BTG 408

PROCESS BIOTECHNOLOGY

GB AKANNI (PhD)

Contents

- Introduction to bioreactor
- Description of various types of vessels for cell cultivation
- Bioreactor design and optimization
- Agitation of bioreactors
- Survey of the applications of biotechnology, emphasizing the pharmaceutical industry and the operation of fermentation systems.

BIOREACTOR
&
FERMENTOR

What are bioreactors?

- A Bioreactor – a vessel which has provision of cell cultivation under sterile condition & control of environmental conditions e.g., pH, Temperature, Dissolved oxygen etc.
- It can be used for the cultivation of microbial, plant or animal cells.
- This process can either be aerobic or anaerobic.
- The bioreactors are commonly cylindrical, ranging in size from litres to cubic metres, and are often made of stainless steel.

What are Fermentors?

- A **fermentor** is used for commercial production in fermentation industries and is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value.

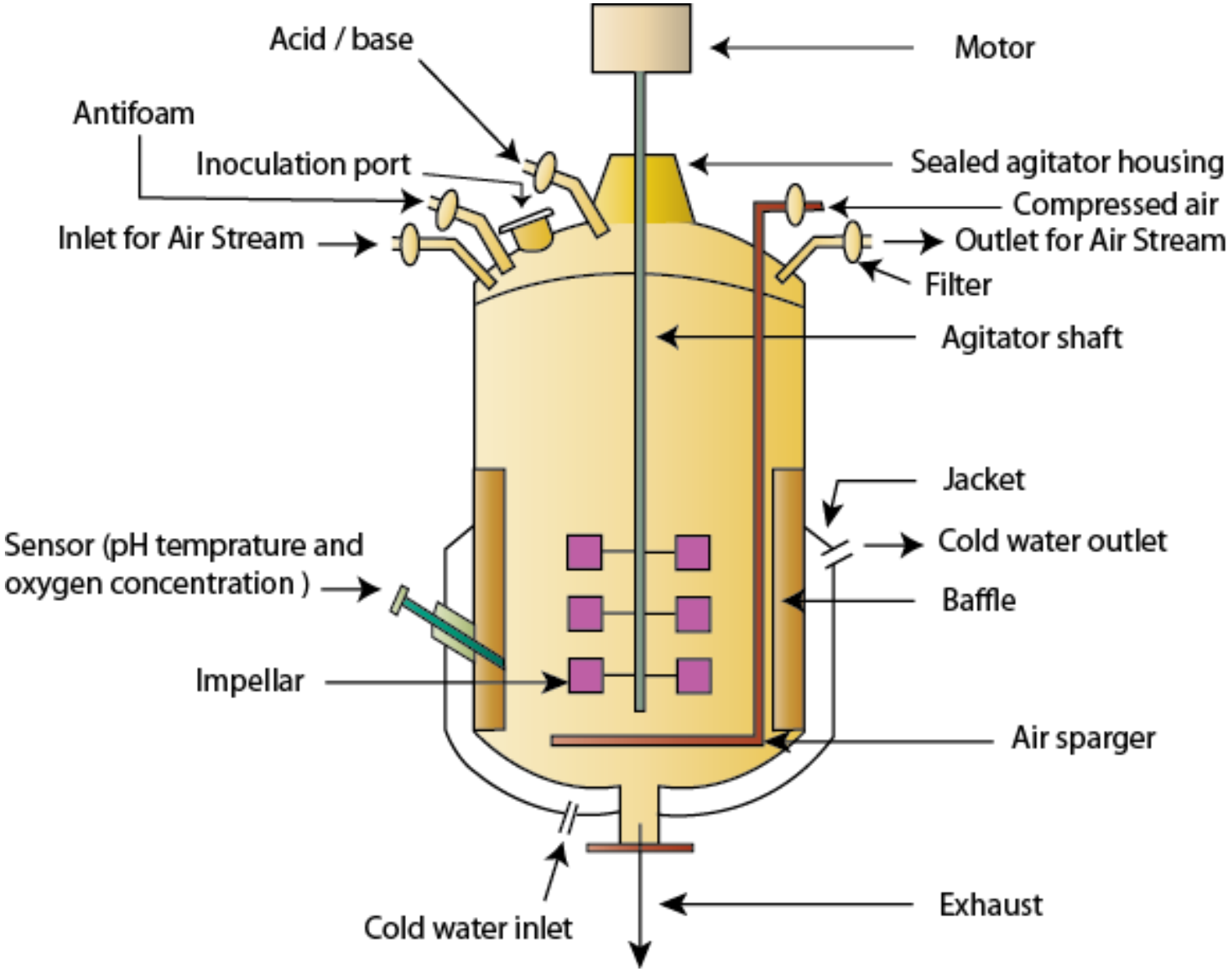
- **Fermentors** are extensively used for food processing, fermentation, waste treatment, etc.

Bioreactor vs Fermentor

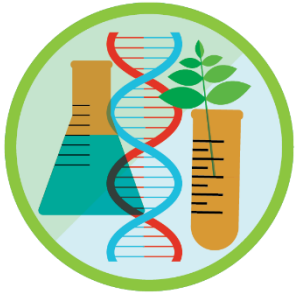
- The main **difference between bioreactor** and **fermentor** is that the **bioreactor** is the vessel that facilitates various types **of** biochemical reactions whereas the **fermentor** is the vessel that facilitates **fermentation**.

- Therefore, **fermentor** is a type **of bioreactor**.

DIAGRAM OF A TYPICAL BIOREACTOR



SPECIFICATIONS OF A BIOREACTOR



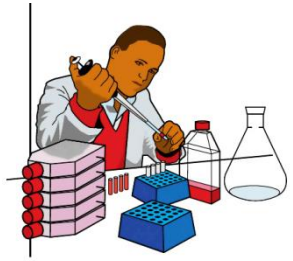
A typical bioreactor consists of following parts:

- **Agitator** – used for the mixing of the contents of the reactor which keeps the "cells" in the perfect **homogenous** condition for better transport of nutrients and oxygen to the desired product(s).

- **Baffle** – used to break the ***vortex formation*** in the vessel, which is usually highly undesirable as it changes the center of gravity of the system and consumes additional power.

- **Sparger** – In aerobic cultivation process, the purpose of the sparger is to ***supply adequate oxygen*** to the growing cells.

- **Jacket** – The jacket provides the annular area for circulation of constant temperature of water which ***keeps the temperature of the bioreactor at a constant value***



OPERATIONAL STAGES IN A BIO-PROCESS

A bioprocess is composed mainly of three stages — upstream processing, bioreaction, and downstream processing — to convert raw material to finished product.

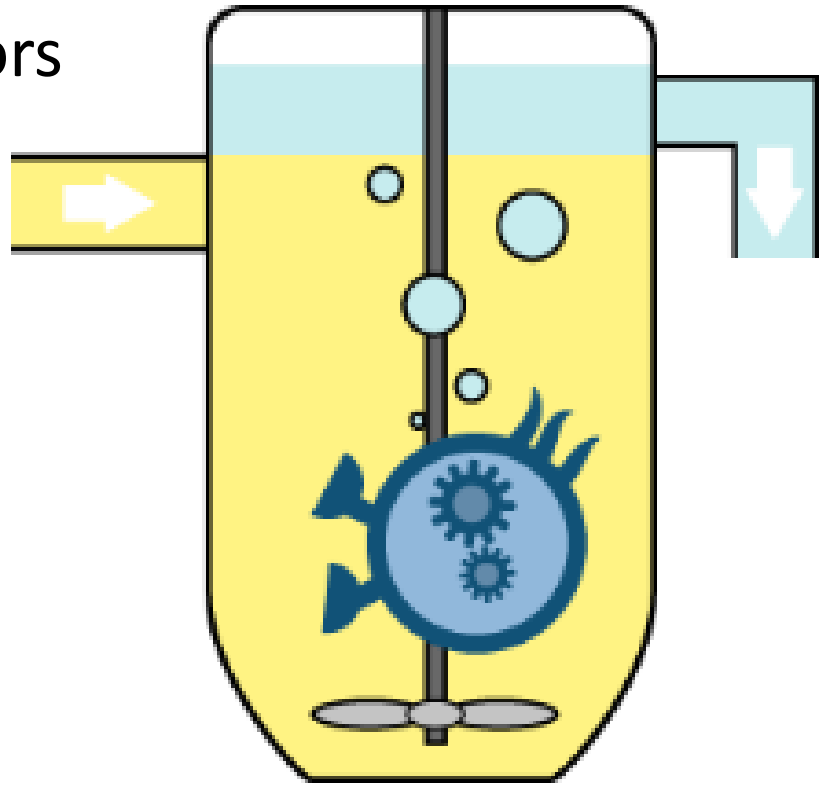
The raw material can be of biological or non-biological origin. It is first converted to more suitable form for processing. This is done in upstream processing step which involves chemical hydrolysis, preparation of liquid medium, separation of particulate, air purification and many other preparatory operations.

After upstream processing step, the resulting feed is transferred to one or more Bioreaction stages. The Biochemical reactors or bioreactors form the base of the Bioreaction step. This step is mainly consists of three operations namely, production of biomass, metabolize biosynthesis and biotransformation.

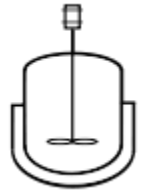
Finally, the material produced in the bioreactor must be further processed in the downstream section to convert it into more useful form. The downstream process is mainly consists of physical separation operations which includes, solid liquid separation, adsorption, liquid-liquid extraction, distillation, drying etc

BIOREACTORS TYPES

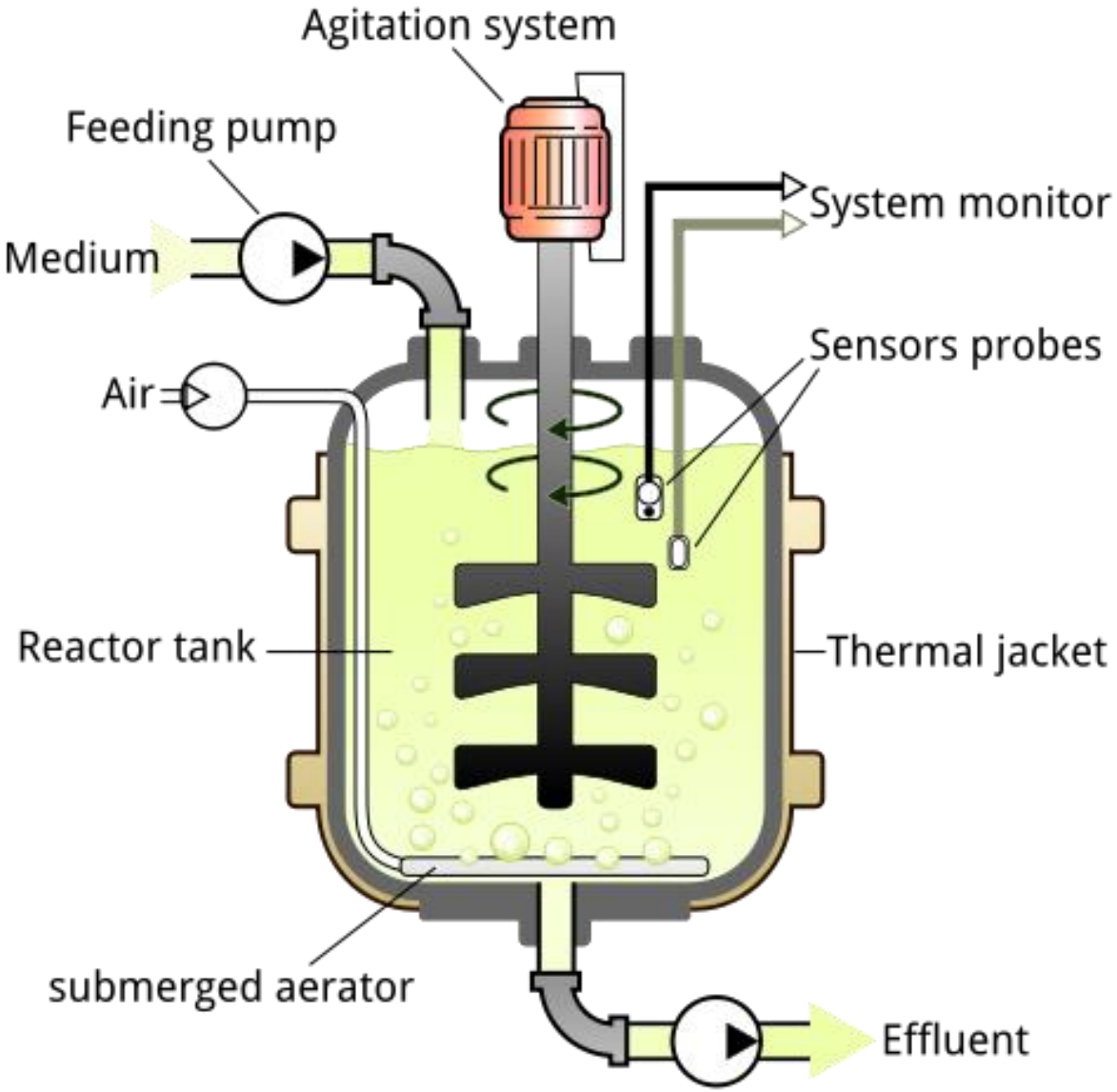
1. Continuous Stirred Tank Bioreactors
2. Bubble Column Bioreactors
3. Airlift Bioreactors
4. Fluidized Bed Bioreactors
5. Packed Bed Bioreactors
6. Photo-Bioreactors



CONTINUOUS STIRRED TANK BIOREACTORS



- A continuous stirred tank bioreactor consists of a cylindrical vessel with motor driven central shaft that supports one or more agitators (impellers).
- The shaft is fitted at the bottom of the bioreactor.
- The number of impellers is variable and depends on the size of the bioreactor i.e., height to diameter ratio, referred to as aspect ratio.
- The aspect ratio of a stirred tank bioreactor is usually between 3-5. However, for animal cell culture applications, the aspect ratio is less than 2.
- The diameter of the impeller is usually 1/3 rd of the vessel diameter.
- The distance between two impellers is approximately 1.2 impeller diameter. Different types of impellers (Rustom disc, concave bladed, marine propeller etc.) are in use.



General structure of a continuous stirred-tank type bioreactor

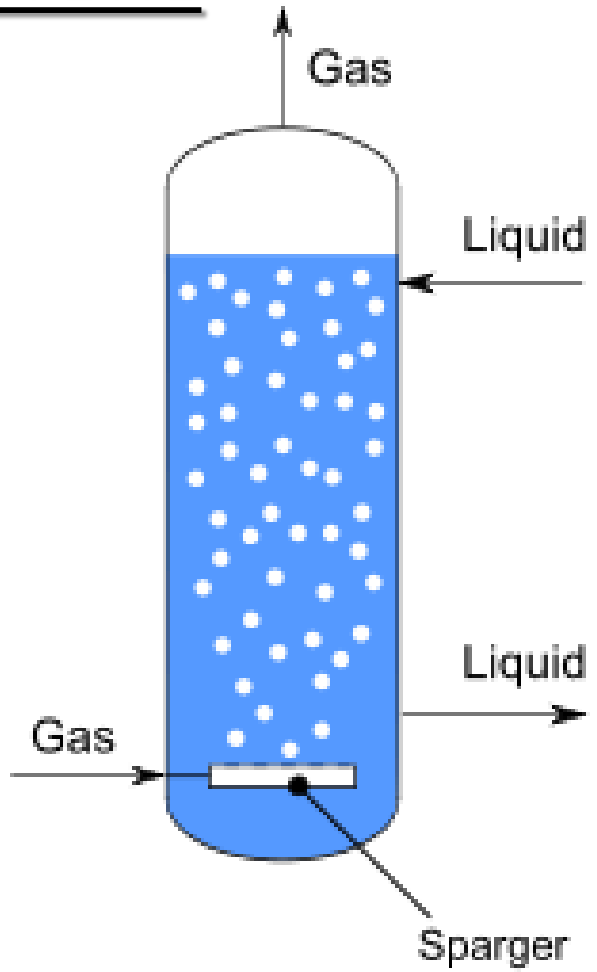
- In stirred tank bioreactors or in short stirred tank reactors (STRs), the air is added to the culture medium under pressure through a device called sparger.
- The sparger may be a ring with many holes or a tube with a single orifice.
- The sparger along with impellers (agitators) enables better gas distribution system throughout the vessel.
- The bubbles generated by sparger are broken down to smaller ones by impellers and dispersed throughout the medium.
- This enables the creation of a uniform and homogeneous environment throughout the bioreactor.

ADVANTAGES OF STRS

- There are many advantages of STRs over other types. These include the efficient gas transfer to growing cells, good mixing of the contents and flexible operating conditions, besides the commercial availability of the bioreactors.

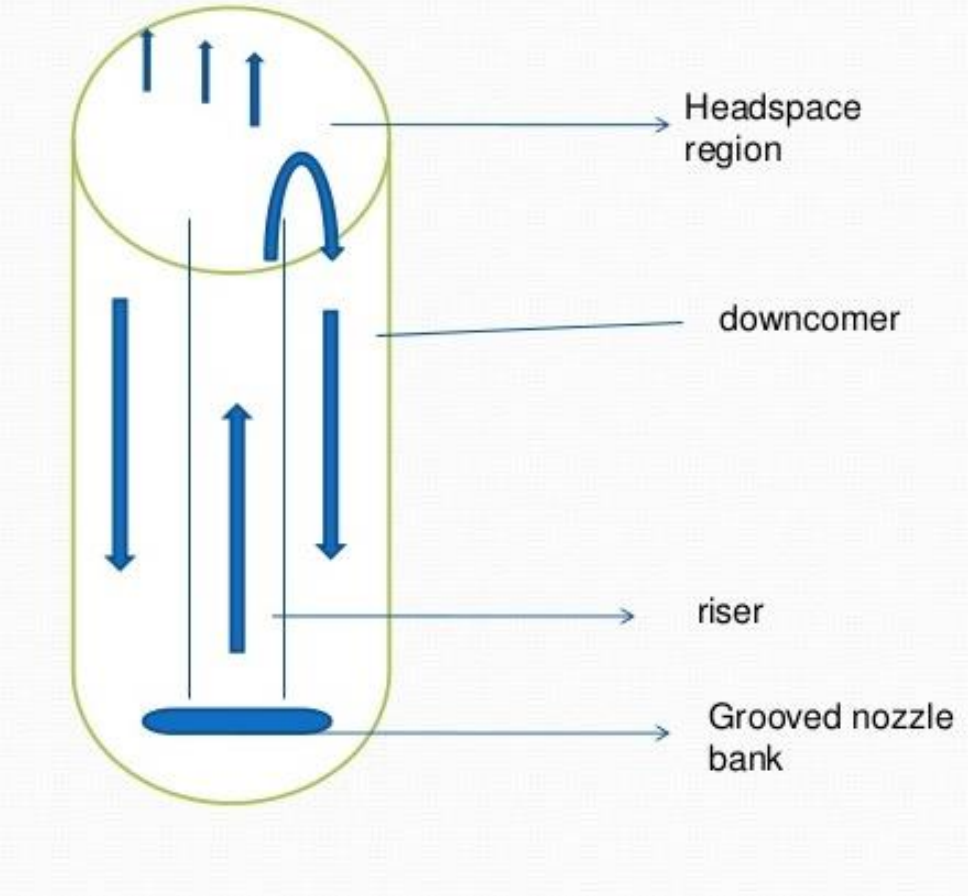
BUBBLE COLUMN BIOREACTORS

- In the bubble column bioreactor, the air or gas is introduced at the base of the column through perforated pipes or plates, or metal micro porous spargers.
- The flow rate of the air/gas influences the performance factors —O2 transfer, mixing.
- The bubble column bioreactors may be fitted with perforated plates to improve performance.
- The vessel used for bubble column bioreactors is usually cylindrical with an aspect ratio of 4-6 (i.e., height to diameter ratio).



AIRLIFT BIOREACTORS

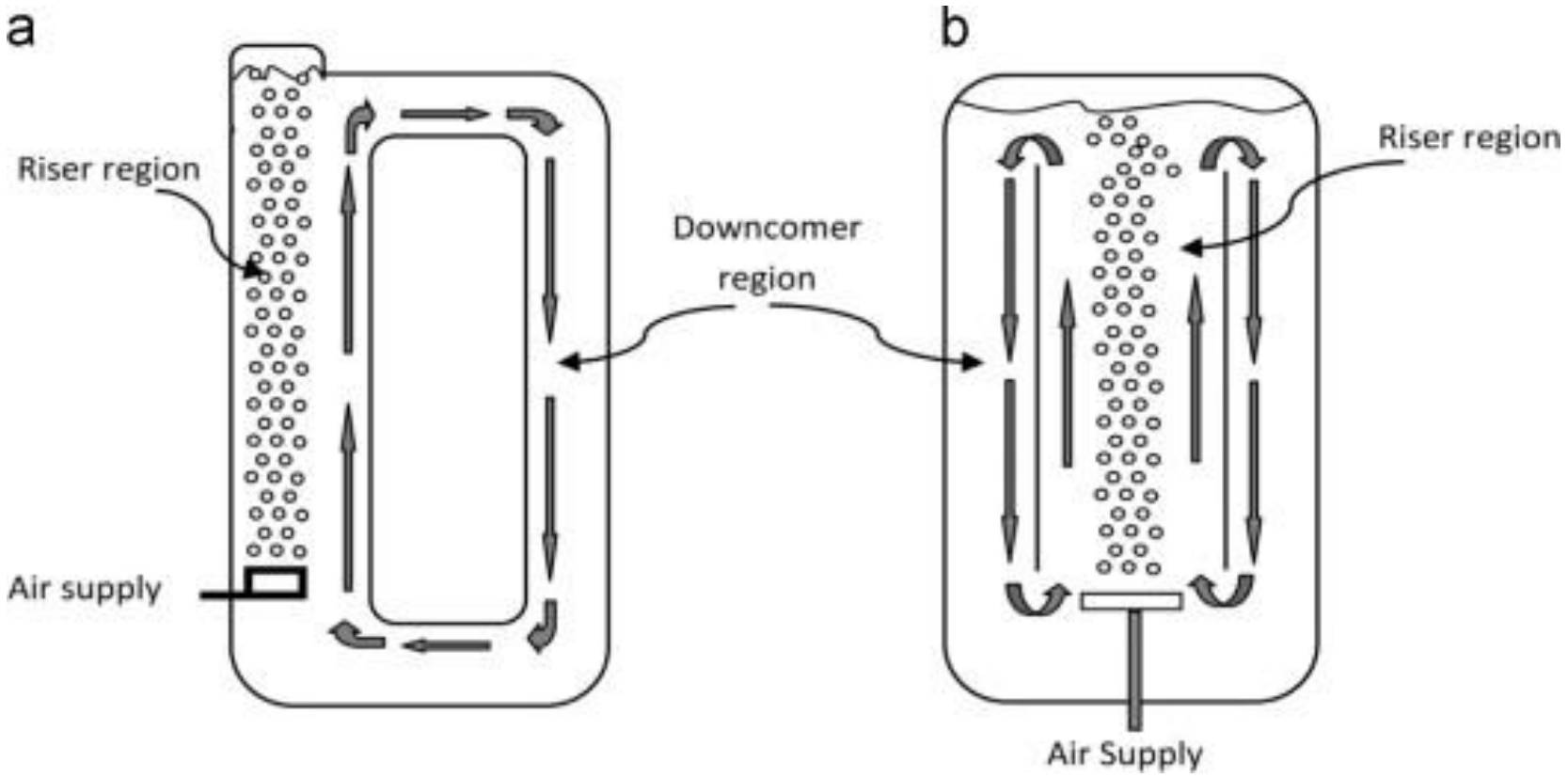
- In the airlift bioreactors, the medium of the vessel is divided into two interconnected zones by means of a baffle or draft tube.
- In one of the two zones referred to a riser, the air/gas is pumped. The other zone that receives no gas is the down comer.
- The dispersion flows up the riser zone while the down flow occurs in the down comer.



TYPES OF AIRLIFT BIOREACTORS

There are two types of airlift bioreactors.

- **Internal-loop airlift bioreactor** has a single container with a central draft tube that creates interior liquid circulation channels.
- These bioreactors are simple in design, with volume and circulation at a fixed rate for fermentation.
- **External loop airlift bioreactor** possesses an external loop so that the liquid circulates through separate independent channels.
- These reactors can be suitably modified to suit the requirements of different fermentations.
- In general, the airlift bioreactors are more efficient than bubble columns, particularly for more denser suspensions of microorganisms.
- This is mainly because in these bioreactors, the mixing of the contents is better compared to bubble columns.



