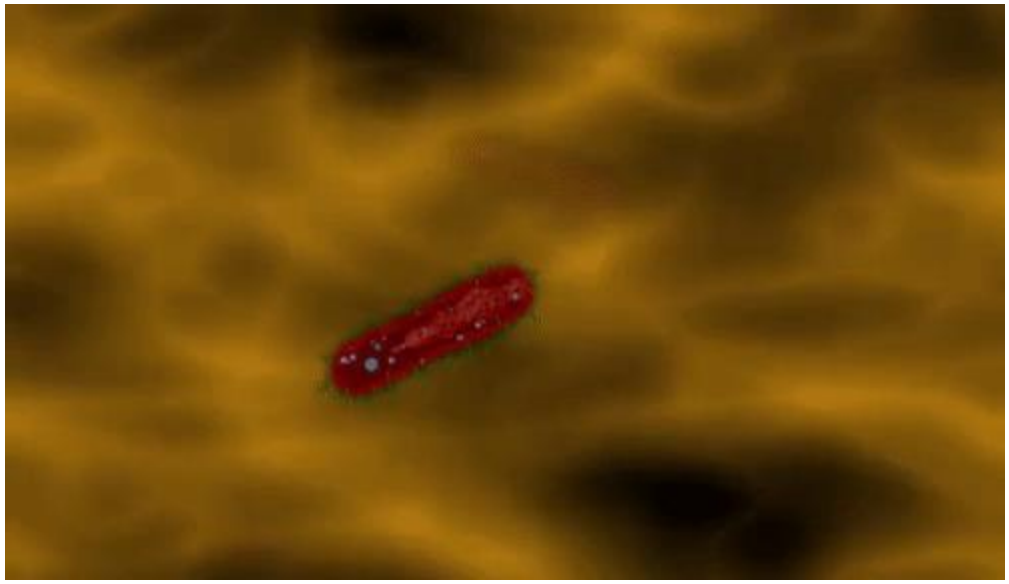
Fermentation



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Learning Objectives

Students should able to understand

- **Fermentation Technology**
- **Fermentation Media**
- **Bioreactor Design**

Fermentation

- Fermentation is the chemical transformation of organic substances into simpler compounds by the action of enzymes, complex organic catalysts, which are produced by microorganisms such as molds, yeasts, or bacteria.

- Fermentation technology is the use of microorganisms to produce food, pharmaceuticals and alcoholic beverages on a large scale industrial basis.

- The science of fermentation is called "zymology".

Principle of Fermentation Process

- The basic principle involved in the industrial fermentation technology is that organisms are grown under suitable conditions, by providing raw materials, meeting all the necessary requirements such as carbon, nitrogen, salts, trace elements and vitamins.

- The end products formed as a result of their metabolism during their life span are released into the media, which are extracted for use by human being and that have a high commercial value.

Major fermentation Products

Group	Product	Organism
Industrial chemicals	Ethanol Lactic acid	*Saccharomyces cerevisiae* *Lactobacillus bulgaricus*
Enzymes	□-amylase Proteases Lipases	*Bacillus subtilis* *Bacillus species* *Saccharomyces lipolytica*
Antibiotics	Penicillin Streptomycin Chlorampenicol	*Penicillium chrysogenum* *Streptomyces griseus* *Streptomyces venezuelae*
Vitamins	Riboflavin Vitamin B12	*Ashbya gossypi* *Pseudomonas dentrificians*

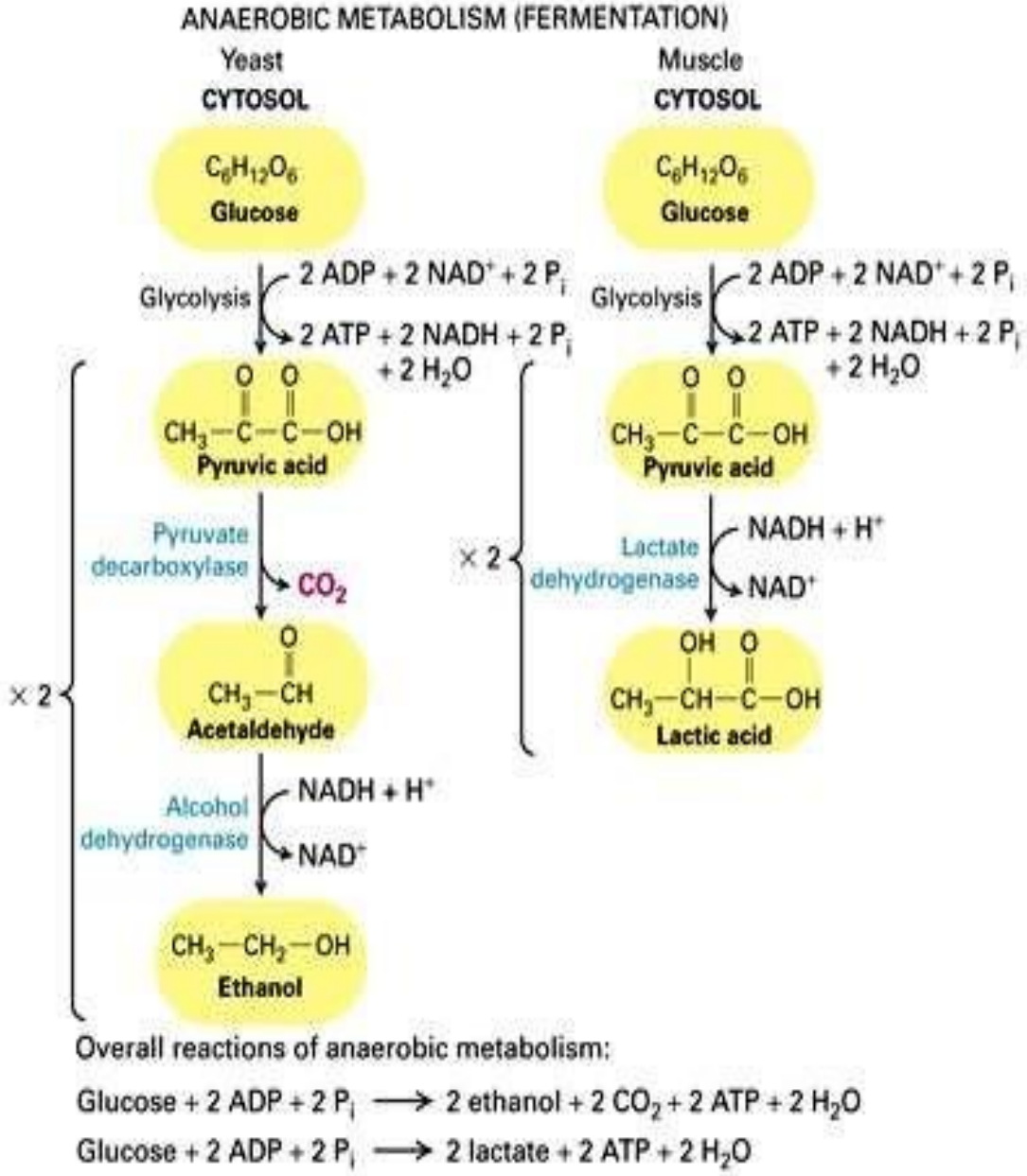
Types Based On Respiration

1.Aerobic Fermentation:

- **Aerobic fermentation** or **aerobic glycolysis** is a metabolic process by which cells metabolize sugars via fermentation in the presence of oxygen and occurs through the repression of normal respiratory metabolism.

- Aerobic fermentation means that oxygen is present. Wine, beer and acetic acid vinegar (such as apple cider vinegar), need oxygen in the "primary" or first stage of fermentation.

- When creating acetic vinegar, for example, exposing the surface of the vinegar to as much oxygen as possible, creates a healthy, flavorful vinegar with the correct pH.



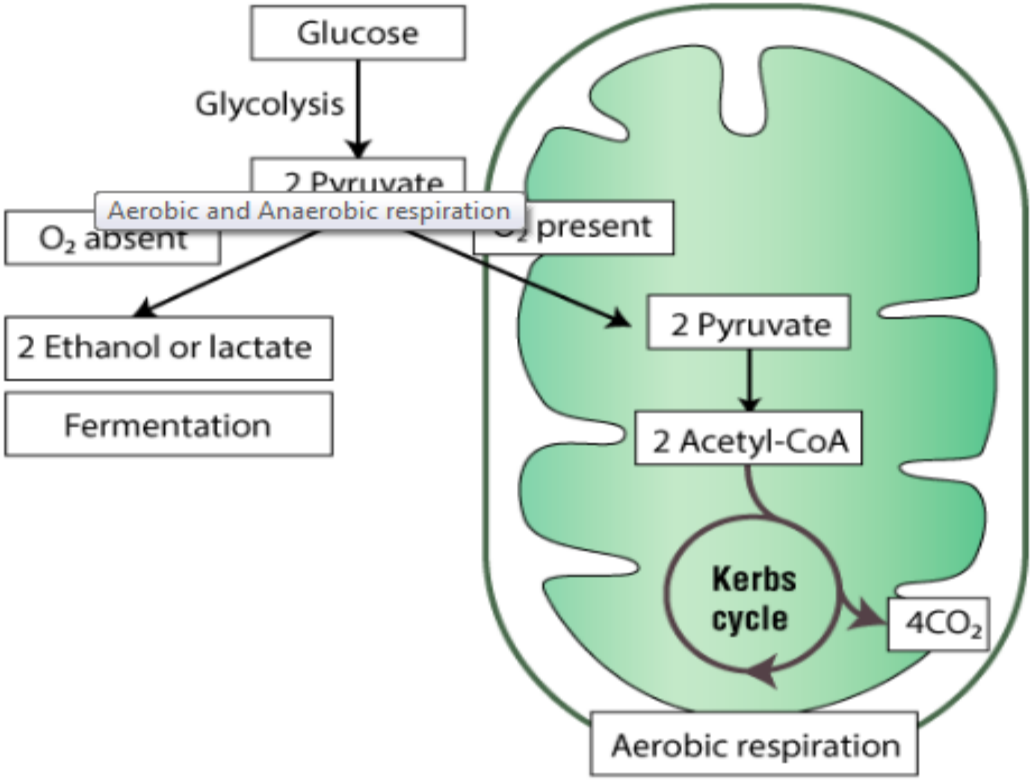
Overall reactions of anaerobic metabolism:

