Fermentation Biotechnology

S N Jain
H. R. Patel Institute of Pharmaceutical Education and Research, Shirpur

Fermentation

- **Definition**

  by biochemist

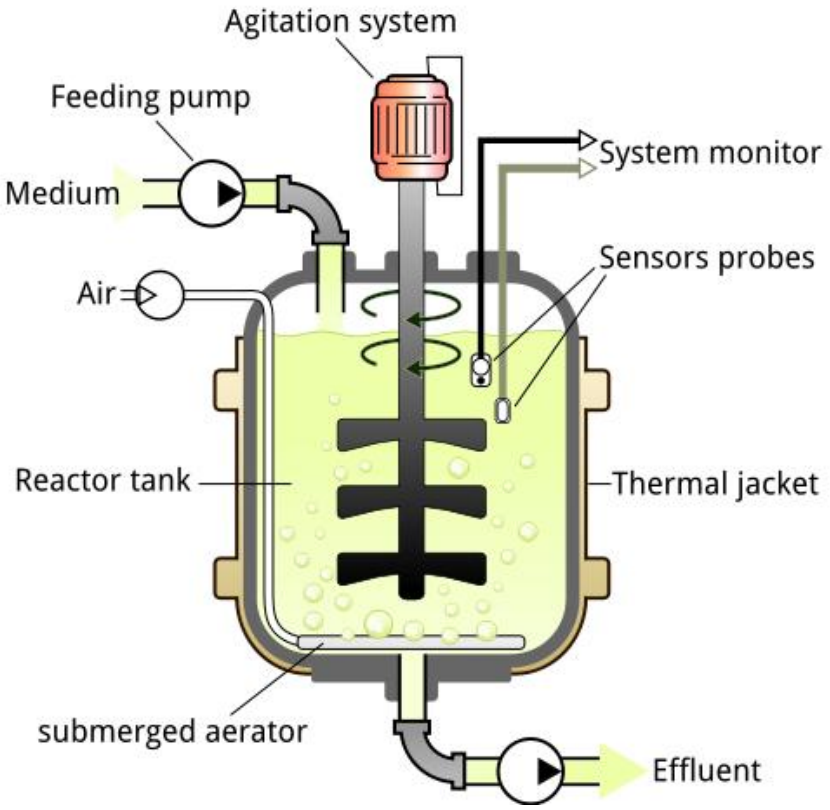
  i. anaerobic process that generate energy by the breakdown of organic compounds.

  ii. Any process that generate bacterial metabolites as end products: lactic acid, enzymes, ethanol, butanol, and acetone.

- Application of modern biotechnology for industrial production, is always referring to Fermentation technology.
  - Less waste generation
  - Reduced energy consumption
- Fermentation biotechnology involves partnership between
  - **Molecular biologists**; responsible for isolating, characterising, modifying and creating effectively expression of industry desirable genes
  - **Biochemical engineers**; to ensure that the microorganisms can be grown in large quantities under the conditions that give optimal product yield.

- **Bioreactor (Inductrial fermenters)**

  Aerobic and anaerobic microorganisms are cultured under controlled conditions in large chamber called **bioreactor** or **fermenter.**



Steps involved in fermentation

1. Sterilization of fermenter and associated equipment.
2. Preparation and sterilization of the culture medium
3. Preparation of the pure culture for inoculation
4. Cell growth and synthesis of the desired product under a specific set of conditions.
5. Product extraction and purification
6. Disposal of expended medium and cell, and cleaning of the fermenter and equipment

Principle of fermentation

- Fermentation technology is linked with the improved productive performance of microorganism by **optimizing their growth conditions.**
- A number of parameters must be precisely regulated to obtain maximum yields either from small (1-10L) or large (>1000L) bioreactors.

How Does Fermentation Work?

- Microorganisms survive using carbohydrates (sugars, such as glucose) for energy and fuel.
- Organic chemicals like adenosine triphosphate (ATP) deliver that energy to every part of a cell when needed.
- Microbes generate ATP using respiration. Aerobic respiration, which requires oxygen, is the most efficient way to do that.
- Aerobic respiration begins with glycolysis, where glucose is converted into pyruvic acid. When there's enough oxygen present, aerobic respiration occurs.

How Does Fermentation Work? (contd…)

- Fermentation is similar to anaerobic respiration—the kind that takes place when there isn't enough oxygen present.
- However, fermentation leads to the production of different organic molecules like lactic acid, unlike respiration, which uses pyruvic acid.
- Depending upon environmental conditions, individual cells and microbes have the ability to switch between the two different modes of energy production.

What Happens During the Fermentation Process?

- During the fermentation process, these beneficial microbes break down sugars and starches into alcohols and acids, making food more nutritious and preserving it so people can store it for longer periods of time without it spoiling.
- Fermentation products provide enzymes necessary for digestion. This is important because humans are born with a finite number of enzymes, and they decrease with age.
- Fermented foods contain the enzymes required to break them down.
- Fermentation also aids in pre-digestion.
- During the fermentation process, the microbes feed on sugars and starches, breaking down food before anyone's even consumed it.

General factors of industrial fermentation

- Most of the organisms used in the fermentation are aerobic; because aerobic metabolism is more efficient than anaerobic metabolism.

  - Oxygen demand
  - consistent pH
  - temperature control
  - Rate and nature of mixing
  - a supply of nutrients for rapid growth
  - antifoaming agent to prevent excess foaming

Types of bioreactor

Type # 1. Continuous Stirred Tank Bioreactors:

Type # 2. Bubble Column Bioreactors:

Type # 3. Airlift Bioreactors:

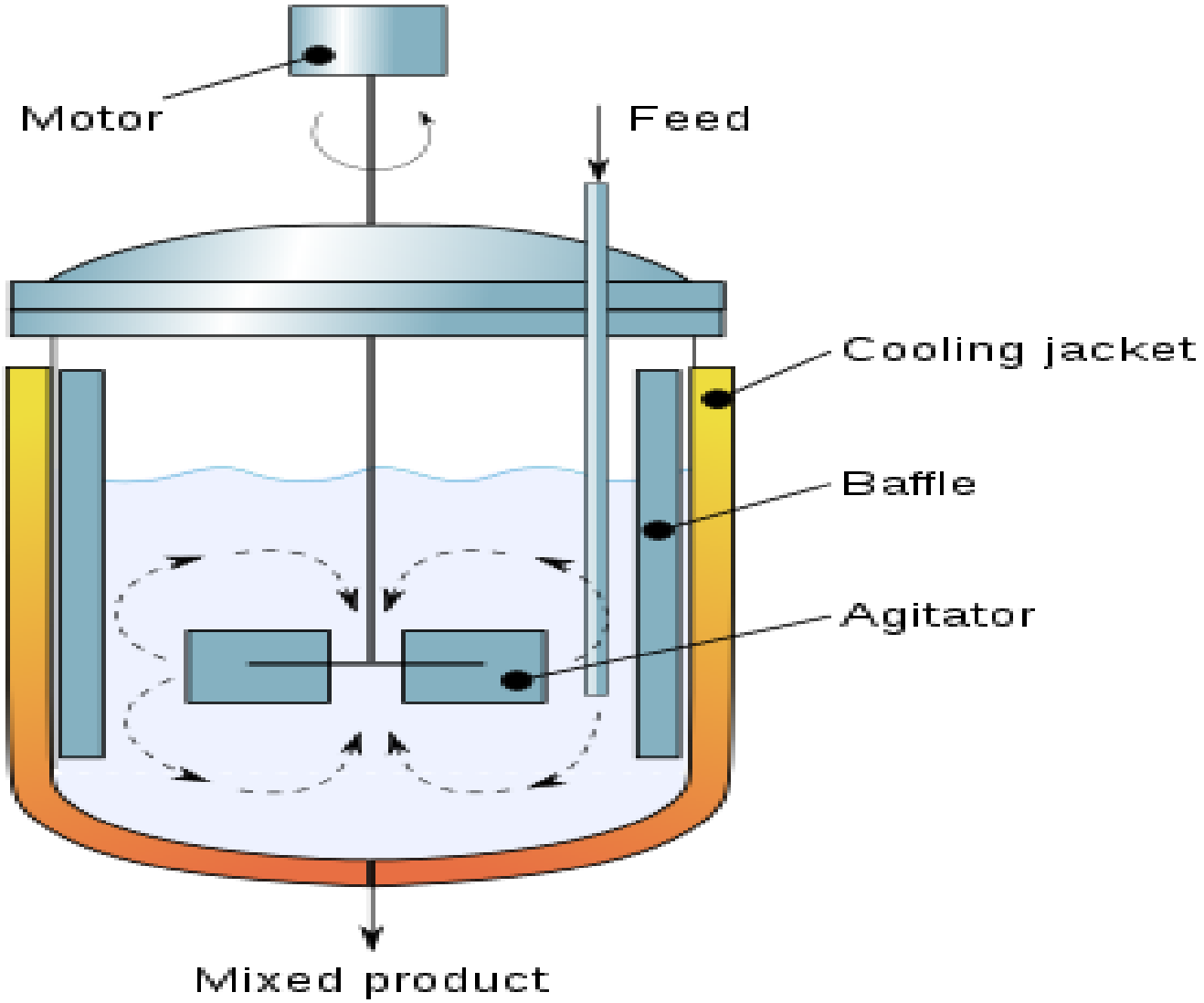
Type # 4. Fluidized Bed Bioreactors:

