B Pharmacy 6th sem
Subject: Pharmaceutical Biotechnology
Subject Code: BP605T

MODULE- 5
FERMENTATION AND BLOOD PRODUCTS

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Objectives of Course

- Appreciate the use of microorganisms in fermentation technology.
- Genetic engineering applications in relation to production of pharmaceuticals.

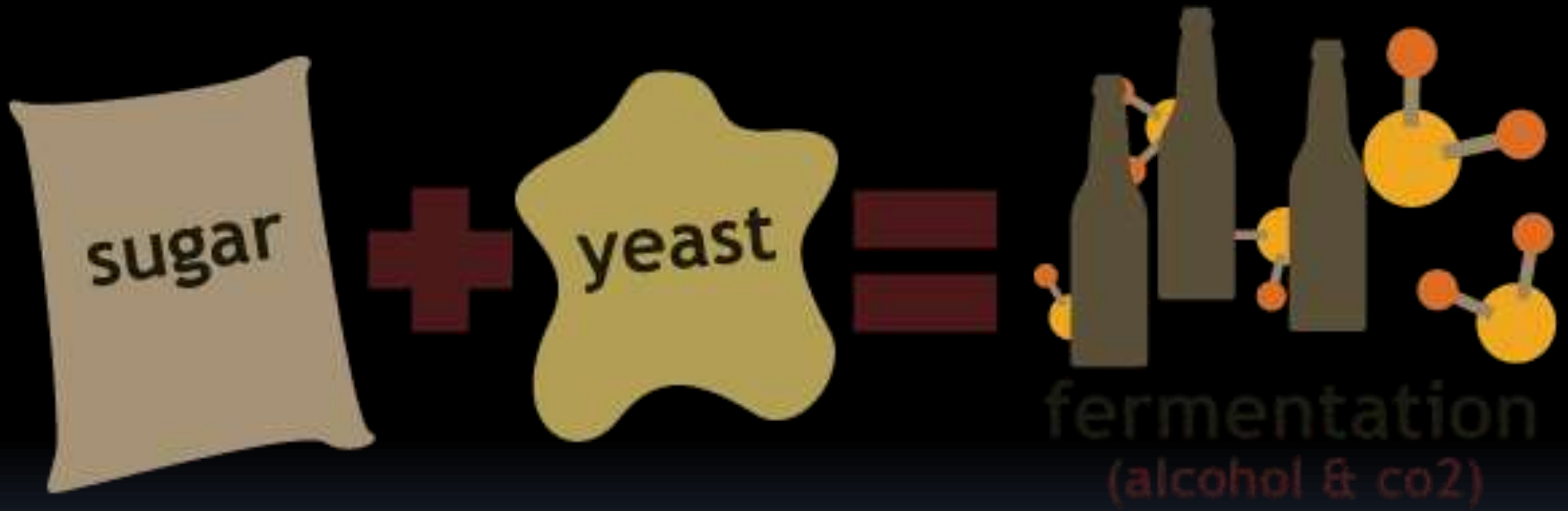
Learning Outcomes

- Students will learn about various fermentation methods including their sterlization methods, aeration and stirring methods.
- They will also learn about various types of fermentor design and parameters used to control fermentation process.

Definition:

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

Enzymes act by hydrolysis, a process of breaking down or predigesting complex organic molecules to form smaller (and in the case of foods, more easily digestible) compounds and nutrients.


sugar
yeast
fermentation
(alcohol & co2)

HISTORY OF FERMENTATION

Fermentation is a natural process. People applied fermentation to make products such as wine, mead (Made of Fermented honey and water), cheese and beer long before the biochemical process was understood. In the 1850s and 1860s Louis Pasteur became the first zymurgist (One who

expert in the field of fermentation) or scientist to study fermentation when he demonstrated fermentation was caused by living cells.