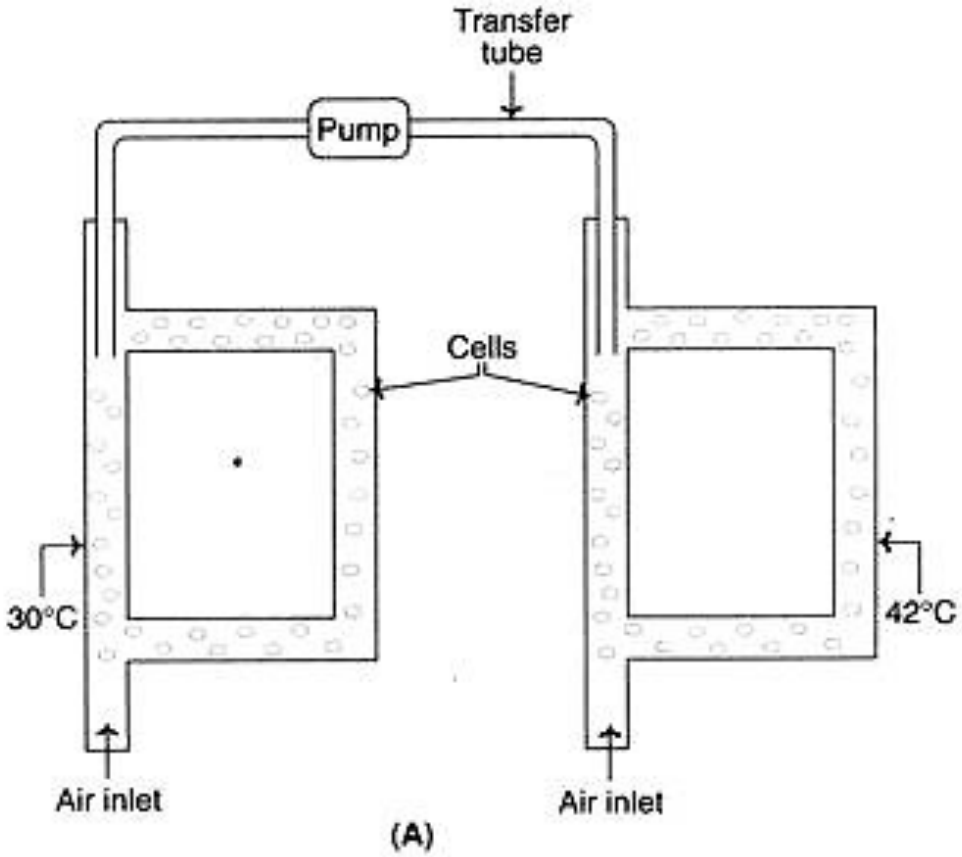
Schematic of airlift bioreactor with (a) external recirculation and (b) internal recirculation

AIRLIFT BIOREACTORS APPLICATION

- Airlift bioreactors are commonly employed for aerobic bioprocessing technology.
- They ensure a controlled liquid flow in a recycle system by pumping.
- Due to high efficiency, airlift bioreactors are sometimes preferred e.g., methanol production, waste water treatment, single-cell protein production.
- In general, the performance of the airlift bioreactors is dependent on the pumping (injection) of air and the liquid circulation.

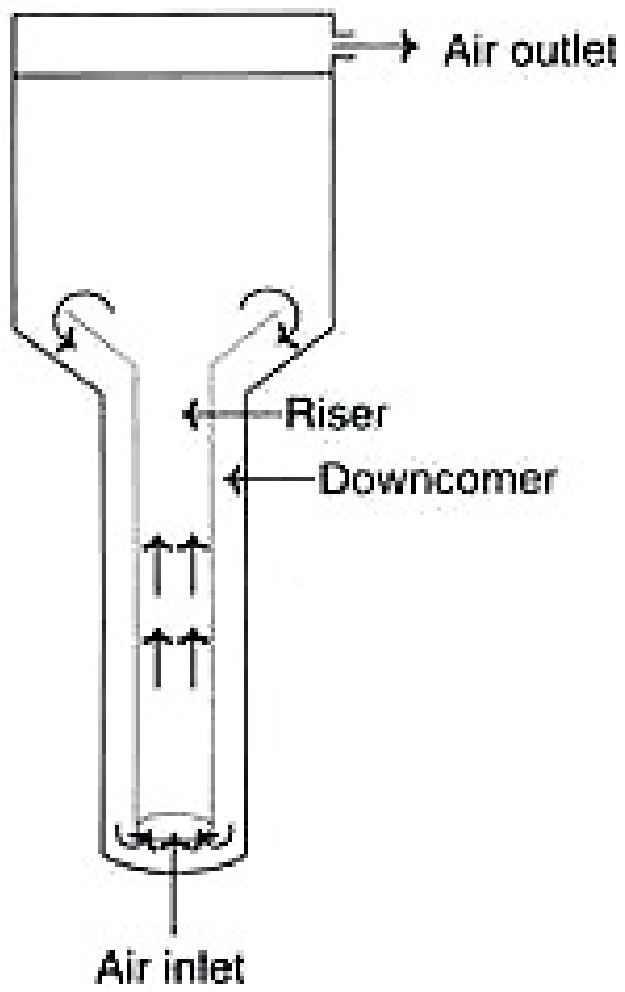
TWO-STAGE AIRLIFT BIOREACTORS

- Two-stage airlift bioreactors are used for the temperature dependent formation of products.
- Growing cells from one bioreactor (maintained at temperature 30°C) are pumped into another bioreactor (at temperature 42°C).
- There is a necessity for the two-stage airlift bioreactor, since it is very difficult to raise the temperature quickly from 30°C to 42°C in the same vessel.
- Each one of the bioreactors is fitted with valves and they are connected by a transfer tube and pump.
- The cells are grown in the first bioreactor and the bioprocess proper takes place in the second reactor.



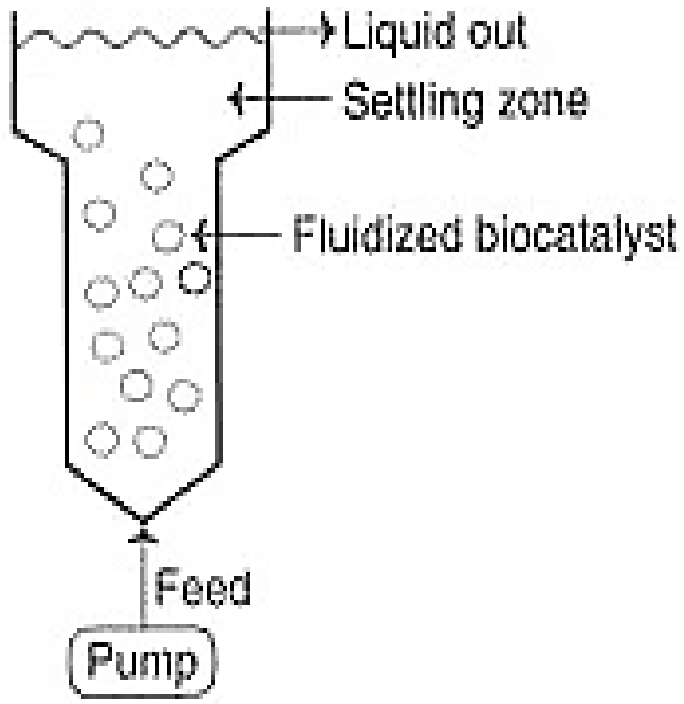
TOWER BIOREACTORS

- A pressure-cycle fermenter with large dimensions constitutes a tower bioreactor.
- A high hydrostatic pressure generated at the bottom of the reactor increases the solubility of O2 in the medium.
- At the top of the riser, (with expanded top) reduces pressure and facilitates expulsion of CO2.
- The medium flows back in the down comer and completes the cycle.
- The advantage with tower bioreactor is that it has high aeration capacities without having moving parts.



FLUIDIZED BED BIOREACTORS

- Fluidized bed bioreactor is comparable to bubble column bioreactor except the top position is expanded to reduce the velocity of the fluid.
- The design of the fluidized bioreactors (expanded top and narrow reaction column) is such that the solids are retained in the reactor while the liquid flows out.
- These bioreactors are suitable for use to carry out reactions involving fluid suspended biocatalysts such as immobilized enzymes, immobilized cells, and microbial flocs.

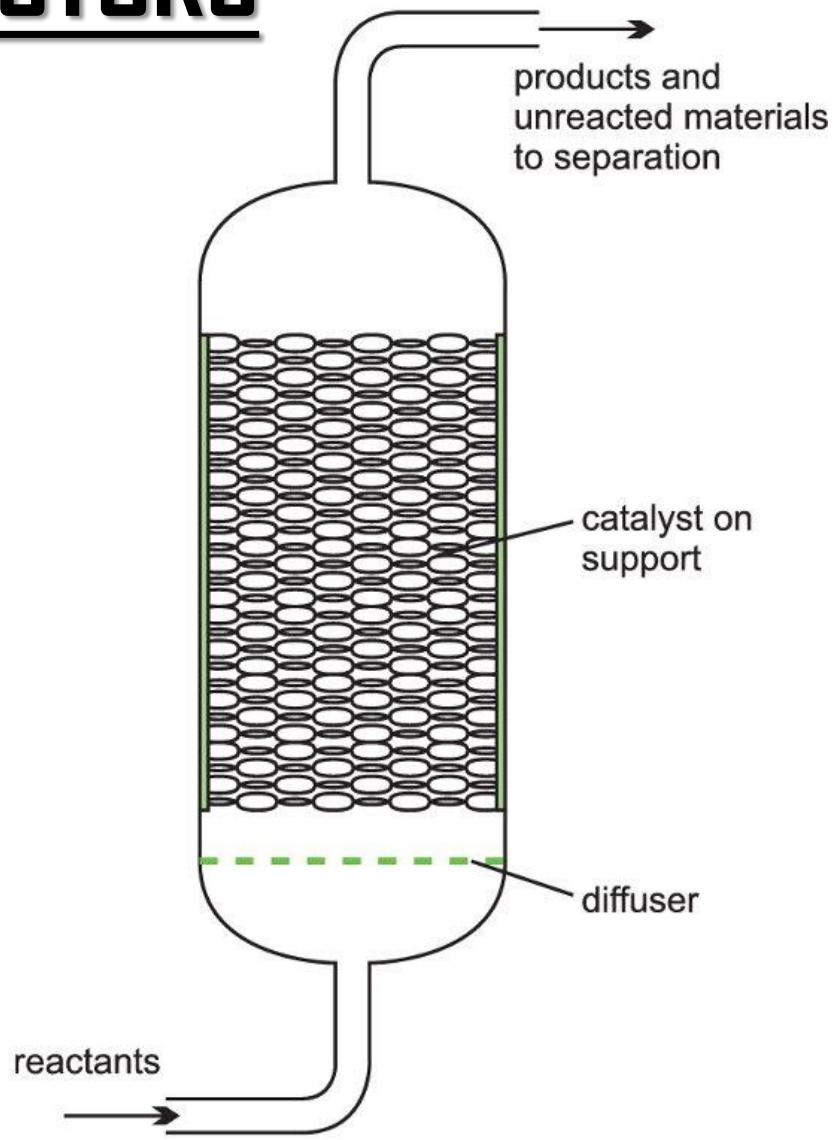


Basic diagram of a fluidized bed reactor

- For an efficient operation of fluidized beds, gas is spared to create a suitable gas-liquid-solid fluid bed.
- It is also necessary to ensure that the suspended solid particles are not too light or too dense (too light ones may float whereas to dense ones may settle at the bottom), and they are in a good suspended state.
- Recycling of the liquid is important to maintain continuous contact between the reaction contents and biocatalysts.
- This enable good efficiency of bioprocessing.

PACKED BED BIOREACTORS

- A bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column constitutes a packed bed.
- The solids used may be porous or non-porous gels, and they may be compressible or rigid in nature.
- A nutrient broth flows continuously over the immobilised biocatalyst.
- The products obtained in the packed bed bioreactor are released into the fluid and removed.
- While the flow of the fluid can be upward or downward, down flow under gravity is preferred.

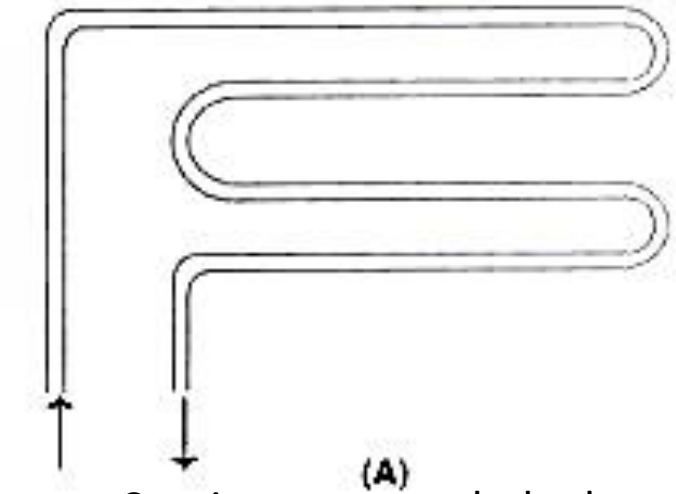


- The concentration of the nutrients (and therefore the products formed) can be increased by increasing the flow rate of the nutrient broth.
- Because of poor mixing, it is rather difficult to control the pH of packed bed bioreactors by the addition of acid or alkali.
- However, these bioreactors are preferred for bioprocessing technology involving product-inhibited reactions.
- The packed bed bioreactors do not allow accumulation of the products to any significant extent.

PHOTO-BIOREACTORS

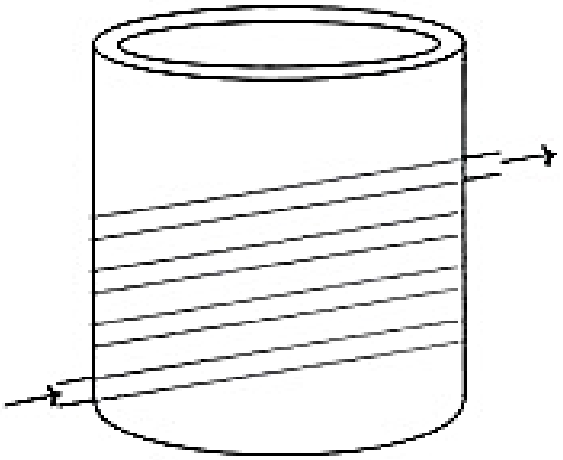
- These are the bioreactors specialised for fermentation that can be carried out either by exposing to sunlight or artificial illumination.
- Since artificial illumination is expensive, only the outdoor photo-bioreactors are preferred.
- Certain important compounds are produced by employing photo-bioreactors e.g., p-carotene, asthaxanthin.
- They are made up of glass or more commonly transparent plastic.
- The array of tubes or flat panels constitute light receiving systems (solar receivers).
- The culture can be circulated through the solar receivers by methods such as using centrifugal pumps or airlift pumps.
- It is essential that the cells are in continuous circulation without forming sediments.
- Further adequate penetration of sunlight should be maintained.
- The tubes should also be cooled to prevent rise in temperature.
- Photo-bioreactors are usually operated in a continuous mode at a temperature in the range of 25-40°C. Microalgae and cyanobacteria are normally used.
- The organisms grow during day light while the products are produced during night.

DIFFERENT TYPES OF PHOTO-BIOREACTORS



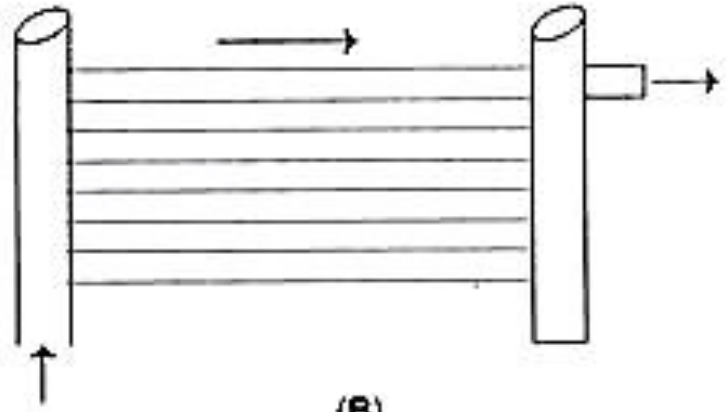
(A)

Continuous run tubular loop



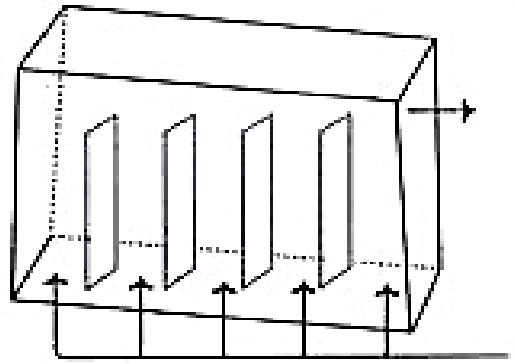
(C)

Helical wound tubular loop



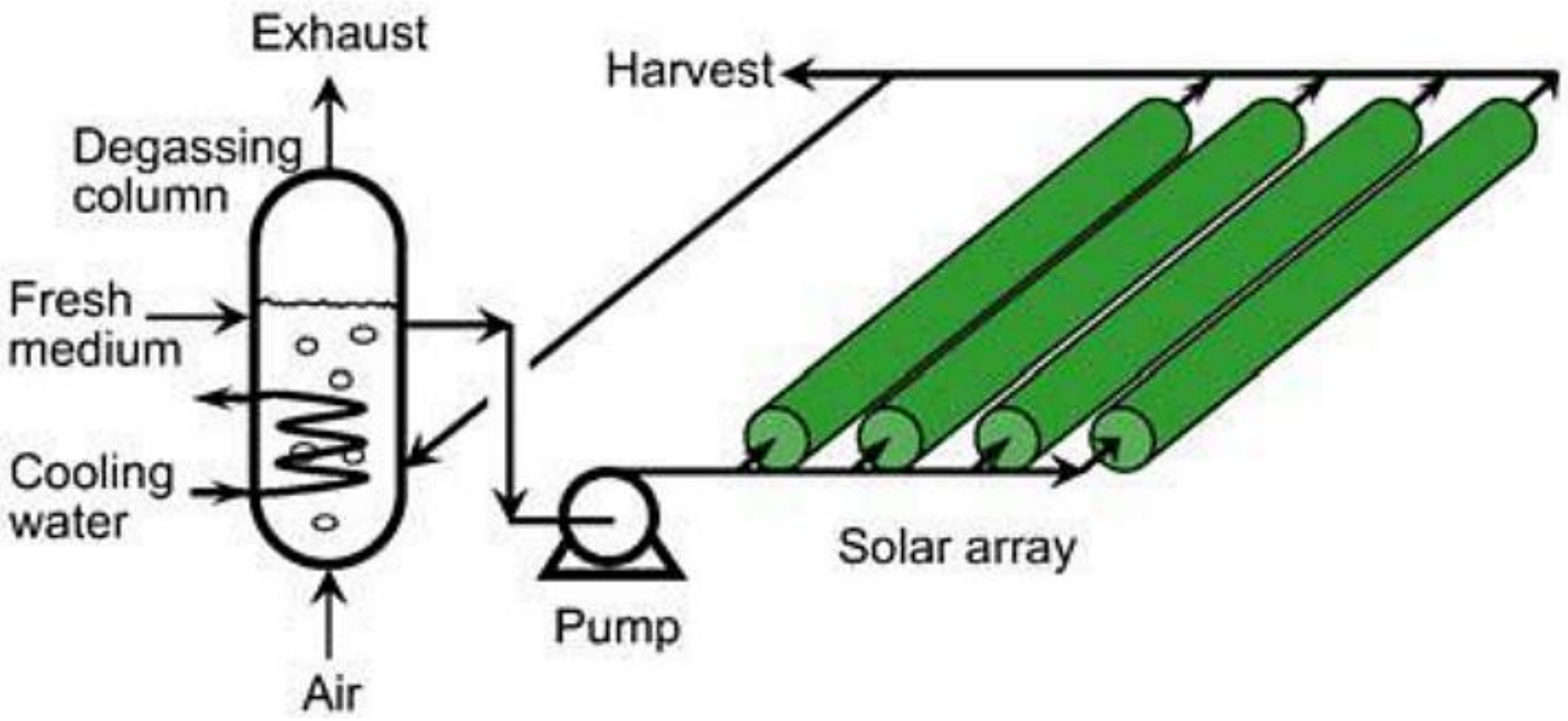
(B)

Multiple parallel tube



(D)

Flat panel configuration



A tubular photobioreactors with parallel run horizontal tubes.

CONCLUSION

High productivity, high product yield and high product concentration are the major objectives of plant tissue process development. A variety of bioreactor types providing growth and expression of bioactive substances are available today for plant cell and tissue cultures. Low biomass and product level can be achieved in any type of bioreactors. However, an improved understanding of the manifold interactions between cultivated cells, product formation and the specific designs for different bioreactor types will enhance and sustain high productivity and also reduce the process costs.