Type # 5. Packed Bed Bioreactors:

Type # 6. Photo-Bioreactors:

Type # 1. 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** are variable and depends on the **size** of the fermenter.

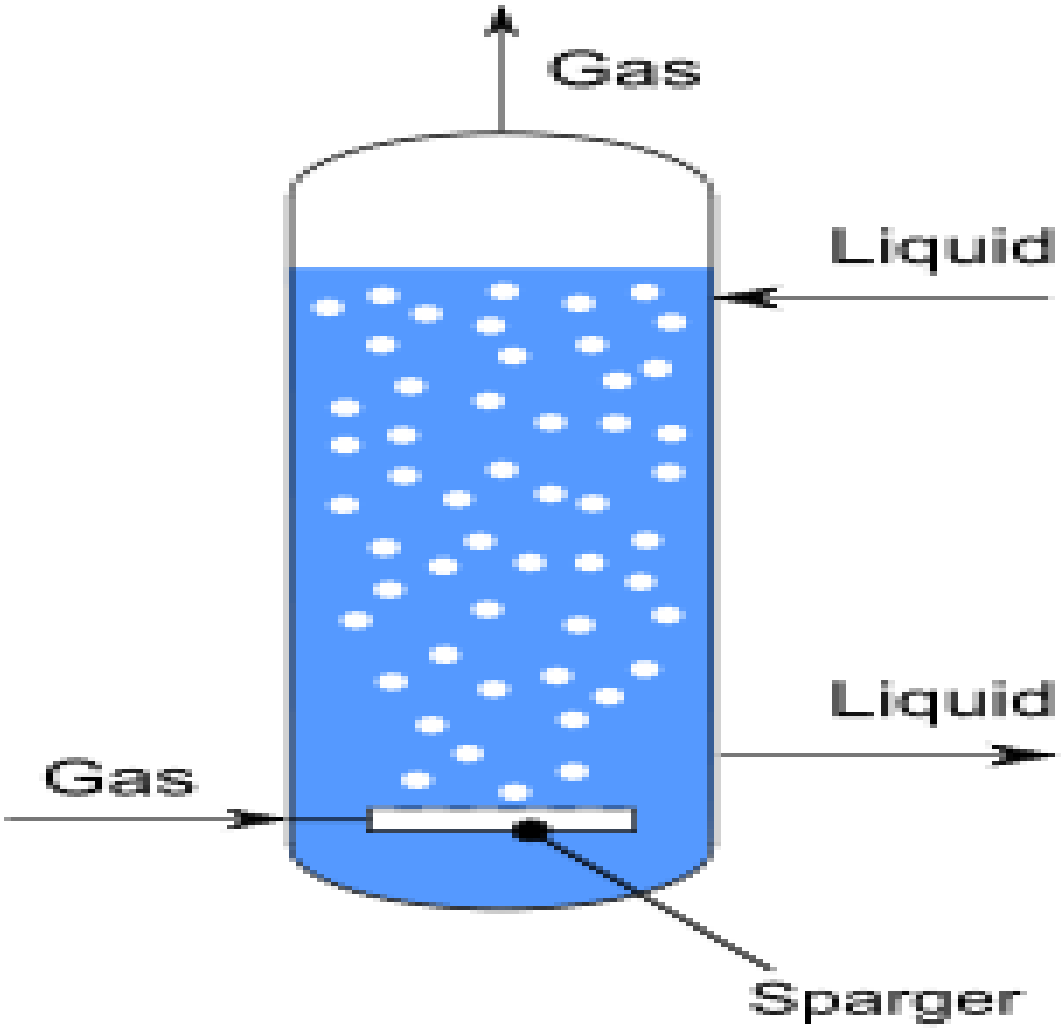

Motor
Feed
Cooling jacket
Baffle
Agitator
Mixed product

- In stirred tank bioreactors, 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.

- There are many advantages of continuous stirred tank bioreactors 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.

Type # 2. Bubble Column Bioreactors:

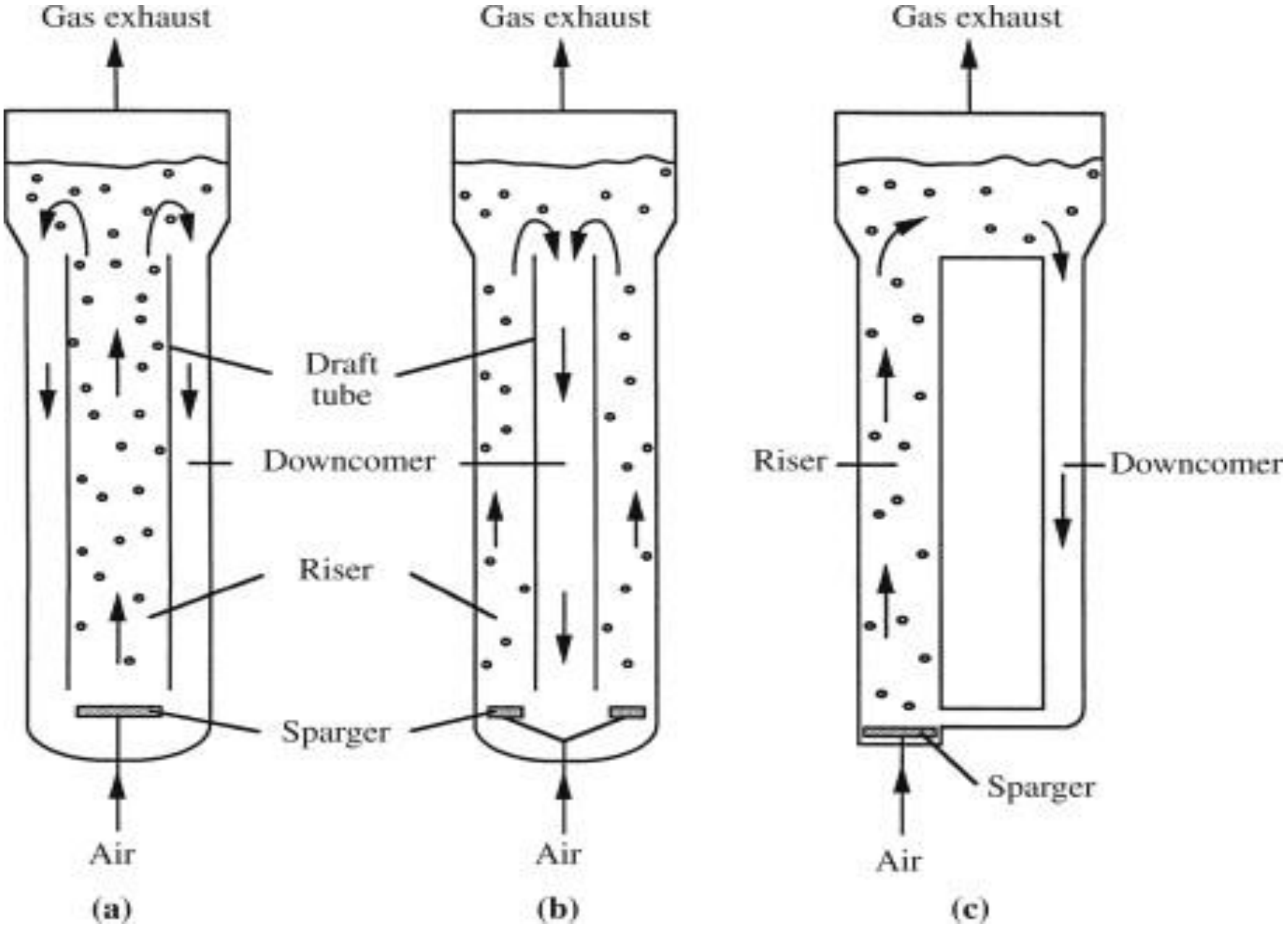
- In the bubble column bioreactor, the air is introduced at the base of the column through **perforated pipes or plates**.
- The flow rate of the air influences the performance factors i.e., **O_2 transfer and mixing.**
- The bubble column bioreactors may be fitted with **perforated plates** to improve performance.
- The vessel used for bubble column bioreactors is usually cylindrical with an aspect ratio **of 4-6** (i.e. height to diameter ratio).