The first solid evidence of the living nature of yeast appeared between 1837 and 1838 when three publications appeared by C. Cagniard de la Tour, T. Swann, and F. Kuetzing, each of whom independently concluded as a result of microscopic investigations that yeast was a living organism that reproduced by budding. The word "yeast," it

should be noted, traces its origins back to the Sanskrit word meaning "boiling." It was perhaps because wine, beer, and bread were each basic foods in Europe, that most of the early studies on fermentation were done on yeasts, with which they were made. Soon bacteria were also discovered; the term was first used in English in the late 1840s, but it did not come into general use until the 1870s, and then largely in connection with the new germ theory of disease.

The view that fermentation was a process initiated by living organisms soon aroused fierce criticism from the finest

chemists of the day, especially Justus von Liebig, J.J. Berzelius, and Friedrich Woehler. This view seemed to give new life to the waning mystical philosophy of vitalism, which they had worked so hard to defeat. Proponents of vitalism held that the functions of living organisms were due to a vital principal (life force, *chi*, *ki*, *prana* , etc.) distinct from physico-chemical forces, that the processes of life were not explicable by the laws of physics and chemistry alone, and that life was in some part self determining. As we shall soon see, the vitalists played a key role in debate on the nature of

fermentation. A long battle ensued, and while it was gradually recognized that yeast was a living organism, its exact function in fermentations remained a matter of controversy. The chemists still maintained that fermentation was due to catalytic action or molecular vibrations.

The debate was finally brought to an end by the great French chemist Louis Pasteur (1822-1895) who, during the 1850s and 1860s, in a series of classic investigations, proved conclusively that fermentation was initiated by living organisms. In 1857 Pasteur showed that lactic acid

fermentation is caused by living organisms. In 1860 he demonstrated that bacteria cause souring in milk, a process formerly thought to be merely a chemical change, and his work in identifying the role of microorganisms in food spoilage led to the process of pasteurization. In 1877, working to improve the French brewing industry, Pasteur published his famous paper on fermentation, *Etudes sur la Biere* , which was translated into English in 1879 as *Studies on Fermentation* . He defined fermentation (incorrectly) as "Life without air," but correctly showed specific types of

microorganisms cause specific types of fermentations and specific end products. In 1877 the era of modern medical bacteriology began when Koch (a German physician; 1843-1910) and Pasteur showed that the anthrax bacillus caused the infectious disease anthrax. This epic discovery led in 1880 to Pasteur's general germ theory of infectious disease, which postulated for the first time that each such disease was caused by a specific microorganism. Koch also made the very significant discovery of a method for isolating microorganisms in pure culture.

TYPES BASED ON RESPIRATION (AEROBIC AND AN-AEROBIC)

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

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

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

Through this method, a cell is able to regenerate nicotinamide adenine dinucleotide (NAD+) from the reduced form of nicotinamide adenine dinucleotide (NADH), a molecule necessary to continue glycolysis. Anaerobic fermentation relies on enzymes to add a phosphate group to an individual adenosine diphosphate (ADP) molecule to produce ATP, which means it is a form of substrate-level phosphorylation. This contrasts with oxidative phosphorylation, which uses energy from an established proton gradient to produce ATP.

