

Chapter-1

Fermentation Technology

By :Yonas D. Kedir Y.

Department of Chemical Engineering
KiOT, Wollo University

Fermentation Definition and its classification

Fermentation:

Definition: The action of a specific microorganism on a substrate to produce the desired chemical compound is called Fermentation.

Classification of Fermentation processes:

Fermentation process is classified into the following types.

1. Batch types fermentation
2. Continuous fermentation
3. Solid state fermentation
4. Anaerobic Fermentation
5. Aerobic Fermentation

Aerobic Fermentation process:

Any Fermentation process is carried out in the presence of Oxygen is called as Aerobic fermentation process.

Anaerobic fermentation process:

The Few fermentation processes are carried out in the absence of air are called as Anaerobic fermentation process.

Fermentation process is carried out either in batch wise or continuous process.

- **Batch Fermentation:** is a discontinuous process and the fermentor has to be cleaned after each process and a fresh batch started.
- It includes the following 5 steps
 1. Medium added
 2. Fermentor sterilized
 3. Inoculum added
 4. Fermentation followed to completion
 5. Culture harvested.

SOLID STATE FERMENTATION

- The growth of microorganisms on moist solid substrate particles in the absence or mere absence of visible liquid water between the particles.
- The moisture content of solid substrate ranges between 12-80%.
- The water content of a typical submentation is more than 95%.
- SSF's are usually used for the fermentation of agricultural products or foods, such as rice, wheat, barley, corn and soybeans. Some food fermentations involving SSF: Wheat by *Aspergillus* Soybean by *Rhizopus* Soybean by *Aspergillus*

phases of solid state fermentation

- There are 3 phases:

1. Solid phase Solid phase of are two type:(i) Natural solid materials(ii) An inert solid support

2. Liquid phase

3. Gaseous phase

Characteristics of solid state fermentation

- 1) The substrate may require preparation or pretreatment like, Chopping or grinding-reduce particle size.
- 2) Microorganism is usually a filamentous fungus requiring aerobic condition.
- 3) The Inoculum is mixed into substrate to fermentation
- 4) Cooking or chemical hydrolysis. Pasteurization or sterilization-reduce contaminants.

Anaerobic Fermentation

In anaerobic fermentation, a provision for aeration is usually not needed.

The air present in the headspace of the fermenter should be replaced by CO₂, H₂, N₂ or a suitable mixture of these.

The fermentation usually liberates CO₂ and H₂

Recovery of products from anaerobic fermenters does not require anaerobic conditions.

- **Aerobic Fermentation**

The main feature of aerobic fermentation is the provision for adequate aeration. In addition, these fermenters may have a mechanism for stirring and mixing of the medium and cells. Aerobic fermenters may be either of the i) stirred tank type in which mechanical motor driven stirrers are

Examples of Fermentation:

i) Ethanol Fermentation (Alcoholic fermentation) is the production of Ethanol and carbondioxide.

ii) Lactic acid fermentation (Homolactic acid fermentation and Heterolactic acid fermentation).

Note: Sugars are the most common substrate of fermentation.

iii) Amino acid fermentation

iv) Cheese, Yogurt production by fermentation process.

Fermentation products:

i) Ethyl alcohol, carbondioxide and water produced from yeast and plant cells.

ii) Lactic acid and Lactate, CO_2 and water produced from bacteria, fungi and animal cells.

iii) Food products:

a) From milk – Yogurt, cheeses

b) From Fruits – wine, vinegar

c) From vegetables – Pickles and sauce

iv) Industrial chemicals – Acetone, amino acids, Butanol, Ethanol.

v) Specialty chemicals – Vitamins, Pharmaceuticals.



