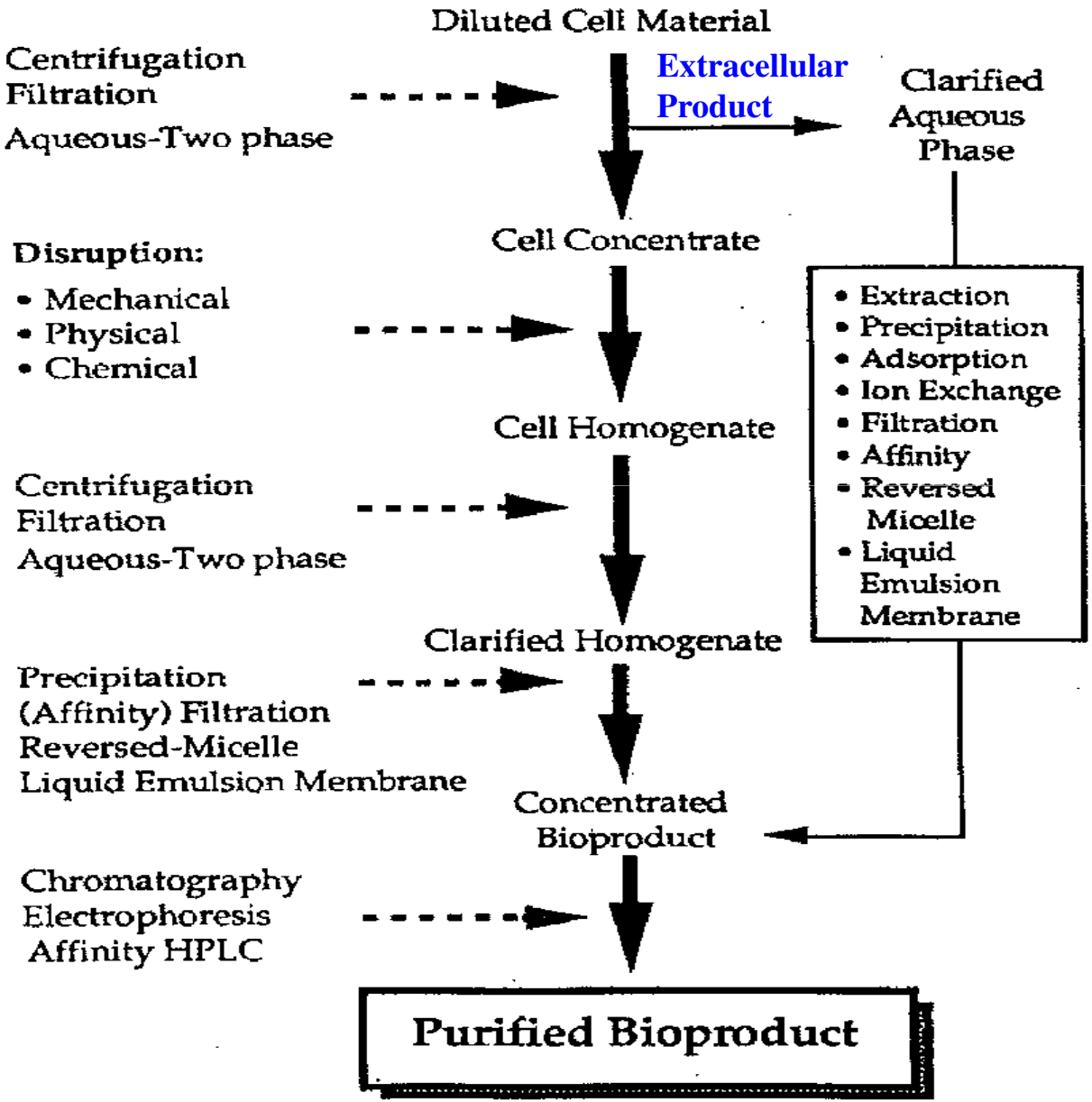
Cell disruption by enzymatic methods has certain advantages i.e., lysis of cells occurs under mild conditions in a selective manner. This is