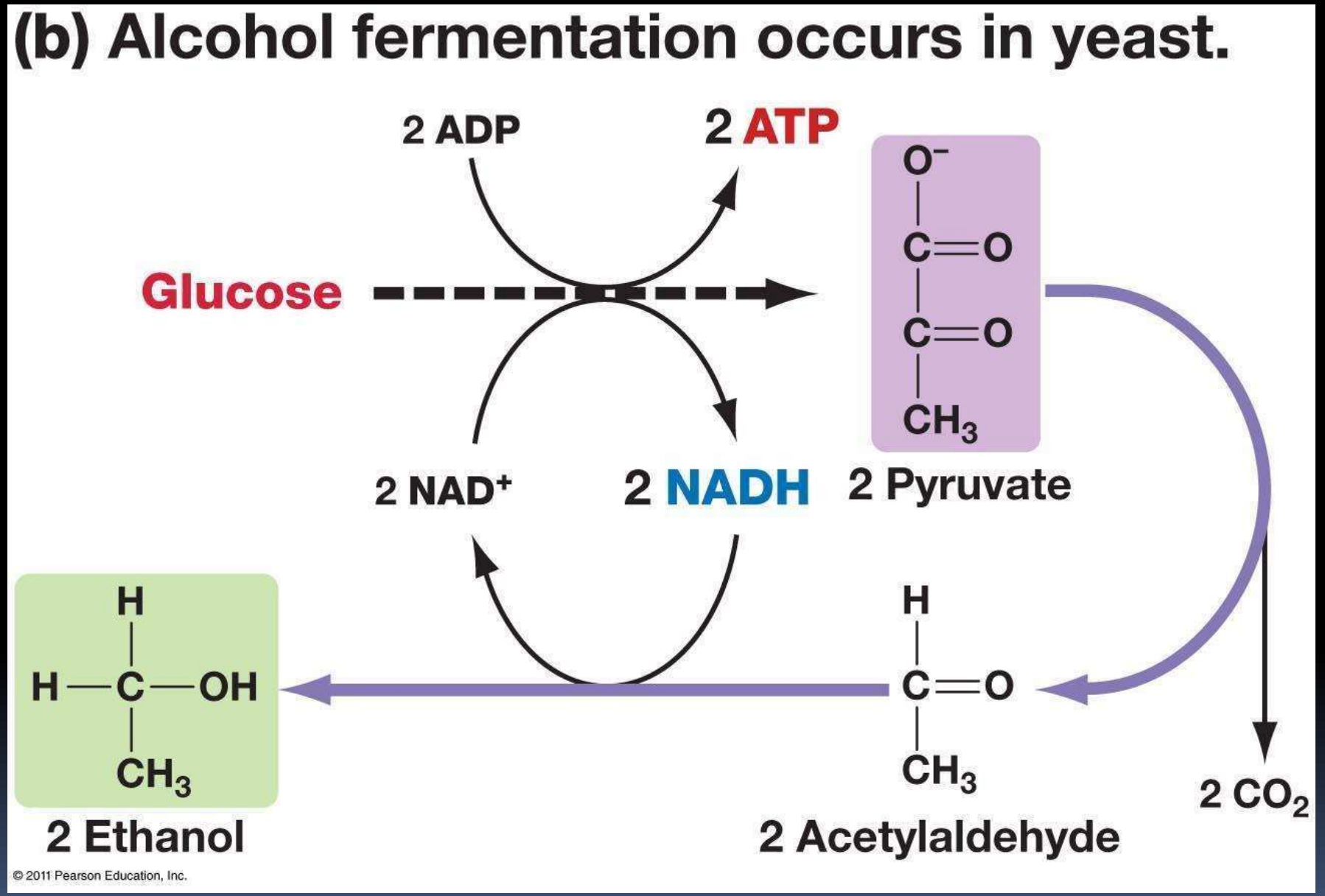
There are two major types of anaerobic fermentation: ethanol fermentation and lactic acid fermentation. Both restore NAD+ to allow a cell to continue generating ATP through glycolysis.

Ethanol fermentation: Ethanol fermentation converts two pyruvate molecules, the products of glycolysis, to two molecules of ethanol and two molecules of carbon dioxide. The reaction is a two-step process in which pyruvate is converted to acetaldehyde and carbon dioxide first, by the enzyme pyruvate decarboxylase.

Yeast and certain bacteria perform ethanol fermentation where pyruvate (from glucose metabolism) is broken into ethanol and carbon dioxide. The net chemical equation for the production of ethanol from glucose is:

$C_6H_{12}O_6$ (glucose) → 2 C_2H_5OH (ethanol) + 2 CO_2 (carbon dioxide)

Ethanol fermentation is used the production of beer, wine and bread. It's worth noting that fermentation in the presence of high levels of pectin result in the production of small amounts of methanol, which is toxic when consumed.