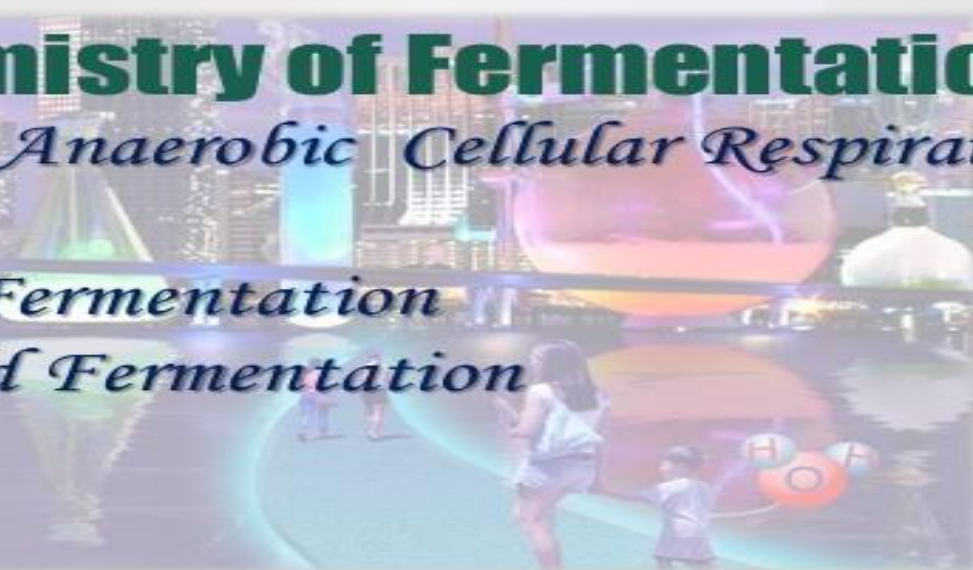
FERMENTATION PROCESSES AND THEIR APPLICATION

"fermentation, far from being a lifeless phenomenon, is a living process..."

- Louis Pasteur

❖ **The Chemistry of Fermentation**

- *Aerobic & Anaerobic Cellular Respiration*
- *Glycolysis*
- *Alcoholic Fermentation*
- *Lactic Acid Fermentation*



What is fermentation?

- Derived from the Latin verb '*fervere*' meaning 'to boil'



- **Louis Pasteur** in the 19th century used the term fermentation in a narrow sense to describe the changes brought about **by yeasts** and other **microorganisms** growing in the **absence of air** (anaerobically);
- he also recognized that ethyl alcohol and carbon dioxide are not the only **products of fermentation.**



The range of fermentation process

There are five major groups of commercially important fermentations:

- (i) Those that produce **microbial cells** (or biomass) as the product.
- (ii) Those that produce **microbial enzymes**.
- (iii) Those that produce **microbial metabolites**.
- (iv) Those that produce **recombinant products**.
- (v) Those that modify a **compound which is added to the fermentation** the transformation process.

Products of Fermentation



The end products of fermentation differ depending on the organism.

- **lactic acid and lactate, carbon dioxide, and water** – produced from many *bacteria, fungi, protists, and animals cells* (notably muscle cells in the body)
- **ethyl alcohol, carbon dioxide, and water** – produced from *yeast and most plant cells*



Products of Fermentation

Fermentation products include:

- **Food products:** from milk (yogurt, kefir, fresh and ripened cheeses), fruits (wine, vinegar), vegetables (pickles, sauerkraut, soy sauce), meat (fermented sausages, salami)
- **Industrial chemicals:** (solvents: acetone, butanol, ethanol, enzymes, amino acids)
- **Specialty chemicals** (vitamins, pharmaceuticals)



FERMENTATION PROCESSES IN FOODS

"fermentation, far from being a lifeless phenomenon, is a living process..."

- Louis Pasteur



Ethyl Alcohol (Ethanol)

Fermentation:

There are three types.

- i) From sucrose substrate
- ii) From waste sulfite substrate of paper mills
- iii) From starch substrate

Ethyl Alcohol properties:

- ❖ Molecular weight = 46.07
- ❖ Density = 0.791 at 20°C
- ❖ Melting point = -112 °C
- ❖ Boiling point = 78.3°C
- ❖ Flash point = 21°C
- ❖ Grades = Anhydrous, 95% denatured

Ethyl Alcohol Production by Fermentation

Raw materials required:

- i) Molasses (50-55% total sugar), ii) Sulfuric acid, iii) Ammonium sulfate, iv) Coal,
- v) Process water, vi) Cooling water

Quantitative requirements:

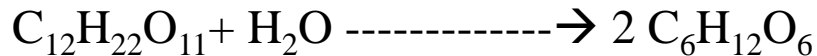
Basis: 1 ton of 100% alcohol

- i) Molasses(50-55% total sugar) =5.6 tons
- ii) Sulfuric acid =27 Kg
- iii) Ammonium sulfate =2.5 Kg
- iv) Coal =1.5 tons
- v) Process water =12 tons
- vi) Cooling water =50 tons
- vii) Plant capacity = 10-100 tons/ day of Ethyl alcohol.