quite advantageous for product recovery. Lysozyme is the most frequently used enzyme and is commercially available (produced from hen egg white). It hydrolyses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

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

- **Separation principles used in various recovery steps:**



Purification Scheme of a Bioproduct



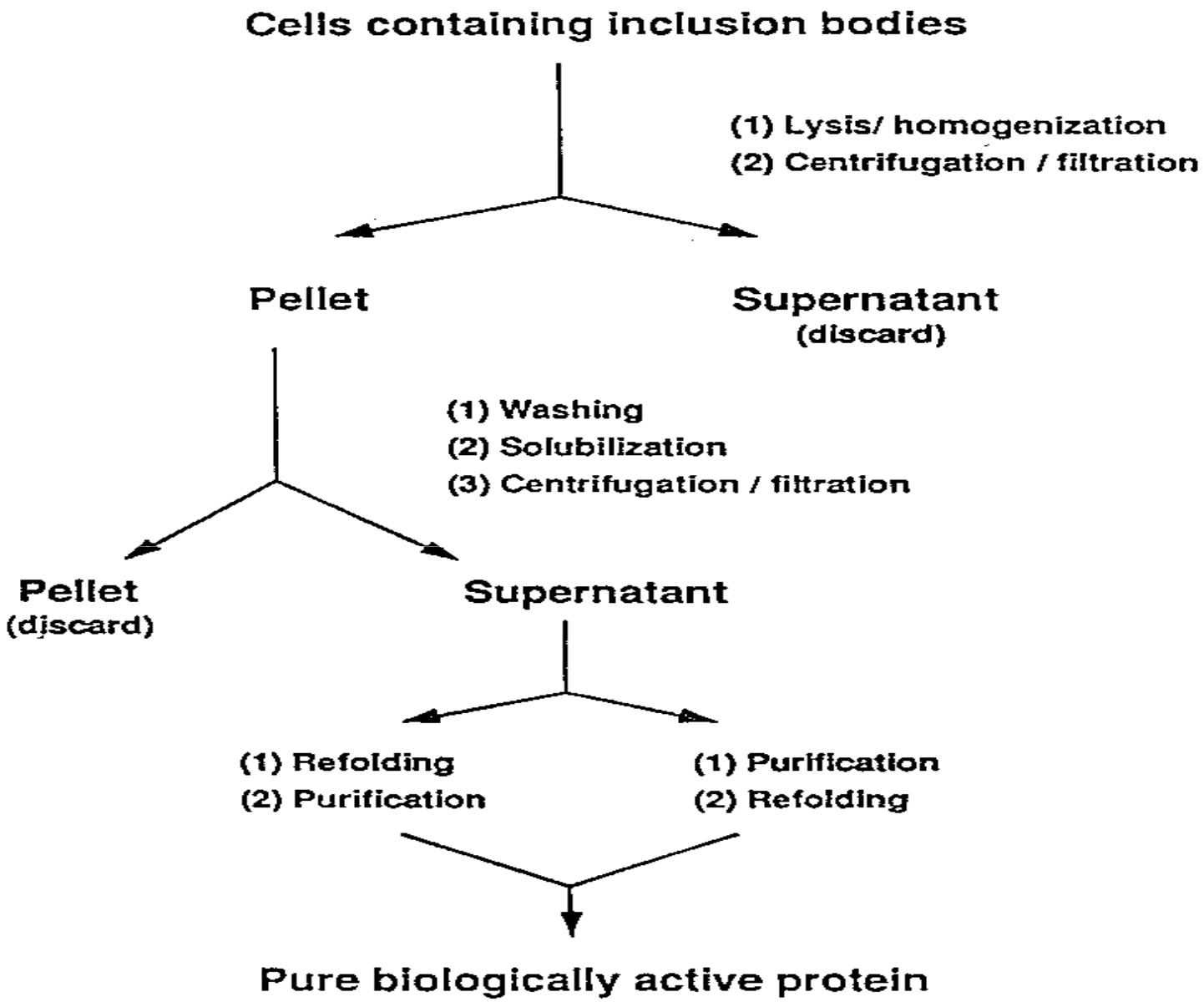
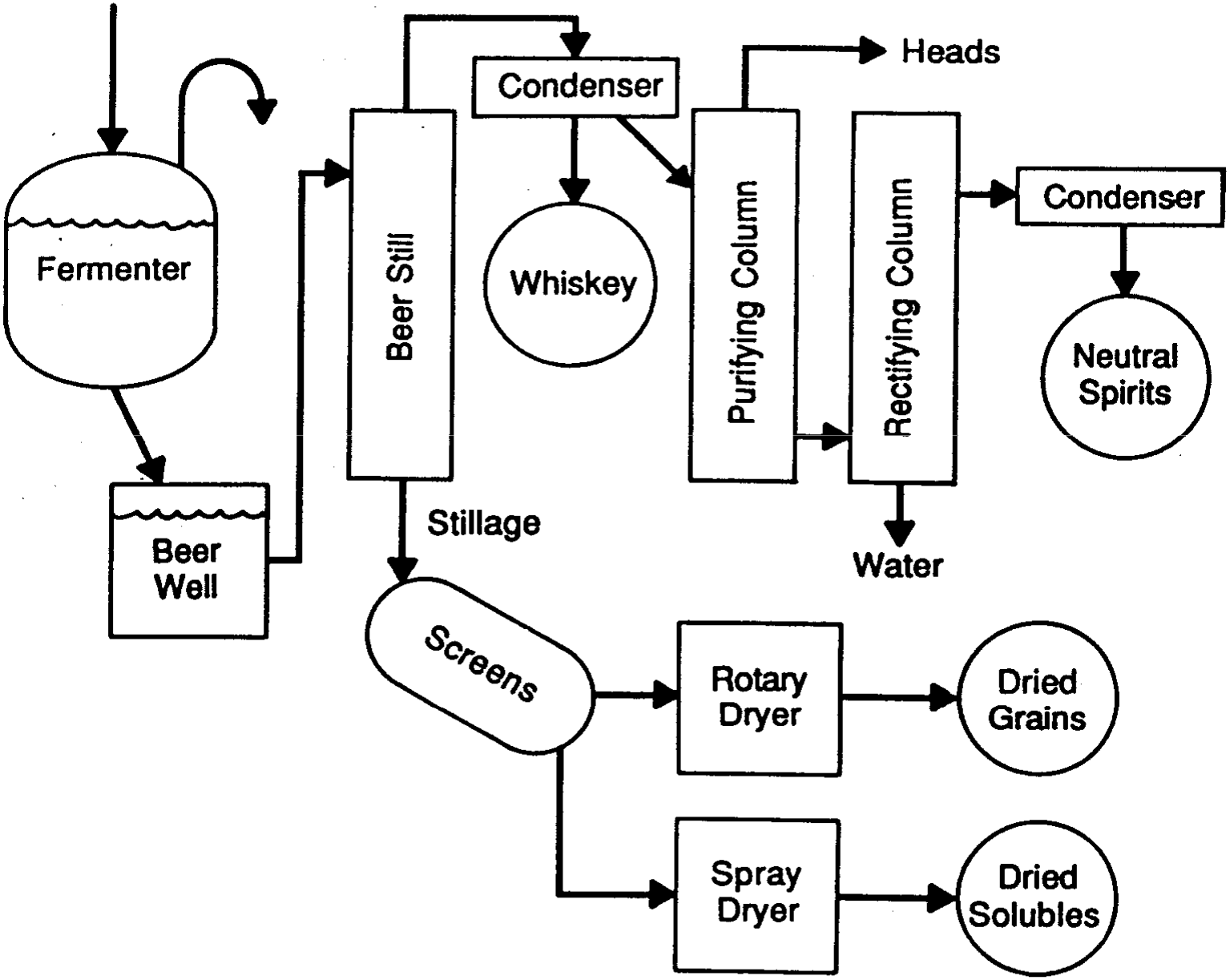
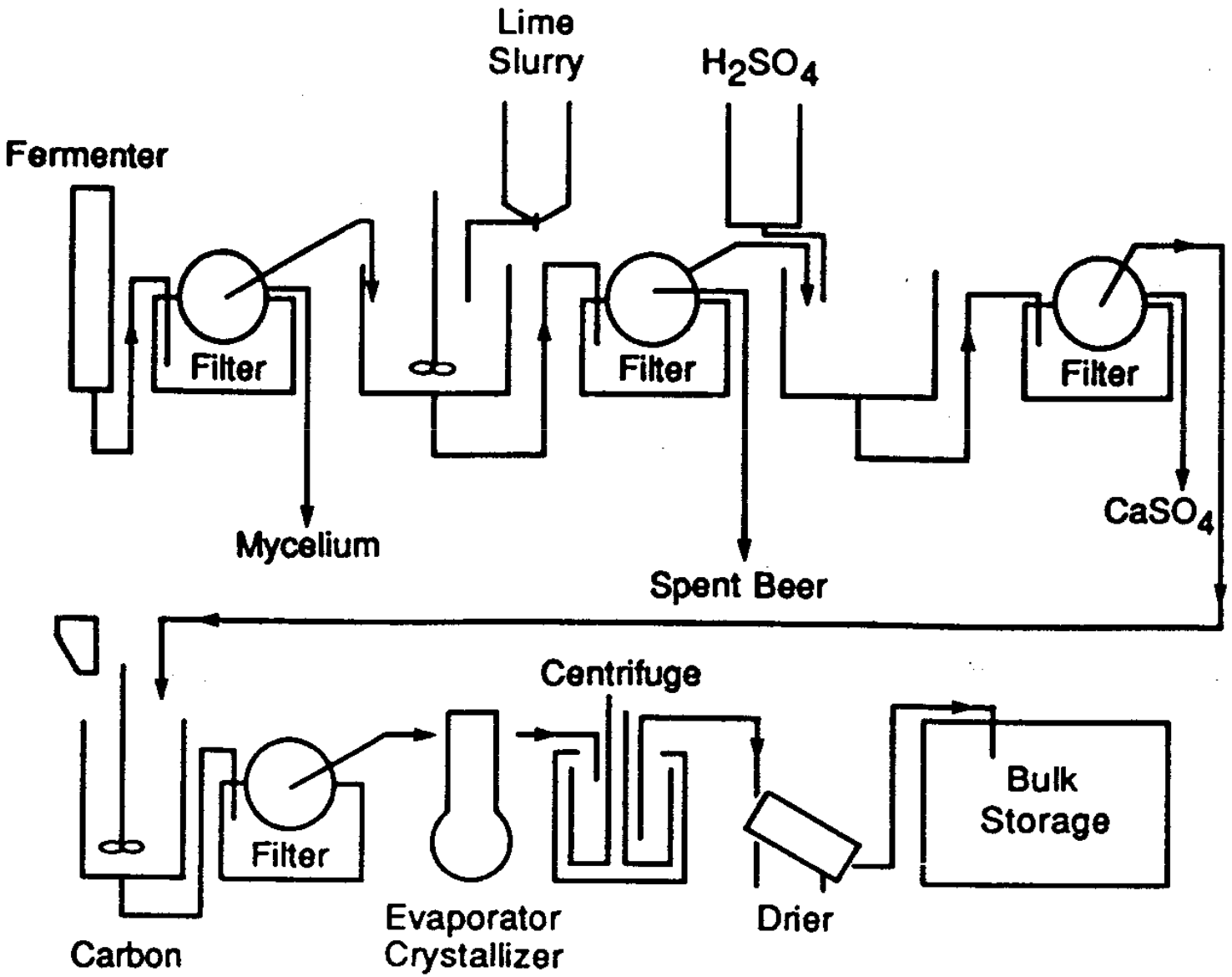


Figure 2. Inclusion body protein recovery scheme.