Glucose + 2 ADP + 2 P_i ⟶ 2 ethanol + 2 CO_2 + 2 ATP + 2 H_2O

Glucose + 2 ADP + 2 P_i ⟶ 2 lactate + 2 ATP + 2 H_2O

2.Anaerobic Fermentation:

- Anaerobic fermentation is a method cells use to extract energy from carbohydrates when oxygen or other electron acceptors are not available in the surrounding environment.
- This differentiates it from anaerobic respiration, which doesn't use oxygen but does use electron-accepting molecules that come from outside of the cell.
- The process can follow glycolysis as the next step in the breakdown of glucose and other sugars to produce molecules of adenosine triphosphate (ATP) that create an energy source for the cell.



Depending upon the end product formed, fermentation can be categorized into various types

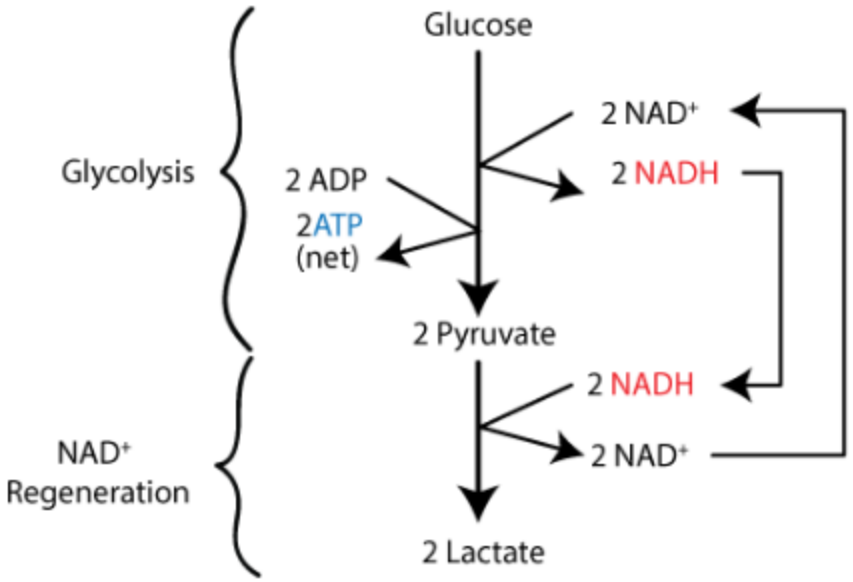
Homo fermentation: When only one type of product is formed

Hetero fermentation: When more than one products are formed

1. Lactic Acid Fermentation

- Lactic acid is formed from pyruvate produced in glycolysis. NAD+ is generated from NADH.
- Enzyme lactate dehydrogenase catalyses this reaction.
- Lactobacillus bacteria prepare curd from milk by this type of fermentation.
- During intense exercise when oxygen supply is inadequate, muscles derive energy by producing lactic acid, which gets accumulated in the cells causing fatigue.

$$C_6H_{12}O_6(Glucose) \xrightarrow[Lactate.dehydrogenase]{Lactobacillus} 2CH_3CHOHCOOH(L$$



2. Alcohol Fermentation

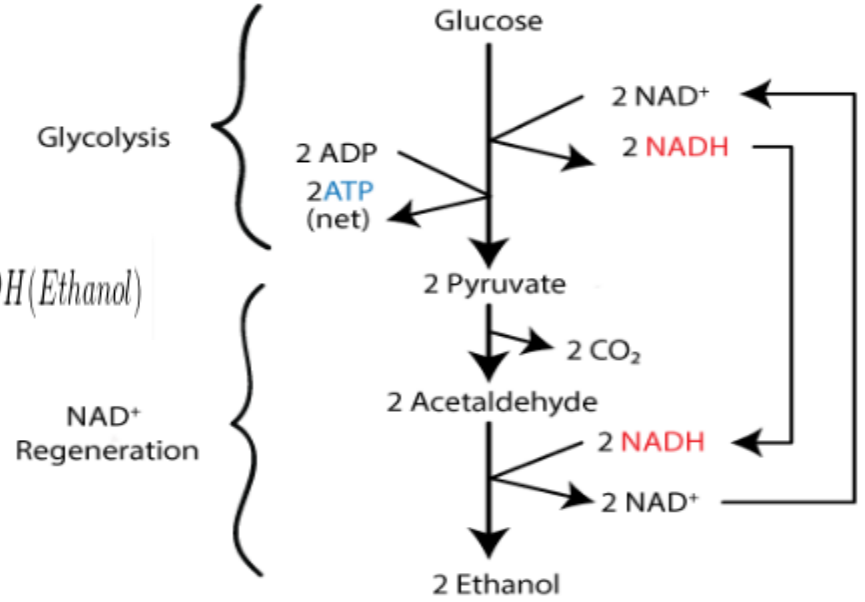
➢This is used in the industrial production of wine, beer, biofuel, etc. The end product is alcohol and CO_2.

➢Pyruvic acid breaks down into acetaldehyde and CO_2 is released. In the next step, ethanol is formed from acetaldehyde.

➢NAD+ is also formed from NADH which is reused in glycolysis. Yeast and some bacteria carry out this type of fermentation.

➢Enzyme pyruvic acid decarboxylase and alcohol dehydrogenase catalyse these reactions.

$$C_6H_{12}O_6(Glucose) \xrightarrow[Decarboxylase]{Dehydrogenase} 2CH_3CHO(Acetaldehyde) + 2CO_2 \rightarrow 2C_2H_5OH(Ethanol)$$



3. Acetic acid Fermentation

- Vinegar is produced by this process. This is a two-step process.
- The first step is the formation of ethyl alcohol from sugar anaerobically using yeast.
- In the second step, ethyl alcohol is further oxidised to form acetic acid using acetobacter bacteria.
- Microbial oxidation of alcohol to acid is an aerobic process.

$$C_2H_5OH(ethanol) + O_2 \xrightarrow{acetobacter} CH_3COOH(acetic\ acid) + H_2O$$

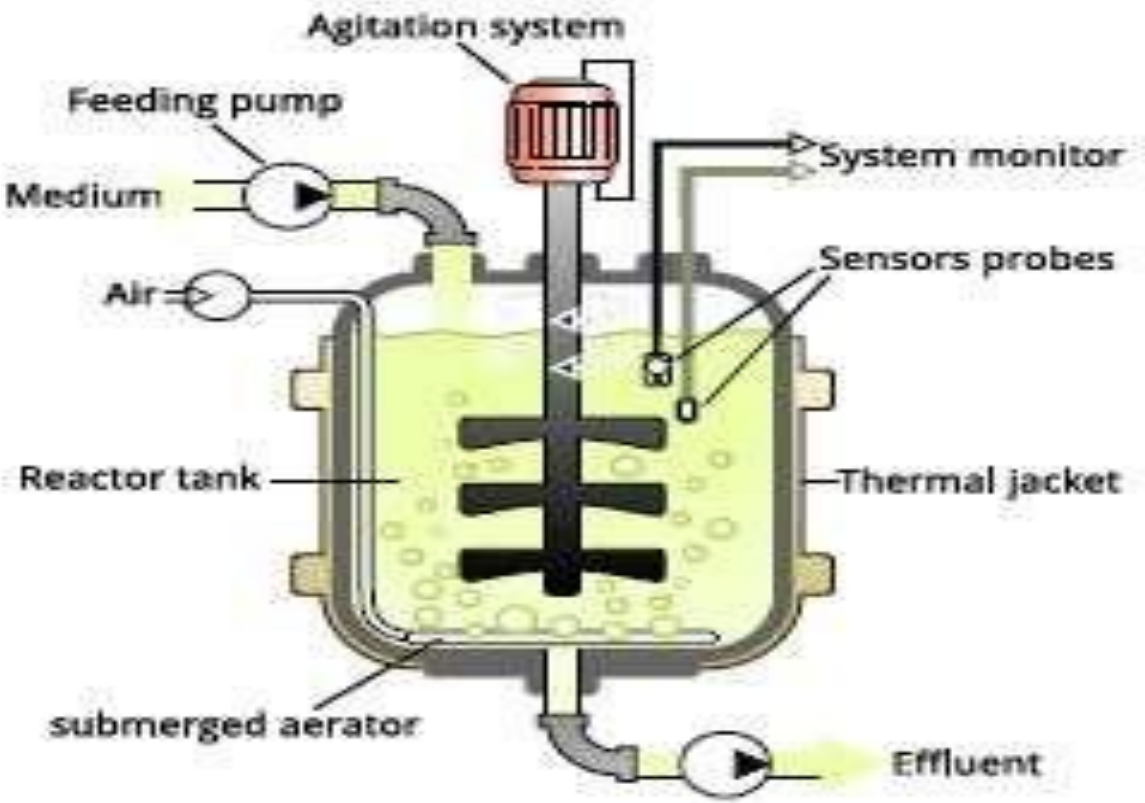
4. Butyric acid Fermentation

- This type of fermentation is characteristic of obligate anaerobic bacteria of genus clostridium.
- This occurs in retting of jute fibre, rancid butter, tobacco processing and tanning of leather.
- Butyric acid is produced in the human colon as a product of dietary fibre fermentation.
- Sugar is first oxidised to pyruvate by the process of glycolysis and then pyruvate is further oxidised to form acetyl-CoA by the oxidoreductase enzyme system with the production of CO_2 and H_2. acetyl-CoA is further reduced to form butyric acid.
- This type of fermentation leads to a relatively higher yield of energy 3 molecules of ATP are formed.