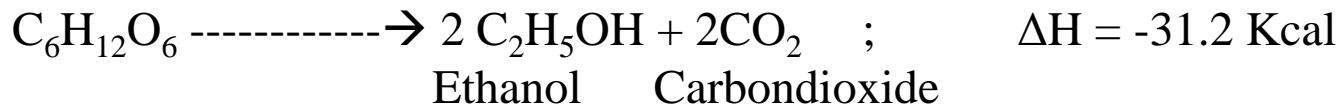
Chemical Reactions:

a) Main Reactions:

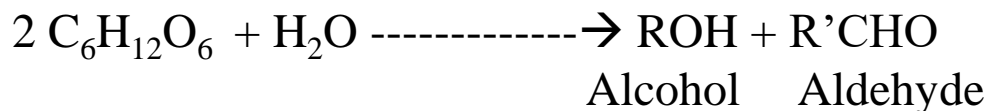
Invertase



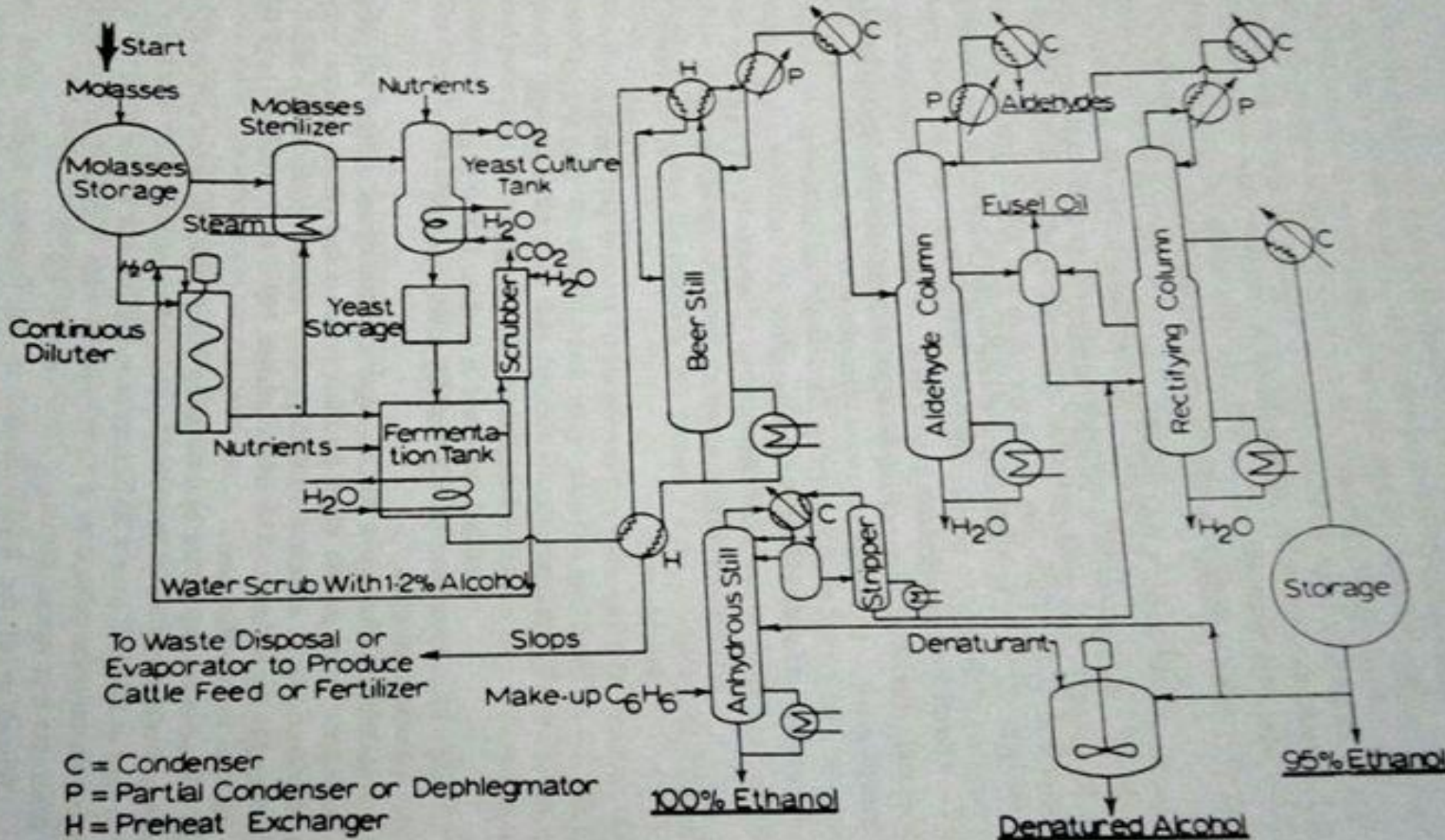
Zymase



b) Side Reactions:



Flow sheet- Ethyl Alcohol by Fermentation



Process Description

- i) Molasses is diluted to 10-15% sugar concentration and pH value is adjusted to 4-5.
- ii) Invertase and Zymase catalytic enzymes are used.
- iii) Nutrients (Ammonium sulfate and Magnesium sulfate) are added in yeast culture tank.
- iv) This diluted mixture is called “mash” passed to steel fermentation tanks.
- v) In Fermentation tank the temperature is kept at 20-30°C for 30-70 hours.
- vi) CO₂ is removed by water scrubbing.
- vii) In the Fermented liquor 8-10% alcohol is separated (Beer).
- viii) In the Beer still 50-60% concentration alcohol is produced and aldehyde is passed to aldehyde still.
- ix) Alcohol is passed to the rectifying column as a side stream.
- x) In this rectifying column, azeotropic alcohol-water mixture of 95% Ethanol is collected as a top side stream.

Ethyl Alcohol Uses:

- i) Denatured alcohol sold for industrial uses.
- ii) High molecular weight alcohols are used as solvents.
- iii) The bottom product from the beerstill is known as “slops” used as cattle feed.
- iv) Direct sale as potable, Government controlled alcohol.