Gas
Liquid
Liquid
Gas
Sparger

- The introduction of gas takes place at the bottom of the column and causes a turbulent stream to enable an optimum gas exchange.
- The mixing is done by the gas sparging and it requires less energy than mechanical stirring.
- The liquid can be in parallel flow or counter-current.

Type # 3. 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**.


Gas exhaust
Gas exhaust
Gas exhaust
Draft tube
Downcomer
Riser
Sparger
Air
Air
Air
Riser
Downcomer
Sparger
(a)
(b)
(c)

3.1 Internal-loop airlift bioreactor

- **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.

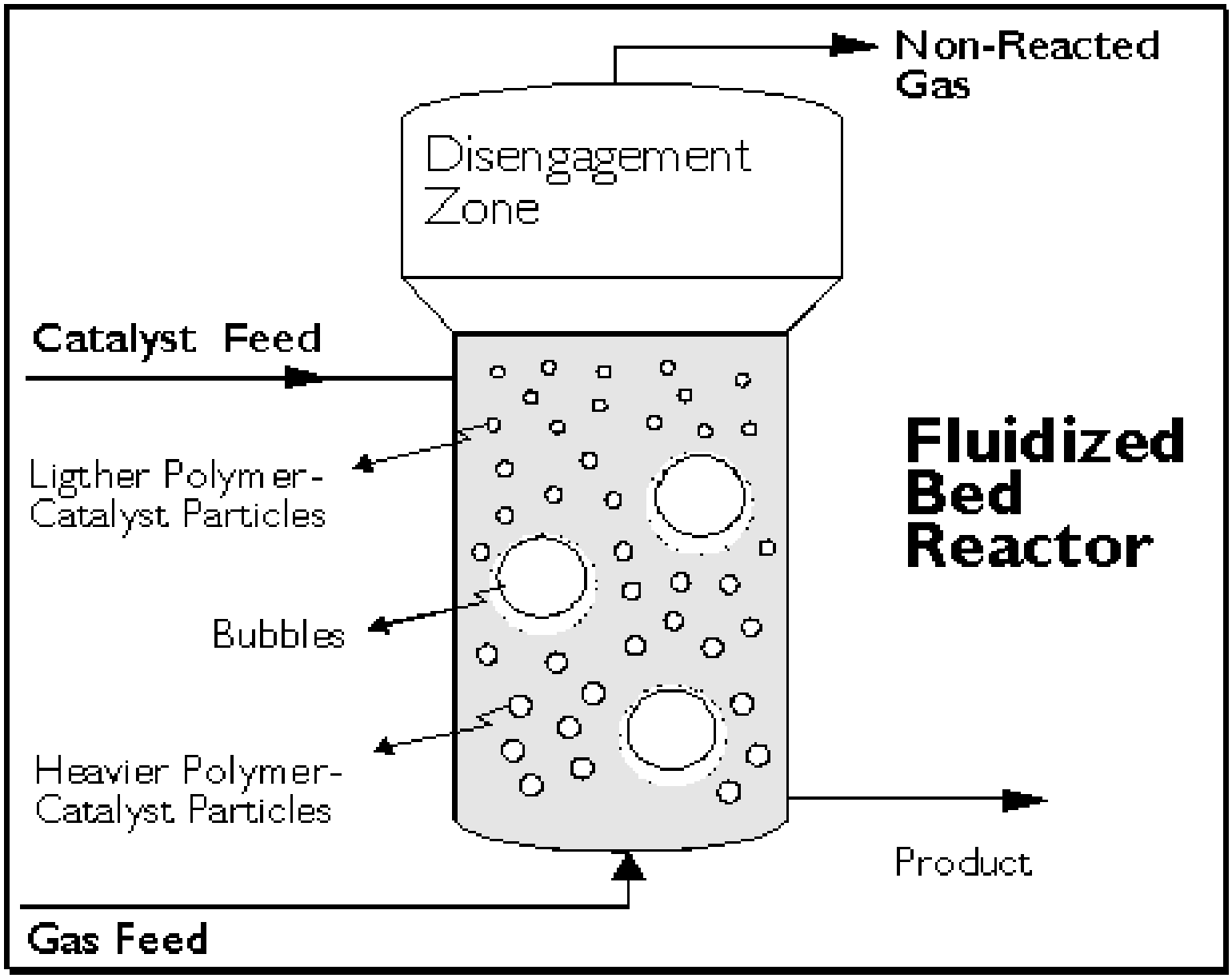
3.2 External loop airlift

- 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.

- 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.
- In general, the performance of the airlift bioreactors is dependent on the **pumping (injection) of air** and the **liquid circulation**.

Type # 4. Fluidized Bed Bioreactors:

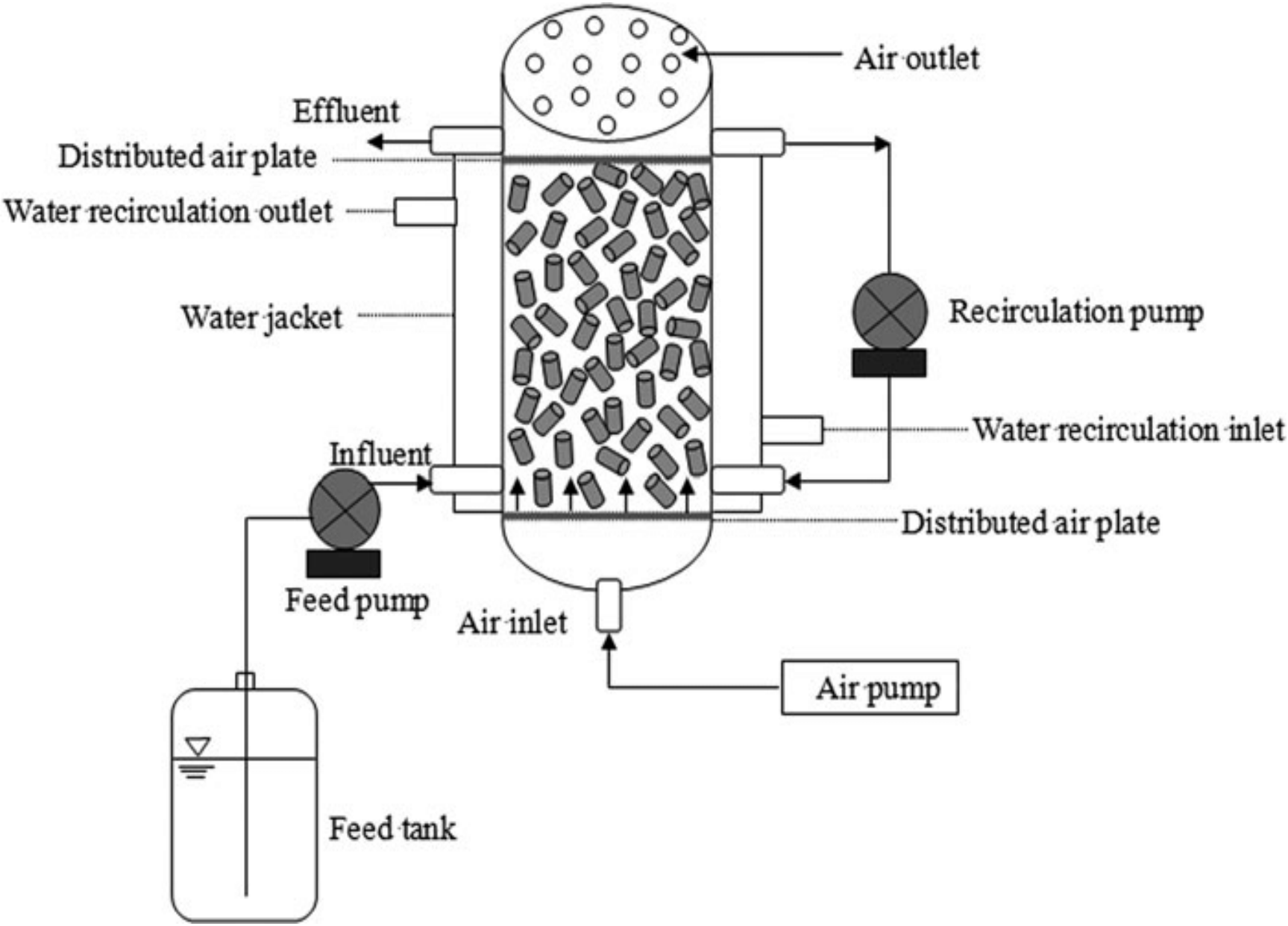
- Fluidized bed bioreactor is comparable to bubble column bioreactor except the **top position** is **expanded** to reduce the velocity of the fluid.
- The design of the fluidized bioreactors (**expanded** top and **narrow reaction** column) is such that the **solids are retained** in the reactor **while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


Non-Reacted Gas
Disengagement Zone
Catalyst Feed
Ligther Polymer-Catalyst Particles
Bubbles
Heavier Polymer-Catalyst Particles
Fluidized Bed Reactor
Product
Gas Feed

- 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 enables good efficiency of bioprocessing.

Type # 5. 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 bioreactor.
- 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 **immobilized 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.


Air outlet
Effluent
Distributed air plate
Water recirculation outlet
Water jacket
Recirculation pump
Water recirculation inlet
Influent
Distributed air plate
Feed pump
Air inlet
Air pump
Feed tank

- 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**.
- The packed bed bioreactors do **not allow accumulation of the products** to any significant extent.

Operation of a Conventional Bioreactor:

1. Sterilization
2. Inoculation and sampling
3. Aeration
4. Control systems
5. Cleaning.

Sterilization:

- **Aseptic conditions** are the basic requirements for successful fermentation.
- That is the bioreactor and its accessories, the growth medium and the **air** supplied during fermentation **must be sterile**.

- **In situ sterilization:**
- The bioreactor filled with the required medium is injected with pressurized steam.
- The whole system is heated to about **120°C** and held at this temperature for about **20 minutes**.
- In situ sterilization has certain limitations. It is not energy-efficient (i.e., energy is wasted) since the bioreactor has to be heated for a long period to rise the temperature of the whole system to 120°C.
- Prolonged heating **may destroy vitamins**.

Inoculation and Sampling:

- The bioreactor with the growth medium under **aseptic conditions** is ready for inoculation .
- The size of the inoculum is generally 1-10% of the total volume of the medium.
- A high yielding production **strain of the organism** taken from **a stock culture** (**lyophilized and stored in a deep freezer or in liquid nitrogen**) is used.
- During the course of fermentation, samples are **regularly drawn** from the bioreactor.
- This is required **to check the contamination** (if any) and measurement of the product formed.

Aeration:

- Aeration of the fermentation medium is required to supply O_2 to the production organisms.
- The aeration system is designed for good exchange of gases.
- Oxygen (stored in tanks in a compressed form) is introduced at the bottom of the bioreactor through a **sparger**.
- The small bubbles of the air pass through the medium and rise to the surface.
- The bioreactor usually has about **20% of its volume as vacant** space on the upper part which is referred to as head space.
- The bioreactor has about **80% working volume**.
- The gases released during fermentation accumulate in the headspace which pass out **through an air outlet.**

Control Systems:

1. **pH**

- It is essential to maintain optimal growth environment in the reaction vessel for maximum product formation.
- Most of the microorganisms employed in fermentation grow optimally between **pH 5.5 and 8.5**.
- In the bioreactor, as the microorganisms grow, they release metabolites into the medium which change pH.
- Therefore, the **pH** of the medium should **be continuously monitored and maintained at the optimal level.**
- This can be done by the addition of acid or alkali base (as needed) and a thorough mixing of the fermentation contents.

Control Systems: (contd…)

2. Temperature:

- Temperature control is absolutely essential for a good fermentation process.
- **Lower** temperature causes **reduced** product formation while **higher** temperature adversely affects the **growth of microorganisms**.
- The bioreactors are normally equipped with **heating and cooling systems** that can be used as per the requirement, to maintain the reaction vessel at optimal temperature.

Control Systems: (contd…)

3. Dissolved oxygen:

- Oxygen is sparingly soluble in water.
- Continuous supply of oxygen in the form of sterilized air is done to the culture medium.
- This is carried out by introducing air into the bioreactor in the form of bubbles.
- Continuous monitoring of **dissolved oxygen concentration** is done in the bioreactor for optimal product formation.

Control Systems: (contd…)

4. Adequate mixing:

- Continuous and adequate mixing of the microbial culture ensures optimal supply of nutrients and O_2, besides preventing the accumulation of toxic metabolic byproducts .
- Good mixing (by agitation) also **creates favorable environment** for optimal and homogeneous growth environment, and good product formation.
- However, **excessive agitation may damage microbial cells** and **increase the temperature** of the medium, besides **increased foam formation.**

Control Systems: (contd…)

5. Nutrient concentration:

- The nutrient concentration in a bioreactor is limited so that its **wastage** is prevented.
- In addition, limiting concentrations of nutrients may be advantageous for **optimal product formation,** since high nutrient concentrations are often associated with inhibitory effect on microbial growth.

Control Systems: (contd…)

6. Foam formation:

- When agitated during aeration, it invariably results in foam formation that builds in head space of the bioreactor.
- **Antifoam** chemicals are used to lower surface tension of the medium, besides causing foam bubbles to collapse. Mineral oils based on **silicone or vegetable oils** are commonly used as antifoam agents.
- Mechanical foam control devices, referred to as **mechanical foam breakers**, can also be used.
- Such devices, **fitted at the top of the** bioreactor break the foam bubbles and the throw back into the fermentation medium.

Cleaning:

- As the fermentation is complete, the bioreactor is harvested i.e. the contents are removed for processing.
- The bioreactor is then prepared for the next round of fermentation after cleaning (technically called turn round).
- Due to large size of the bioreactors, it is not possible to clean manually.
- The cleaning of the bioreactors is carried out by using high-pressure water jets from the nozzles fitted into the reaction vessel.

Penicillin production

- The term antibiotic has been defined by Selman Waksman as being an organic compound produced by one microorganism that, at great dilutions, inhibits the growth of or kills another or even group of other harmful microorganisms.
- Fermentation is the process used for the large-scale production of an antibiotic.
- The first discovered natural antibiotic was **Penicillin**.