Bioreactor design and optimization

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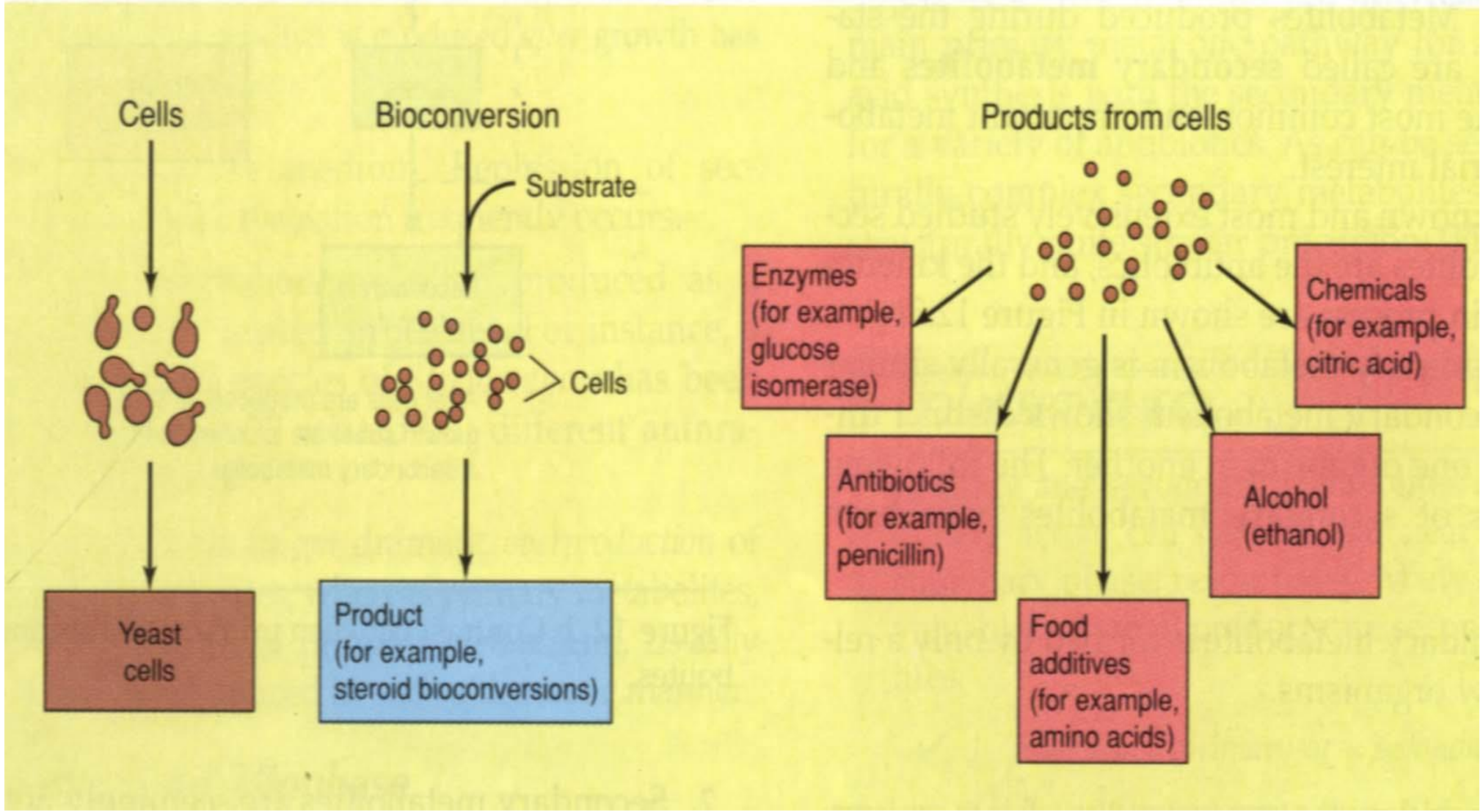
Fermentation

- Fermentation Technology is the technology to grow cells in a large scale with high efficiency, it also includes product recovery processes.
    - Fermentor: Microbial organisms
    - Bioreactor: Microorganisms, Animal or plant cell lines

Microbial products



Microbial cells, enzymes, pharmaceutical products, Specialty Chemicals and food additives

Flowsheet for developing an industrial microbial fermentation process

- Strain selection
- Laboratory process development
- Pilot Scale up
- Industrial Scale up
- Downstream process development
- Product packaging techniques
- Other commercial considerations

UPSTREAM

BIOREACTION/FERMENTATION

DOWNSTREAM

Strain Selection

- Purchase from Culture Collections
- Screening of nature circumstances
- Genetic engineering
- Mutations

Inoculation: usually 1% to 10% of final volume

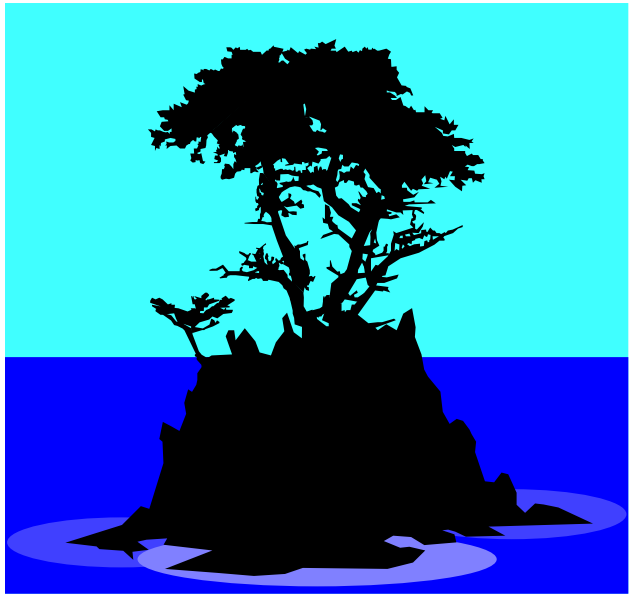
International Culture Collections

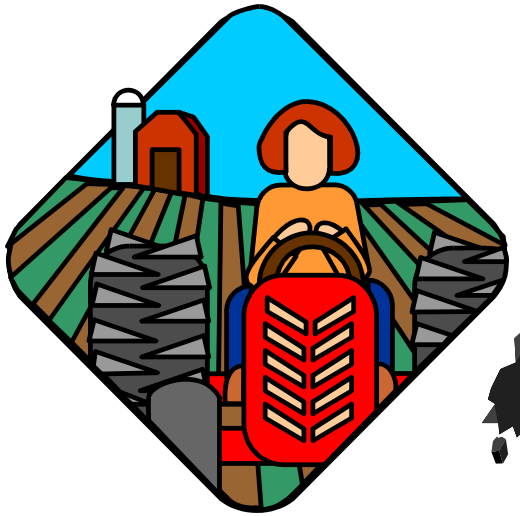
TABLE 12.1 Culture collections that supply cultures of industrial microorganisms[a]

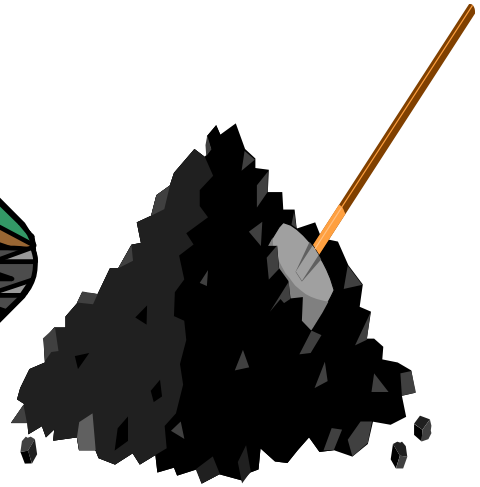
Abbreviation	Name	Location
ATCC	American Type Culture Collection	Rockville, MD, United States
CBS	Centraalbureau voor Schimmelculturen	Baarn, The Netherlands
CCM	Czechoslovak Collection of Microorganisms	J. E. Purkyne University, Brno, Czech Republic
CDDA	Canadian Department of Agriculture	Ottawa, Canada
CMI	Commonwealth Mycological Institute	Kew, United Kingdom
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH	Braunschweig, Germany
FAT	Faculty of Agriculture, Tokyo University	Tokyo, Japan
IAM	Institute of Applied Microbiology	University of Tokyo, Japan
NCIB	National Collection of Industrial Bacteria	Aberdeen, Scotland
NCTC	National Collection of Type Cultures	London, United Kingdom
NRRL	Northern Regional Research Laboratory	Peoria, IL, United States
PCC	Pasteur Culture Collection	Paris, France

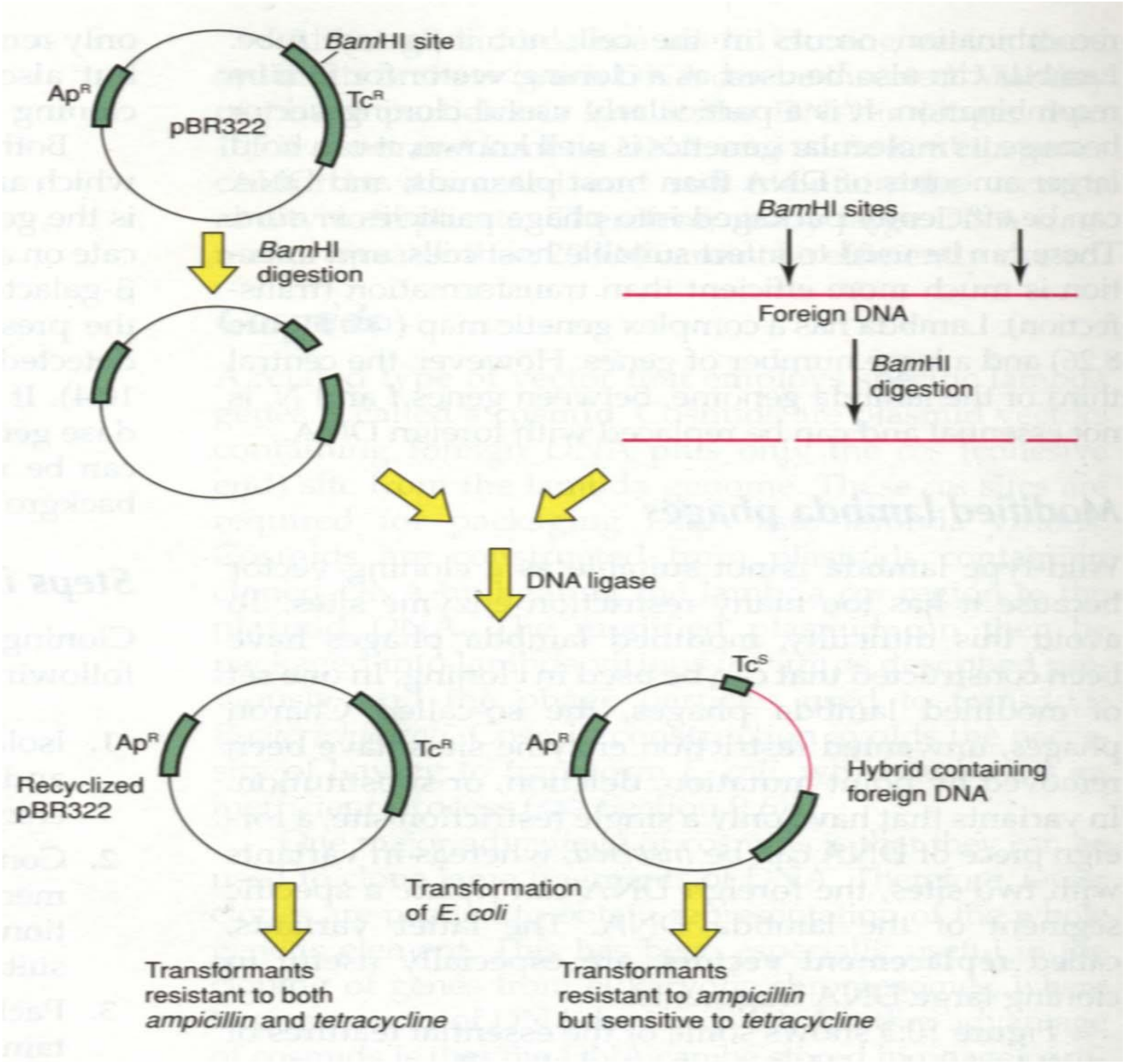
[a]Listed here are just a few of the general culture collections. Many universities and research laboratories maintain collections of specific microbial groups.

Screening of nature circumstances



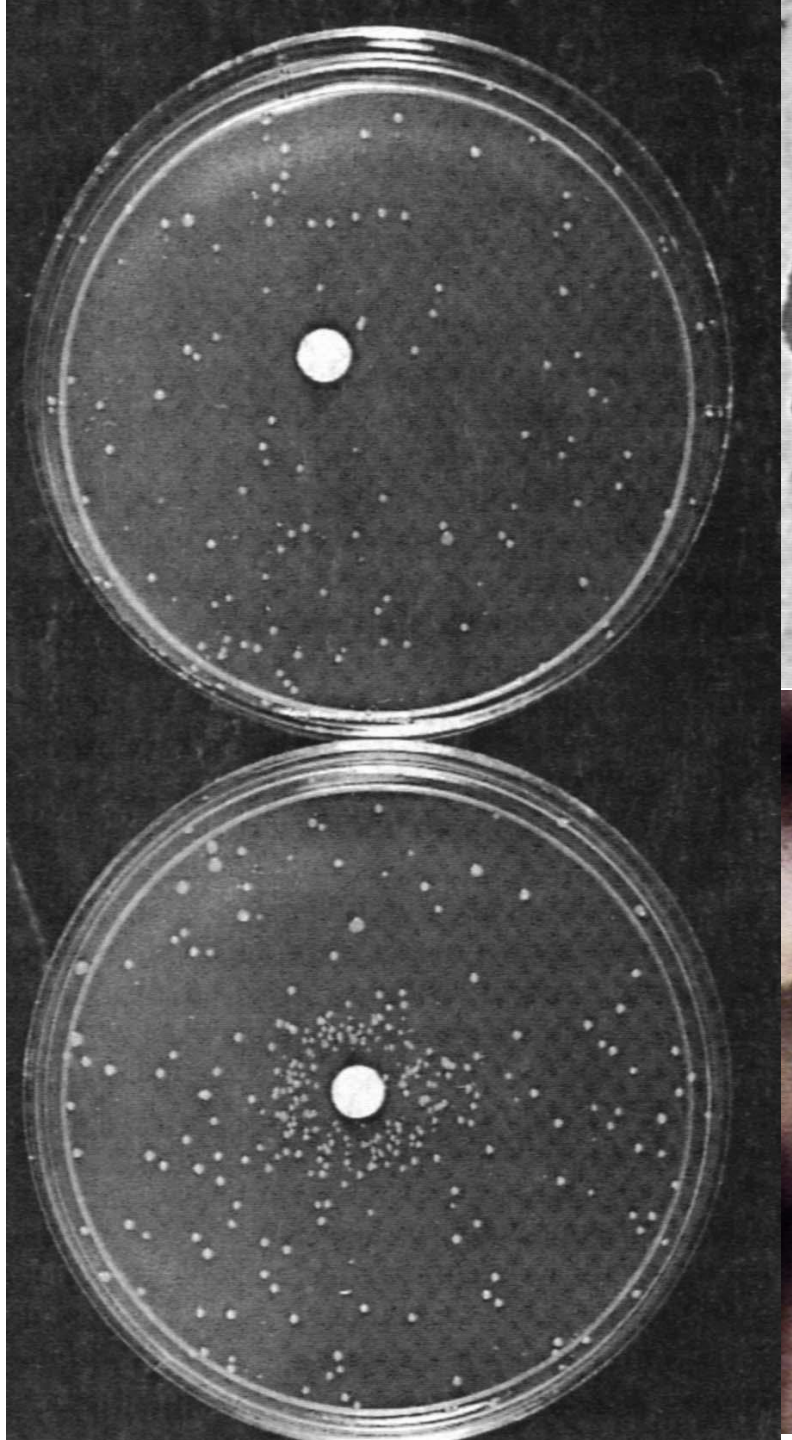


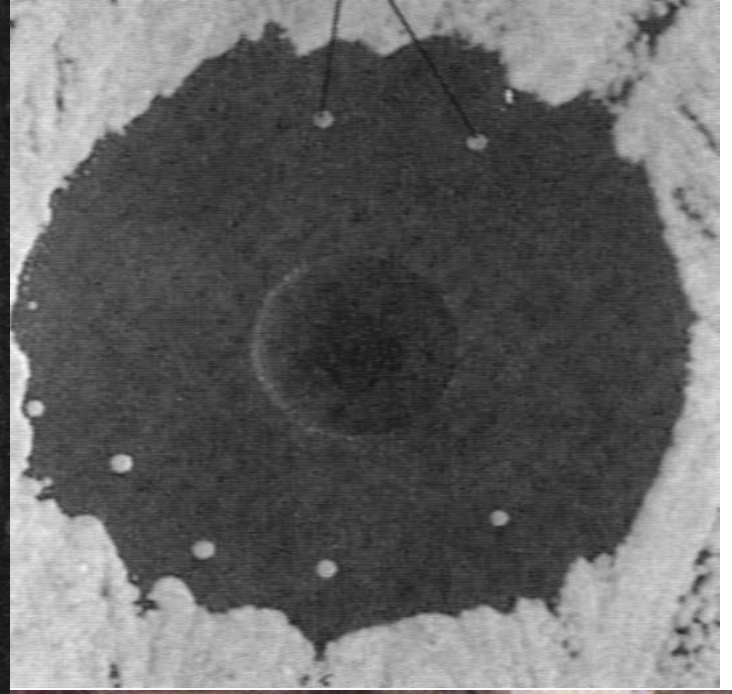




Genetic Engineering

Various high value added products have been produced from the Genetic engineering methods





Mutation

Via chemical or physical, and biological means

Stages needed for transferring an industrial process from the laboratory to the commercial fermentor

- Shake flask Experiments
- Lab scale bioreactor (1-20 L)
- Pilot scale bioreactor (100-3000 L)
- Commercial fermentor (10,000-500,000 L)

1 hectoliter = 100 liters

Input	Output	Table Chart
1 kL	1000 L	kiloliters to liters table
1 ML	1000000 L	megaliters to liters table
1 µL	0.001 mL	microliters to milliliters table
1 qt (US dry)	0.0389 ft³	US dry quarts to cubic feet table
1 µm³	1.0E-12 cm³	cubic micrometers to cubic centimeters table
1 µL	1.0E-6 L	microliters to liters table
1 mL	0.0001 daL	milliliters to dekaliters table
1 cL	0.001 daL	centiliters to dekaliters table
1 kL	10000 dL	kiloliters to deciliters table
1 kL	10 hL	kiloliters to hectoliters table

Laboratory process development
Shake Flask Experiments

Optimization of conditions for cell growth and product formation using shake flask experiments:

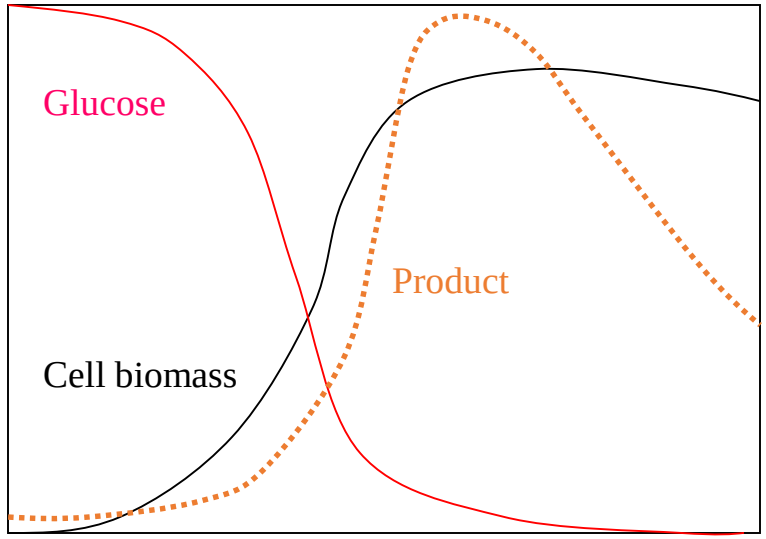
1. **pH**
2. **Temperature**
3. **Dissolved oxygen (DO)**
4. **Substrate choice**
5. **Maximal and optimal substrate concentration**
6. **Others**

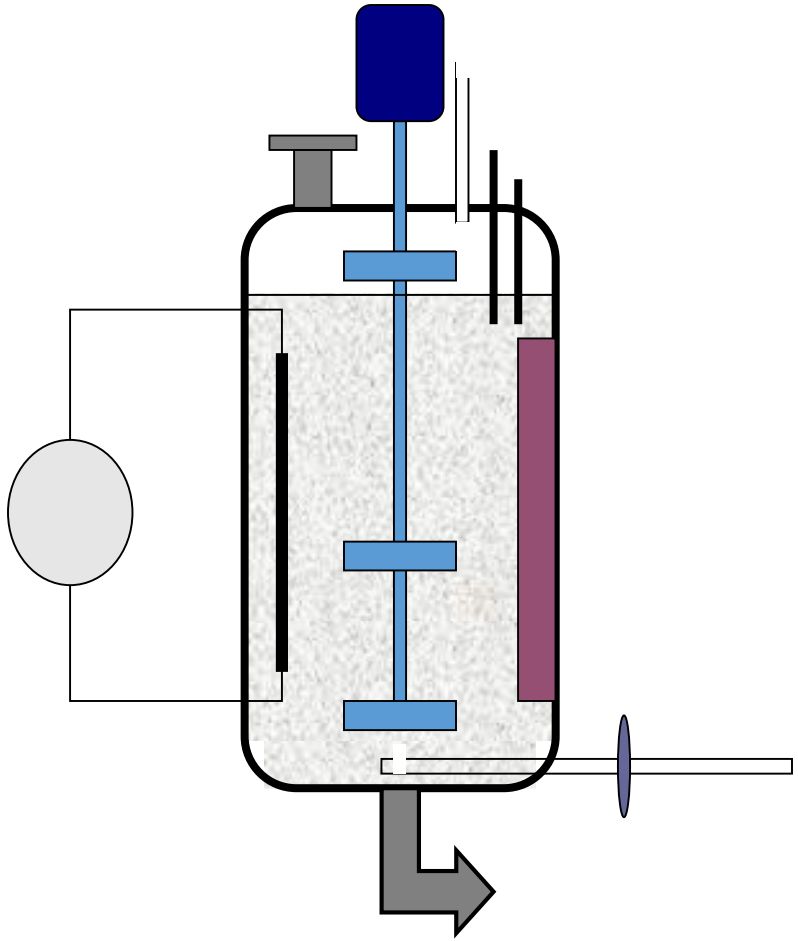
Operating Systems

- Fermentations in liquid media can be carried out under batch, fed-batch or continuous culture conditions
- **Batch fermentor** : a closed system where all nutrients are present at start of fermentation within a fixed volume
- **Fed-batch fermentor**: fresh medium is fed in throughout the fermentation and volume of batch increases with time
- **Continuous culture:** fresh medium is fed into the vessel and spent medium and cells are removed (fixed volume) **CHEMOSTAT**
- In all systems, pH, temperature, aeration etc is monitored and adjusted

Batch Fermentation

1. Medium added
2. Fermentor sterilised
3. Inoculum added
4. Fermentation followed to completion
5. Culture harvested

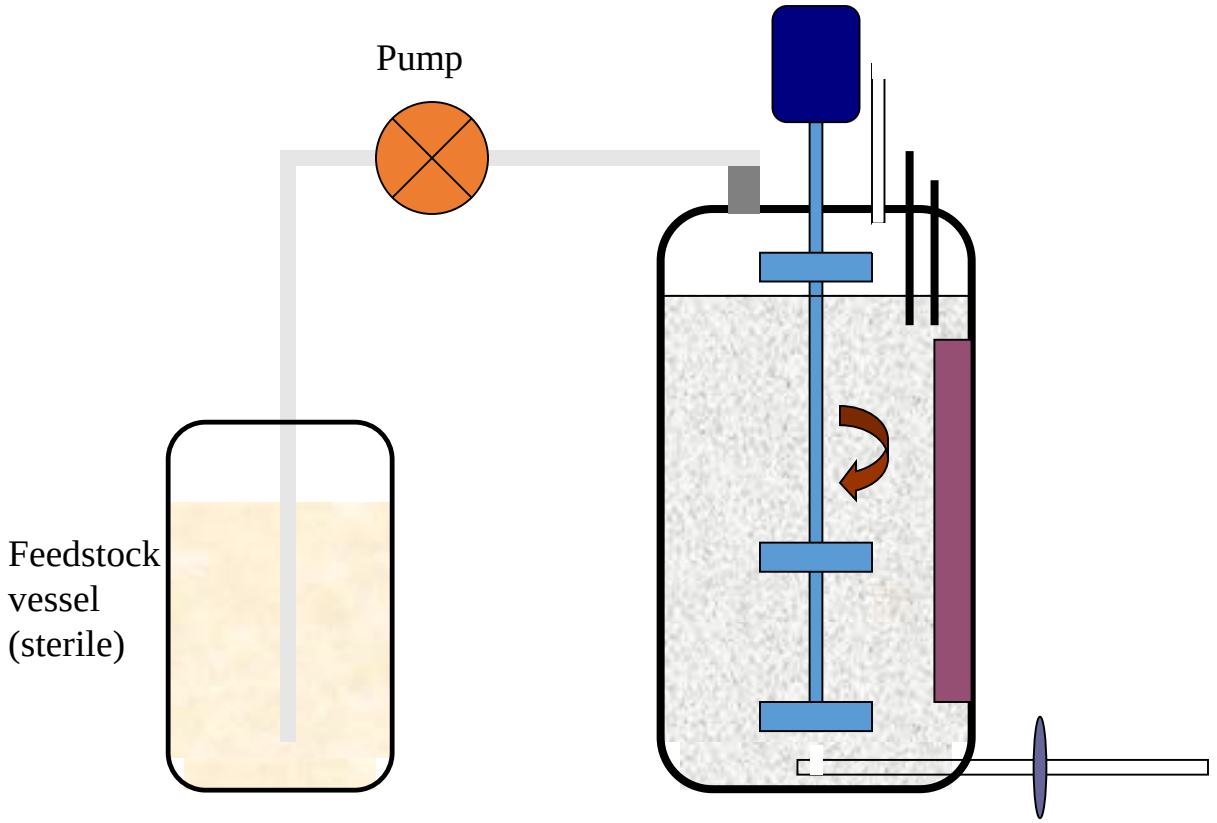




Characteristics of a Batch Fermentation System

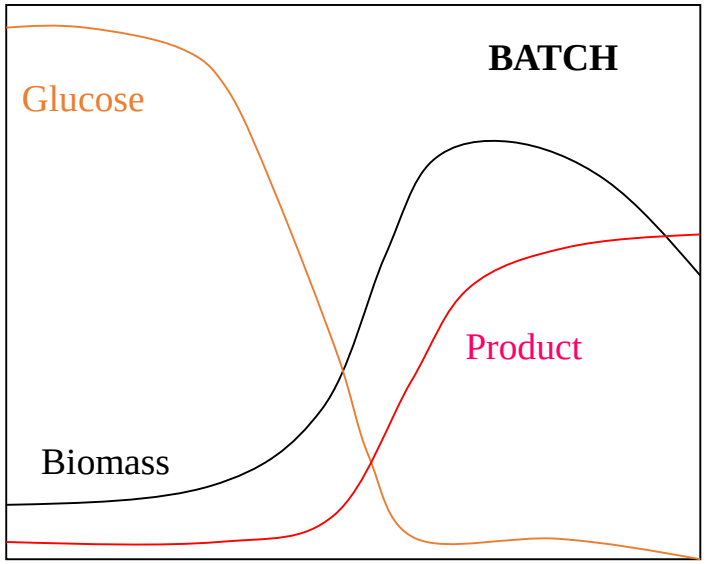
- Simplest fermentor operation
- Sterilisation can be performed in the reactor
- All nutrients are added before inoculation
- Maximum levels of C and N are limited by inhibition of cell growth
- Biomass production limited by C/N load and production of toxic waste products
- Cells are harvested when biomass levels or product levels start to decline