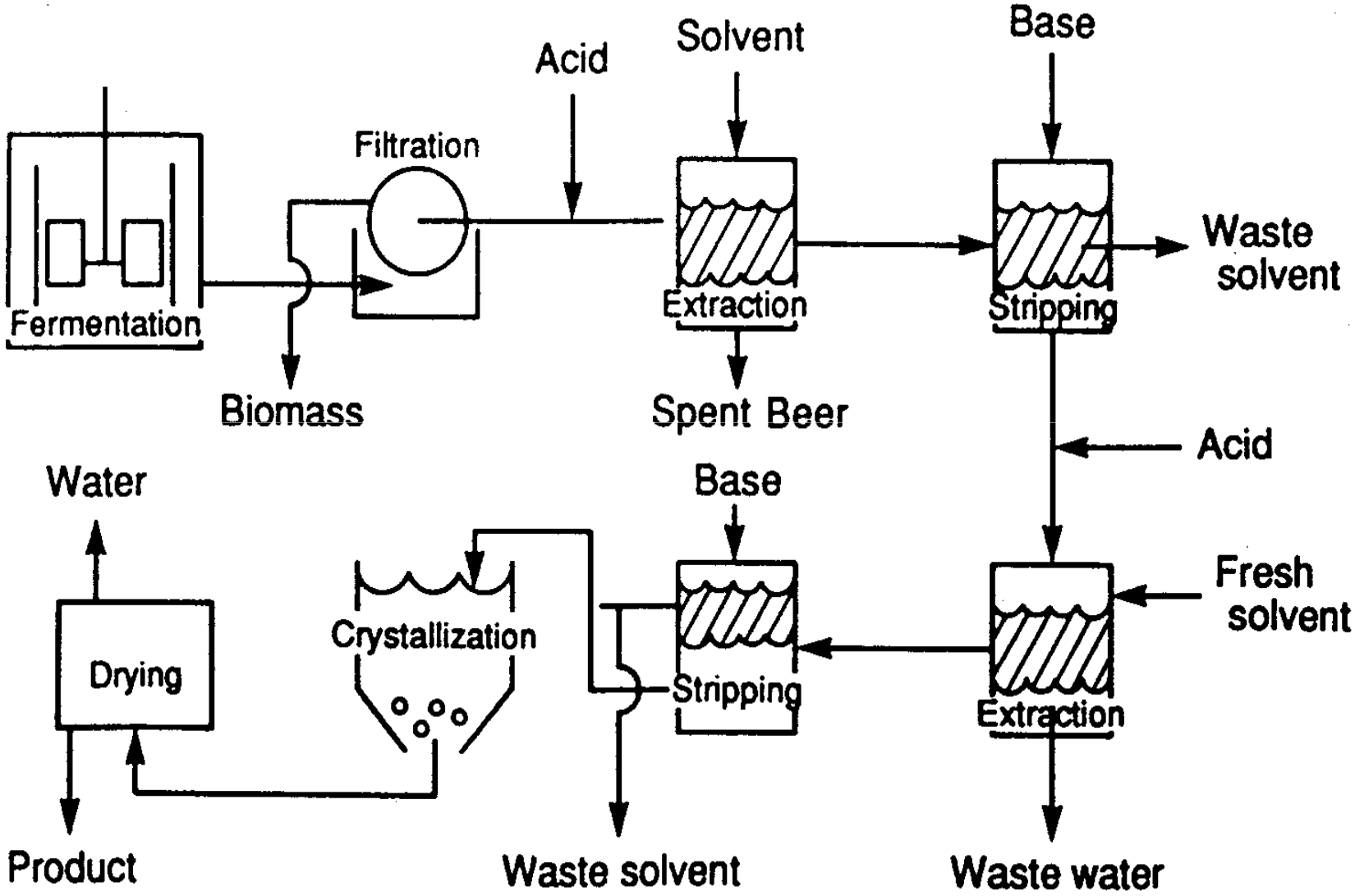
- **Process for ethanol production from fermentation:**