- Penicillin was obtained from multicellular fungi, “Penicillium molds”.
- Penicillin is a group of compounds having common basic nucleus of 6-amino penicillanic acid (6-APA).
- 6-APA contains ring like structure termed as a β-lactam ring.
- Penicillin are of two different types,

  i) Natural Penicillin ii) Semi-synthetic Penicillin
- Natural penicillin is directly harvested from the Penicillium mould.
- Semi-synthetic Penicillin consists of the basic Penicillin nucleus with new side chain can change properties of natural penicillin.

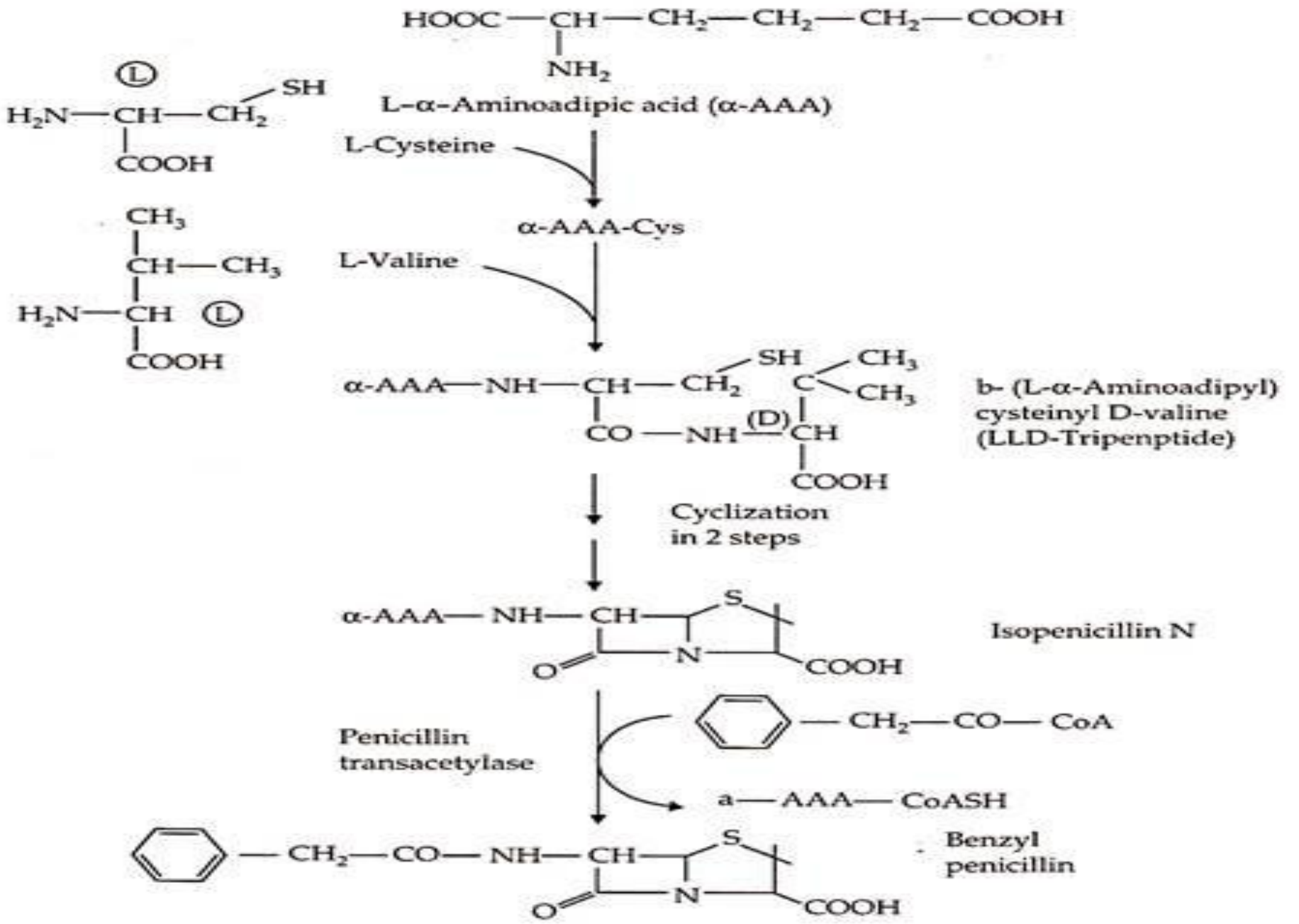


Fig. 6.3: Biosynthesis of Penicillin

1. Microorganisms strain improvement

- Out of various species of the fungus Penicillium mainly two species are used in the fermentation. i.e. P. notatum & P. chrysogenum.
- From these two, P. chrysogenum is high yielding strain and therefore most widely used as production.
- After strain improvement the production strain should be carefully maintained because P. chrysogenum is genetically unstable.

2. Bio parameters

- PH: near 6.5
- Temperature: 26°C to 28°C
- Aeration: a continuous stream of sterilized air is pumped into it.
- Agitation: have baffles which allow constant agitation

3. Raw materials

- Raw materials are primary requirement to design the fermentation broth for antibiotic production.
- Fermentation broth contains all the necessary elements required for the proliferation of the microorganisms.
- Generally, it contains a carbon source, nitrogen source, mineral source, precursors and antifoaming agents (if necessary)

4. Carbon Source

- Lactose acts as a very satisfactory carbon compound if it is used in a concentration of 6%.
- Other carbohydrates like glucose & sucrose may be used but it has to provide with slow feeding rate.
- Complex as well as cheap sources like molasses, or soy meal can also be used which are made up of lactose and glucose sugars.

5. Nitrogen Source

- Another essential compound for metabolism of organisms is nitrogen.
- Ammonium salts such as ammonium sulphate, ammonium acetate, ammonium lactate or ammonia gas are used.

6. Mineral Source

- Additionally, some minerals are necessary for the proper growth of these organisms are included.
- These elements include phosphorus, sulphur, magnesium, zinc, iron, and copper which generally added in the form of water soluble salts.

7. Production medium components

Production medium	Components Percent(%)
Lactose:	3.5 to 6
Calcium Carbonate:	1.0
Cornsteep Liquor:	3.5
Glucose:	1.0
Phenyl acetic acid:	0.5
Sodium hydrogen phosphate:	0.4
Antifoaming Agent: Edible oil:	0.25

4. Recovery

- The recovery of penicillin is carried out in three successive stages:

    1.Removal of mycelium

    2.Counter current solvent extraction of penicillin

    3.Treatment of crude extracts.

4. Recovery (contd…)

- The fermentation broth is filtered on a rotatory vacuum filter to remove the mycelium and other solids.
- Phosphoric or sulphuric acids are added to lower the pH in order to transform the penicillin to the anionic form.
- Then the broth is directly extracted in a Counter Current Solvent Extractor with an organic solvent.
- The filtration is carried out under such conditions which avoid contamination of the filtrate.

4. Recovery (contd…)

- Penicillin is then again extracted into water from the organic solvent by adding an adequate amount of potassium or sodium hydroxide to form a salt of the penicillin.
- The resulting aqueous solution is again acidified & re-extracted with methyl isobutyl ketone.
- The resulting crystalline penicillin salts are then washed and dried.

4. Recovery (contd…)

- Final product must pass rigorous government standards.
- Spent solvents resulting from the above procedure are recovered for re use.
- Sometimes the crude extract of penicillin is passed out from charcoal treatment to eliminate pyrogens; even sterilization can also be done.
- Sterile vials are used for packaging of an antibiotic either as a powder or suspension.
- For oral use it is tableted usually with a film coating.

Citric acid production

- Citric acid (2-hydroxy-propane-1,2,3-tricarboxylic acid) derives its name from the Latin word *citrus*, a tree whose fruit is like the lemon.
- Although many microorganisms can be employed to produce citric acid, *A. niger* is still the main industrial producer.