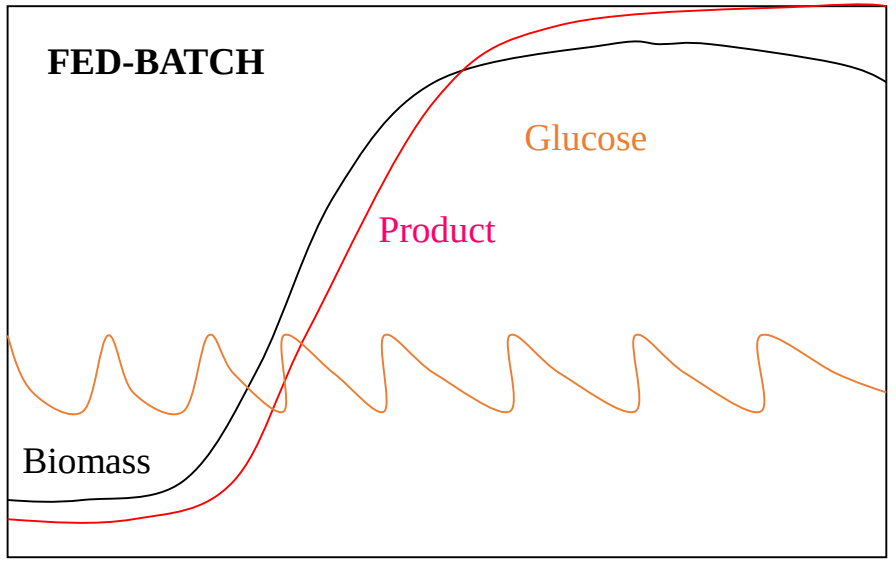
Fed-Batch Fermentation



Characteristics of Fed Batch Fermentors

- Initial medium concentration is relatively low (no inhibition of culture growth)
- Medium constituents (concentrated C and/or N feeds) are added continuously or in increments
- Controlled feed results in higher biomass and product yields
- Fermentation is still limited by accumulation of toxic end products

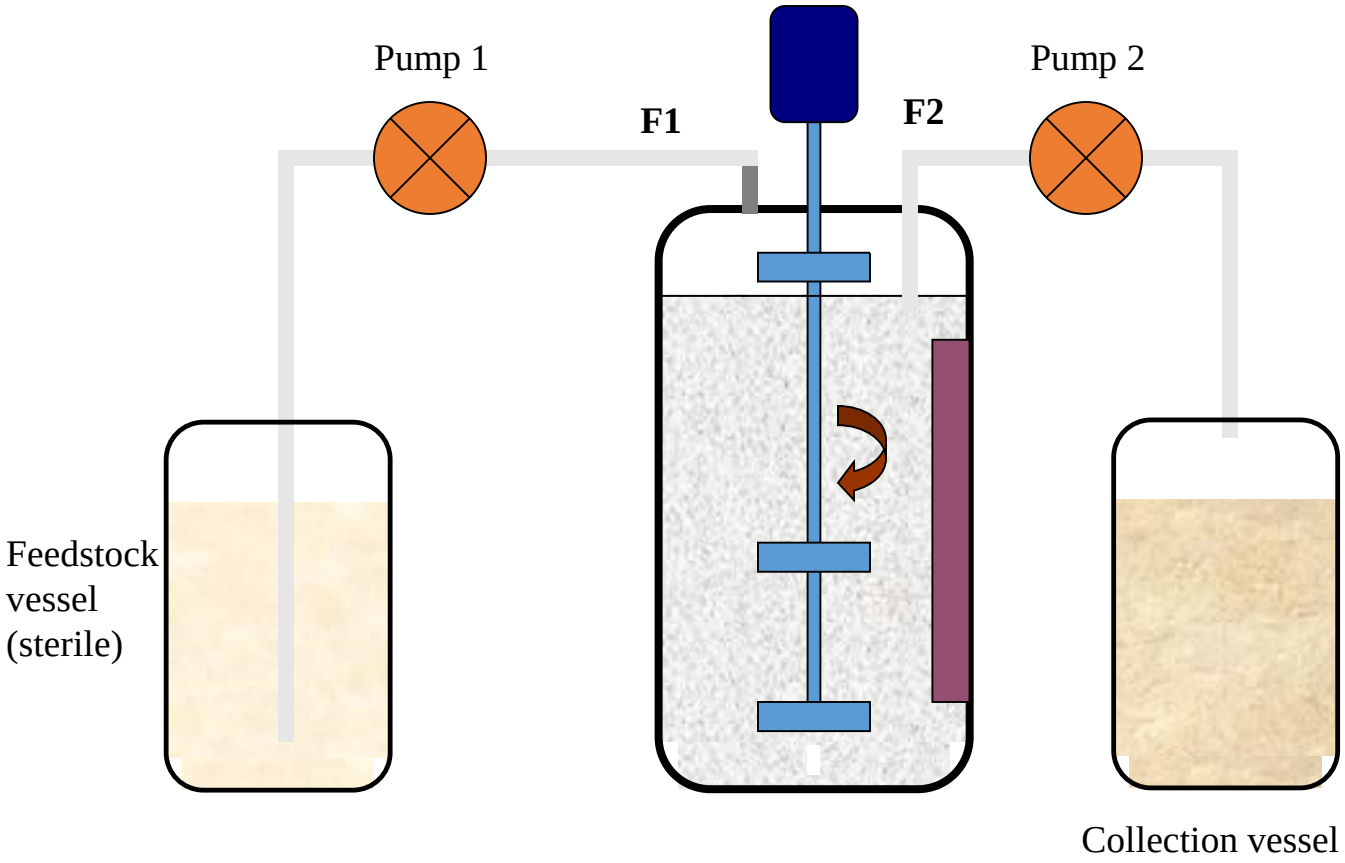




Continuous Fermentation (*Chemostat*)

Flow rate$_1$ = Flow rate$_2$

F1 **F2**



Characteristics of Continuous (*Chemostat*) Fermentation

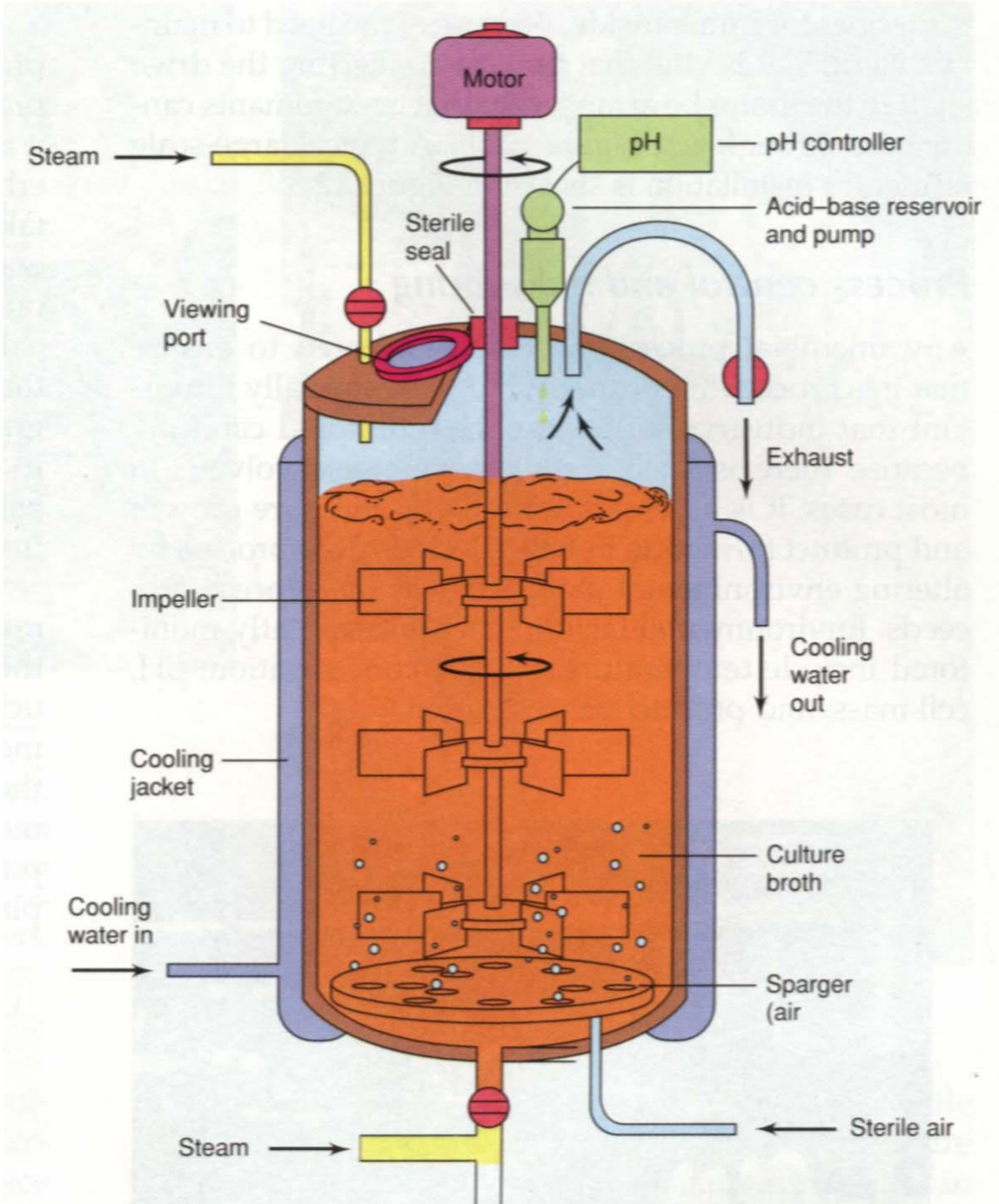
- Input rate = output rate (volume = const.)
- Flow rate is selected to give steady state growth (growth rate = dilution rate)
  - Dilution rate > Growth rate ➞ culture washes out
  - Dilution rate < Growth rate ➞ culture overgrows
  - Dilution rate = Growth rate ➞ steady state culture
- Product is harvested from the outflow stream
- Stable chemostat cultures can operate continuously for weeks or months.

Bioreactor/Fermentor design and construction

Laboratory fermentations: shake flasks

- Industrial fermentors are custom designed
- Design, quality, mode of operation depends on:
  - Production organism
  - Optimal operating conditions for product formation
  - Product value
  - Scale of production
  - Reliability
  - Economics: must minimise running costs and capital investment

Pilot Fermentor



- **Mixing**
- **Air inlet and outlet**
- **Cooling and heating**
- **pH control**
- **Nutrient addition**
- **Inoculation**
- **Viewing port**

Bioreactor/Fermentor Controls

- **Mixing**
  - **Dependent on fermentor dimensions, paddle design and flanging**
  - **Controls aeration rate**
  - **May cause cell damage (shear)**
- **Temperature control**
  - **Critical for optimum growth**
- **Aeration**
  - **Related to flow rate and stirrer speed**
  - **Dependent on temperature**
- **pH control**
  - **Important for culture stability/ survival**
- **Foaming control**

Agitation/Mixing

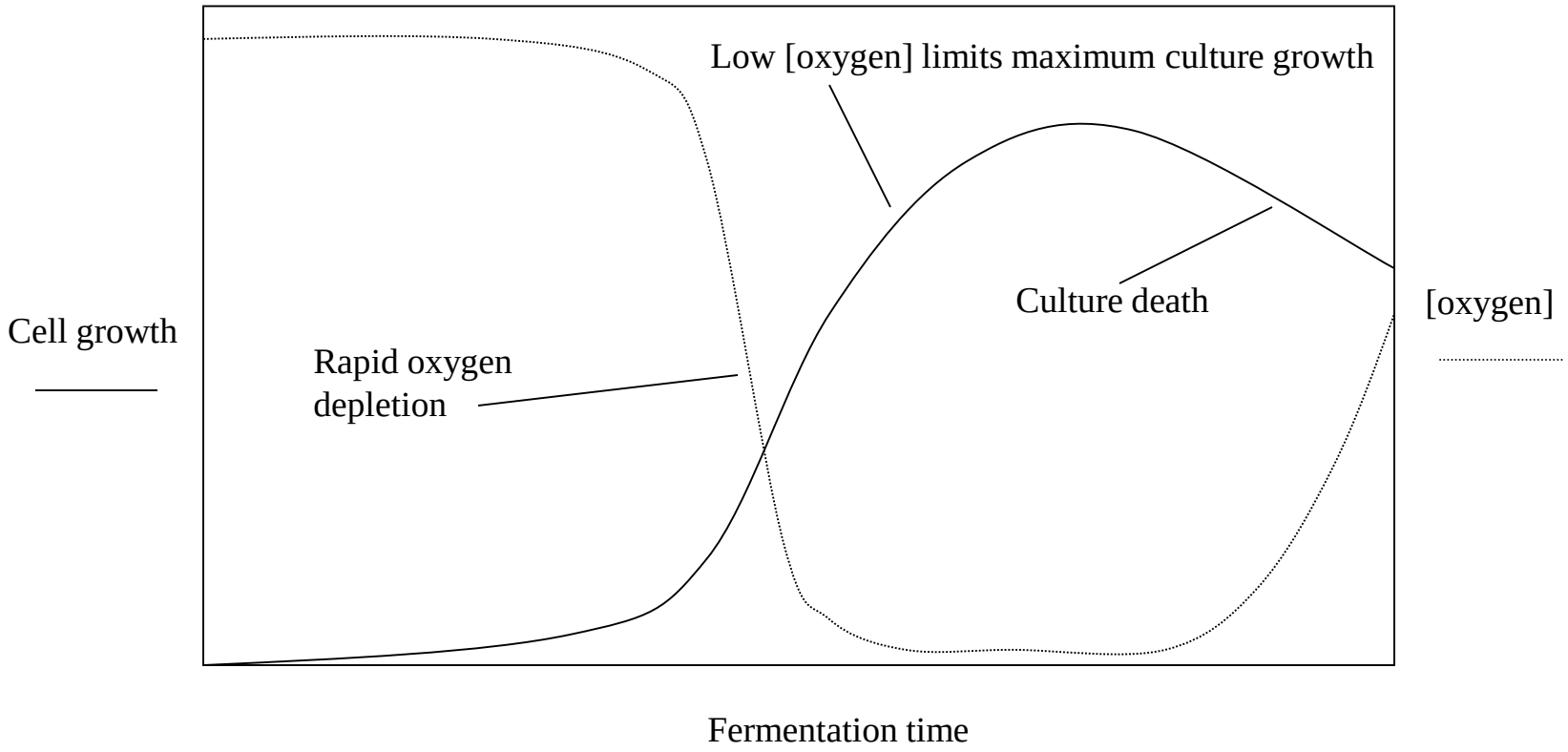
- Efficient mixing is critical for:
  - Homogeneous distribution of nutrients
  - Even temperature distribution
  - Rapid pH adjustment
  - Retention of air bubbles
- Different designs of stirrer blades give different circulation patterns
- Mixing is assisted by the presence of baffles on the fermentor walls
- Stirrer tip speed dictates the degree of shear stress
- Some cells types are very susceptible to shear stress
- Excessive stirring can promote foaming

Aeration

Filtered air supplied by forced airflow at the base of the fermentor

- Size of air bubble dictated by air supply tube hole diameter
- Time taken for air bubbles to rise to surface (residence time) is dependent on bubble size and stirrer rate
- Rate of oxygen dissolution depends on surface area (bubble size) and residence time
- dO_2 concentration is dependent on dissolution rate and microbial uptake rate
- O_2 electrode used to give feed-back information to air supply pump (must maintain constant dO_2 concentrations)

Typical dO_2 profile in batch fermentor without oxygen monitoring

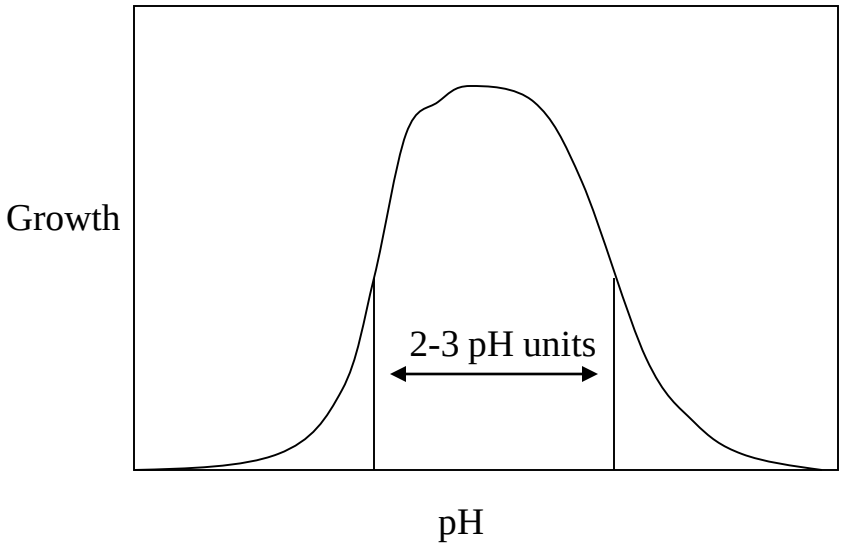


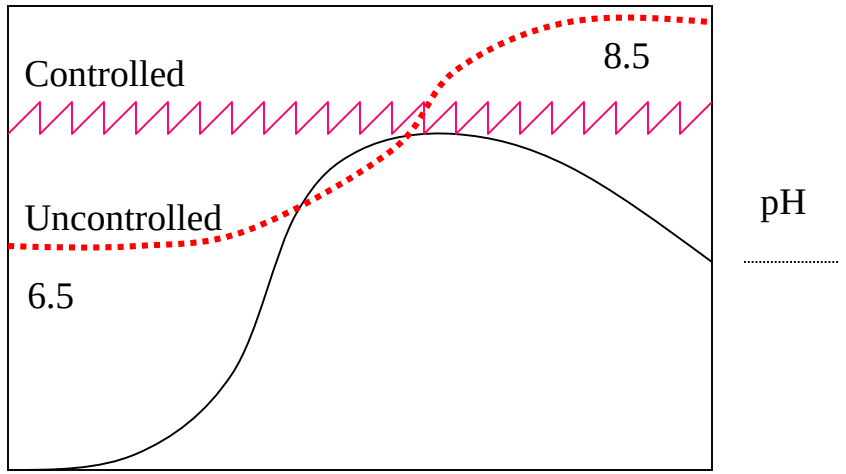
Temperature control

- Microbial growth sensitive to temperature changes
- Heat supplied by direct heating probes or by heat exchange from an outer jacket
- T control by injecting cold/hot H_2O
- These systems sterilise the system prior to inoculation (inject pressurised steam)
- Heat generated during fermentation due to
  - Metabolic heat
  - Mechanical agitation

pH control

- Most cultures have narrow pH growth ranges
- The buffering in culture media is generally low
- Most cultures cause the pH of the medium to rise during fermentation
- pH is controlled by using a pH probe, linked via computer to NaOH and HCl input pumps.





Foaming

- Foaming is caused when
  - Microbial cultures excrete high levels of proteins and/or emulsifiers
  - High aeration rates are used
- Excess foaming causes loss of culture volume and may result in culture contamination
- Foaming is controlled by use of a foam-breaker and/or the addition of anti-foaming agents (silicon-based reagents)

Culture Volume: 1/5th of the volume (Headspace)

Sterilisation

- The fermentor and all additions (medium, air) must be completely sterile
- Sterilisation is performed by:
  - Small fermentors (<10L); autoclaving
  - Large fermentors;
    - Vessel:steam sterilisation; gas (ethylene oxide)
    - Medium: autoclaving or ultrafiltration
    - Air: ultrafiltration

Fermentation Media

- Media must satisfy all nutritional requirements of the organism and fulfil the objectives of the process
- Generally must provide
  - a carbon source (for energy and C units for biosynthesis)
  - Sources of nitrogen, phosphorous and sulfur
  - Minor and trace elements
  - Some require added vitamins e.g. biotin and riboflavin
- Media generally contain buffers or pH controlled by adding acids / alkalis
- Potential problems
  - Compounds that are rapidly metabolized may repress product formation
  - Certain compounds affect morphology

Factors affecting final choice of raw materials

- Costs and availability
- Ease of handling, transporting and storing
- Sterilization requirements and denaturation problems
- Formulation, mixing and viscosity characteristics
- Concentration of product produced / rate of formation/ yield per gram of substrate
- Levels and ranges of impurities which may produce undesirable by products
- Health and safety considerations

Carbon sources

- Molasses
  - Byproduct of cane sugar production
  - a dark viscous syrup containing 50% CHO (sucrose) with 2% nitrogen, vitamins and minerals
- Malt extract
  - Use aqueous extracts of malted barley to produce C sources for cultivation of fungi and yeasts
  - Contain 90% CHO, 5% nitrogen and proteins, peptides and amino acids

Carbon sources

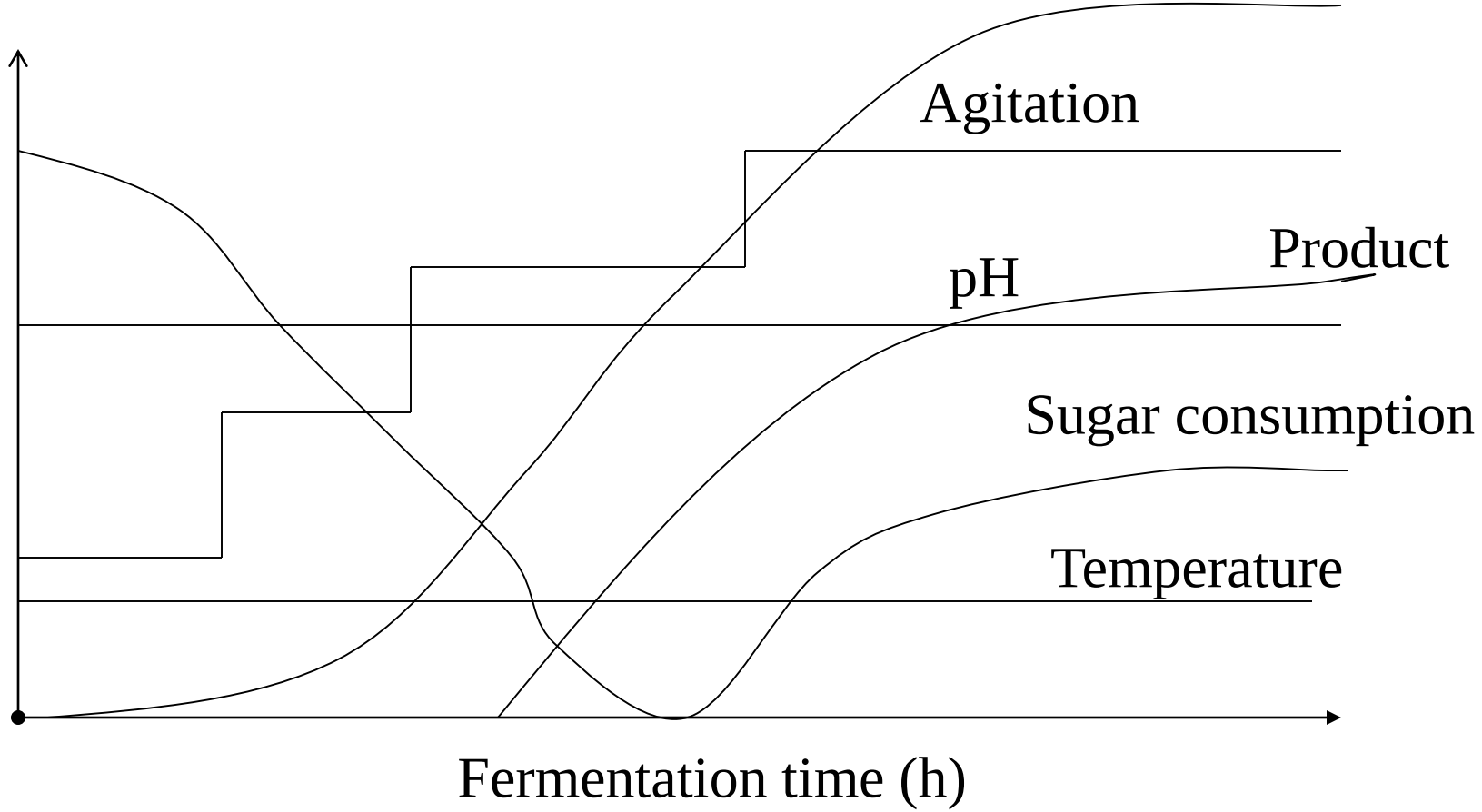
- Whey
  - Aqueous byproduct of dairy industry
  - Contains lactose and milk proteins
  - Difficult to store (refrigerate) so freeze dried
  - Many MO's won't metabolize lactose but whey is used in production of penecilluin, ethanol, SCP, xanthan gum etc
- Alkanes and alcohols
  - $C_{10} – C_{20}$ alkanes, metane and methanol used for vinegar and biomass production
  - Use is dependent on prevaling petroleum price