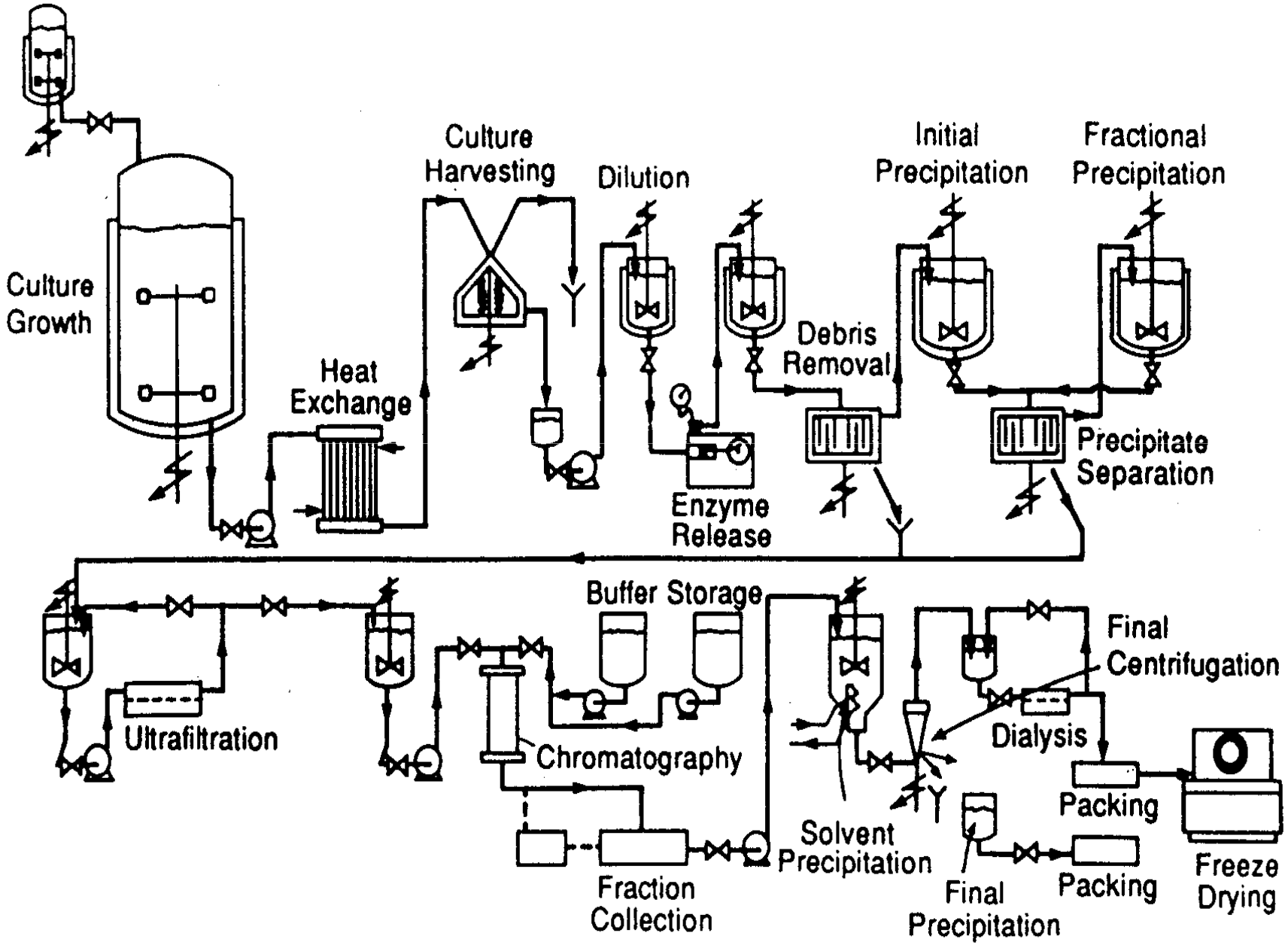
- **Manufacture of citric acid:**



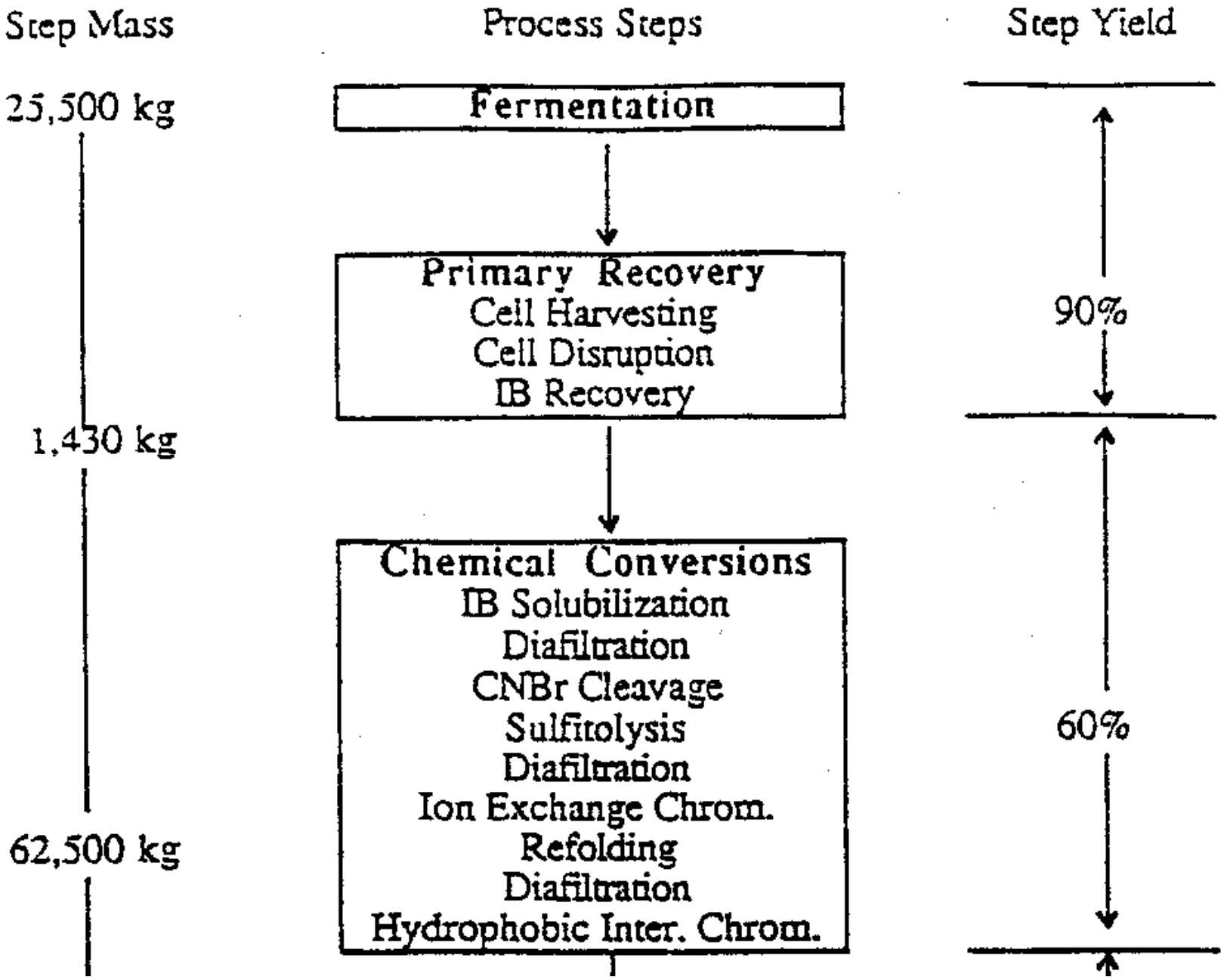