Anaerobic fermentation -1

Lactic acid fermentation: Lactic acid fermentation is a biological process by which glucose and other six-carbon sugars (also, disaccharides of six-carbon sugars, e.g. sucrose or lactose) are converted into cellular energy and the metabolite lactate.

The pyruvate molecules from glucose metabolism (glycolysis) may be fermented into lactic acid. Lactic acid fermentation is used to convert lactose into lactic acid in yogurt production. It also occurs in animal muscles when

the tissue requires energy at a faster rate than oxygen can be supplied. The next equation for lactic acid production from glucose is:

$C_6H_{12}O_6$ (glucose) $\rightarrow$ 2 $CH_3CHOHCOOH$ (lactic acid)

The production of lactic acid from lactose and water may be summarized as:

$C_{12}H_{22}O_{11}$ (lactose) + H_2O (water) $\rightarrow$ 4 $CH_3CHOHCOOH$ (lactic acid)

Yogurt is made by fermenting milk. It's high in protein, calcium, and probiotics ("good" bacteria). Here's how to make yogurt and a look at the chemistry of yogurt.

TYPES OF FERMENTATION

Homo Lactic fermentation: The fermentation in which only the lactic acid is produced. There is no any side product formed after the reaction.

Hetero-Lactic Fermentation: The Fermentation in which the lactic acid is produced along with some by products like gases.

MECHANISM OF FERMENTATION

Fermentation takes place when the electron transport chain is unusable (often due to lack of a final electron receptor, such as oxygen), and becomes the cell's primary means of ATP (energy) production.[1] It turns NADH and pyruvate produced in glycolysis into NAD+ and an organic molecule (which varies depending on the type of fermentation; see examples below). In the presence of O2, NADH and pyruvate are used to generate ATP in respiration. This is called

oxidative phosphorylation, and it generates much more ATP than glycolysis alone. For that reason, cells generally benefit from avoiding fermentation when oxygen is available, the exception being obligate anaerobes which cannot tolerate oxygen.

The first step, glycolysis, is common to all fermentation pathways this is the cause of fermentation:

$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ Pi \rightarrow 2\ CH_3COCOO^- + 2\ NADH + 2\ ATP + 2\ H_2O + 2H^+$

Pyruvate is CH_3COCOO^-. Pi is inorganic phosphate. Two ADP molecules and two Pi are converted to two ATP and two water molecules via substrate-level phosphorylation. Two molecules of NAD^+ are also reduced to NADH.