Nitrogen Sources

- Microorganisms generally can use inorganic or organic N
  - Inorganic sources: ammonia, ammonium salts
  - Organic sources: amino acid, proteins and urea
    - Corn steep liquor
    - Yeast extract
    - Peptones
    - Soya bean meal

Process control and monitoring

- Process parameters to be monitors

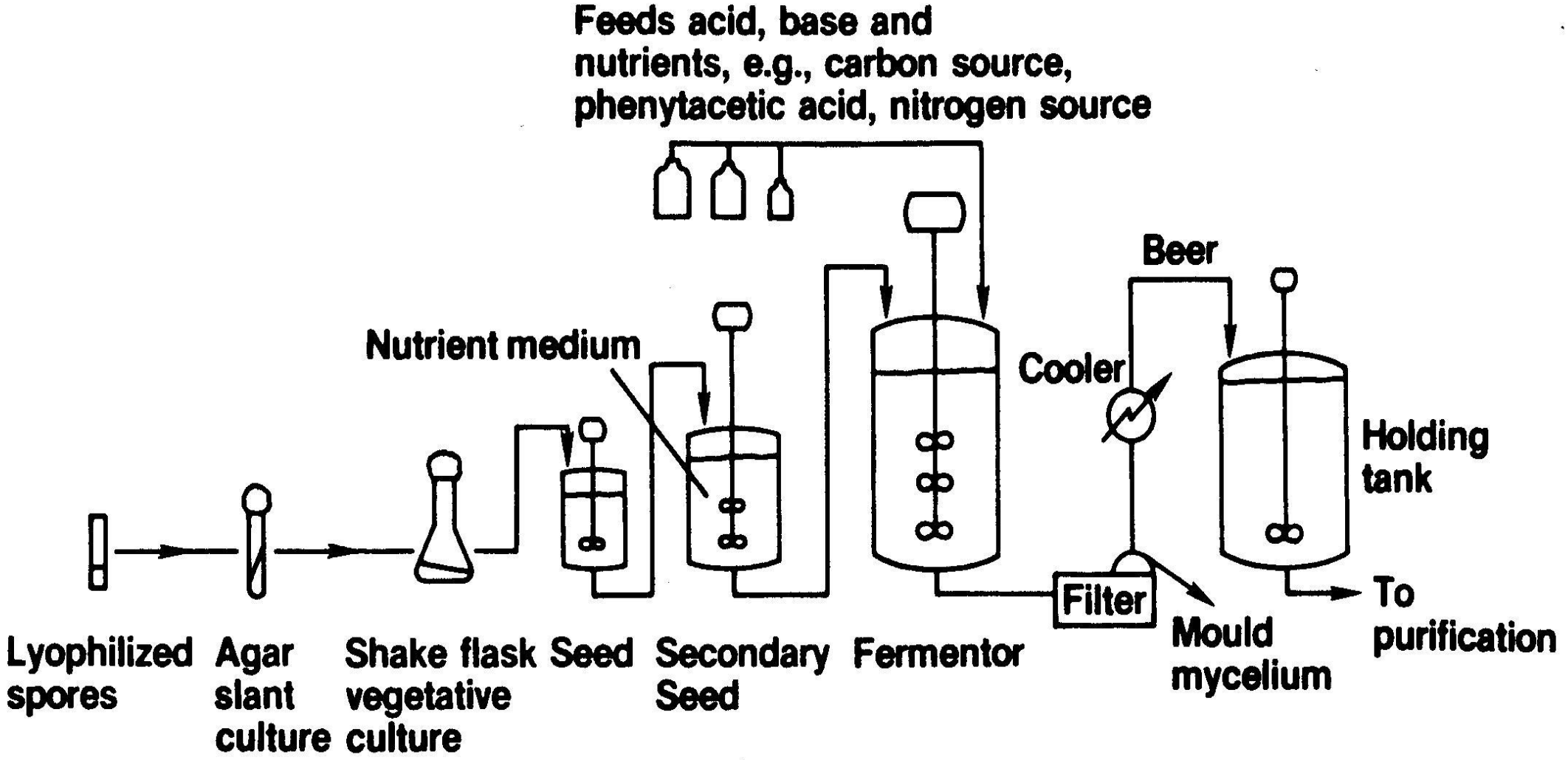


Computer softwares have been developed to monitor and change the process on line

Scale Up

- **Scale up: The transfer of a process from small-scale laboratory equipment to large-scale commercial equipment**
- Pilot experiment
  - To test the feasibility of the lab scale fermentation process in a ***semi-industrial*** scale
  - Pilot fermentors normally have a size ranging from 100 L to 3,000 L, depending on the products to be mass produced later.

The Scale-up Fermentation Process



Fermentor sizes for various purposes

TABLE 12.2 Fermentor sizes for various industrial processes

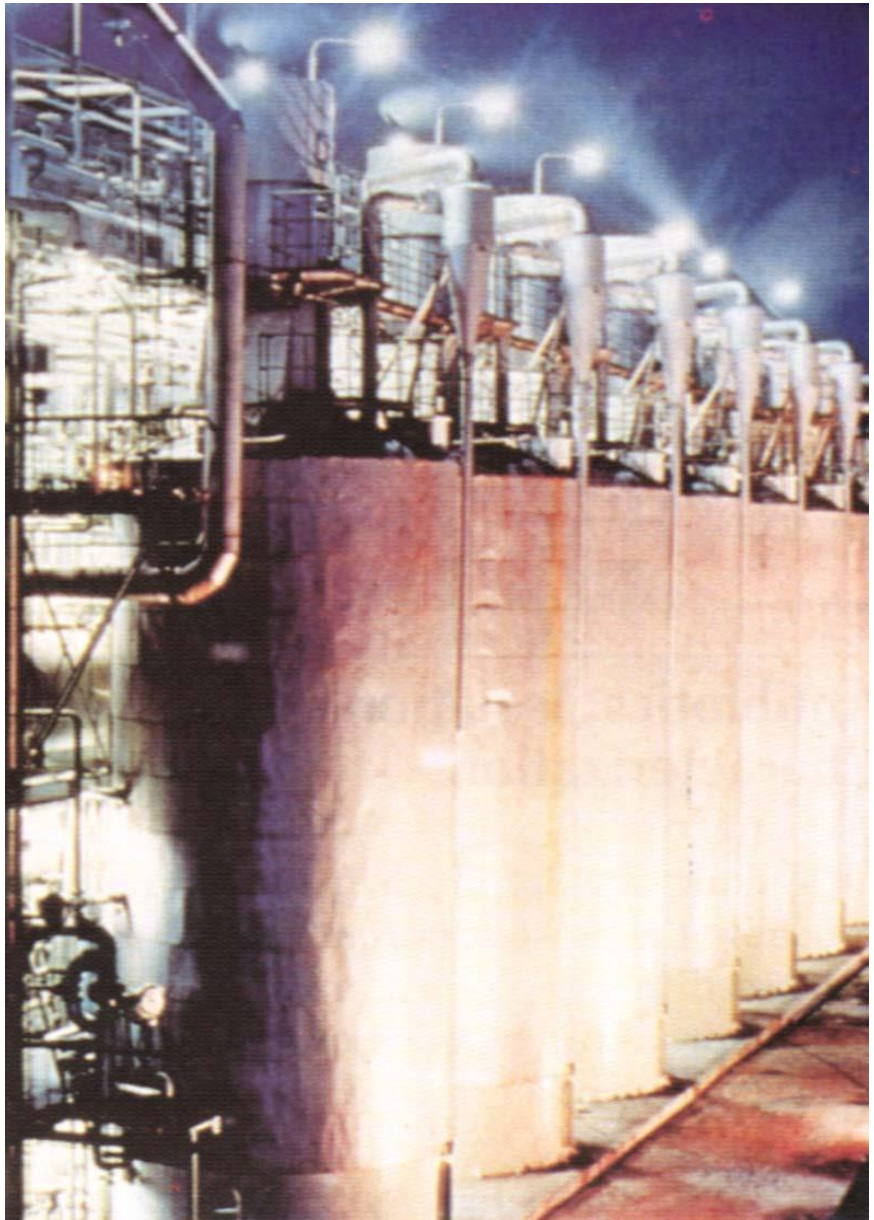
Size of fermentor (liters)	Product
1–20,000	Diagnostic enzymes, substances for molecular biology
40–80,000	Some enzymes, antibiotics
100–150,000	Penicillin, aminoglycoside antibiotics, proteases, amylases, steroid transformations, amino acids
200,000–500,000	Amino acids (glutamic acid)

http://babubkmj.bravehost.com/Animation%20which%20i%20made%20for%20fermentation.swf

Problems emerging during the scale up

- As the size of the equipment is increased, the surface-volume ratio changes
- Large fermentor has much more volume for a given surface area, it is obviously more difficult to mix the big tank than the small flask
- In scale up studies on aerobic fermentations, oxygen rate in the fermentor is best kept constant as the size of the fermentor is increased.
    - How to keep DO constant?
        - Increase stirring rate
        - Increase air pressure
        - Use pure oxygen
        - Increase air inlet

Industrial Scale up



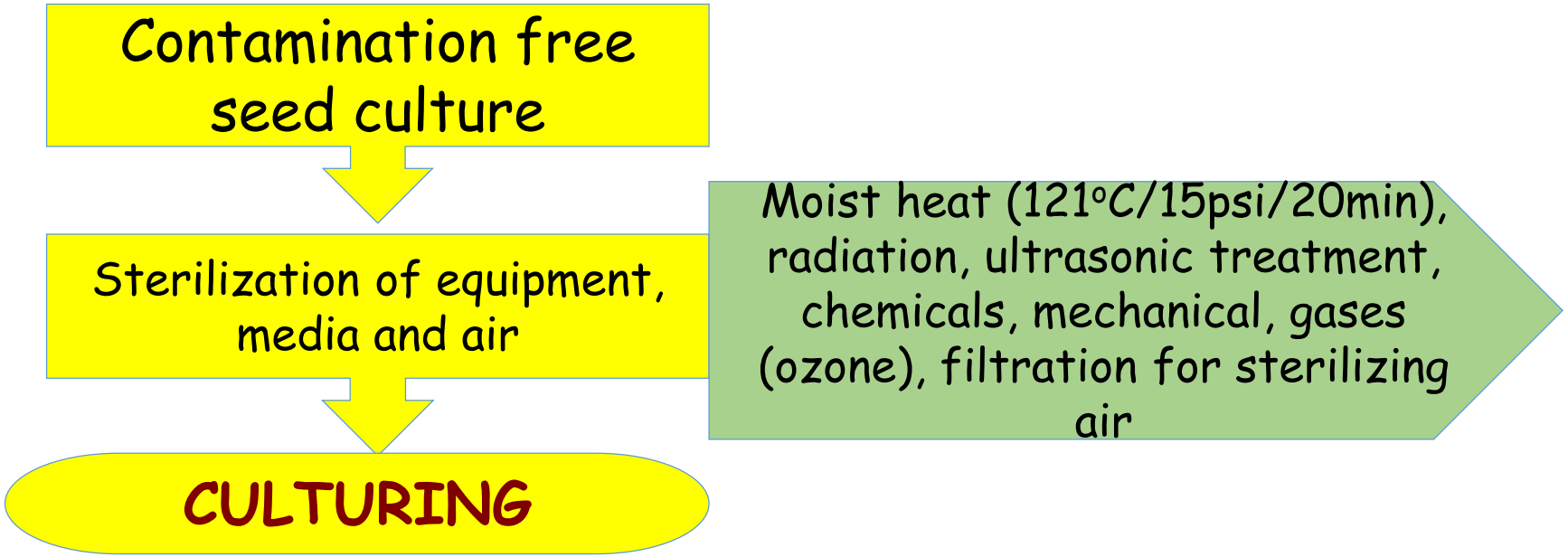
- To transfer the pilot scale results into a commercially feasible production setting.
- Fermentor sizes range from 100 L to 500,000 L, depending on products.

APPLICATIONS OF INDUSTRIAL BIOTECHNOLOGY

Fermentation Technology

Culturing methods for Micro-organisms

Sterilization: devoid of MO (aseptic conditions)



Avoidance of contamination can be achieved by

- Use pure inoculum to start fermentation
- Sterilize the media
- Sterilize fermenter vessel
- Sterilize all materials to be added to the fermentation during the process
- Maintaining aseptic conditions during the fermentation

Control of environmental conditions for Microbial growth

Temperature
pH
Agitation
O2 conc

} To be carefully monitored and maintained

Acidic pH: fungi and yeast
Psychrophiles, acidophiles etc

Fermentation

In Microbiology

Any process for the production of useful products through mass culture of Micro Organisms

In Biochemistry

-The numerous Oxidation-Reduction reactions in which organic compounds used as carbon and energy act as acceptors and donors of H_2 ion. The organic compound gives rise to various products of fermentation which accumulate in the growth medium

-Takes place in absence of O_2

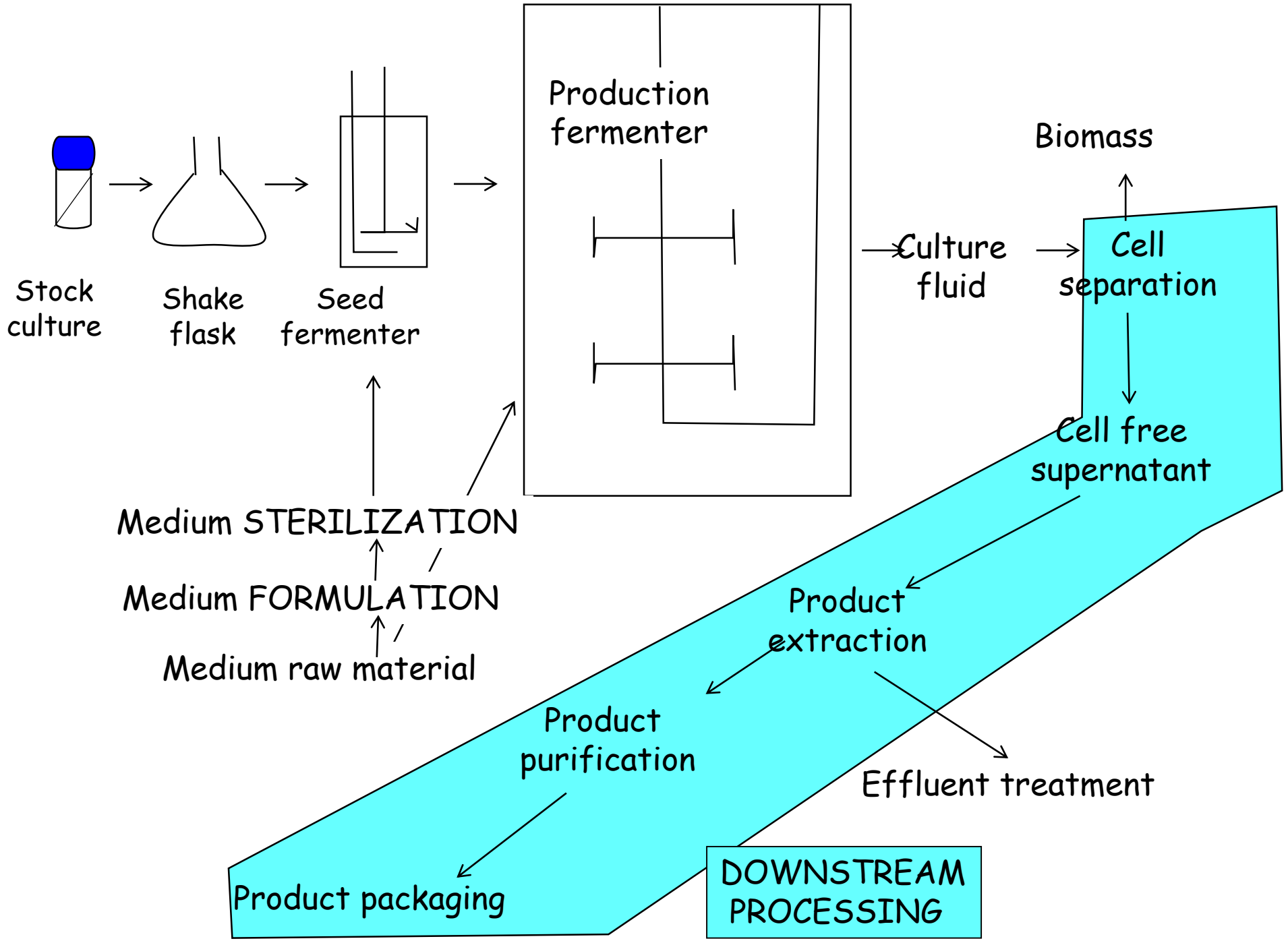
-Now term industrial fermentation for large scale cultivation of micro-organisms…most of them is aerobic

Some important submerged fermentation products

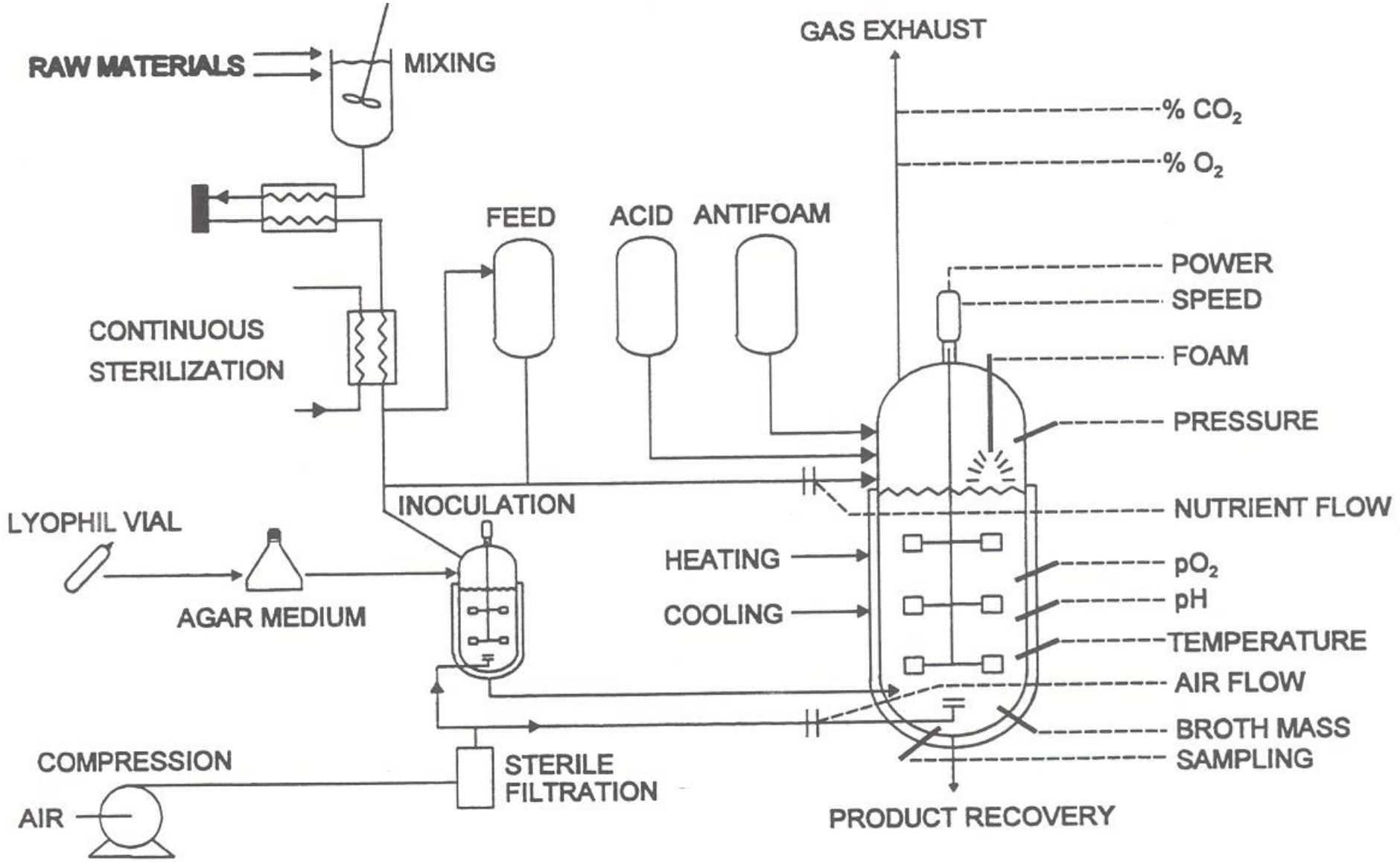
Product	**Organism**	**Use**
Ethanol	*Saccharomyces cerevisiae*	Industrial solvents, beverages
Glycerol	*Saccharomyces cerevisiae*	Production of explosives
Lactic acid	*Lactobacillus bulgaricus*	Food and pharmaceutical
Acetone and butanol	*Clostridium acetobutylicum*	Solvents
α-amylase	*Bacillus subtilis*	Starch hydrolysis

Process of commercial fermentation

1. **Formulation of media** to be used in culturing the organism during development of inoculum and in the production fermenter
2. **Sterilization** of the medium, fermenter and ancillary equipment
3. **Production of** an active, **pure culture** in sufficient quantity to inoculate the production vessel
4. The **growth of the organism in** the production **fermenter** under optimum conditions for product formation
5. The **extraction of the product** and its purification
6. **Disposal of effluents** produced by the process


Production fermenter
Biomass
Stock culture
Shake flask
Seed fermenter
Culture fluid
Cell separation
Cell free supernatant
Medium STERILIZATION
Medium FORMULATION
Medium raw material
Product extraction
Product purification
Effluent treatment
Product packaging
DOWNSTREAM PROCESSING

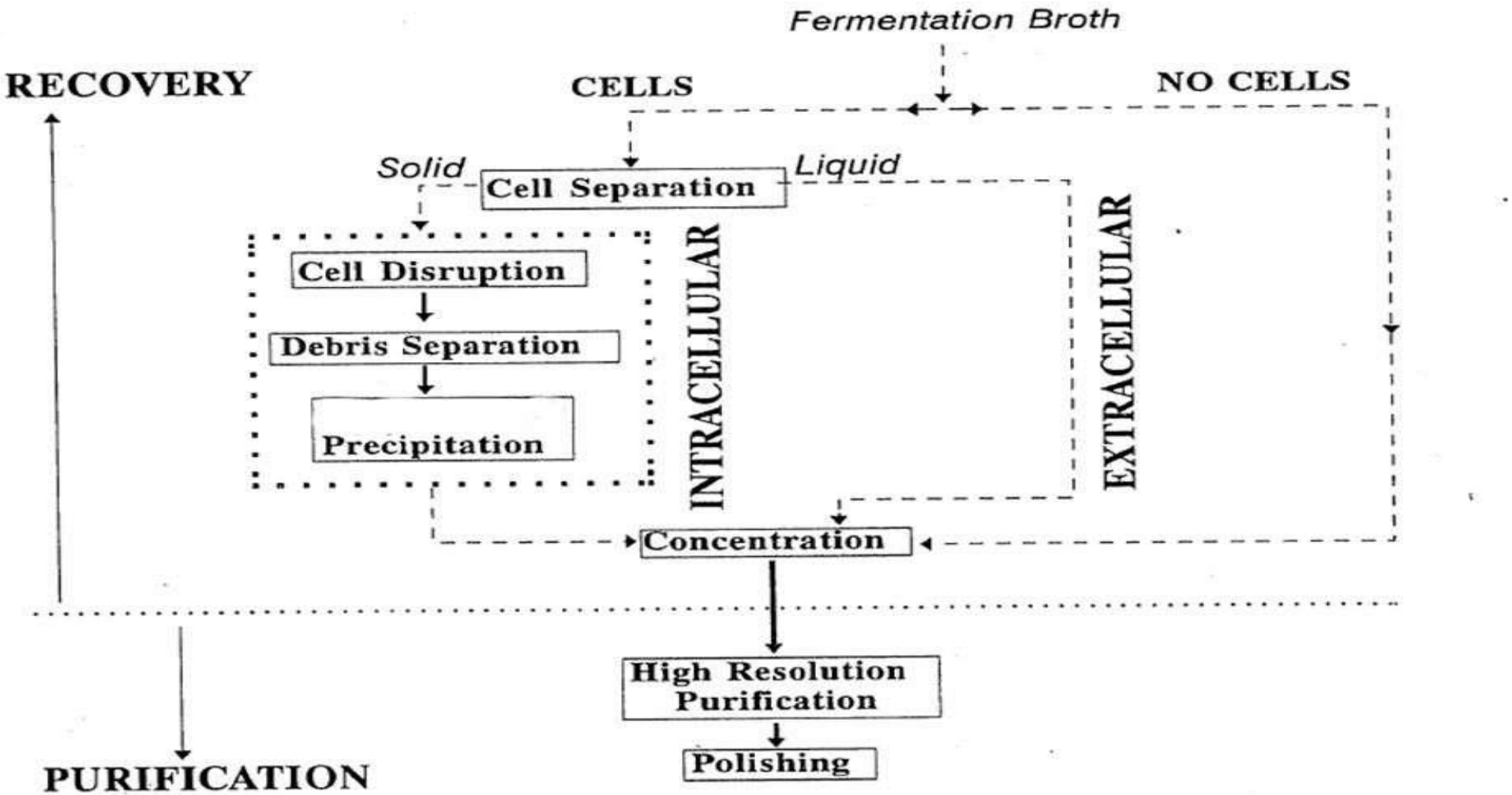
Flow sheet of a multipurpose fermenter and its auxiliary equipment



Downstream Processing

BTG 408

General Steps in Downstream Purification



Downstream processing

- The various stages of processing that occur after the completion of the fermentation or bioconversion stage, including separation, purification, and packaging of the product.