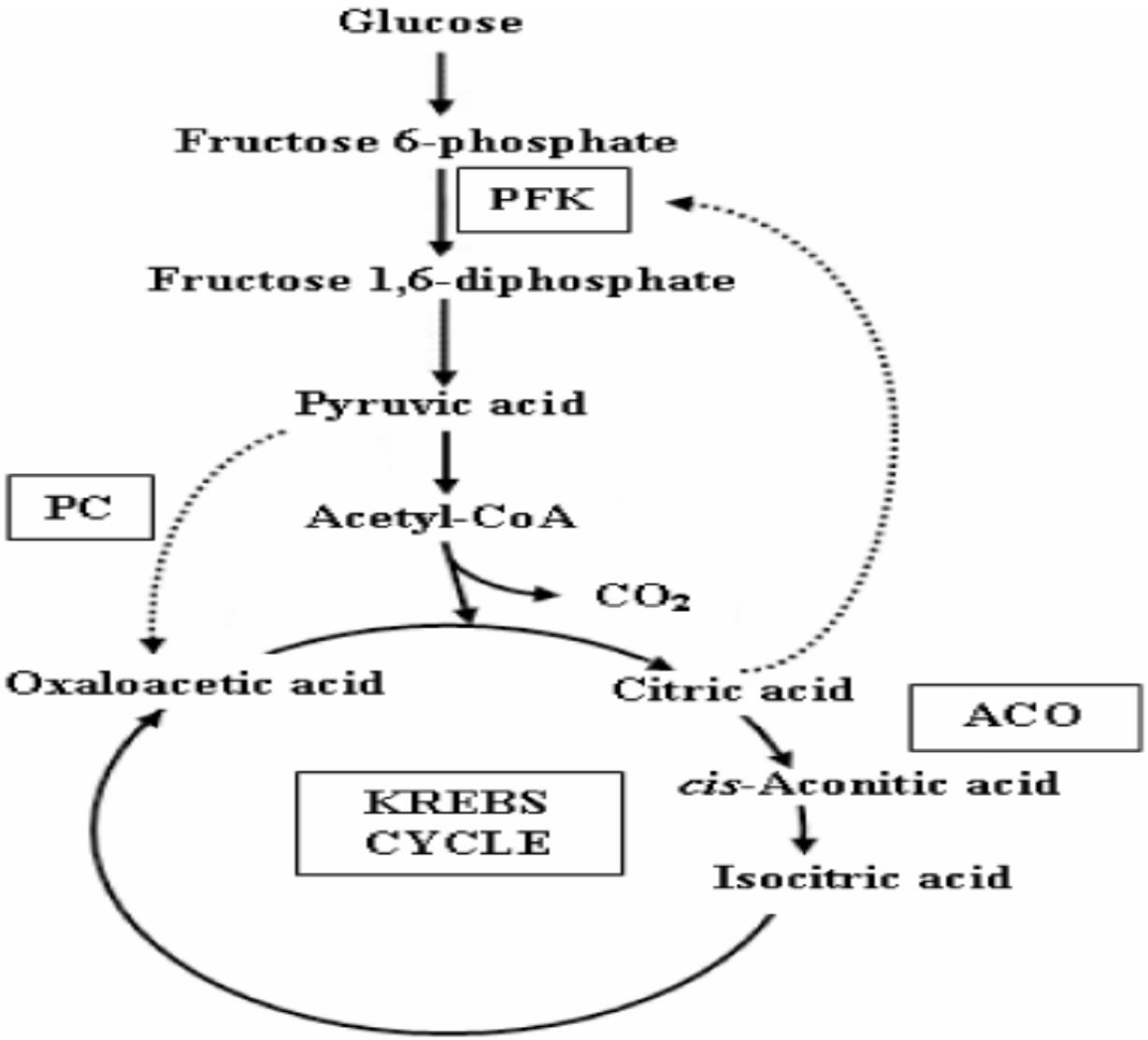


Figure 2. Schematic representation of the main metabolic reactions involved in the production of citric acid by *A. niger* (Manzoni, 2006). PFK = phosphofructokinase, PC = pyruvate carboxylase, ACO = aconitase.

Solid State Fermentation

The solid substrate is soaked with water up to 65 - 70 % of water content. After the removal of excess water, the mass undergoes a steaming process

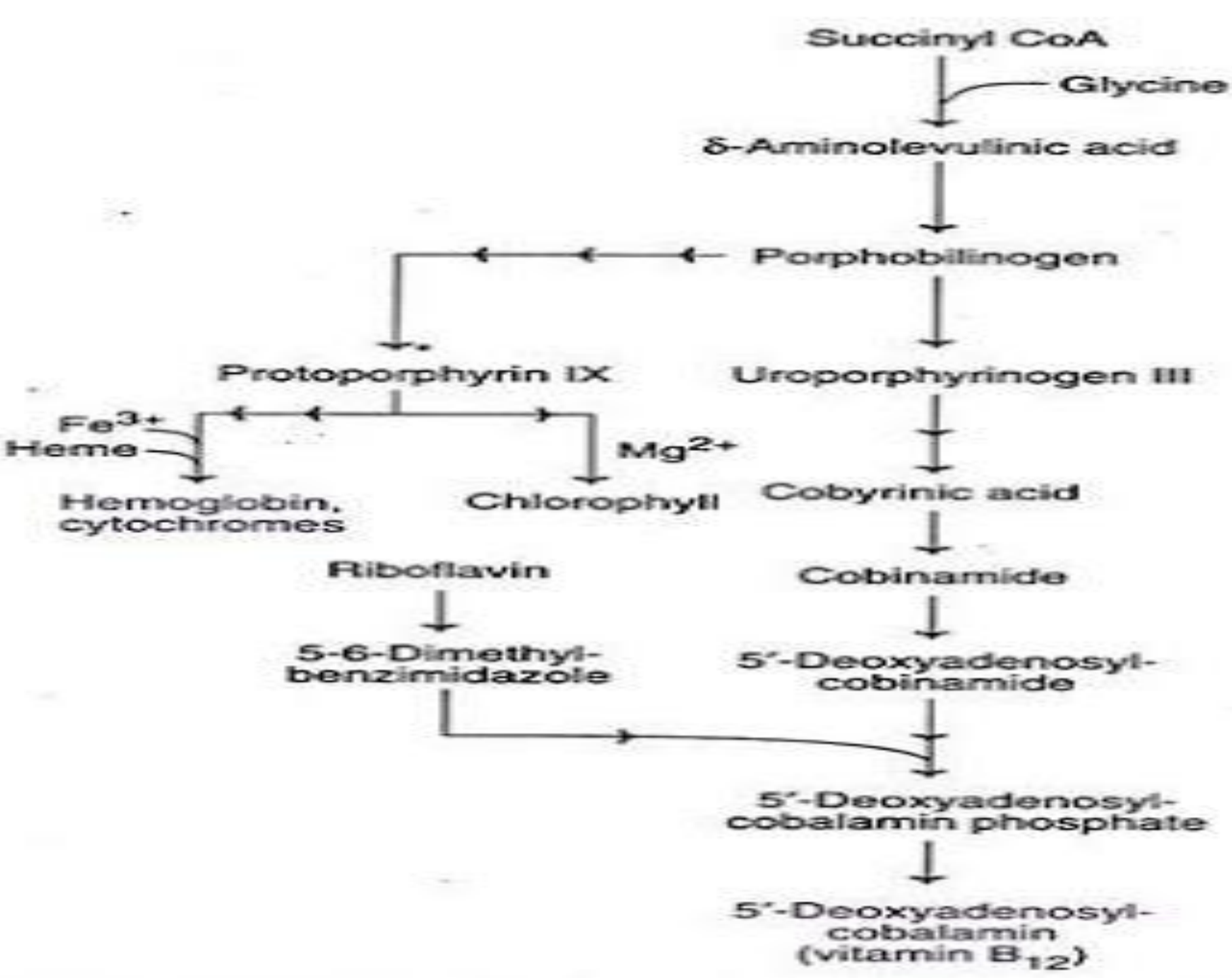
Sterile starch paste is inoculated by spreading *Aspergillus niger* conidia in the form of aerosol or as a liquid conidia suspension on the substrate surface

The pH of the substrate is about 5 to 5.5, and incubation temperature 28 to 30 ºC. Growth can be accelerated by adding α-amylase, although the fungus can hydrolyze starch with its own α-amylase. During the citric acid production pH dropped to values below 2