Major Engineering problems:

- i) Collection and storage of Molasses.
- ii) Maintenance of sterile & Yeast culture conditions.
- iii) Batch Vs Continuous operation
- iv) Waste disposal problems.

Beverages

- ❖ Beverages are liquids specifically prepared for human consumption. A beverage is any type of drink and most beverages, including juice, soft drinks and carbonated drinks have some form of water in them.
- ❖ Alcoholic Beverages – It is a drink containing ethanol commonly known as alcohol.
- ❖ Cider – Cider is a fermented alcoholic beverage made from apple juice. Cider alcohol content varies from 1.2% to 8.5%.
- ❖ Distilled beverages – It is also known as liquor and spirits and it is an alcoholic beverage produced by distillation of a mixture produced from alcoholic fermentation.
- ❖ Hard soda- It is a type of alcoholic beverage that is manufactured in the style of a soft drink.
- ❖ Wine – wine is an alcoholic beverage made from fermented grapes or other fruits.

Examples of Beverages

❖ Beer:

❖ Cider : is an alcoholic beverage made from the fermented juice of apple

❖ **Distilled liquor:** an alcoholic liquor (as brandy, whiskey, gin, rum, or arrack) obtained by distillation from wine or other fermented fruit juice or plant juice or from a starchy material (as various grains) that has first been brewed. — called also hard liquor.