Stages in Downstream Processing

(A) Removal of Insolubles

(B) Product Isolation

(C) Product Purification

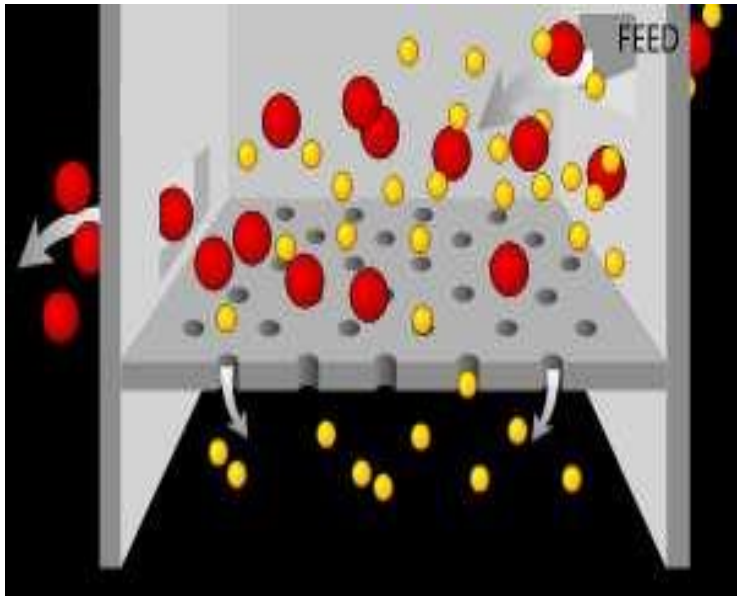
(D) Product Polishing

- A few product recovery methods may be considered to combine two or more stages.

For example, expanded bed adsorption accomplishes removal of insolubles and product isolation in a single step. Affinity chromatography often isolates and purifies in a single step.

(A) Removal of Insolubles

- Separation of cells, cell debris or other particulate matter

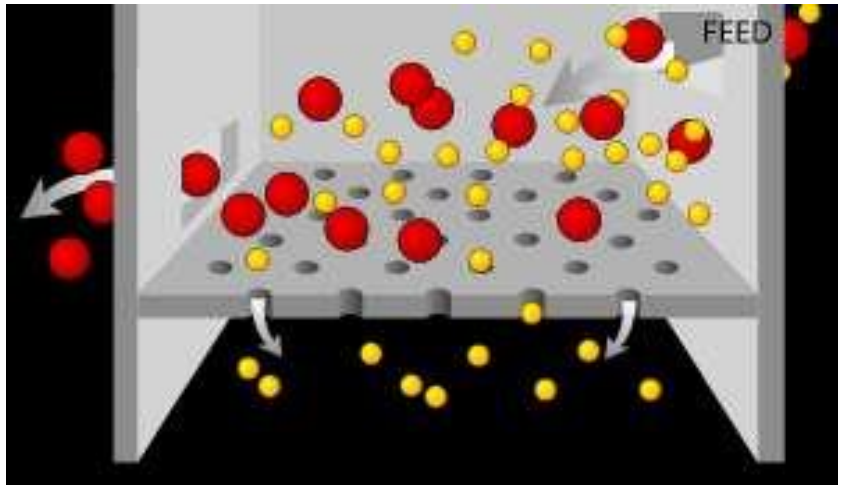


- Typical operations to achieve this:

1) Filtration
2) Centrifugation
3) Sedimentation
4) Flocculation a process where a solute comes out of solution in the form of floc or flakes.
5) Gravity settling

1. Filtration

• A mechanical operation used for the separation of solids from fluids (liquids or gases) by interposing a medium to porous membrane through which the fluid can pass, but the solids in the fluid are retained.



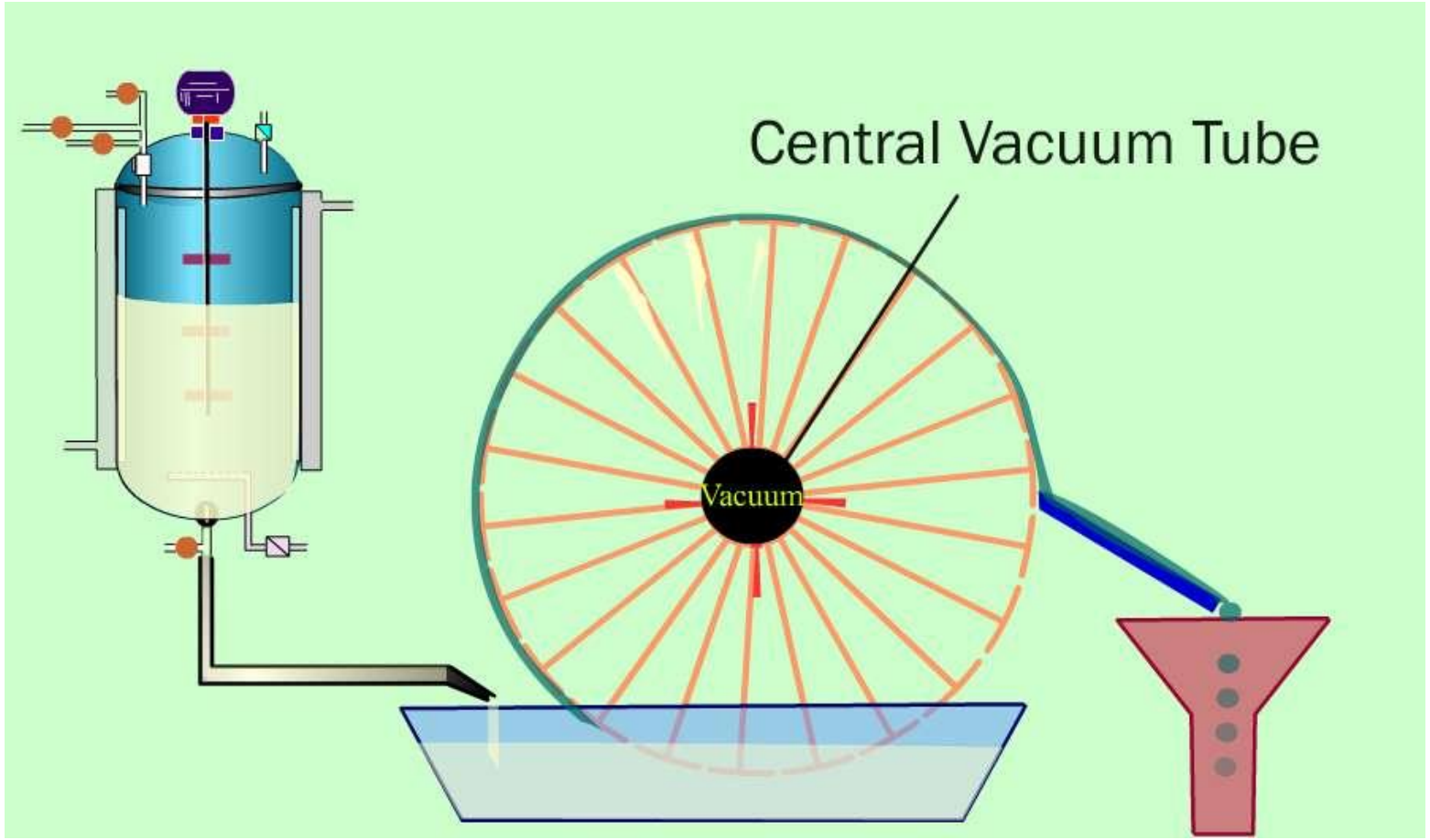
Filtration

- The solid particles deposited on the filter form a layer, which is known as filter cake.

- All the solid particles from the feed are stopped by the cake ,and the cake grows at the rate at which particles are bought to its surface.

- All of the fluid goes through the cake and filter medium.

Continuous Rotary filter



Continuous Rotary Vacuum filter

- It is one of the most commonly used type of filter in fermentation.
- The drum is pre-coated prior to filtration.
- A small agent of coagulating is added to the broth before it is pumped into the filter.
- The drum rotates under vacuum and a thin layer of cells sticks to the drum.
- The thickness of the layer increases in the section designed for forming the cake.

Points to be considered while selecting the filter medium:

- Ability to build the solid
- Minimum resistance to flow the filtrate
- Resistance to chemical attack
- Minimum cost
- Long life

2. Centrifugation

• Centrifugation is used to separate particles of 100 – 0.1µm from liquid by gravitational forces.

• It depends on particles size, density difference between the cells and the broth and broth viscosity.
• Use of the centrifugal force for the separation of mixtures

• More-dense components migrate away from the axis of the centrifuge
• Less-dense components migrate towards the axis.

• Types of centrifuges used are Tubular bowl centrifuge, multichamber centrifuge, disc bowl centrifuge etc.

3. Sedimentation

- It is apllicable only for large particles >100 µm flocs.
- It is a slow process and takes ~3 hours.
- It is used in process like activated sludge effluent treatment.
- It’s a free settling process depends only on gravity.
- Particles settling is a high particle density suspension(hindered settling).

4. Flocculation

- Process where a solute comes out of solution in the form of flocs or flakes

- Particles finer than 0.1µm in water remain continuously in motion due to electrostatic charge which causes them to repel each other

- Once their electrostatic charge is neutralized (use of coagulant) the finer particles start to collide and combine together

- These larger and heavier particles are called flocs

(B) Product Isolation

• Removal of those components whose properties vary markedly from that of the desired product

• Water is the chief impurity

a) Isolation steps are designed to remove it (i.e.dialysis)
b) Reducing the volume
c) Concentrating the product.
d) i. Liquid –liquid extraction,

ii. adsorption,

iii. ultrafiltration

iv.precipitation → are some of the unit operations involved

i. Liquid -Liquid extraction

- It is a separation process that takes the advantage of the relative solubilities of solute in immiscible solvents

- Solute is dissolved more readily and becomes more concentrated in the solvent in which it has a higher solubility.

- A partial separation occurs when a number of solutes have different relative solubilities in the two solvents used

- Solvent should be non toxic, selective, inexpensive and immiscible with broth and should have a high distribution coefficent for the product.

ii. Adsorption

- A surface phenomenon

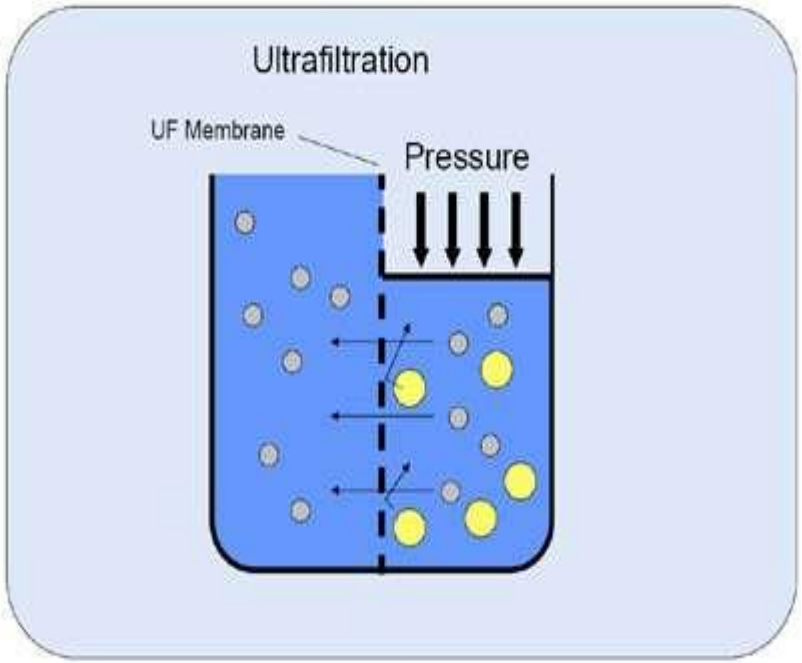
- It is the binding of molecules to the surface and different from absorption

- The binding to the surface is weak and reversible

- Compounds containing chromogenic group are usually strongly adsorbed on activated carbon

- Common adsorbent used are **Activated Carbon**, **Silica gel**, **Alumina** because they present enormous surface areas per unit weight.

iii. Ultrafiltration



UF is basically a pressure-driven separation process

The operating pressure is usually between 0.1 and 1 MPa

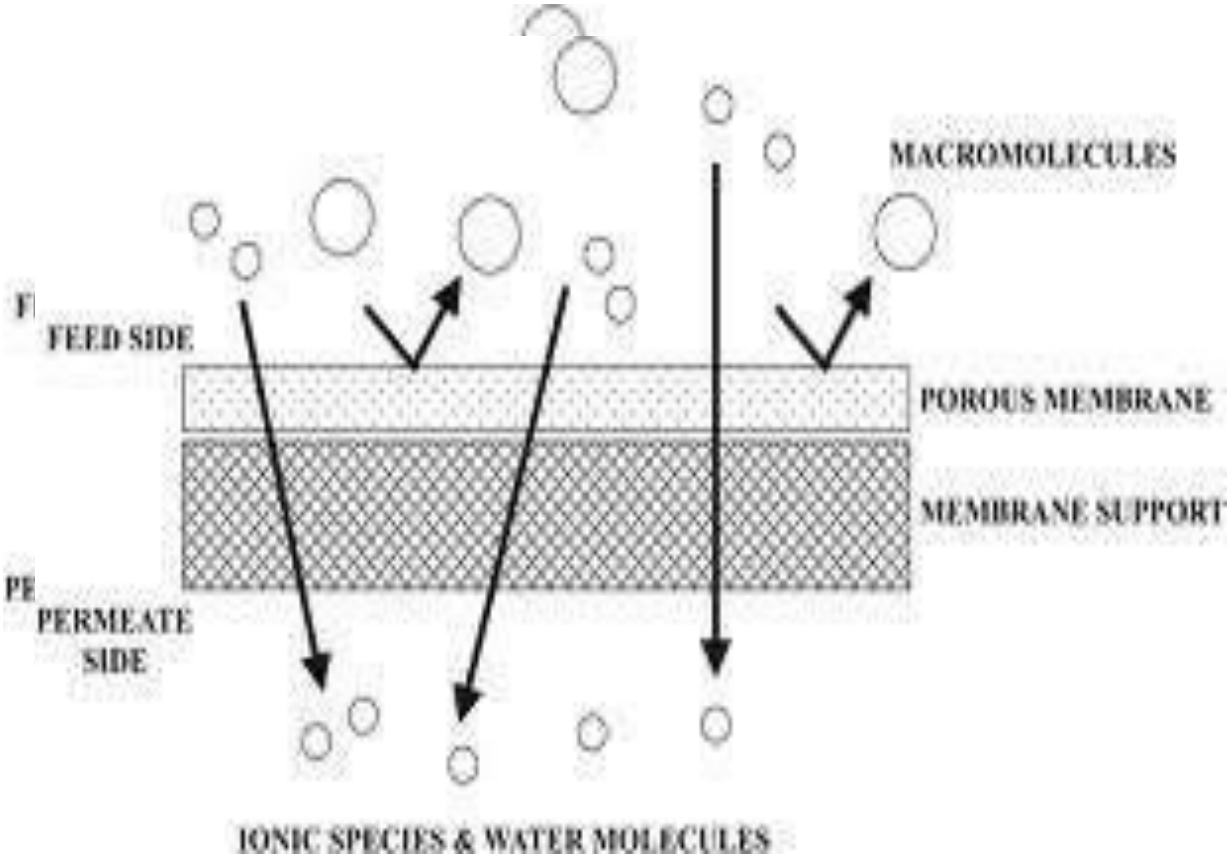
Ultrafiltration

- UF is governed by a screening principle and dependent on particle size

- UF membranes have a pore size between 1 nm and 100 nm (10 and 2000 Å), thus allowing retention of compounds with a molecular weight of 300 to 500 000 Dalton.

- Typically, the process is suitable for retaining biomolecules, bacteria, viruses, polymers, colloidal particles and sugar molecules.

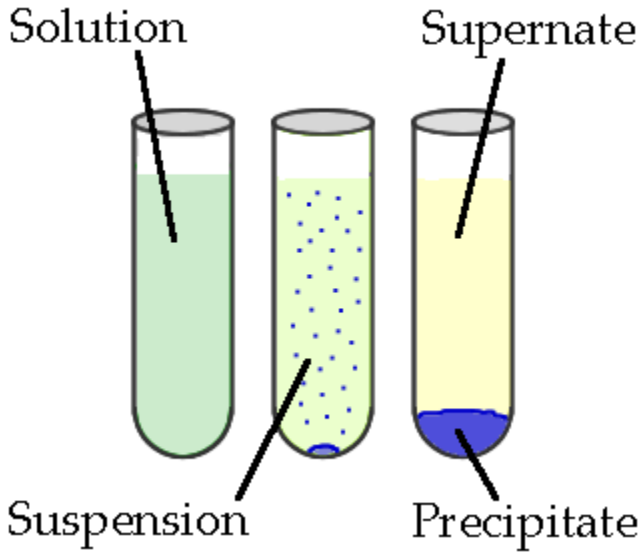
Ultrafiltration



iv. Precipitation

- Formation of a solid in a solution during a chemical reaction

- Solid formed is called the **precipitate** and the liquid remaining above the solid is called the **supernate.**



Precipitation

- Salts such as ammonium & sodium sulphate are used for proteins to precipitate
- Organic solvents methanol used to precipitate dextrans
- Chilled ethanol and acetone used for protein precipitation
- Non ionic polymer such as polyethylene glycol used in precipitation

(C) Product Purification

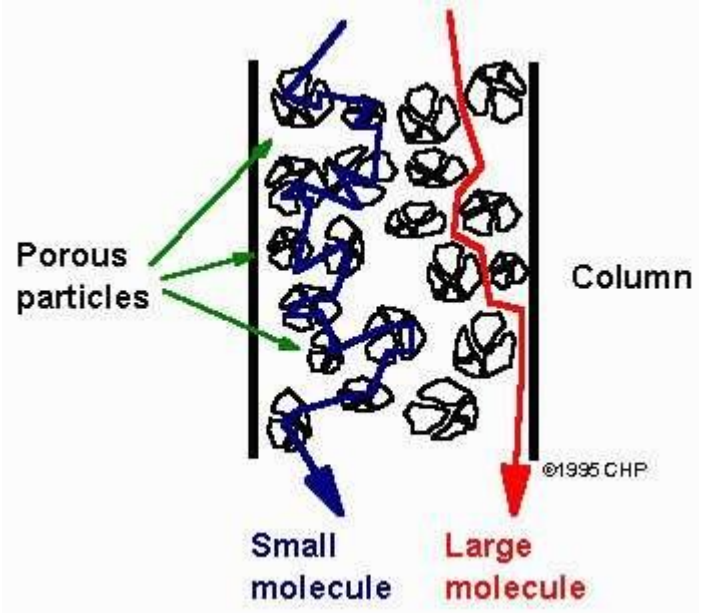
- Done to separate those contaminants that resemble the product very closely in physical and chemical properties
- Expensive to carry out
- Require sensitive and sophisticated equipment
- Significant fraction of the entire downstream processing expenditure
- Examples of operations include affinity, size exclusion, reversed phase chromatography,crystallization and fractional precipitation

Chromatography

- Separation of mixtures
- Passing a mixture dissolved in a "mobile phase" through a *stationary phase, which* separates the analyte to be measured from other molecules in the mixture and allows it to be isolated.

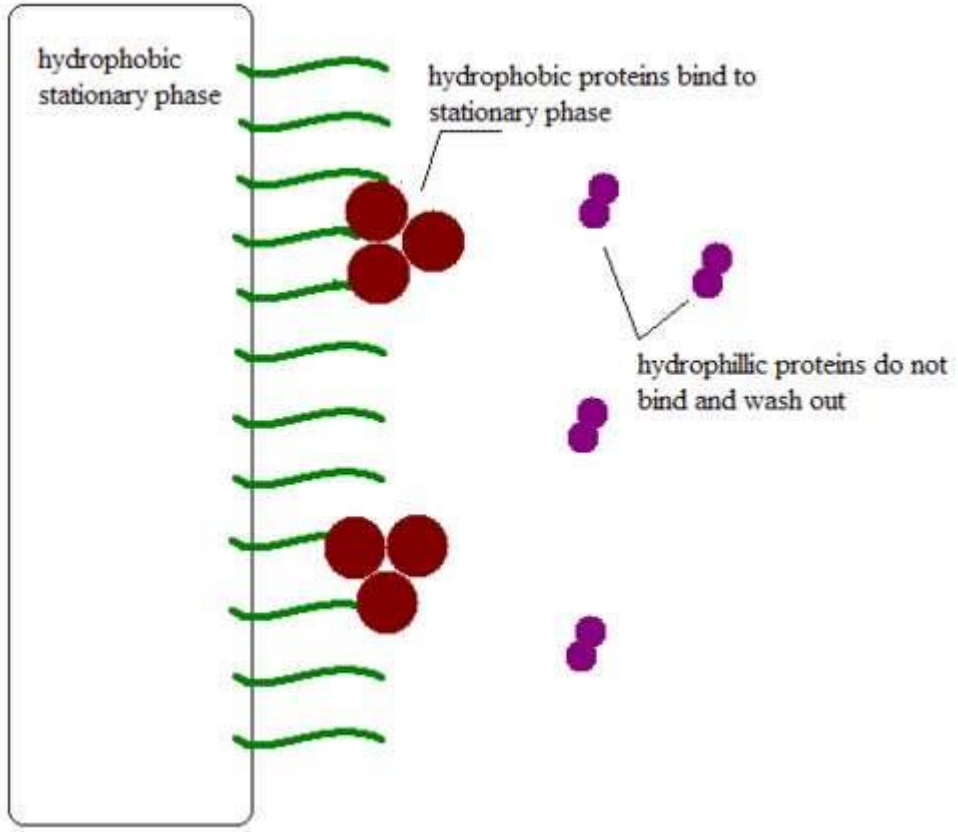
Size exclusion chromatography

- Gel permeation/filtration
- chromatography (GPC)
- Separates molecules
- according to their size
- Low resolution"polishing"
- Tertiary/Quaternary structure(native)



Reversed phase chromatography

Reversed-phase chromatography is an elution procedure used in liquid chromatography in which the mobile phase is significantly more polar than the stationary phase.