The solid state surface process takes 5 to 8 days at the end of which the entire is extracted with hot water. On other cases, mechanical passes are also used to obtain more citric acid from the cells

Vitamin B12 production

- Vitamin B_{12} also known as cyanocobalamin, belongs to the cobalamin family of compounds.
- Vitamin B_{12} is synthesized by prokaryotes and inhibits the development of pernicious anemia in animals.
- Large scale industrial production of vitamin B_{12} occurs via microbial fermentation, predominantly utilizing *Pseudomonas denitrificans*, *Propionibacterium shermanii*, or *Sinorhizobium meliloti.*


Succinyl CoA
Glycine
δ-Aminolevulinic acid
Porphobilinogen
Protoporphyrin IX
Uroporphyrinogen III
Fe^{3+}
Heme
Mg^{2+}
Hemoglobin, cytochromes
Chlorophyll
Cobyrinic acid
Riboflavin
Cobinamide
5-6-Dimethyl-benzimidazole
5′-Deoxyadenosyl-cobinamide
5′-Deoxyadenosyl-cobalamin phosphate
5′-Deoxyadenosyl-cobalamin (vitamin B_{12})

FERMENTATION PROCESS OF VITAMIN

MEDIA PREPARATION
Medium is basically corn steep liquor and glucose and for nitrogen source ammonium phosphate is used

INOCULATION
Inoculum from the slant culture is done contained in the flask

PRODUCTION MEDIUM
Cobalt Salt is added for max yield of cobalamin.

STERILIZATION
the medium prepared is sterilized by an autoclave

FERMENTATION
Fermentation is done in Batch Mode process

CENTRIFUGAL EXTRACTION
The culture broth is then harvested & centrifuged to get a concentrated amount of mass of cells.

BIOMASS REMOVAL
The impurities are separated from the culture medium. For removal of such impurities a vacuum filter is commonly used.

SOLVENT ADDITION

Recrystallization

DRYING
The solvent portion is then evaporated by exposing to normal air as a result of which crystals of cyanocobalamine is left in the vessel which is stored for further use.

Glutamic acid production

- Vitamin B_{12} also known as cyanocobalamin, belongs to the cobalamin family of compounds.
- Vitamin B_{12} is synthesized by prokaryotes and inhibits the development of pernicious anemia in animals.
- Large scale industrial production of vitamin B_{12} occurs via microbial fermentation, predominantly utilizing *Pseudomonas denitrificans*, *Propionibacterium shermanii*, or *Sinorhizobium meliloti.*

(a) Carbon Source:

- A wide variety of carbohydrates are used as carbon source in the fermentation process.
- Glucose and sucrose are frequently used.

(b) Nitrogen Source:

- Ammonium sulphate, ammonium chloride, ammonium phosphate, aqueous ammonia, ammonia gas and urea have been used as nitrogen source.
- Although large amount of ammonium ions are necessary, a high concentration of it inhibits the growth of the microorganism as well as the yield of L-glutamic acid

(c) Growth Factors:

- The important growth factor is biotin.
- Its optimal concentration depends upon the carbon source used. In media with 10% glucose, its requirement is 5 mg liter^{-1}.
- In media with lower glucose concentration, it is considerably lower.
- Some strains require L-cystine as an additional growth factor.

(d) Oxygen Supply:

- The oxygen concentration should neither be too low nor too high.

(e) pH:

- Optimum pH for growth and glutamic acid production is 7.0-8.0 and it is controlled by the addition of ammonium salts.

The fermentation process of L-glutamic acid is described below:

(i) Inoculum production

(ii) Preparation of medium

(iii) Fermentation process

(iv) Harvest and recovery

(i) Inoculum Production:

- A suitable strain of *Corynebacterium glutamicum* from a stock culture is selected.

(ii) Preparation of Medium:

- **A production medium is prepared with the following composition:**

Chemical	Concentration (in %)
Glucose	10
K_2HPO_4	0.05
KH_2PO_4	0.05
$MnSO_4 \cdot 7H_2O$	0.025
$FeSO_4 \cdot 7H_2O$	0.001
$MnSO_4 \cdot 4H_2O$	0.001
Urea	0.5
Biotin	2.5 mg $liter^{-1}$

(iii) Fermentation Process:

- The fermentation is carried out, approximately, for 40-48 hours at 30°C temperature.
- The pH is adjusted to 7.0-8.0.
- The urea is added intermittently during the fermentation.
- Approximately 50% of the supplied carbohydrate is converted into L-glutamic acid.

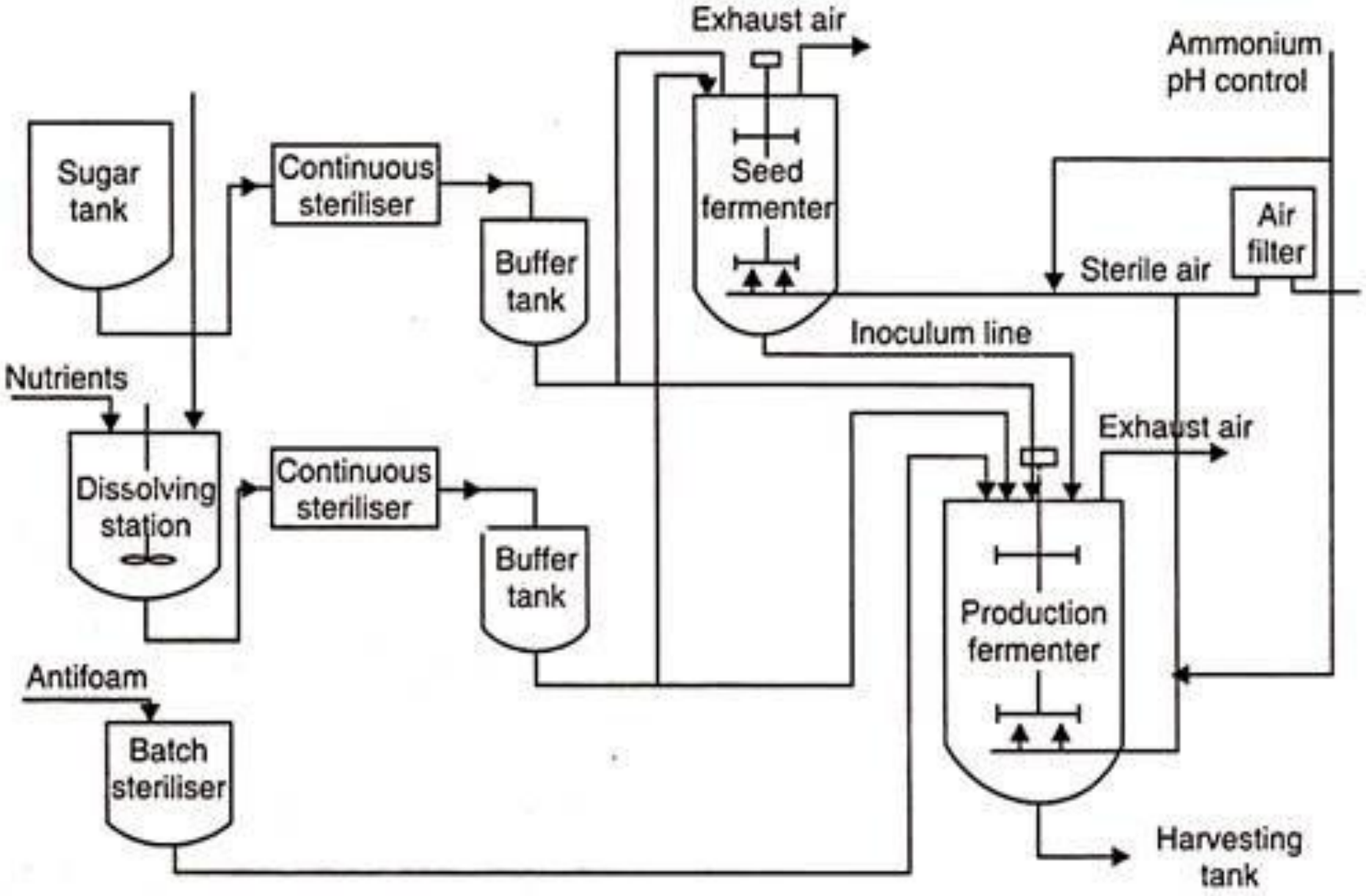


Fig. 5.8: Flow diagram of L-Glutamic acid production

Griseofulvin production

- Griseofulvin is an antifungal antibiotic first isolated from a Penicillium species in 1939. It is a secondary metabolite produce by the fungus *Penicillium griseofulvum*.
- The compound is insoluble in water, and slightly soluble in ethanol, methanol, acetone, benzene, $CHCl_3$, ethyl acetate, and acetic acid.