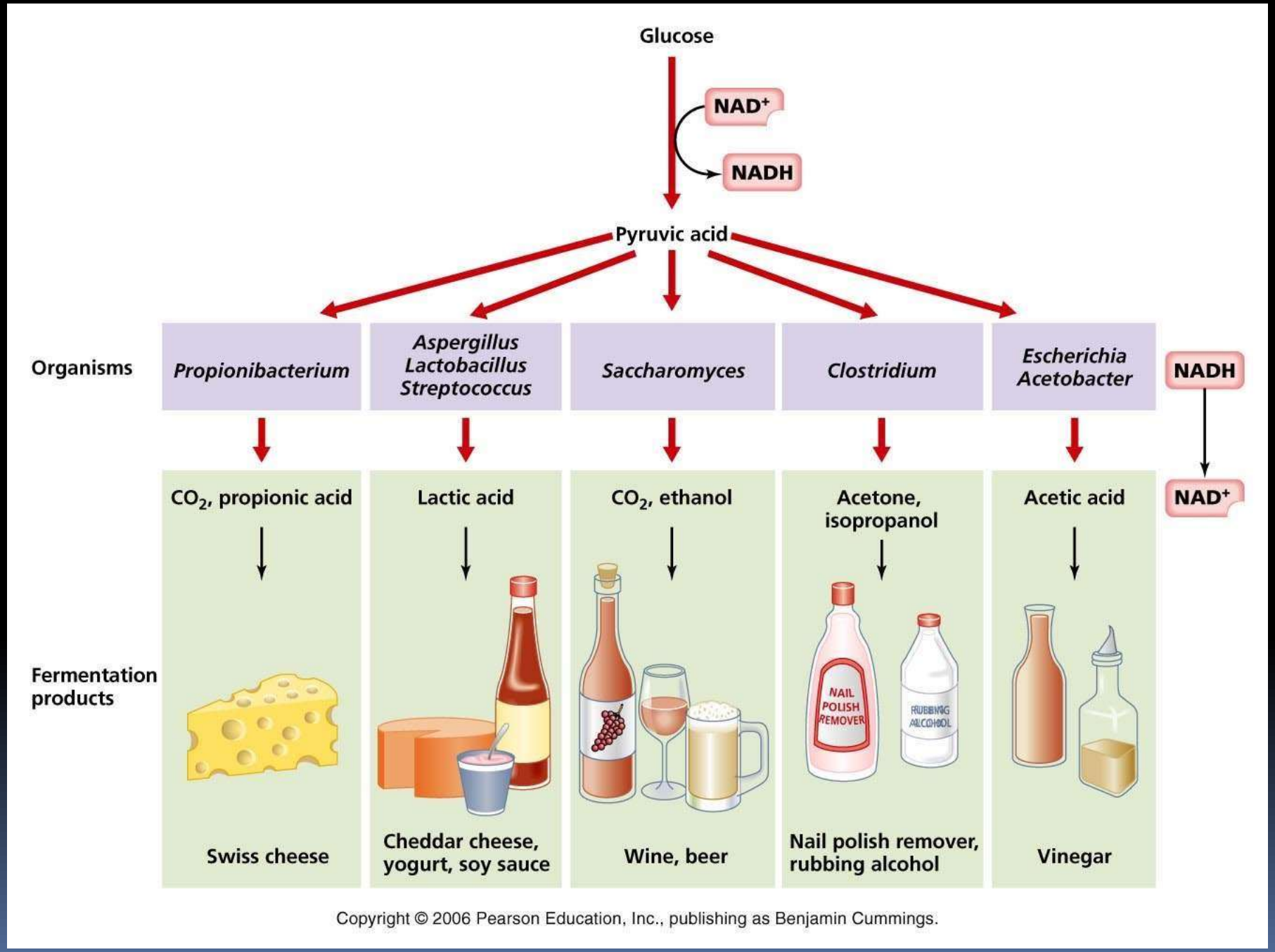
In oxidative phosphorylation the energy for ATP formation is derived from an electrochemical proton gradient generated across the inner mitochondrial membrane (or, in the case of bacteria, the plasma membrane) via the electron transport chain. Glycolysis has substrate-level phosphorylation (ATP generated directly at the point of reaction).

Humans have used fermentation to produce food and beverages since the Neolithic age. For example,

fermentation is used for preservation in a process that produces lactic acid as found in such sour foods as pickled cucumbers, kimchi and yogurt (see fermentation in food processing), as well as for producing alcoholic beverages such as wine (see fermentation in winemaking) and beer. Fermentation can even occur within the stomachs of animals, such as humans.

PRODUCTS OF FERMENTATION

➔Wine
➔Beer
➔Lactic acid
➔Vinegar
➔ Yogurts
➔ cheese
➔ Sauerkraut
➔ Kimchi
➔ Pepperoni
ETC……..