- **Penicillin production:**



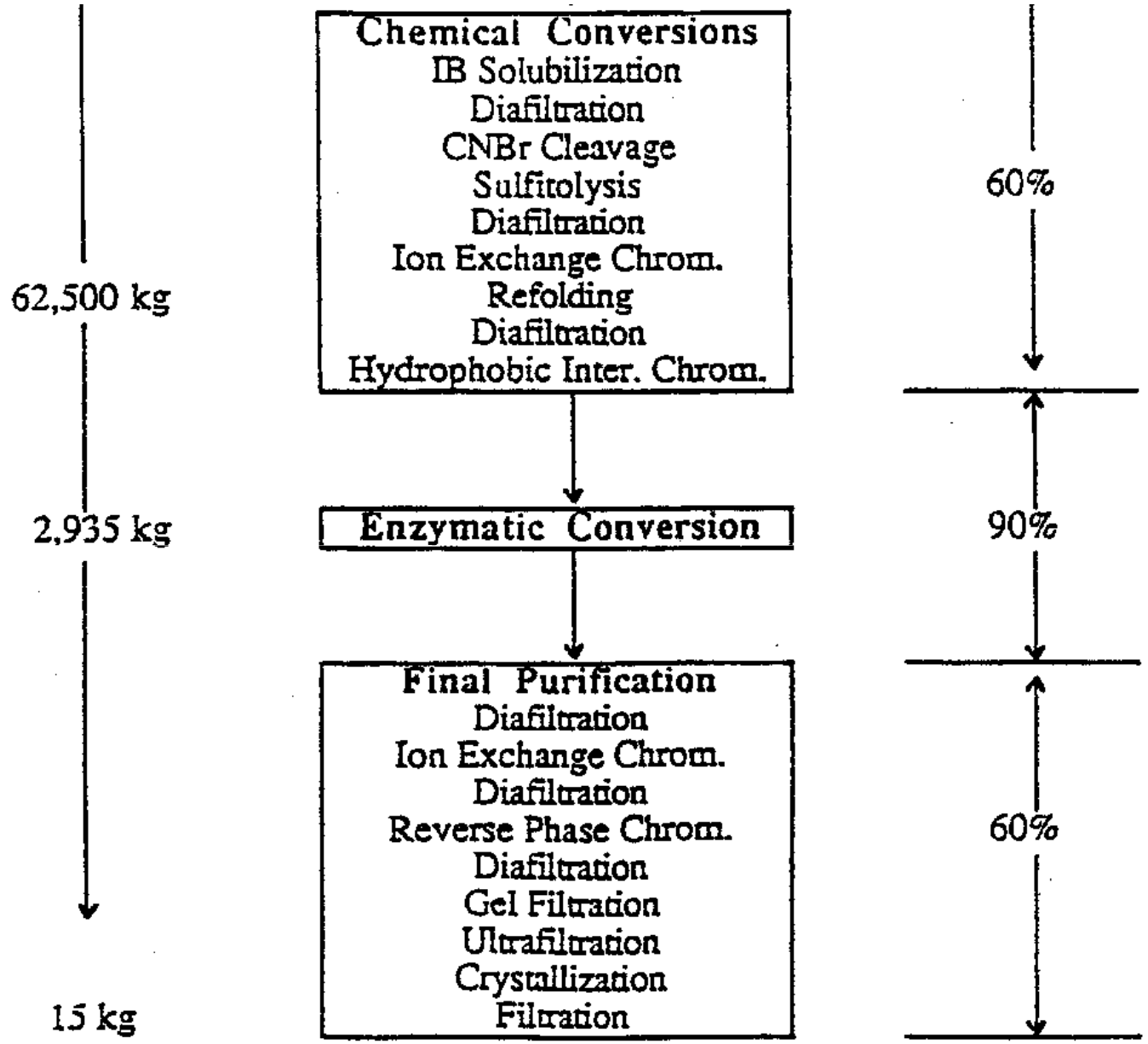
• Production of an intracellular enzyme:



- **Processing scheme for recombinant insulin:**



• Processing scheme for recombinant insulin (2/2):



* IB: inclusion body