$$C_6H_{12}O_2 \xrightarrow[butyricum]{clostridium} C_4H_8O_2 + 2CO_2 + 2H_2$$

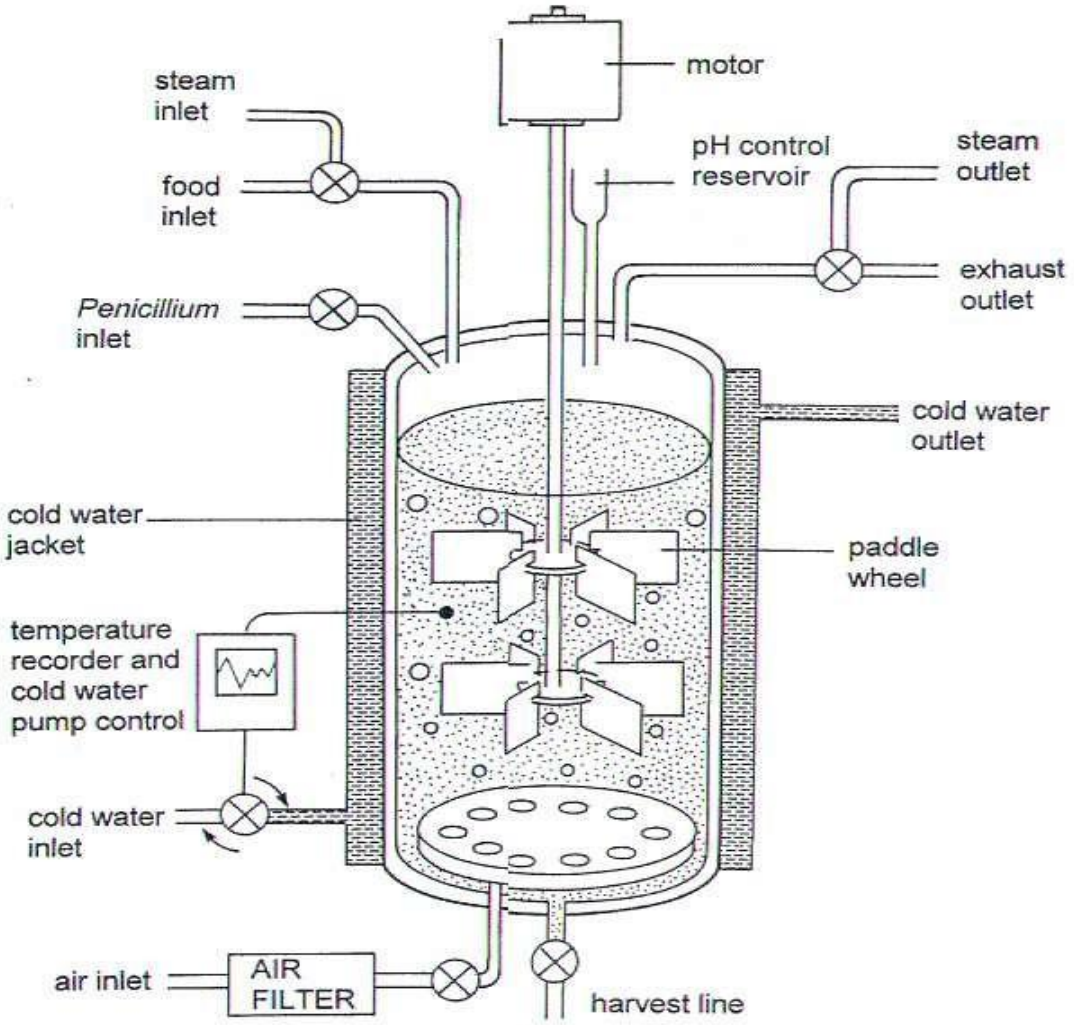
Fermenter ?

"A specially designed vessel in which large quantity fermentation media is added with fermentation microorganisms which provides best possible environment control and process control for the biosynthesis of fermentation products."

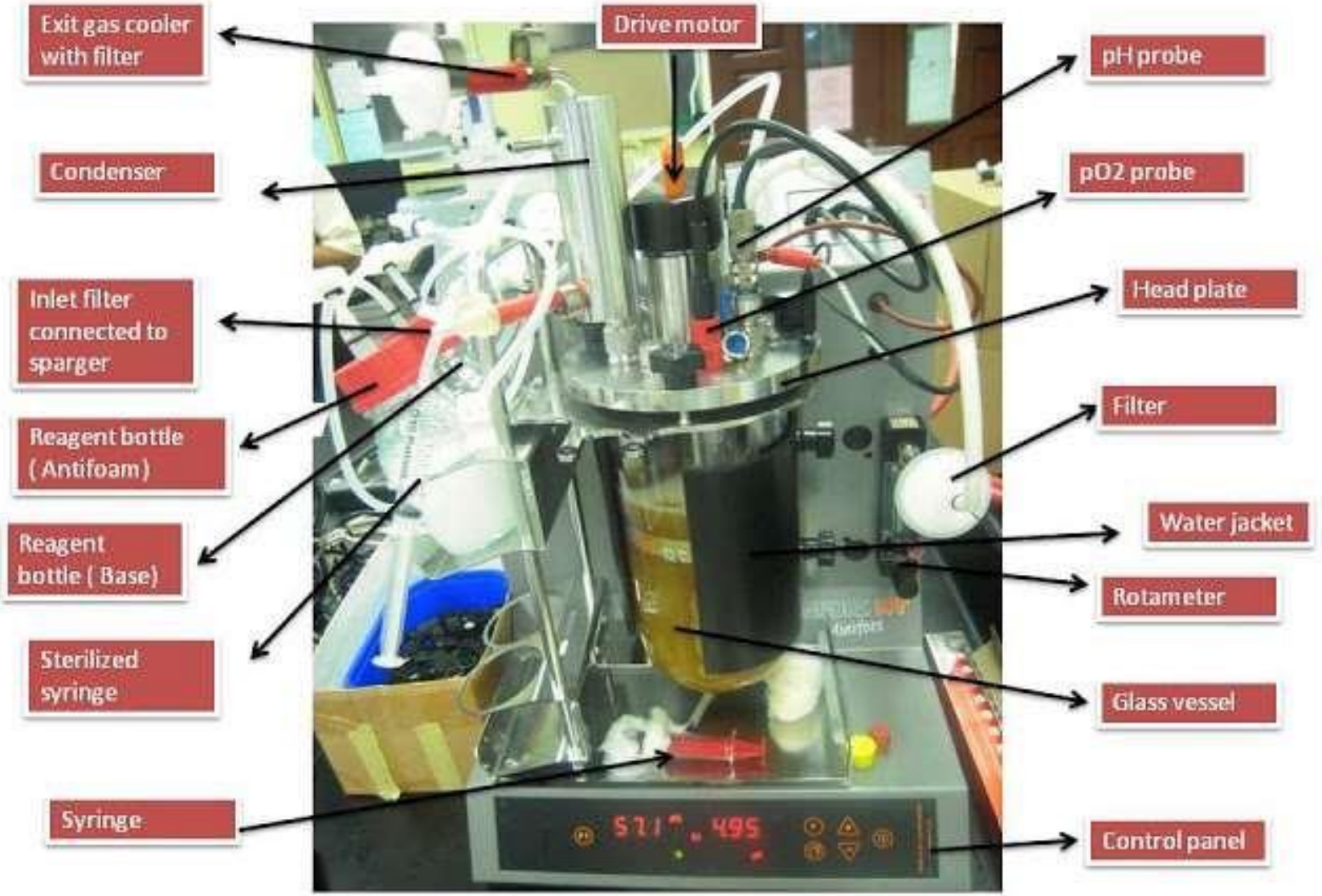


General structure of a continuous stirred-tank type bioreactor

Design of Fermenter



components of a typical fermenter



FERMENTOR

- Closed vessels
- Used for fermentation
- Production of large scale process

- Fermenter is a closed vessels now it is called as bioreactor
- Fermenter is a old process used for cultivate the
  - microorganisms
- Fermenter used for cultivate Prokaryotic cells (fungi bacteria)
- Bioreactors used for cultivate eukaryotic cells (mammalian and insects)