FERMENTER DESIGN

Basic Design of a Fermenter

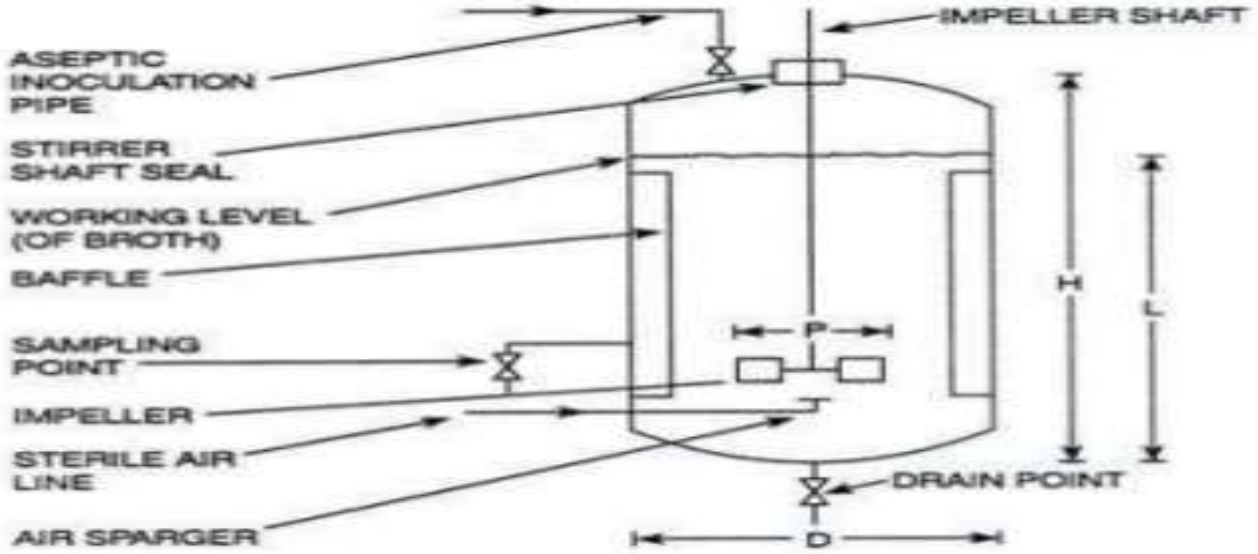


FIG. 14.3. Diagram of a fermenter with one multi-bladed impeller. H, fermenter height; L, liquid height; D, tank diameter; P, impeller diameter.