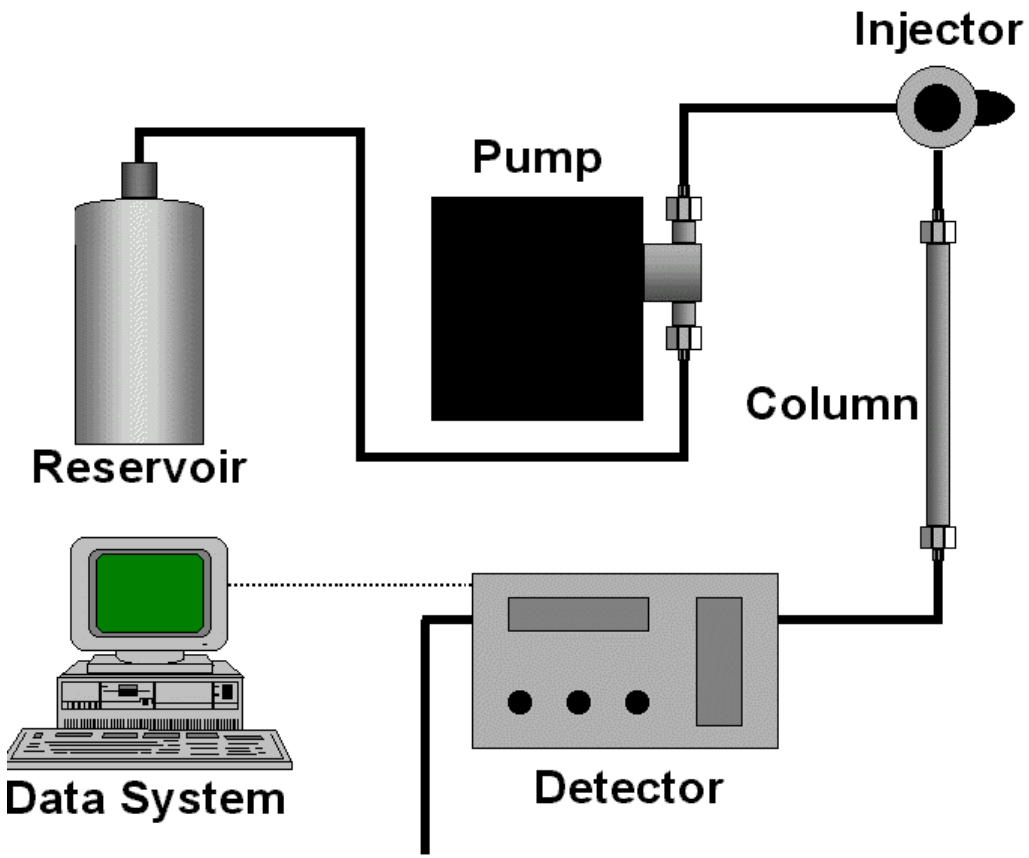
Definitions: Polarity

- The dipole-dipole intermolecular forces between the slightly positively-charged end of one molecule to the negative end of another or the same molecule.
- Molecular polarity is dependent on the difference in electronegativity between atoms in a compound and the asymmetry of the compound's structure.

Liquid Chromatography

- Mobile phase is a liquid.
- Carried out either in a column or a plane.
- HPLC
- In the HPLC technique, the sample is forced through a column that is packed with irregularly or spherically shaped particles or a porous monolithic layer (stationary phase) by a liquid (mobile phase) at high pressure.

HPLC Configuration



(D) Product Polishing

- End with packaging of the product in a form that is stable, easily transportable and convenient
- Crystallization
- Desiccation
- Lyophilization
- Spray drying
- May include:
- Sterilization of the product
- Remove or deactivate trace contaminants which might compromise product safety viruses or depyrogenation

Crystallization

- process of formation of solid crystals precipitating from a solution, melt or more rarely deposited directly from a gas.
- chemical solid-liquid separation technique, in which mass transfer of a solute from the liquid solution to a pure solid crystalline phase occurs.

lyophilization

- freezing the material
- reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas.

Industrial Production of Penicillin using *Penicillium chrysogenum*

GB AKANNI (PhD)

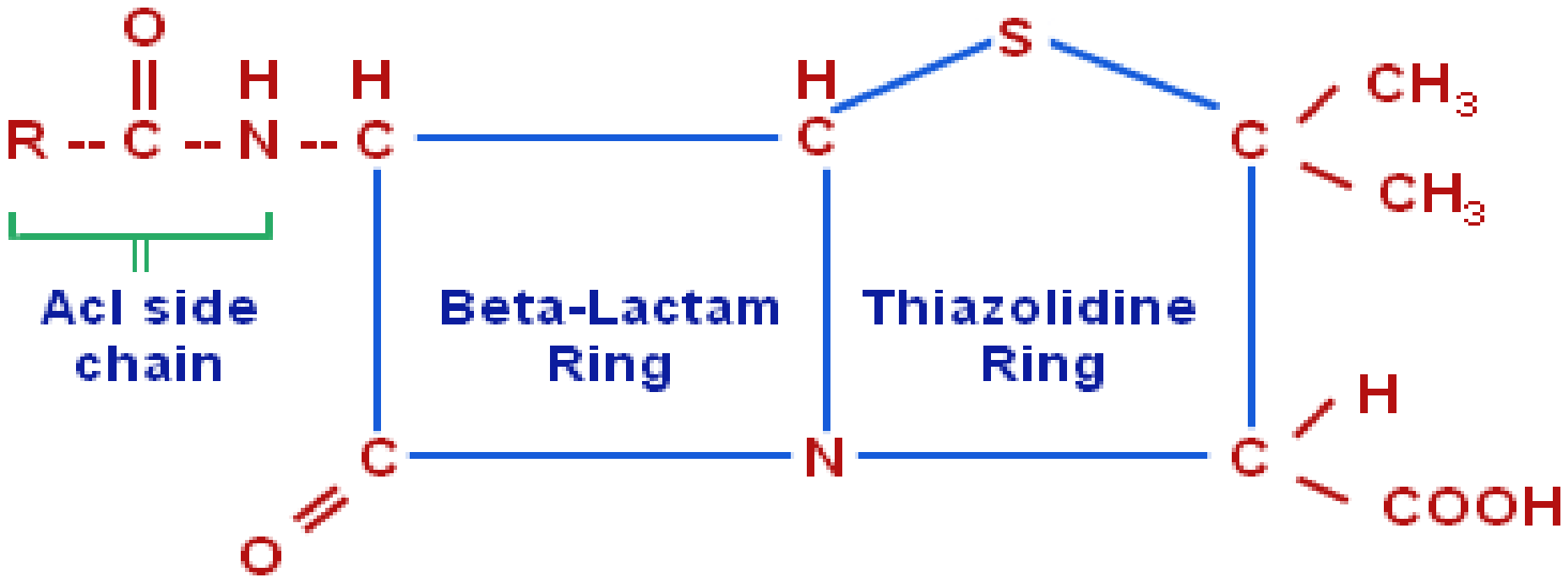
BTG 408

PROCESS BIOTECHNOLOGY

What is Penicillin?

- First true naturally-occurring antibiotic ever discovered: a great medical breakthrough.
- Group of antibiotics produced by the *Penicillium* fungi.
- **Secondary metabolite** of the microbial growth curve
- It is a group of closely related compounds, not a single compound.

  Examples: Amoxicillin, ampicillin, phenoxymethylpenicillin.
- Around 50 drugs that are penicillins.



General Structure of Penicillins

General Structure of Penicillins

- Have β-Lactam functional group, thus belong to the β-Lactam antibiotic group.

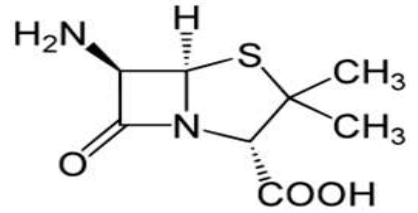
- They all have a basic ring-like structure (a β-Lactam) derived from two amino acids (valine and cysteine) via a tripeptide intermediate. The third amino acid of this tripeptide is replaced by an acyl group (R).

- The nature of this acyl group produces specific properties on different types of penicillin.

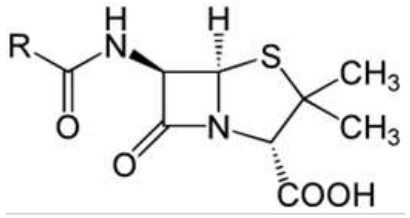
Penicillin Derivatives

- Derivatives produced to deal with the problem of bacterial resistance to penicillin.

- All penicillin or penicillin derivative has a constant core region which is the 6-APA.



- The only region that is different from different types of penicillin derivative is its R group.



How Does Penicillin Work?

- Inhibits the synthesis of peptidoglycan in cell walls.
- β-Lactam of penicillin binds to the enzyme - transpeptidase, that is used in the formation of peptidoglycan cross linking.
- The enzyme is inhibited, thus inability to form cross linking.
- Cell wall is weakened causing osmotic imbalance in the cell. This leads to cell death.
- As human cells do not have cell walls, penicillin does not affect them.

Spectrum of Activity

- Effective against actively growing Gram positive bacteria - which have thick peptidoglycan.

- Some penicillins like amoxicillin are also effective against Gram negative bacteria, except *Pseudomonas aeruginosa*.

Fermentors

- Purpose of fermentor: provide contained, controlled and homogeneous environment in which the fermentation can proceed in a manner that is both safe and practical and which optimises the particular objectives of the fermentation.

- Other primary factors include cost, reliability and safety.

- For reactor being designed for specific purpose, there are a number of important parameters that will greatly affect performance:

1. *Reactor Size:* optimum rates of production?
2. *Reactor Configuration:* mechanical agitation or will a bubble column.
3. *Mode of operation:* Will it be batch fed or continuously fed?
4. *Conditions inside the reactor:* how will conditions (pH, temperature, …) be controlled?

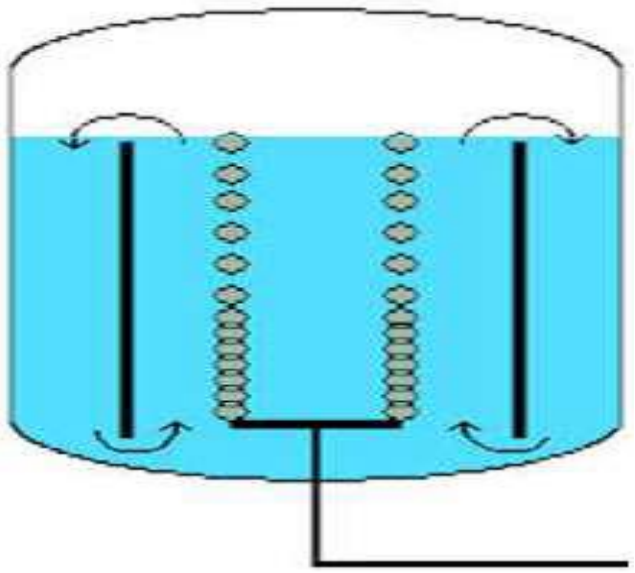
Economic requirements:

- Easy to operate aseptically.
- Reasonably flexible regarding process requirements.
- Low power consumption.
- Stable under fluctuating conditions.
- Cheap, robust, simple and well understood for scale-up.

Specific Conditions for Penicillin Production

- Most penicillins form filamentous broths. This means they can be difficult to mix due to their high viscosity.
- Also the increasing viscosity of the broth can hinder oxygen transfer.

- A solution for the viscosity and the filamentous growth of penicillium species could be bubble columns (air lift reactors) which would distribute the oxygen equally and also to agitate the medium.



- Penicillin is an aerobic organism; oxygen supply is critical-reactor must have an efficient oxygen supply system.
- The optimum pH for penicillin growth is 6.5-maintain pH efficiently (pH controller and acid-base reservoir).
- Strain Stability problems (mutations) - careful strain maintenance is required.
- Biomass doubling is about 6h-provisions must be made.

Media Consideration

The aim of the media is to:

- provide all the elements required for the synthesis of cell materials and the formation of the desired product.
- provide favourable environment for the culture in question.
- be cost effective.

Media Formulation

- pH 6.5
- Temperature 20-24 °C
- Oxygen
- Nitrogen: corn steep liquor 8.5 %
- Glucose 1%
- 80% ethanol
- phenylacetic acid
- Probenecid
- Lactose 1%
- Calcium Carbonate 1%
- Sodium hydrogen phosphate 0.4%
- Antifoaming agent: vegetable oil

- Microorganisms require C, H, O, S and N for cell growth and cell maintenance.
- Also require small amounts of trace elements such as Cu, Mn and Co (frequently depend on the water source) or growth factors such as vitamins or amino acids.
- Certain organisms such as *Penicillium chrysogenum* that produce antibiotics, enzymes or other secondary metabolites frequently require precursors like purine/pyrimidine bases or organic acids to produce metabolites.

Primary and Secondary Metabolism

- Primary metabolism is the metabolism of energy production for the cell and for its own biosynthesis. In aerobic organisms (such as *Penicillium chrysogenum*) it involves the conversion of sugars such as glucose to pyruvic acid and the production of energy.

- Secondary metabolism regards the production of metabolites that are not used in energy production for example penicillin from *Penicillium chrysogenum*. The metabolite is being utilized as a defence mechanism against other microorganisms in the environment.

- *Penicillium chrysogenum* can kill off the competition to allow itself to propagate efficiently.

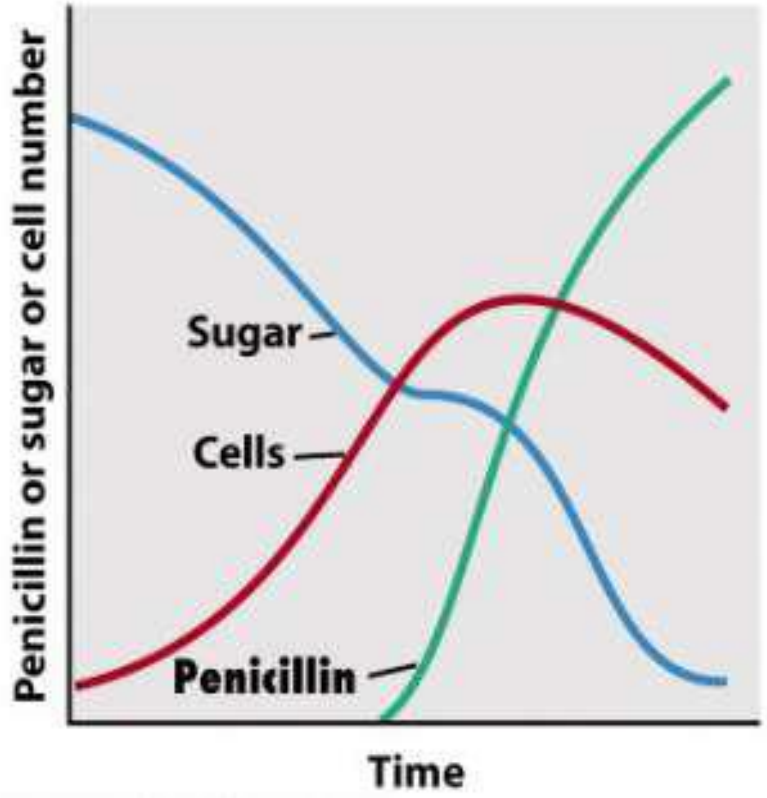
Production Method

- Secondary metabolites are only produced in times of stress when resources are low and the organism must produce these compounds to kill off its competitors to allow it to survive.

- It is these conditions that we wish to duplicate

in order to achieve the maximum amount

of product from ourfermentation.



Stages of Production

1. Primary metabolism will be emphasised. Media for this stage will be focussed on achieving maximum growth and biomass production.

2. Once the desired biomass has been achieved, starve (Limiting the amount of C and N available to the culture) the culture and induce the kind of stress conditions that trigger the production of the antibiotic.

★ Use the fed-batch method to feed the culture. This allows us to add the substrate to the reactor in small increments and to even change the substrate if we so desire.

Assignment 1

- You have been employed as a Fermentation Biotechnologist at GlaxoSmithKline (GSK) for the production of antibiotics (Penicillin).

Describe the following:

- Design the fermentation procedure (process design in a flowchart)
- How do you maintain the seed culture
- Seed culture/strain improvement methods
- Fermentation media composition
- Fermentation parameter
- Product recovery

Hand in date: 20th May, 2020; Time: 12 noon

Email: gbakanni@mtu.edu.ng

TERMINOLOGY

- **Bioprocess**
  – Any process that uses living cells or their components (e.g., bacteria, enzymes, chloroplasts) to obtain desired products
- **Bioprocessing**
  – R&D and manufacturing of products prepared from or used by biological systems (food, feed, biopharmaceuticals, and cosmetics)
- **Biochemistry**
  – Study of chemistry and biological processes of living organisms and the molecular basis for the changes occurring in living cells
- **Biotechnology**
  – Use of biological systems to make/modify products/processes (plant regeneration, gene manipulation/transfer). In the past, producers used cross-hybridization to alter a plant's genetic makeup. With biotechnology, DNA can be altered directly.

- **Bioengineering**
  – Application of engineering in biological sciences. Bioprocess engin. (biocatalysis, bioseparation, bioinformatics, bioenergy), genetic engin. (sub-set of biotechnology), cellular engin. (tissue culture), and biomedical engin. are sub-sets of bioengineering.

- **Biomedical engineering**

– It combines chemical, electrical, and mechanical engineering to improve medical diagnosis (ultrasound, MRI), monitoring, and therapy (artificial hips, knees and other joints). Sometimes, biomedical engineering is also referred to as bioengineering.

- **Biophysics**

– Use of physics to study biological systems. Overlaps with biochem., nanotechnology, bioengin., agrophysics. May involve molecular level issues (DNA, RNA, microscopy), structural biology, enzyme kinetics etc. Biomedical physics is a sub-set.

- **Biopharmaceutical**
  – Medical drugs produced using biotechnology. They are proteins (including antibodies), nucleic acids (DNA, RNA or antisense oligonucleotides), recombinant vaccines, and monoclonal antibodies used for therapeutic or in vivo diagnostic purposes, and are produced by means other than direct extraction from a native (non-engineered) biological source.

- **Biofuel**

– Solid, liquid or gaseous fuel derived from organic (relatively recently dead biological matter) material and is distinguished from fossil fuels, which are derived from long dead biological material. They can be produced from any (biological) carbon source, with the most common source being photosynthetic plants.

- **Biodiesel (Fatty acid alkyl ester)**
  – Biofuel produced from vegetable oils (corn, cotton, rapeseed, soybean, palm, mustard), restaurant waste oils, animal fats or algae
  – A non-petroleum-based diesel fuel consisting of long chain alkyl (methyl, propyl or ethyl) esters, made by transesterification of vegetable oil or animal fat (tallow), which can be used (alone, or blended with conventional petrodiesel) in unmodified dieselengine vehicles (resulting in better emissions in the form of decreased particulates, CO, and hydrocarbons)
  – 100 lbs of oil + 10 lbs of methanol –> 100 lbs of biodiesel + 10 lbs of glycerol

- **Bioethanol**

– An alcohol made by fermenting sugars from biological materials (starch crops -- corn) or cellulosic biomass (trees, grasses)
– Can be used as vehicle fuel in its pure form or as a gasoline additive to increase octane & improve vehicle emissions (CO_2)

- **E10**
  – Fuel that contains 10% ethanol, mixed with 90% gasoline
  - **E85**
  – A mixture of 85% ethanol and 15% gasoline. This will not work in most cars.
  - **B20**
  – A blend of 20% biodiesel and 80% petroleum diesel
  - **Biomass**
  – Biological material (C, H, O, N, alkali, alkaline earth, heavy metals) derived from living, or recently living organisms (plant or animal based)

- **Substrate**
  – The surface a plant or animal lives upon (serves as food source for cells). It can be biotic or abiotic (non-living) materials.
- **Reaction (chemical) kinetics**
  – Mechanism and factors influencing the speed of chemical reactions
- **Catalyst**
  – A substance that increases the rate of a chemical reaction (by providing a lower activation energy reaction mechanism to occur) without being consumed or produced by the reaction. Proteins that act as catalysts in biochemical reactions are called enzymes.
- **Enzyme**
  – A specialized chemical that helps living organisms perform a task. In bioreactors, enzymes created by microorganisms attack the plant cell wall and break it up to get the glucose out.

Yeast

• Unicellular fungus (more than 1000 species identified)
– Commonly used to leaven bread and ferment alcoholic beverages
– Most yeasts belong to the division Ascomycota
– A few yeasts (eg., *Candida albicans*) can cause infection in humans
– Saccharomyces cerevisiae (most commonly used yeast), was domesticated for wine, bread, and beer production 2000+ yrs ago

• Yeast physiology can be either obligately aerobic orfacultatively anaerobic (fermentative)
– There is no known obligately anaerobic yeast

• In absence of OXYGEN
2, fermentative yeasts produce energy by converting sugars into CO_2 and ethanol (alcohol)
• In brewing, ethanol is the desired product, while in baking, CO_2 raises the bread and the ethanol evaporates

Yeast (contd.)

• Many yeasts can be isolated from sugar-rich environmental
samples such as fruits and berries (grapes, apples, peaches
etc.) and exudates from plants (such as plant saps or cacti)

• The most common mode of vegetative growth in yeast is the
asexual reproduction by budding or fission
– A small bud (daughter cell), is formed on the parent cell
– The nucleus of the parent cell splits into a daughter

Yeast (contd.)