Sr.	Parts of fermenter	Function
1	Impellor (agitator)	To stir the media continuously and hence prevent cells from settling down, and distribute oxygen throughout the medium
2	Sparger (Aerator)	Introduce sterile oxygen to the media in case of aerobic fermentation process
3	Baffles (vortex breaker)	Disrupt vortex and provide better mixing
4	Inlet Air filter	Filter air before it enter the fermenter
5	Exhaust Air filter	Trap and prevent contaminants from escaping
6	Rotameter	Measure flow rate of Air or liquid
7	Pressure gauge	Measure pressure inside the fermenter
8	Temperature probe	Measure and monitor change in temperature of the medium during the process
9	Cooling Jacket	To maintain the temperature of the medium throughout the process
10	pH probe	Measure and monitor pH of the medium
11	Dissolve Oxygen Probe	Measure dissolve oxygen in the fermenter
12	Level probe	Measure the level of medium
13	Foam probe	Detect the presence of the foam
14	Acid	Maintain the required pH of the medium by neutralizing the basic environment
15	Base	Maintain the required pH of the medium by neutralizing the acidic environment
16	Antifoam	Breakdown and prevent foams
17	Sampling pint	To obtain samples during the process
18	Valves	Regulation and control the flow liquids and gases
19	Control panel	Monitor over all parameters

1. Vessel

- Function of a fermenter is to carryout process under appropriate aseptic and pre-defined environmental conditions.

- A fermentation vessel is designed in such a way that it requires minimal labour operation and maintenance.

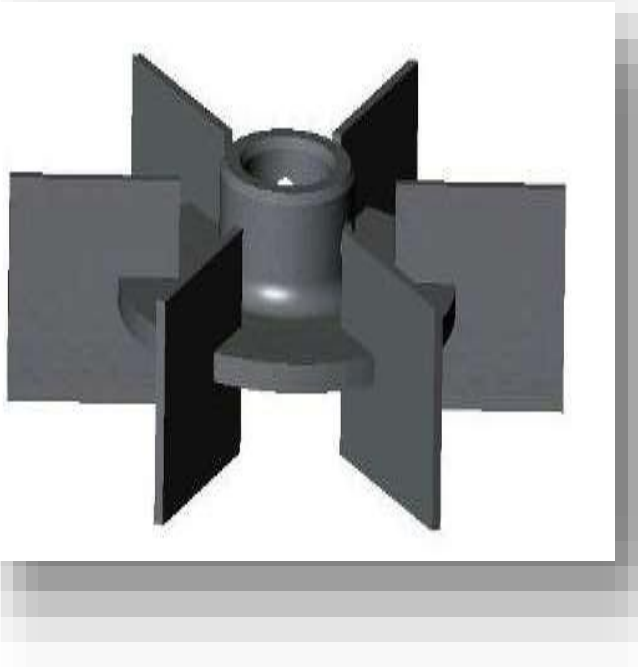
- There are mainly two types of vessels base on the type fermentation process :

- **Small scale fermenter (Laboratory scale fermenter)** These are made up of glass

- **Large scale fermenter (Industrial scale fermenter)**

As, stainless steel is the most satisfactory material, it is used to manufacture vessels of high volume

2.Impeller (Agitator)



- Mounted to a shaft through abearing in the lid

- Driven by an external power source

- The agitator is required to achieve a number of mixing objective.

- Bulk fluid and gas-phase mixing
- Air dispersion,
- Oxygen transfer,
- Heat transfer,
- Suspension of solid particles and maintain a uniform environment throughout the vessel contents.

3. Sparger

- A device that introduce air into medium
- Has a pipe with minute holes (1/64 - 1/32 inch or large)
- Hole – allows air under pressure to escape into medium
- Depending on volume of medium in the fermentation vessel, different types of spargers are installed in the fermenter.

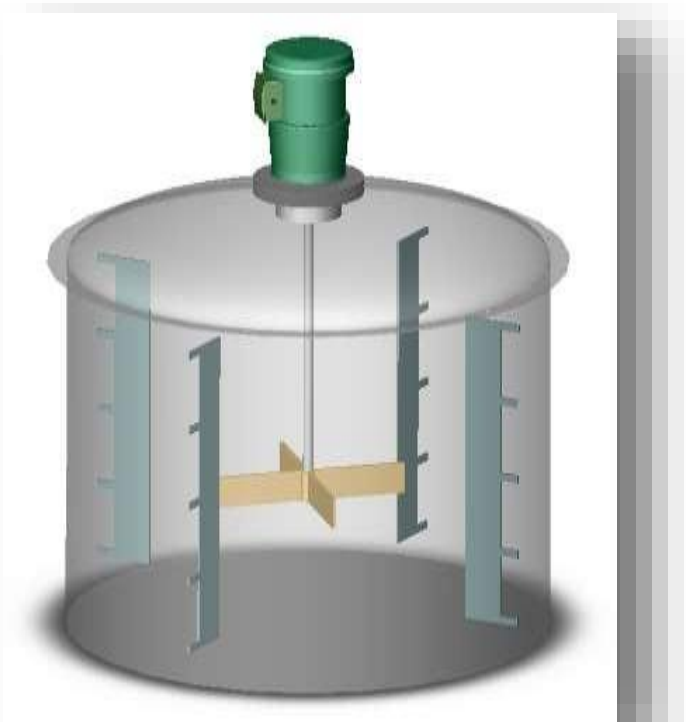
1. Porous
2. Orifice
3. Nozzle

4.Baffles

- Baffles are metal strips roughly one-tenth of vessel diameter and attached radially to the wall of bioreactor

- Generally four to eight baffles are incorporated.

- They are normally incorporated into agitated vessels of all sizes to prevent vortex and to improve aeration efficiency



5.Temperature controlling (heating and cooling) devices

- Mechanical agitation and exothermic microbial metabolic activity generates heat during the fermentation process.
- Endothermic microbial metabolic activity lower down the temperature of the fermentation medium
- To maintain this temperature, heat is to be either added to or removed from the system
- The cooling system is used to remove excess heat from the system
- Internal heating coils are used for providing heat (Note: *In case of lab scale process, the fermenter is placed in thermostatically controlled bath)*

Temperature probe

6.pH control: -

- Certain microorganisms grow in particular pH only. In fermentation it is very essential to control

- pH in order to grow the desired microorganisms for product formation.

- pH control sensors are used in fermenter for periodically checking of pH.

7.Feed ports

- Feed ports are the tubes (for Lab scale fermenter) and pipelines (for large scale fermenter) connected to the nutrient reservoir

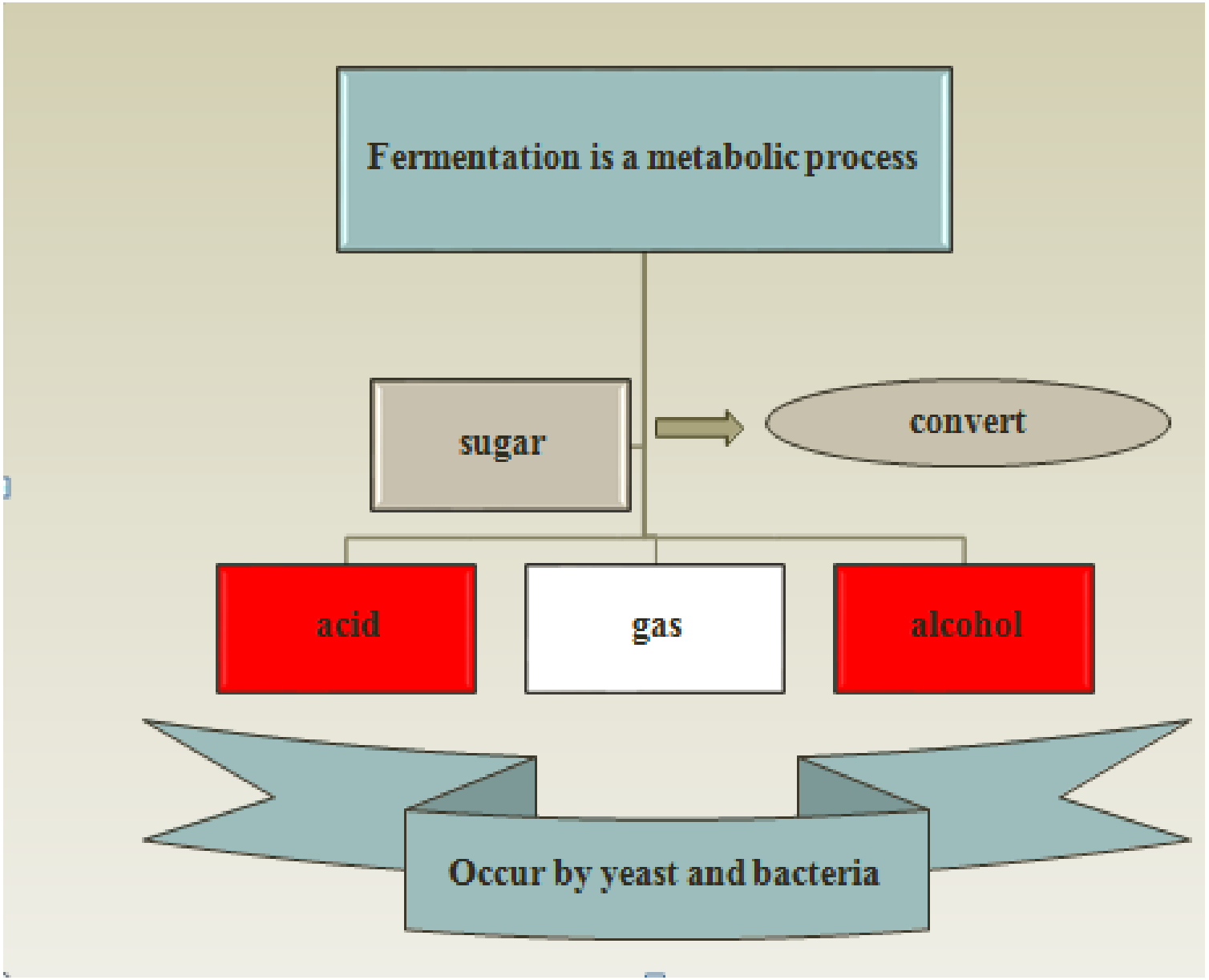
- These tubes or pipelines are used to add nutrients and acid/alkali in the fermenter before and during the fermentation process