PRODUCTION OF PENICILLIN

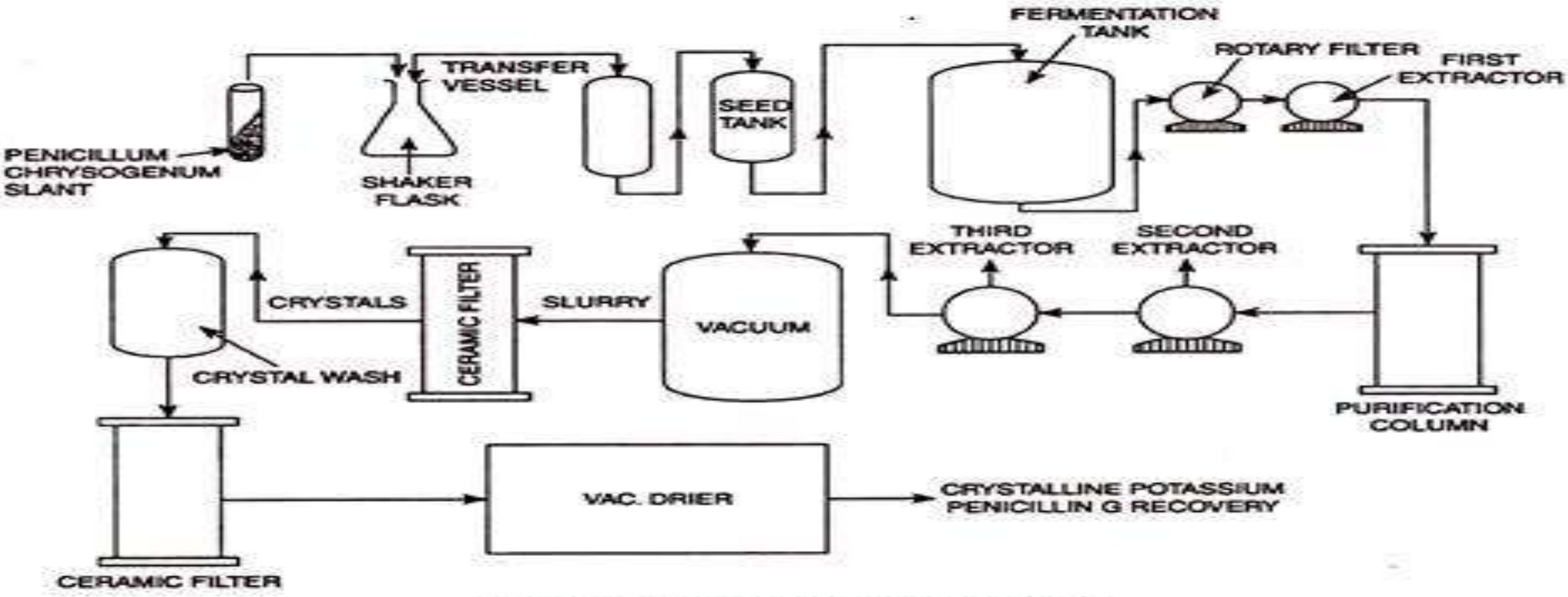


FIG. 40.7. Commercial production of penicillin