• In brewing beer, top-fermenting yeasts (float to the top of the beer) produce higher alcohol concentrations and prefer higher temperatures (15-25 °C)
– Eg., *Saccharomyces cerevisiae* (known to brewers as ale yeast)

• They produce fruitier, sweeter type ale beers

• Bottom-fermenting yeasts ferment more sugars, leaving a crisper taste and work well at low temperatures (5-10 °C)
– Eg., *Saccharomyces uvarum* (formerly known as *Saccharomyces carlsbergensis*)
• They are used in producing lager-type beers

• Brewers of wheat beers often use varieties of *Torulaspora*

Fermentation

• Conversion of carbohydrate (eg. sugar) into acid or alcohol by yeast or bacteria
• It is used in brewing and wine making for the conversion of sugars to alcohol (ethanol – CH_3CH_2OH)
– This process, followed by distillation, can be used to obtain pure ethanol (bioethanol) for use as a transport biofuel

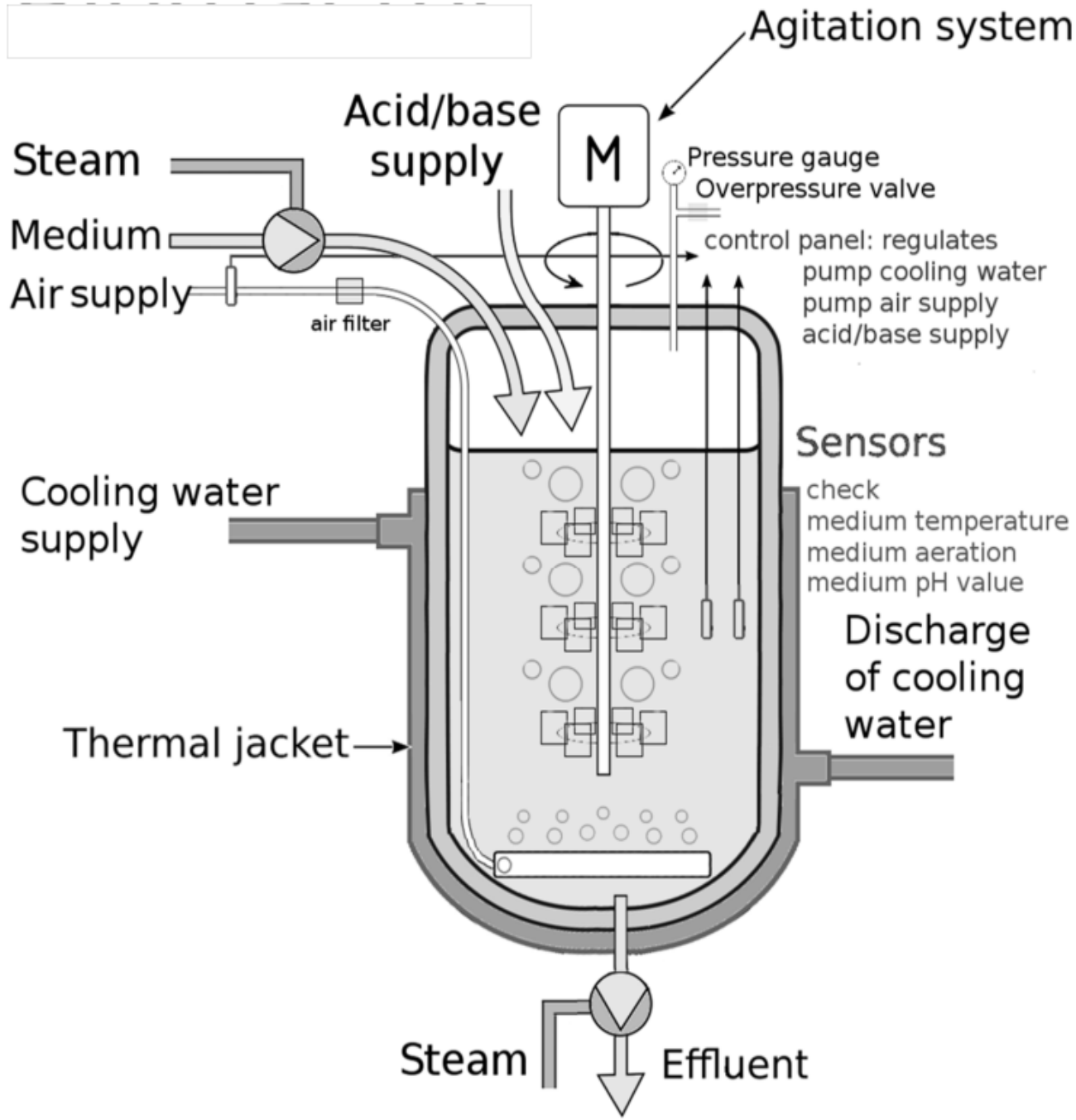
• It can also be viewed as the energy-yielding anaerobic metabolic breakdown (respiration) of a nutrient molecule such as glucose, without net oxidation (eg., in muscle cells)

• Fermentation typically refers to the fermentation of sugar to alcohol using yeast, but other fermentation processes include making of yogurt, souring of milk, rising of dough

Bioreactor

• An apparatus (usually jacketed cylindrical SS vessel) for growing organisms such as bacteria, viruses, or yeast that are used in the production of pharmaceuticals, antibodies, or vaccines, or for the bioconversion of organic wastes

• Under optimum conditions of gas (air, oxygen, nitrogen, and carbon dioxide) flow rates, temperature, pH, dissolved oxygen level, and agitation speed, the microorganisms or cells will reproduce at a rapid rate



Controls

Temperature

Pressure

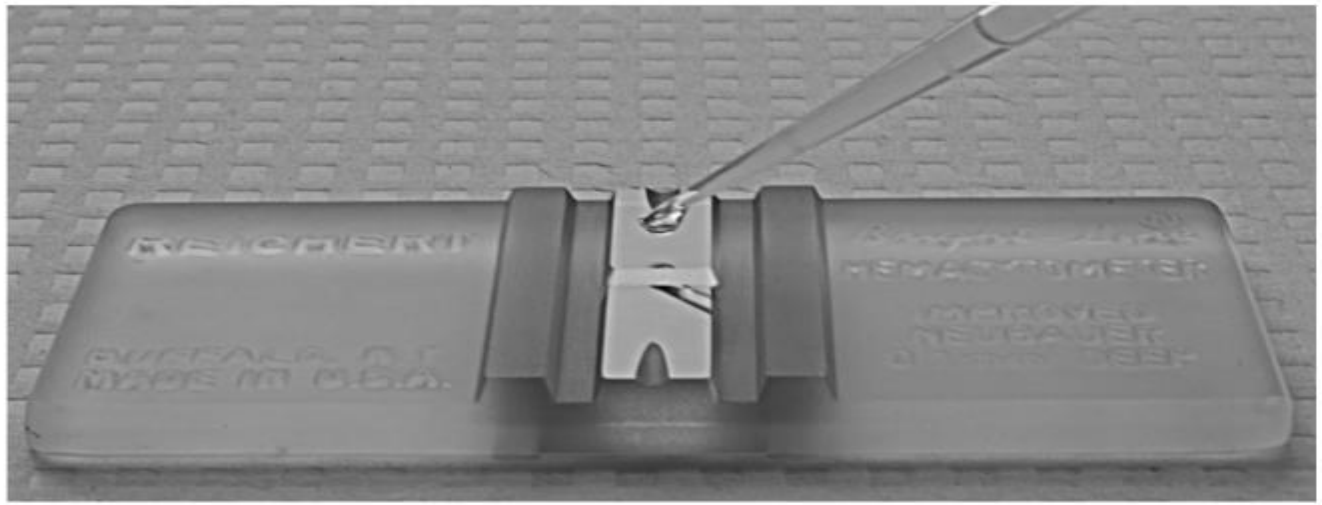
pH

Agitation speed

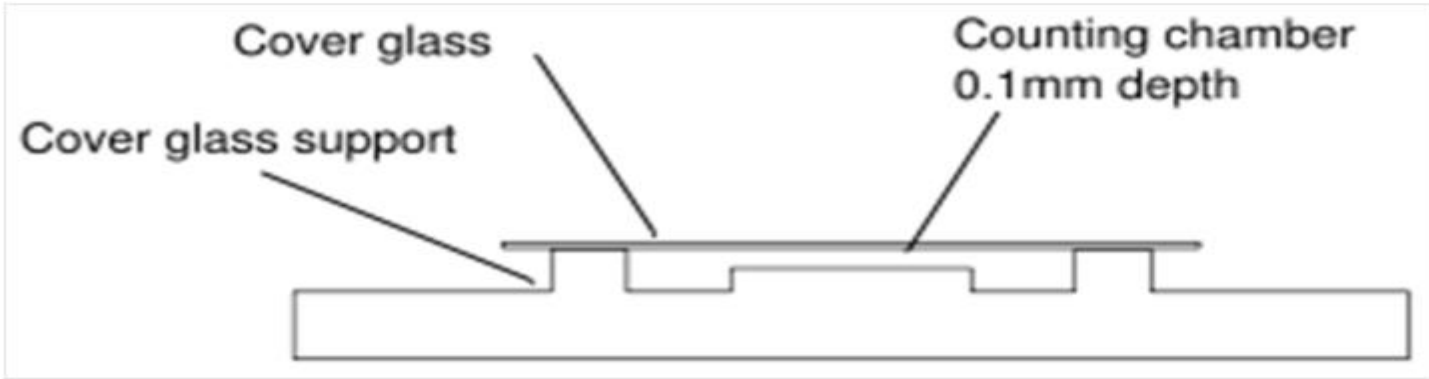
Air flow rate

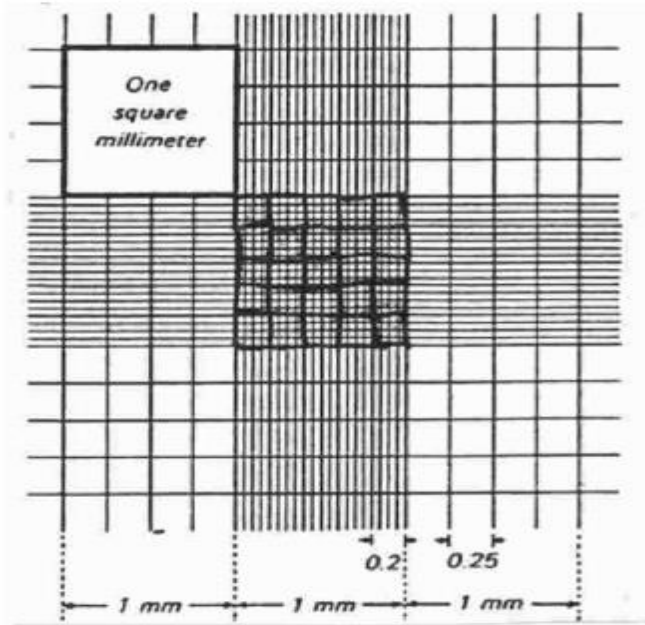
Hemacytometer

A device used to count cells



A thick glass microscope slide with a rectangular indentation that creates a chamber that is engraved with a laser-etched grid of perpendicular lines. The area bounded by the lines and depth of chamber are known. Thus, by counting the number of cells in that volume of fluid, we can calculate the concentration of cells in the fluid.

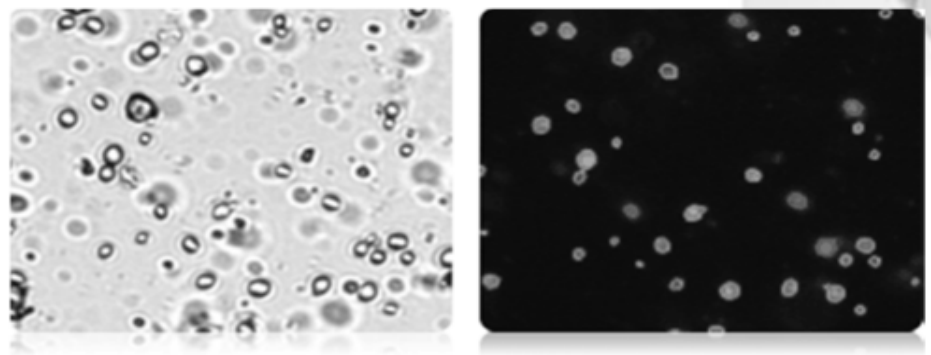




Cellometer

Automated cell counting – no need for hemacytometer.

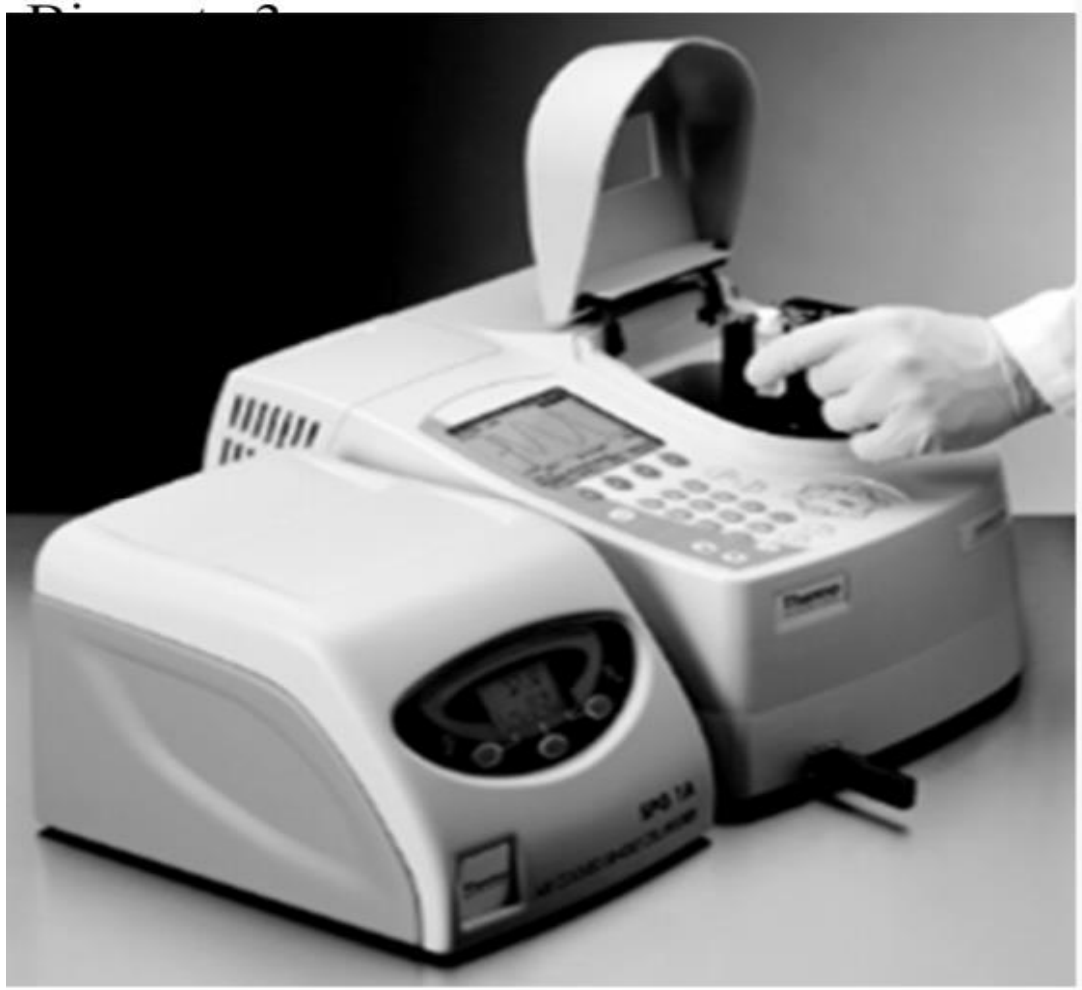
Disposable Counting Chambers consist of two enclosed chambers with a precisely controlled height. 20 µl is loaded into the chamber and inserted into the Cellometer. It utilizes bright field imaging and pattern-recognition software to identify and count individual live & dead cells stained with Trypan Blue.

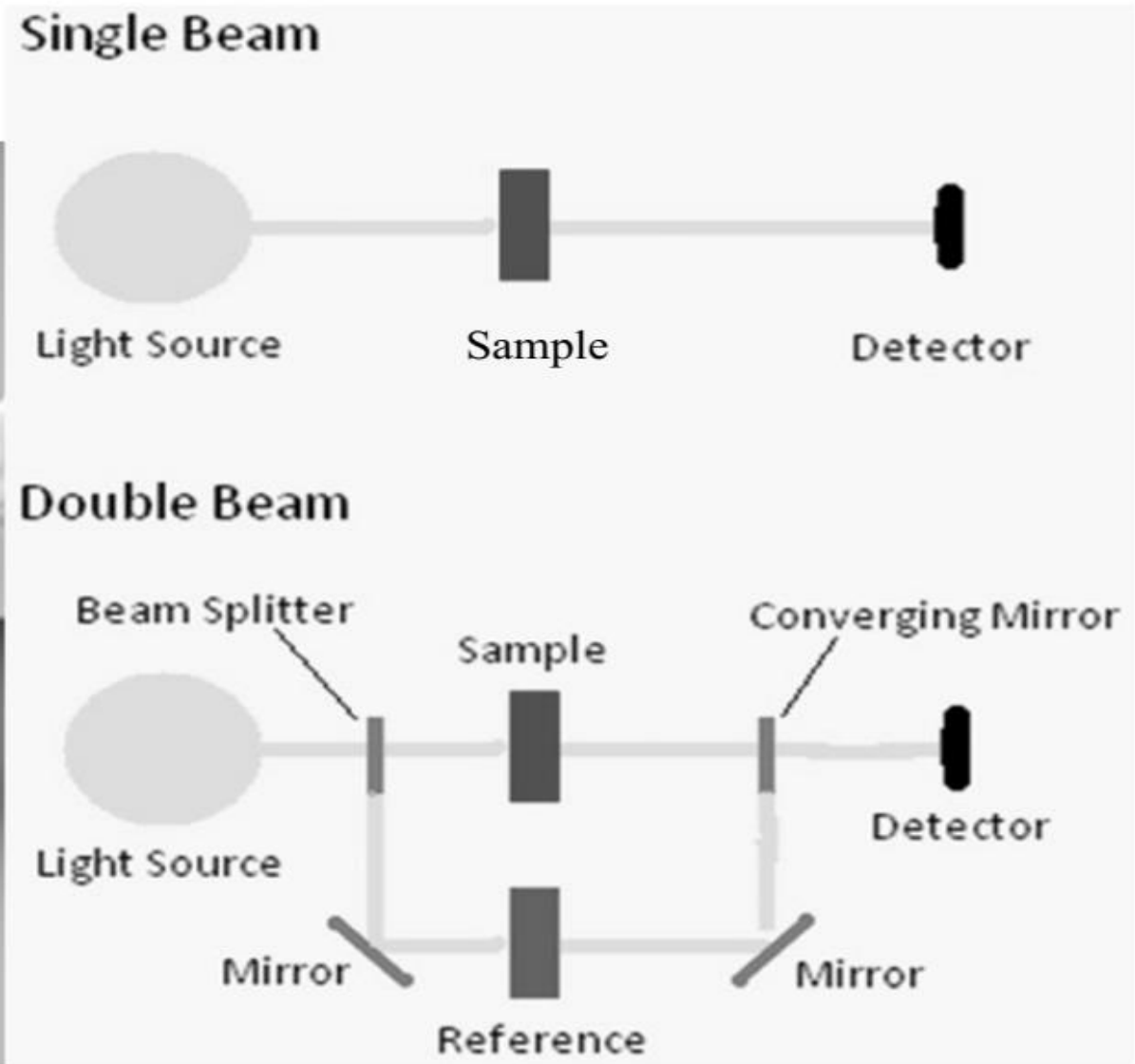


Green: Live cells
Red: Dead cells

Spectrophotometer

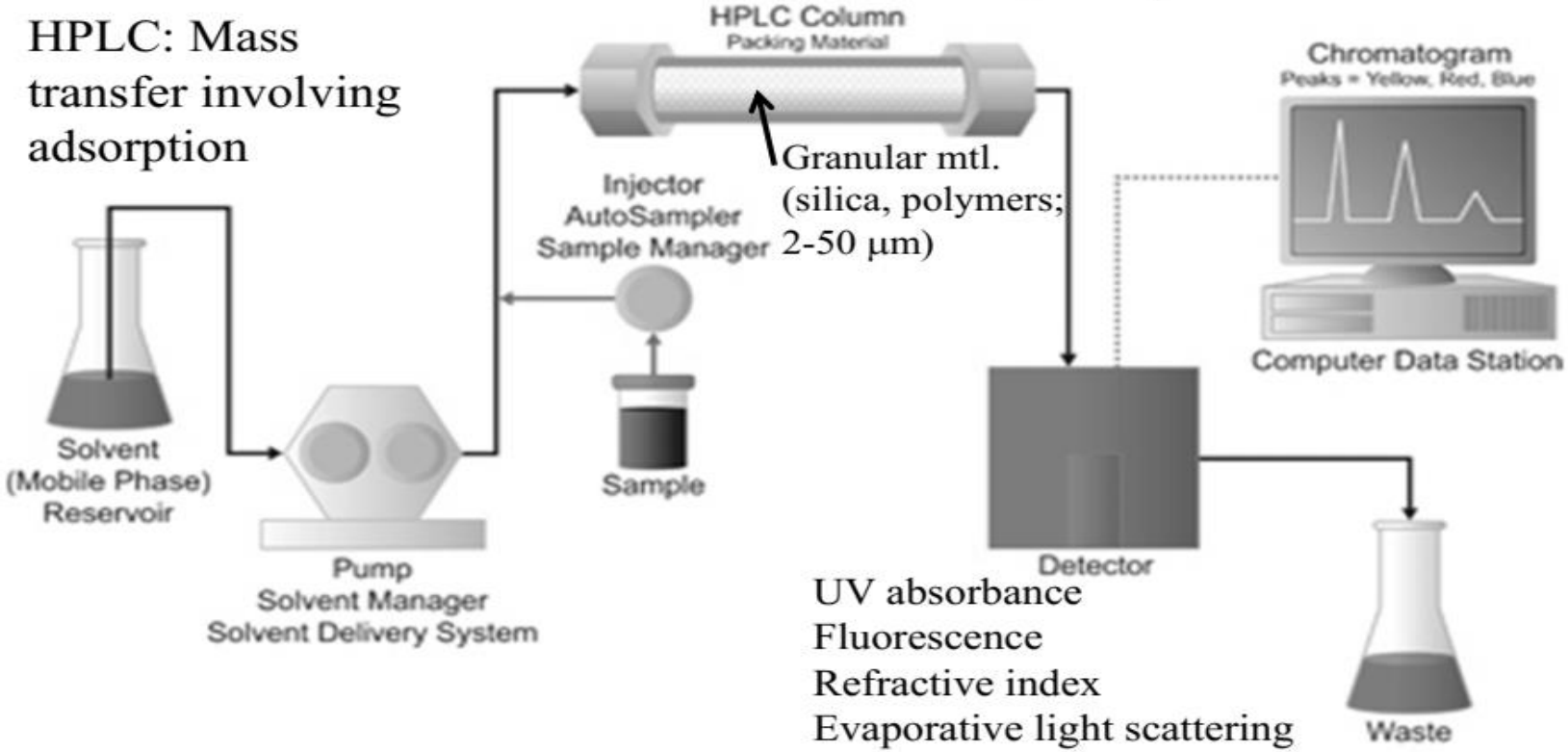
Measures amount of light reflected from an object or amount of light absorbed by an object





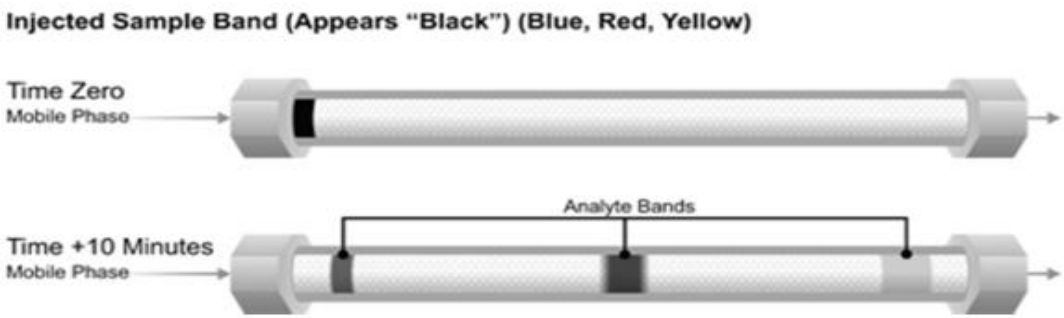
High Performance Liquid Chromatography (HPLC) System

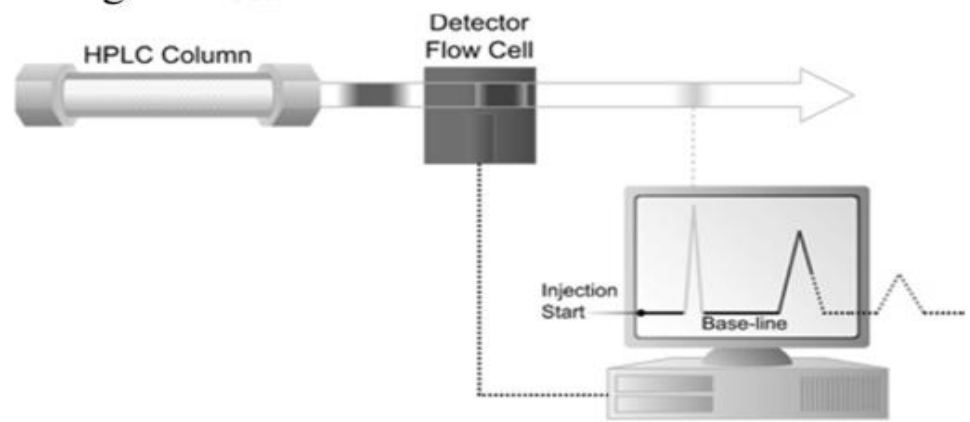
HPLC: Mass transfer involving adsorption



UV absorbance
Fluorescence
Refractive index
Evaporative light scattering

Separate, identify, quantify components in a mixture. Pump a solvent with the sample through solid column of adsorbent material. Each component diffuses at diff. rate, thereby separating. Detector identifies & quantifies each component.





Summary

- **Bioprocess**
  – Any process that uses living cells or their components (e.g., bacteria, enzymes, chloroplasts) to obtain desired products
- **Bioprocessing**
  – R&D and manufacturing of products prepared from or used by biological systems (food, feed, biopharmaceuticals, and cosmetics)
- **Yeast**
  – Obligate aerobe or facultative anaerobic (fermentative)
  – In absence of O_2, fermentative yeasts produce energy by converting sugars into
  CO_2 and ethanol (alcohol)
- **Fermentation**
  – Conversion of carbohydrate (eg. sugar) to acid/alcohol by yeast/bacteria
- **Bioreactor**
  – Growing bacteria/virus/yeast by controlling temperature, pH, air flow
- **Analysis equipment**