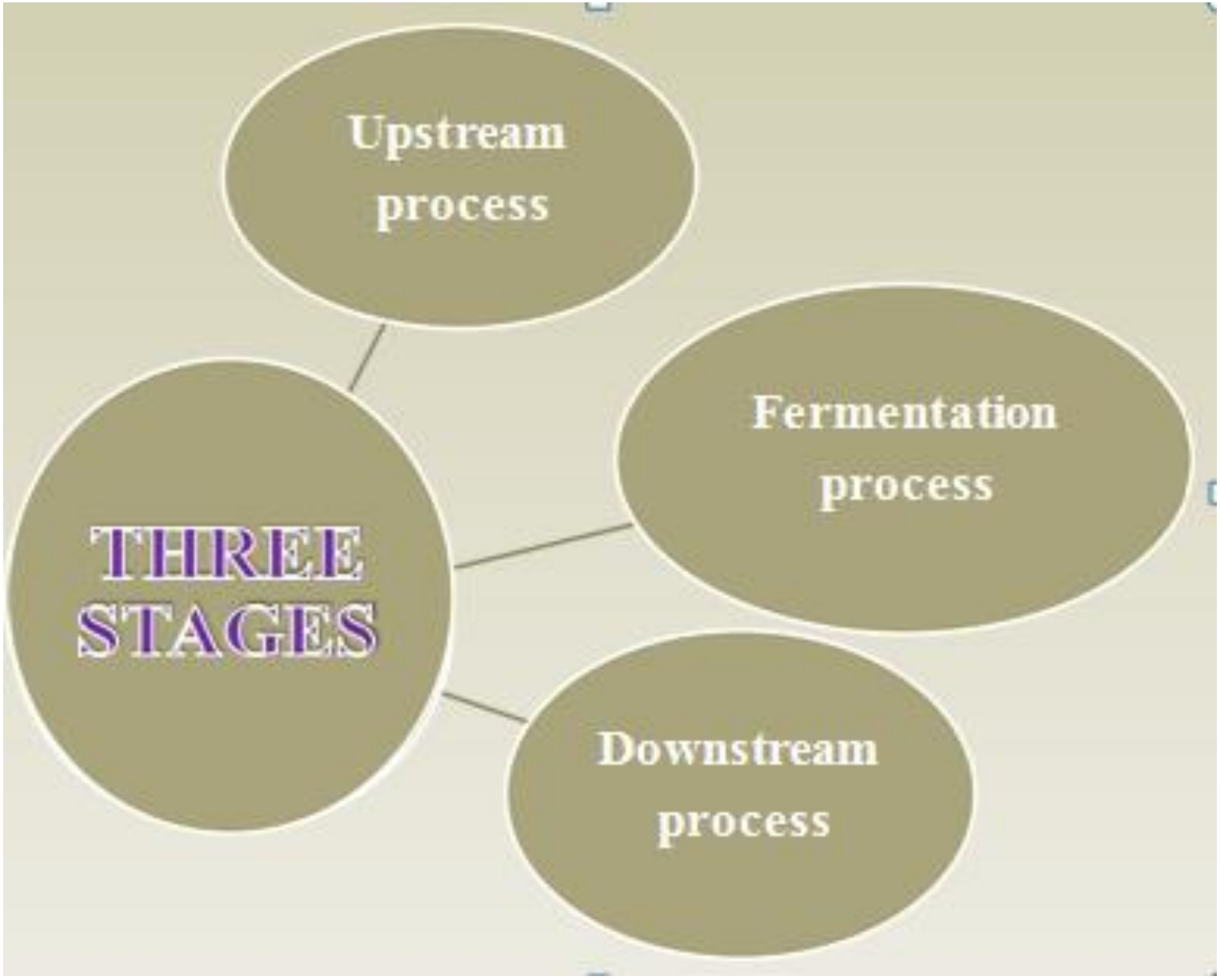
- They are heat sterilized in situ and /or ex situ with stem

- It is advisable to sterilize after connection has been done and before any additions are made

Monitoring and controlling parts of fermenter are:

S.No	Part	Use
1	Pt100	temperature sensor (platinum resistance electrode)
2	Foam probe	kept above the medium level to sense foam formation
3	pH electrode	senses pH
4	O_2 sensor	Monitors dissolved oxygen level
5	Heater pad	directly heats the medium
6	Cold finger	after direct heating – used to cool the vessel contents (closed coil/pipe to pass cool water)
7	Rotameter	variable air flow meter – indicates rate of air flow into vessel – attached to air sparger
8	Pressure valve	attached to rotameter for safer operation
9	Air pump	supply of air
10	Peristaltic pump	to pump in medium, acids, bases, antifoam


Fermentation is a metabolic process
sugar
convert
acid
gas
alcohol
Occur by yeast and bacteria


Upstream process
Fermentation process
THREE STAGES
Downstream process

Upstream Process

- The pre-fermentation stage
  - Isolation
  - Improvement
  - Producing of microorganisms
- Screening method; isolate microbes to produce decreed products Two methods;
  - primary screening checking the quality of microbes done in agar plate.
  - Secondary screening checking the quntative of microbes done in liquid media

Upstream Process

- Microbes isolated from natural sources thus is improved to get product
- **strains** by using
  - Recombination
  - Mutations
  - Cell fusion
  - Gene cloning
- Media formulation : growth medium must have essential nutrients for microbial growth for successful fermentation process
- Two kind media :
  - Inoculum media : enrich the culture
  - Production media: contain carbon and nitrogen
- Raw materials : corn molasses, cellulose, corn, streep liquor soybean, sugar, beet molasses, malt extract etc.,

Upstream Process

➢**Upstream processing** includes **formulation** of the fermentation medium, sterilization of air, fermentation medium and the Fermenter, inoculum preparation and inoculation of the medium.

➢The fermentation medium should contain an energy source, a carbon source, a nitrogen source and micronutrients required for the growth of the microorganism along with water and oxygen, if necessary.

➢A medium which is used for a large scale fermentation, in order to ensure the sustainability of the operation, should have the following characteristics;

1. It should be cheap and easily available
2. It should maximize the growth of the microorganism, productivity and the rate of formation of the desired product
3. It should minimize the formation of undesired products

Upstream Process

- Usually, waste products from other industrial processes, such as molasses, lignocelluloses wastes, cheese whey and corn steep liquor, after modifying with the incorporation of additional nutrients, are used as the substrate for many industrial fermentations.

- **Sterilisation** is essential for preventing the contamination with any undesired microorganisms.