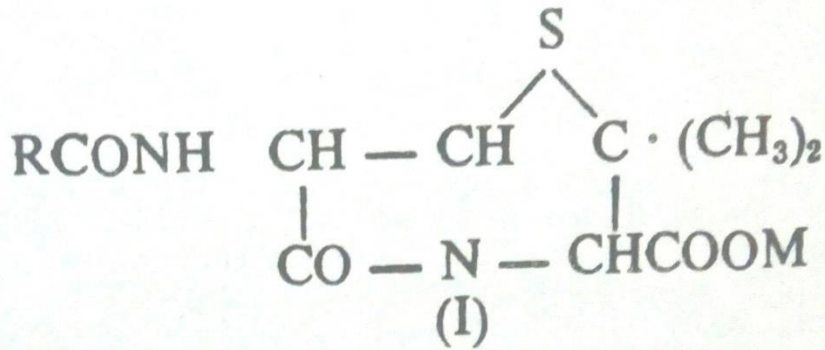
❖ **Hard soda:** a type of alcoholic beverage and craft beer that is manufactured in the style of a soft drink. It has gained recent prominence in the United States after the success of the Not Your Father's Root Beer brand manufactured by Small Town Brewery.

❖ **Wine:** the fermented juice of grapes, made in many varieties, such as red, white, sweet, dry, still, and sparkling, for use as a beverage, in cooking, ...

❖ **Hot beverages:** Also known as hot cocoa, it typically consists of shaved chocolate, melted chocolate or cocoa powder, heated milk or water, and sugar. Hot egg chocolate is a type of hot chocolate. Hot toddy. Mixed drink made of liquor and water with sugar and spices and served hot. Irish coffee.

Penicillin Production

Structure of Penicillin



Variations in R and M

Types of Penicillin:

| Name | Chemical Name | R in side chain |
|----------------|------------------|---|
| Penicillin I | 2-Pentenyl | $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2$ |
| Penicillin II | Benzyl | $\text{C}_6\text{H}_5\text{CH}_2-$ |
| Penicillin III | P-Hydroxy benzyl | $\text{HOC}_6\text{H}_4\text{CH}_2-$ |
| Penicillin IV | n-Heptyl | $\text{n-C}_7\text{H}_{15}$ |
| Penicillin V | Phenoxy Methyl | $\text{C}_6\text{H}_5-\text{OCH}_2-$ |

i) Direct rapid action injection:

M= Na or K

ii) Oral tablets : M= K, Ca, Al

iii) Delayed action oil base Injection:

Procaine

Quantitative Requirements:

Basis: 1 kg of Penicillin

Broth – 135 kg

Lactose-100 kg

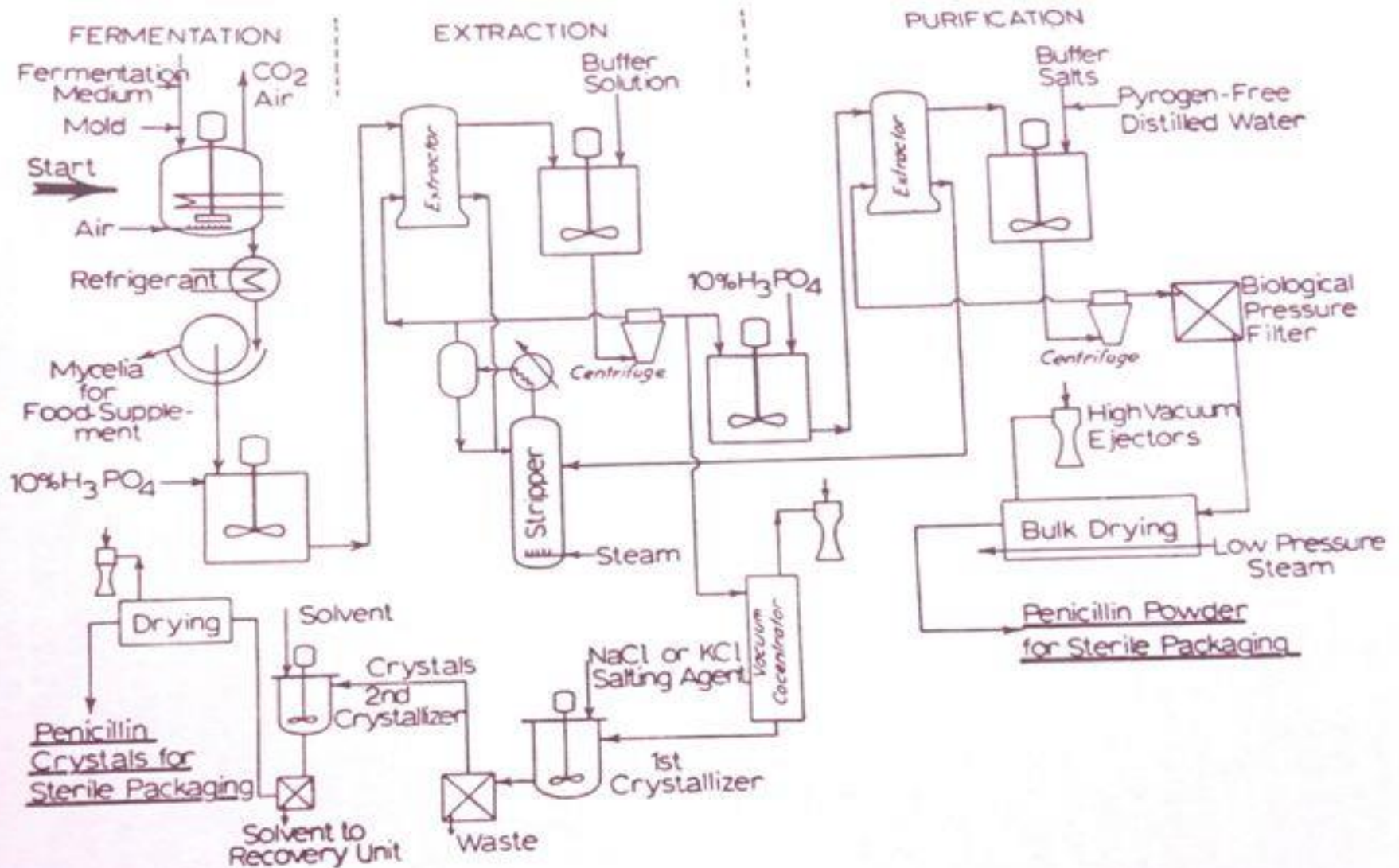
Calcium carbonate-35 kg

Process water-3 tons

Air- 7000 Nm³

Others- Precursors, Nutrients

Penicillin Production



Penicillin production by fermentation.

Process description

- i) Aerobic batch fermentation is carried out.
- ii) Maize steep liquor is diluted to 4- 5% solids with water and prepared with 4-5% lactose solids.
- iii) Nutrients such as Magnesium sulfate, Zinc sulfate (or) sodium sulfate, ammonium acetate and potassium dihydrogen sulfate are added.
- iv) Calcium carbonate is added to adjust the pH value as 5-6.
- v) Phenyl acetic acid precursor is used to control the type and yield of Penicillin II.
- vi) Batch fermentation Medium is sterilized with steam at 115°C for 30 minutes and cooled with water to 25°C.
- vii) Penicillin chrysogenum mold culture is inoculated and sterile air is blown for 4-5 days into the tank.
- viii) Solvent extraction is used to separate Penicillin from the fermented broth.
- ix) Suspended solids are removed by continuous filtration at 1-2°C and Dilute Phosphoric acid is used to adjust the pH of clear filtrate.
- x) Penicillin salt is extracted with amyl acetate and raffinate is sent to solvent recovery still.
- xi) Penicillin is purified in second acidic extraction and freeze drying is applied.
- xii) Crystalline penicillin salt and Oil-soluble procaine penicillin are also produced.
- xiii) Composition of oil-soluble procaine penicillin: 20-30% Conc. Penicillin with 50% Procaine hydrochloride aqueous solution.