Mode of action

- Griseofulvin inhibit fungal cell mitosis and nuclear acid synthesis.
- It also binds to and interferes with the function of spindle and cytoplasmic microtubules by binding to alpha and beta tubulin.
- It binds to keratin in human cells, and then once it reaches the fungal site of action, it binds to fungal microtubules thus altering the fungal process of mitosis.

Uses

- Ringworm of the Beard
- Ringworm of Scalp
- Fungal Disease of the Nails
- Ringworm of Groin Area
- Athlete's Foot
- Ringworm of the Body.

Preparation of the medium

- **Medium**

Czapek Dox Medium

- **Chemicals**

• Glucose	5%
• Sodium Nitrate	0.2%
• Potassium Hydrogen Phosphate	0.1%
• Magnesium Sulphate $7H_20$	0.05%

INDUSTRIAL PREPARATION OF GRISEOFULVIN BY SUBMERGED FERMENTATION

STEPS INVOLVED IN THE MANUFACTURING PROCESS

- Fermentation
- Pre treatment of fermentation broth
- Filtration
- Extraction
- Decolourization
- Isolation and separation
- Precipitation and purification

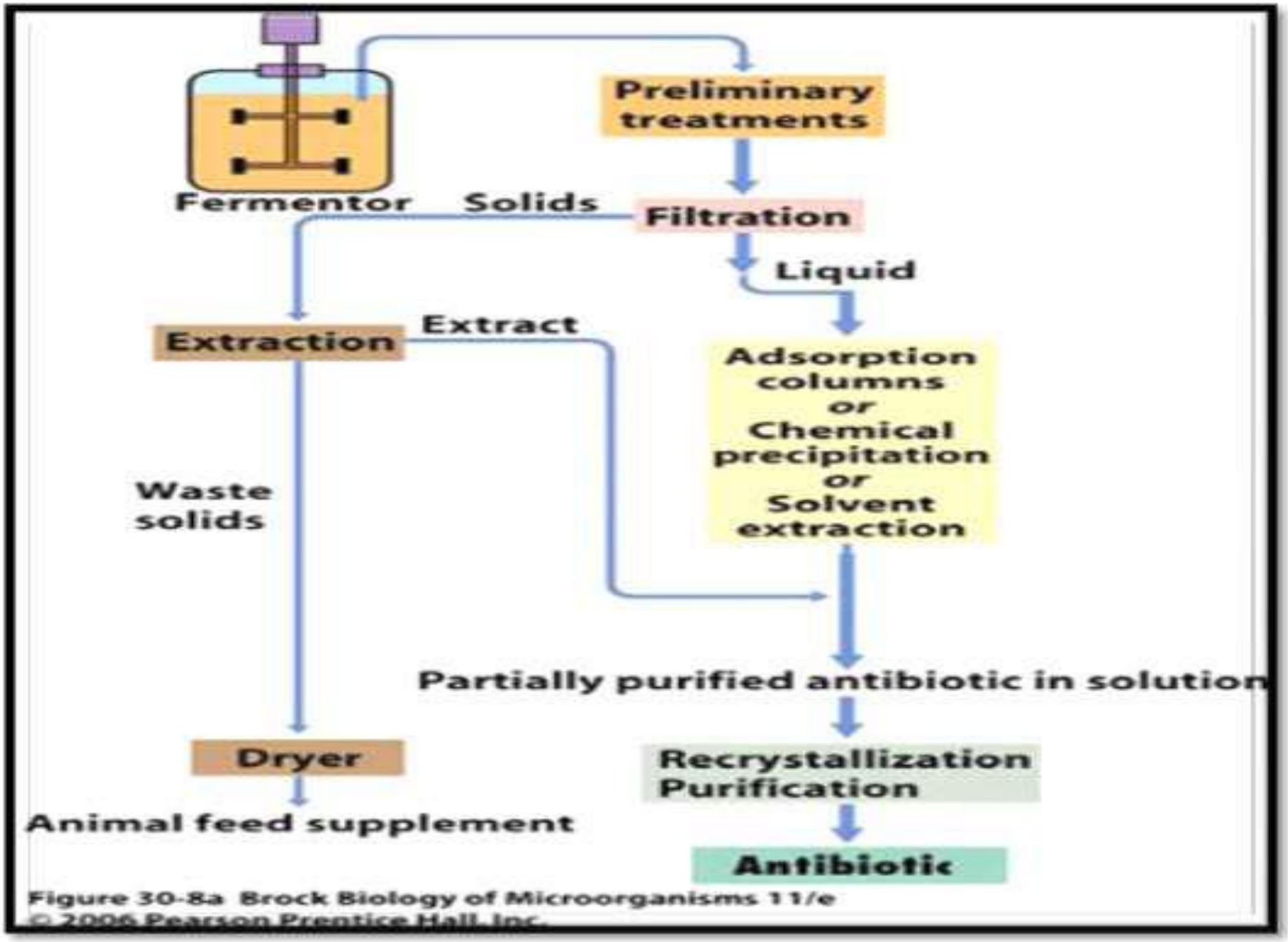


Figure 30-8a Brock Biology of Microorganisms 11/e

Fermentation

- The pH of Czapek-Dox medium was adjusted between 6.0-7.2.
- The medium was dispensed in the fermenter.
- The fresh sample of mycelial suspension of fungus
- *Penicillium griseofulvum* from the fresh slope on raper steep agar (Czapek-Dox medium + corn steep+ agar) was obtained.
- The solution was autoclaved for 20 minutes at 120°C at 15lbs pressure and fermented for 14 days at 24°C.

Pre-treatment of Fermentation broth

- The broth is heated above 60°C for 20-30minutes. After heating, sufficient coagulation of material occurs to produce a valuable improvement in separation characteristics of the broth.
- The period of heating may be short, 5-10 minutes at 80°C having been found to provide a satisfactory increase in filtration rate.

Filtration

- Drum covered with diatomaceous earth matter and allowed to rotate under vacuum with half immersed in the slurry tank. Small amount of coagulation agent added to broth and pumped into the slurry tank. As drum rotates in the slurry tank under vacuum thin layer of coagulated particles adhere to drum.
- The layer thickens to from cake. As the cake portion in the drum comes to the upper region which is not immersed in the liquid it is washed with water and dewatered immediately by blowing air over it.
- Then before the dried portion is again immersed into the liquid it is cut off from drum by knife.

Extraction

- Griseofulvin is extracted in the cold acetone when it is used as an extraction agent.
- The extractions with the cold acetone may be carried out with the efficiencies between 75-96% or even upto 99.5%. the quantity of the solvent used in the extraction at large scale production should be kept minimum.
- The volume of acetone should be 3-5 times of the mycelial felt.

Isolation and Separation

- The impurities or waxy substances are removed by washing the extract with a solvent in which extract is immiscible and also griseofulvin is insoluble.
- Hydrocarbon solvents, generally aliphatic hydrocarbons such as hexane or petroleum containing a high portion of hexane are in general suitable for this step.

Precipitation and purification

- Griseofulvin can be precipitated from the solvent extract in various ways.
- One of the method is using the liquid solvent in which griseofulvin is substantially insoluble.
- Griseofulvin non-solvent is preferably water. The alkaline water is more effective for the removal of colored impurities present in the crystals of the griseofulvin.