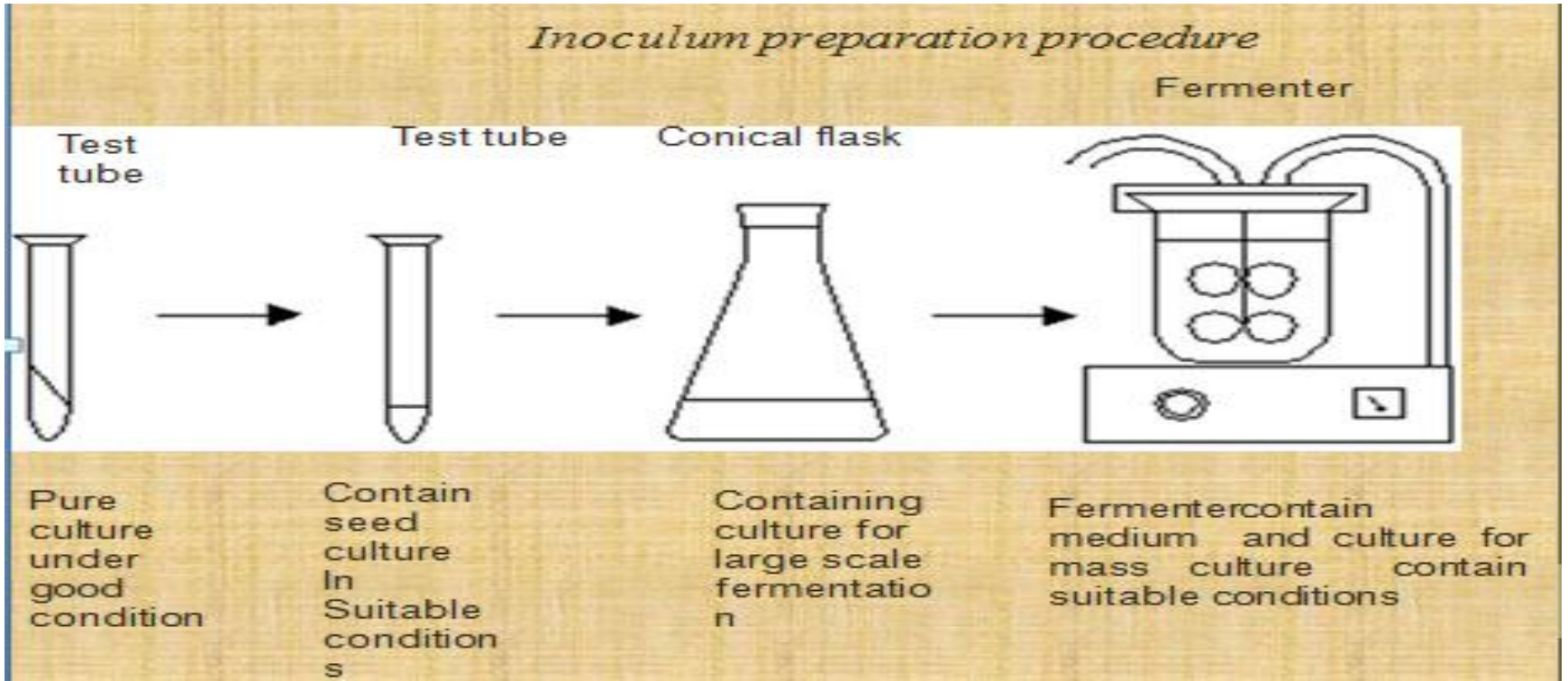
- **Air** is sterilised by **membrane filtration** while the **medium** is usually **heat sterilised**.
- Any nutrient component which is **heat labile** is **filter-sterilised**
- and later added to the sterilised medium.
- The fermenter may be sterilised together with the medium or
- separately.

➢Inoculum build up is the preparation of the seed culture in amounts sufficient to be used in the large Fermenter vessel.

➢This involves growing the microorganisms obtained from the pure stock culture in several consecutive Fermenter.

➢This process cuts down the time required for the growth of microorganisms in the Fermenter, thereby increasing the rate of productivity.

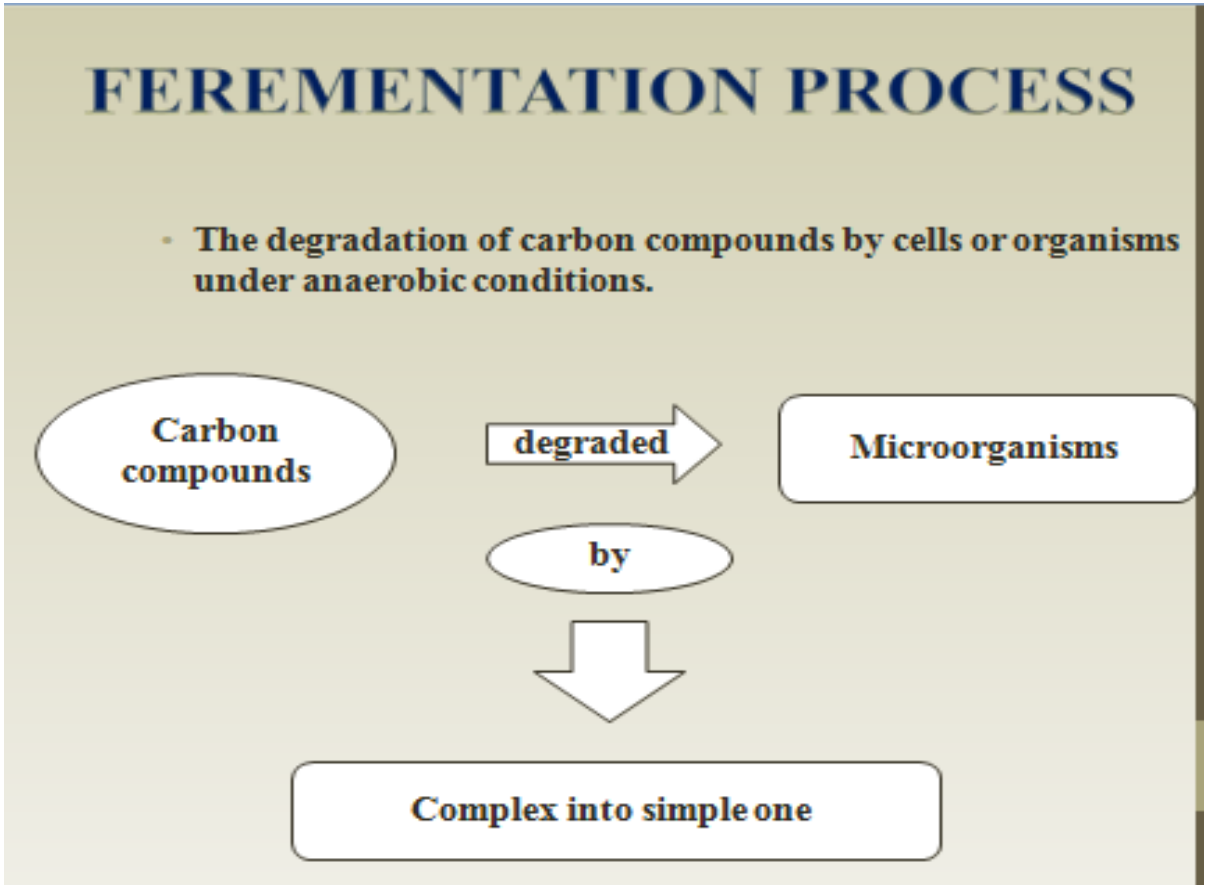
➢Then the seed culture obtained through this process is used to inoculate the fermentation medium.



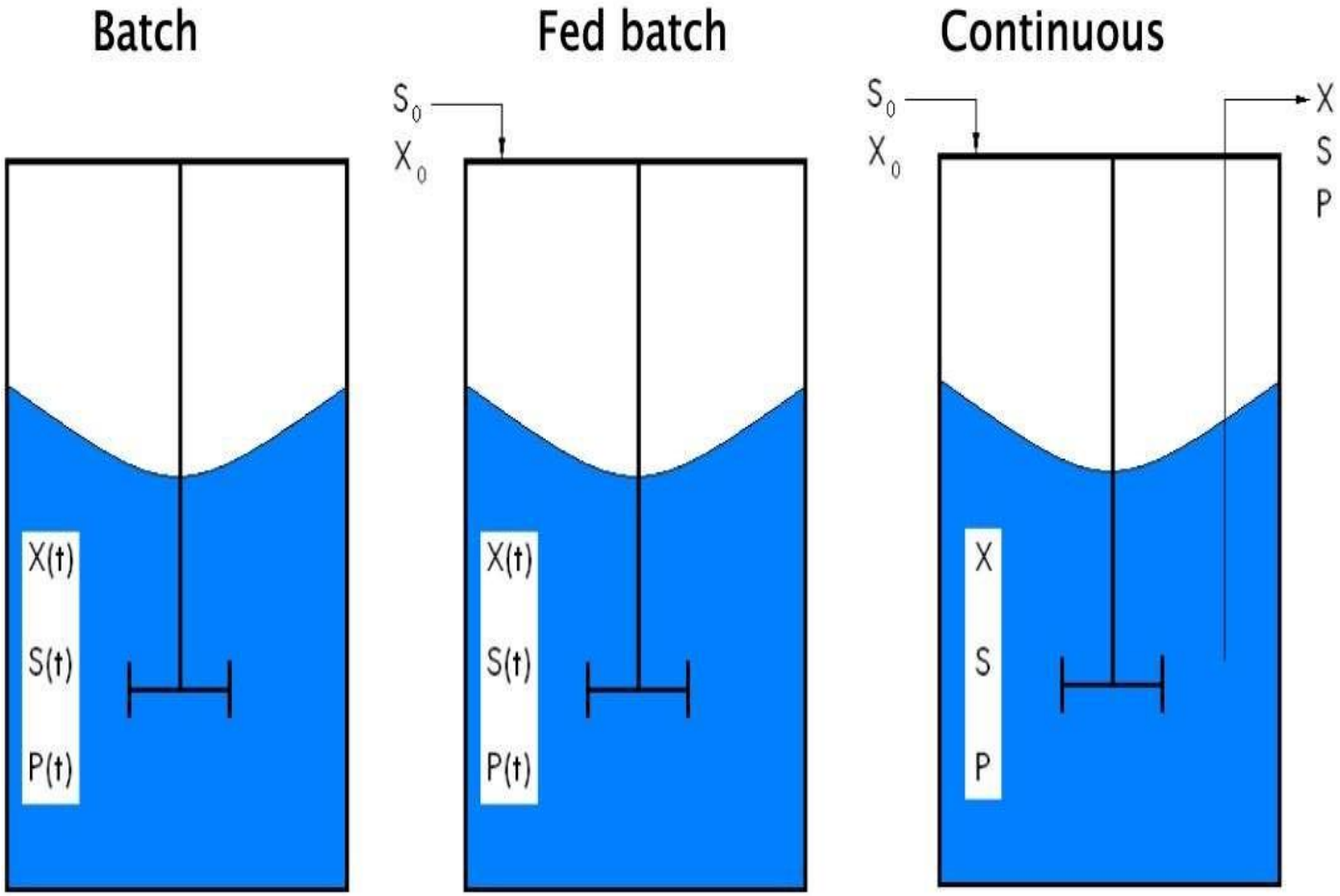
Micro-organisms

- Micro-organisms used for fermentation process grow on or in growth medium which satisfies the nutritional needs of microbes.
- Complete analysis is needed to be done to establish the most favourable medium for the growth of the microbe used for fermentation.
- Formulating medium at lab scale can be done by adding main ingredients like water, carbon source, nitrogen source, minerals and other supplements in pure form and in required quantities is very easy which supports the growth of the microbe whereas, the same may not support the satisfactory growth of the same organism at industrial level
- Following criteria need to be satisfied for the material to be treated as medium at industrial level.
- It should give maximum yield of product.
- It should give minimum yield of undesired product.
- It should be consistently available throughout the year.
- It should be cheap.