CHAPTER 2

Modern Biotechnology

- ❖ **Biotechnology = bios (life) + logos (study)**
 - Literally ‘the study of tools from living things’
- ❖ **Biotechnology is the application of genetic engineering and DNA technology to produce therapeutic and medical diagnostic products and processes.**

What are the stages of biotechnology?

i) Ancient Biotechnology

- **Early history as related to food and shelter including domestication**

ii) Classical Biotechnology

- **Built on ancient biotechnology**
- **Fermentation promoted food production**
- **Medicine**

iii) Modern Biotechnology

- **Manipulates genetic information in micro-organism**
- **Genetic Engineering**

Cont...d

iv) Cell biology

- Structure, organization and reproduction

v) Biochemistry

- Synthesis of organic compounds
- Cell extracts for fermentation (enzymes versus whole cells)

vi) DNA Revolution

- DNA → RNA → Protein

- **What are the benefits of biotechnology?**

i) Medicine

- human
- veterinary
- Bio-pharming

ii) Environment

iii) Agriculture

iv) Food products

v) Industry and manufacturing

Industrial Enzymes

To break down a protein into its amino acids, enzymes are needed. Enzymes are biological molecules (proteins) that act as catalysts and help complex reactions occur everywhere in life.

Definition

- Enzymes are biocatalyst that speeds up digestion and metabolism.
- They are located in the cells, cytoplasm, mitochondria, tissues and body fluids.
- **Endoenzymes**
- Enzymes that function within the cells. Most of the enzymes are these types. Eg. metabolic enzymes (cytochrome oxidase)
- **Exoenzymes**
- Enzymes that are liberated by cells and catalyse reactions outside the cell. Eg. digestive enzymes (amylase, lipase, protease)

Why are enzymes called Biological catalysts?

Enzymes are soluble protein molecules that can speed up chemical reactions in cells. These reactions include respiration, photosynthesis and making new proteins. For this reason **enzymes** are sometimes **called biological catalysts**.

Classification of Industrial enzymes:

There are three types of enzymes. They are as follows.

❖ Lipases

❖ Proteases

❖ Carbohydrases

Most industrial enzymes are produced recombinantly. Why?

A. Higher expression B. Higher purity (% protein)

C. Cheap, D. It can be engineered protein

E. It can express enzymes which are found in pathogenic organisms.

❖ Heterologous Protein Expression

❖ Homologous Protein Expression

Application of Industrial Enzymes

Application of Protease

❖ Protease **enzyme** –It is used in the manufacturing of baby foods to pre-digest proteins.

Application of Lipase

❖ Lipase –It can be used in conjunction with protease in biological detergents in order to break down and digest the substances in stains into smaller and more water soluble substances.

Application of Carbohydrases

Cellulase - hydrolyzes cellulose

Cellulose is a polysaccharide (glucose units)

Biocatalysis & Biocatalysts

- Biocatalysis can be defined as the bioprocess in which the use of natural substances to speed up or catalyze chemical reactions.

What is bio catalyst?

- ❖ In biocatalytic processes natural catalysts such as protein enzymes perform chemical transformations on organic compounds.
- ❖ Enzymes that have been more or less isolated and enzymes still residing inside living cells.

Types of biocatalysts

- **Microbes:** e.g. yeast, and other anaerobic bacterias
- **Lipases:** These are the most widely used class of enzymes in organic synthesis, they are preferred widely because of their better stability as compared to others.
- **Proteases:** Enzymes which break down proteins.
- **Cellulases:** Enzymes which break down cellulose.
- **Amylases:** which break down starch into simple sugars

Merits of Biocatalysts

- i) Biocatalysts can operate at room temperature and pressure.
- ii) It creates higher quality products.
- iii) Biocatalysts are pollution less and easily decomposable at any stage.
- iv) It can be easily disposed.
- v) Biocatalysts can be practically used in Industries.

Biotech Drug Products

- **Anticoagulant drug:** Lepirudin
- **Antisense drugs:** Fomivirsen sodium
- **Colony stimulating factors:**
 - i) Erythropoietin
 - ii) Interferon
 - ❖ Clotting factors - Kogenate, Recombinate
 - ❖ Growth factor – Becaplemin
 - ❖ Antibiotic drugs:
Penicillin, Terramycin, streptomycin, chloromycin, Neomycin,
Aureomycin, Tetracycline

Thank you