Fermentation Process



Types Of Fermentation



1.Batch fermentation:

- Nutrients are added in the fermentation for the single time only and growth continues until the particular nutrients are exhausted
- In the batch process when the microorganism is added into a medium which supports its growth, the culture passes through number of stages known as 'growth curve'

A typical growth curve consists of following stages

a) Lag phase
b) Acceleration phase
c) Log or exponential phase
d) Deceleration phase
e) Stationary phase
f) Death phase

(a) Lag phase:

Immediately after inoculation, there is no increase in the numbers of the microbial cells for some time and this period is called lag phase. In this is phase the organisms adjust to the new environment in which it is inoculated into.

(b) Acceleration phase:

The period when the cells just start increasing in numbers is known as acceleration phase.

(c) Log phase:

This is the time period when the cell numbers steadily increase.

(d) Deceleration phase:

The duration when the steady growth declines.

(e) Stationary phase:

The period where there is no change in the microbial cell number is the stationary phase. This phase is attained due to depletion of carbon source or accumulation of the end products.

(f) Death phase:

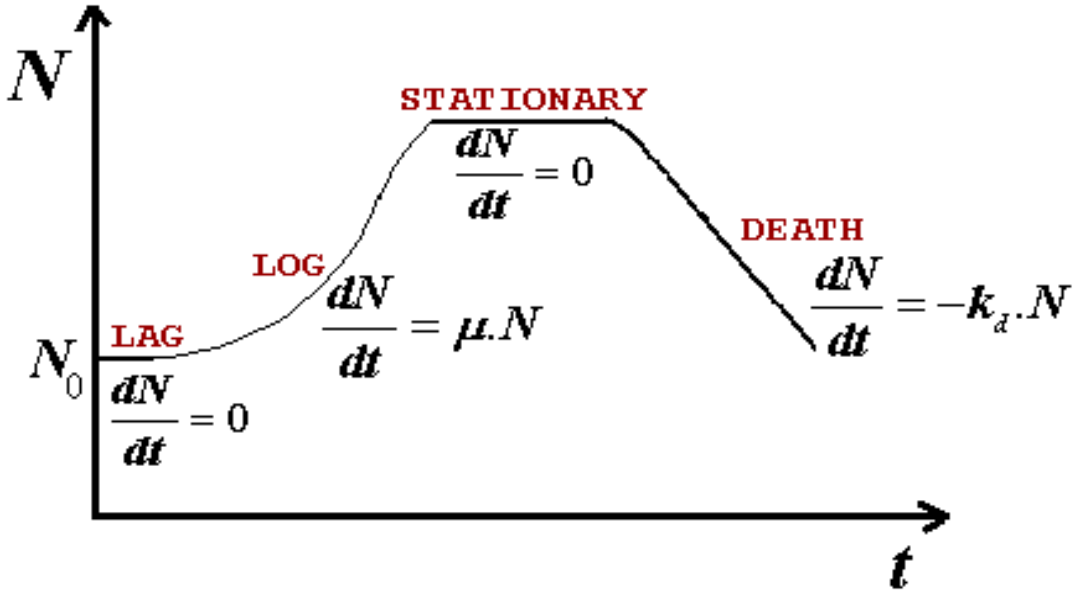
The period in which the cell numbers decrease steadily is the death phase.

This is due to death of the cells because of cessation of metabolic activity and depletion of energy resources.

Depending upon the product required the different phases of the cell growth are maintained. For microbial mass the log phase is preferred. For production of secondary metabolites i.e. antibiotics, the stationary phase is preferred.

Growth kinetics of batch culture

The number of living cells (population of growth rate dN/dt)varies with time in a batch system as shown below:



where;

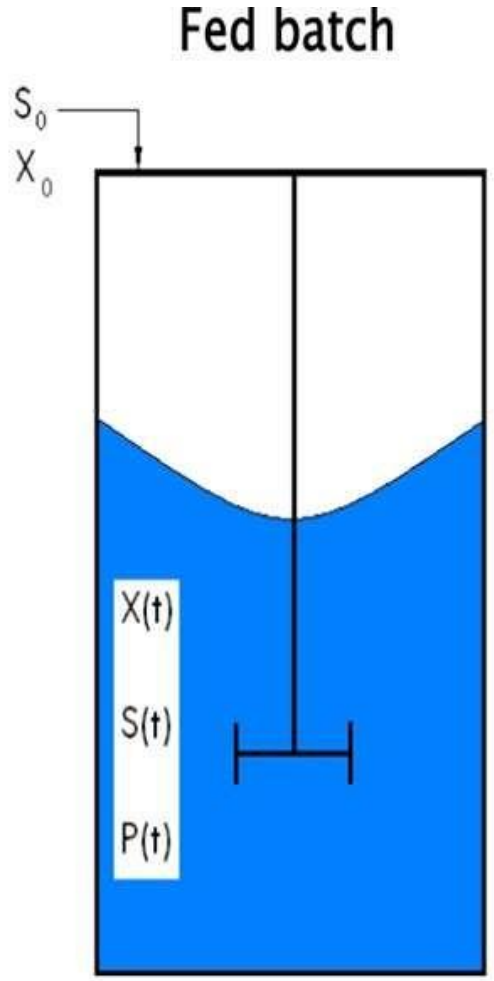
N = number of bacteria at any time t in the reactor

t = time

N_0 = initial number of bacteria in the reactor after inoculation

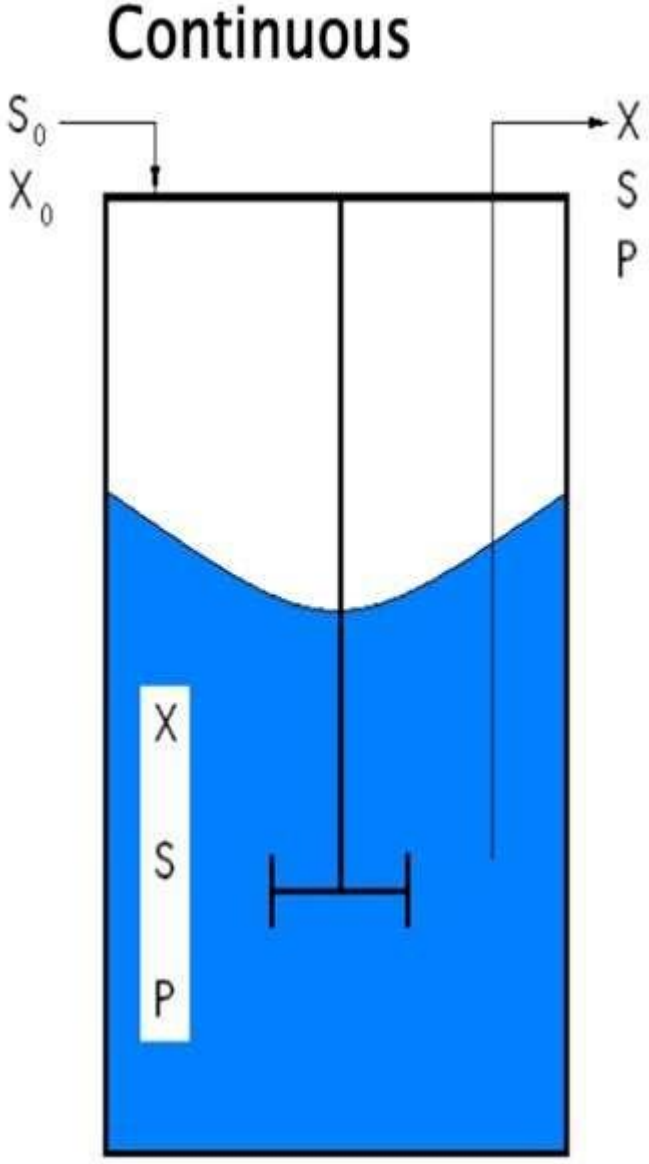
2.Feb-batch fermentation:

- In this type of fermentation, freshly prepared culture media is added at regular intervals without removing the culture fluid.
- This increases the volume of the fermentation culture.
- This type of fermentation is used form production of proteins from recombinant microorganisms.
- The total amount of the biomass in the vessel increases but biomass concentration is maintained constant



3.Continuous fermentation:

- The growth rate and physiological conditions of microorganisms can be maintained by using a process of continuous culture (chemostat)
- In this the products are removed continuously along with the cells and the same is replenished with the cell girth and addition of fresh culture media.
- This results in a steady or constant volume of the contents of the fermenter.
- type of fermentation is used for the production of single cell protein (S.S.P), antibiotics and organic solvents.



Advantages and disadvantages of batch and continuous operations

BATCH SYSTEMS

- Easy To Operate And Control
- Genetic Stability Of Organism Could Be Controlled If It Is Genetically Engineered Biocatalyst.
- Lower Contamination Risk
- Non-productive Down Time Is A Disadvantage
- Batch To Batch Variability Is Problem
- Accumulation Of Inhibitory Products Is Problem

CONTINUOUS SYSTEMS

- degeneration of biocatalyst
- higher contamination risk is a disadvantage
- efficient, higher productivity
- product is obtained with uniform characteristics; quality of the product is almost same from time to time
- no accumulation of

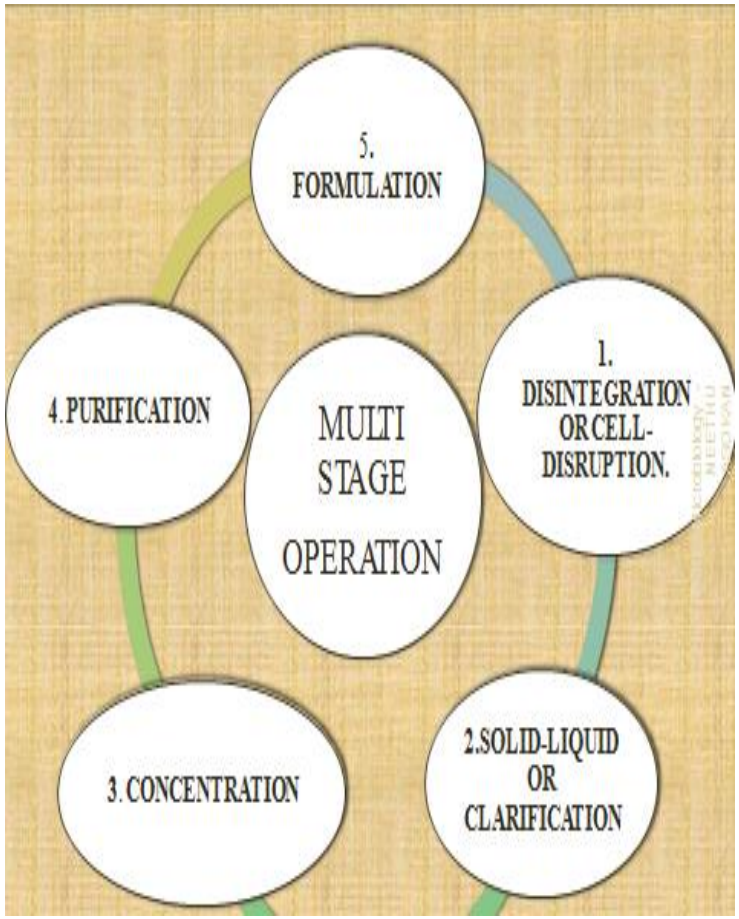
Down stream Process

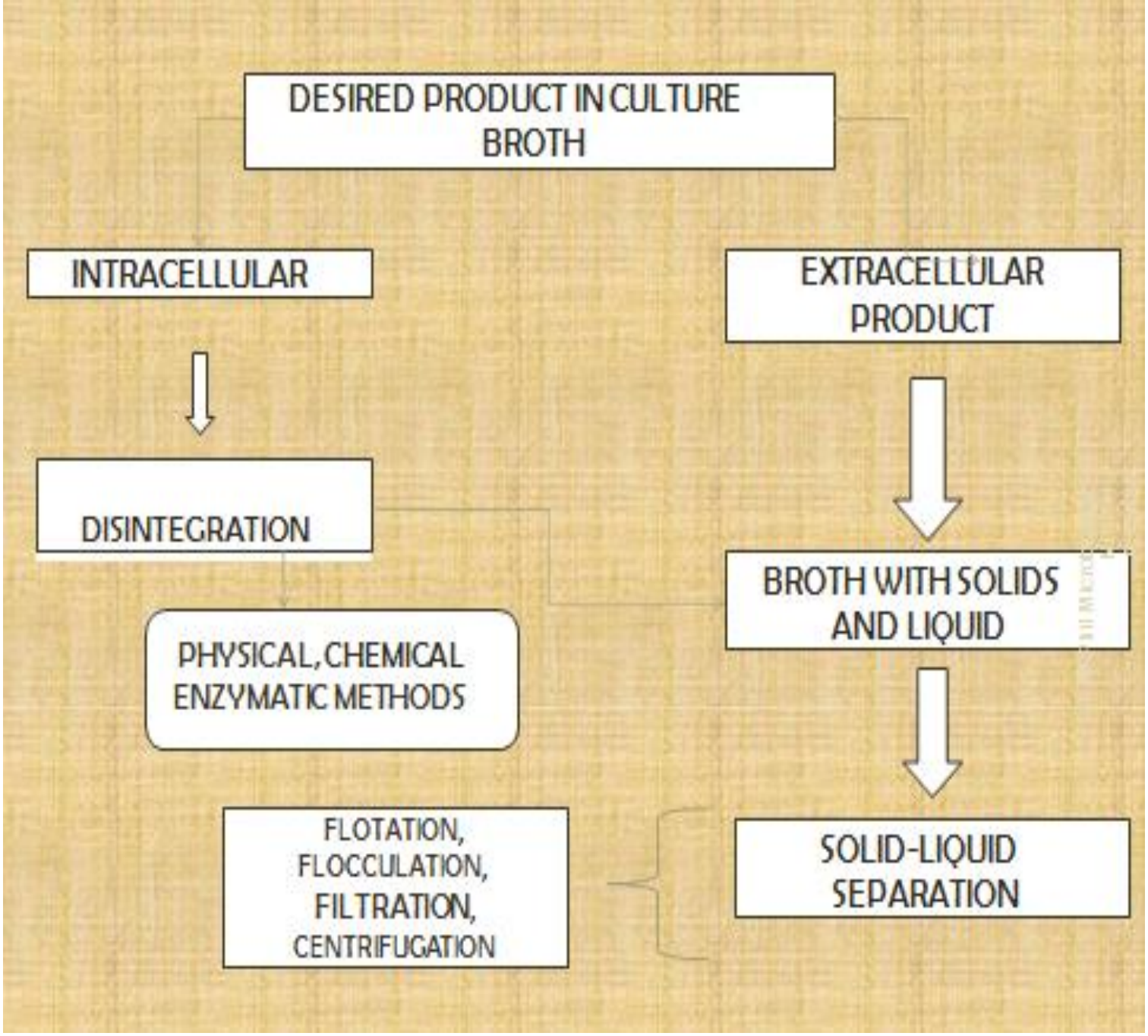
- when fermentation is over, the desired product is recovered from the growth medium.

- Then the product is Extraction Purification and Packed of a biotechnological product from fermentation is refered to as DSP or product recovery or downstream processing.

- The end products include Antibiotics, Amino acid, Vitamins,Organic acid, Industrial enzyme, vaccines etc.

- It is complex and important as fermentation process




DESIRED PRODUCT IN CULTURE BROTH
INTRACELLULAR
EXTRACELLULAR PRODUCT
DISINTEGRATION
PHYSICAL, CHEMICAL ENZYMATIC METHODS
BROTH WITH SOLIDS AND LIQUID
FLOTATION, FLOCCULATION, FILTRATION, CENTRIFUGATION
SOLID-LIQUID SEPARATION

CELL -DISRUPTION OR DISINTEGRATION

Mechanical methods

- Shear forces in solid matter and solution.

Non mechanical methods:

- Lysis
  - ✓Physical – freezing and thawing : now high osmotic pressure, shock
  - ✓Chemical – surface active agents, solvents, antibiotics etc.,
  - ✓Enzymatic – lysozyme
- Drying
  - ✓Freeze-drying, Air drying, Pressure release, Drying with solvents

SOLID-LIQUID SEPARATION OR CLARIFICATION

- **Primary operation**
  - ✓ Separation of whole cells
  - ✓ Removal of cell debris
  - ✓ Collection of protein precipitate
  - ✓ Collection of inclusion bodies etc.,

Solid-liquid separation (clarification)

- Coagulation
  - ✓ Colloids into small flocs using simple electrolytes
- Flocculation
  - ✓ Agglomeration of these small flocs into larger settle-able particles using polyelectrolytes.
- Flotation
  - ✓ Enrichment of microorganisms
- Filtration
  - ✓ The separation of suspended particles from liquid
- Centrifugation
  - ✓ Gravitational force used for separate the particles

CONCENTRATION METHODS

- The purity or concentration of metabolite
  - **Evaporation** –steam as heat source
  - **Extraction** – the cell mass and more or less clear solution obtained
  - **Adsorption** – special polymer resins (chemical) used for the isolation of hydrophilic metabolites that cannot be extract with organic solvents.
  - **Filtration** – separation of biomolecules and particles [pore size]
  - **Precipitation** – removal of product from the solvent
  - **Dialysis** – semi permeable membrane

PURIFICATION

- Purify relatively low concentration of metabolic products
- The chromatography technique are used
- Purification is a main process in fermentation
- Desired product purification is important
- Many techniques are used for the purification

Thank You