PRODUCTION OF GLUMATIC ACID

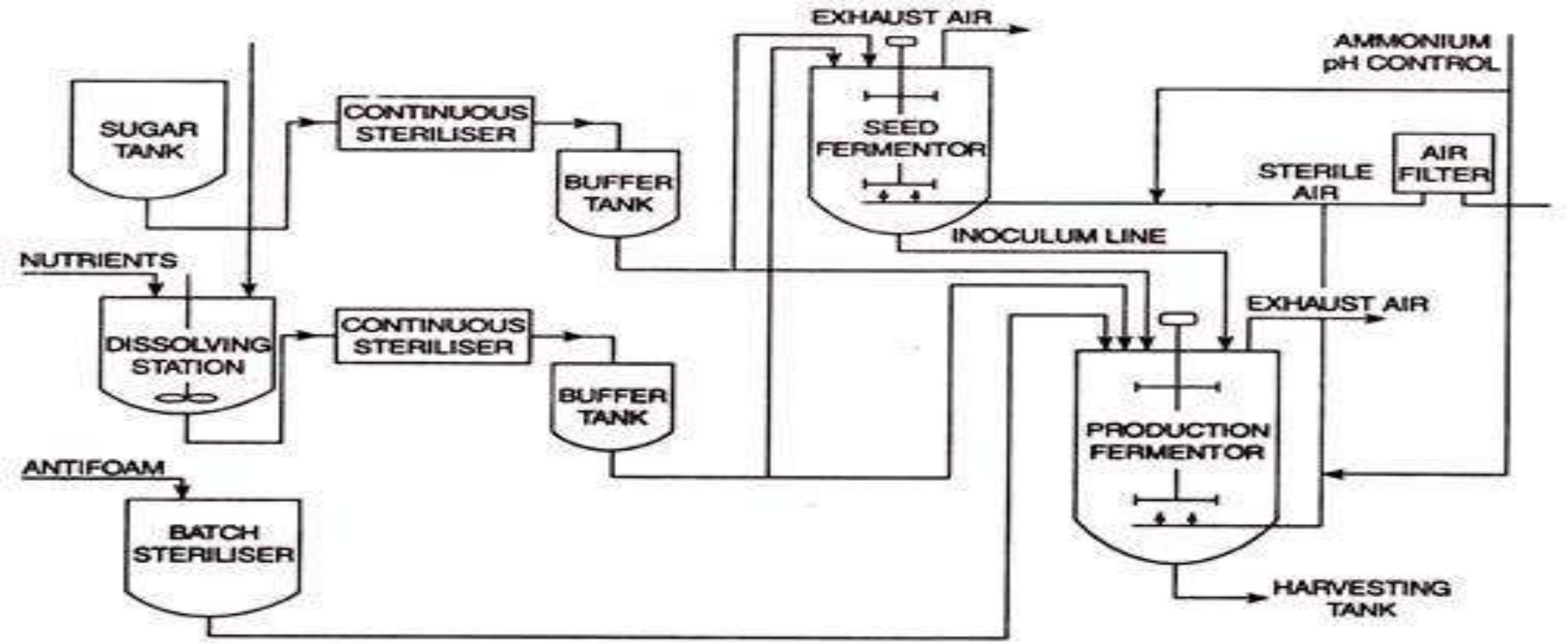
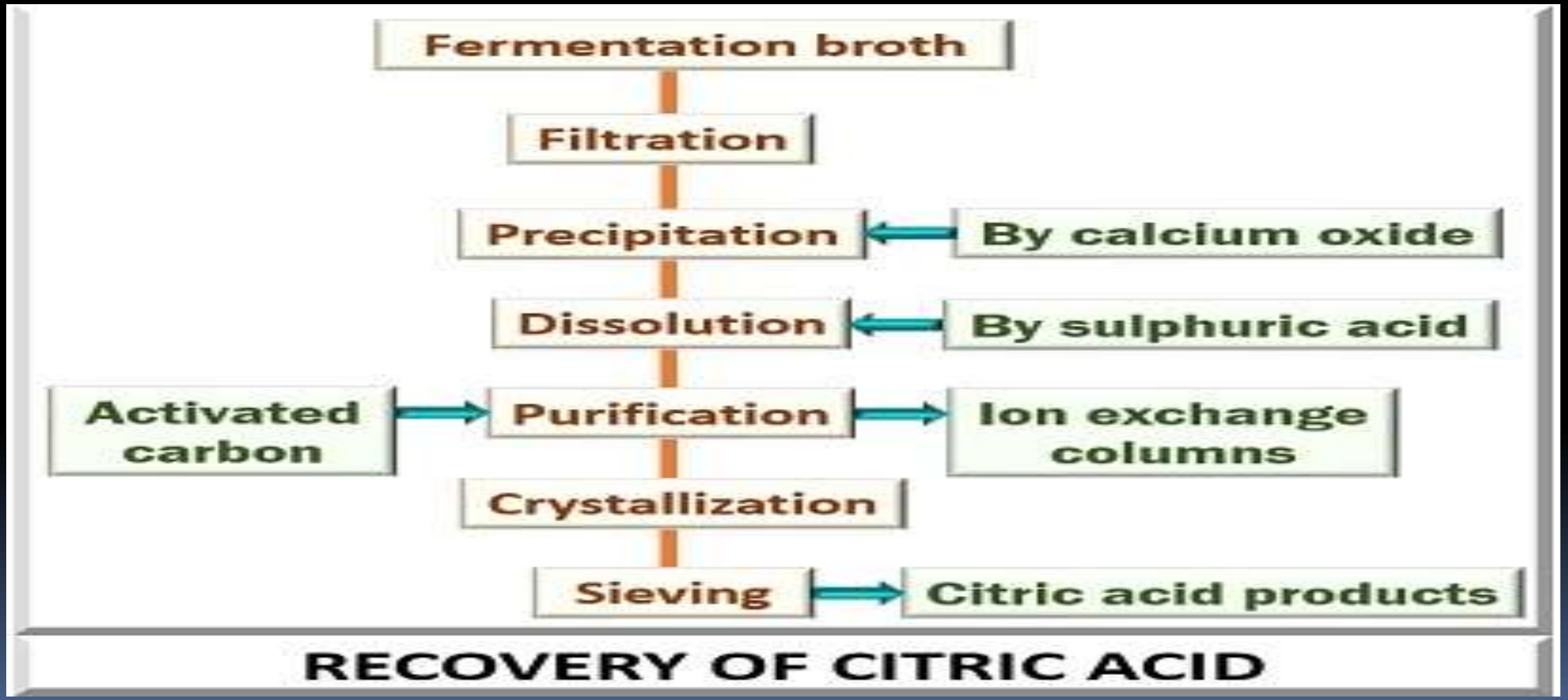
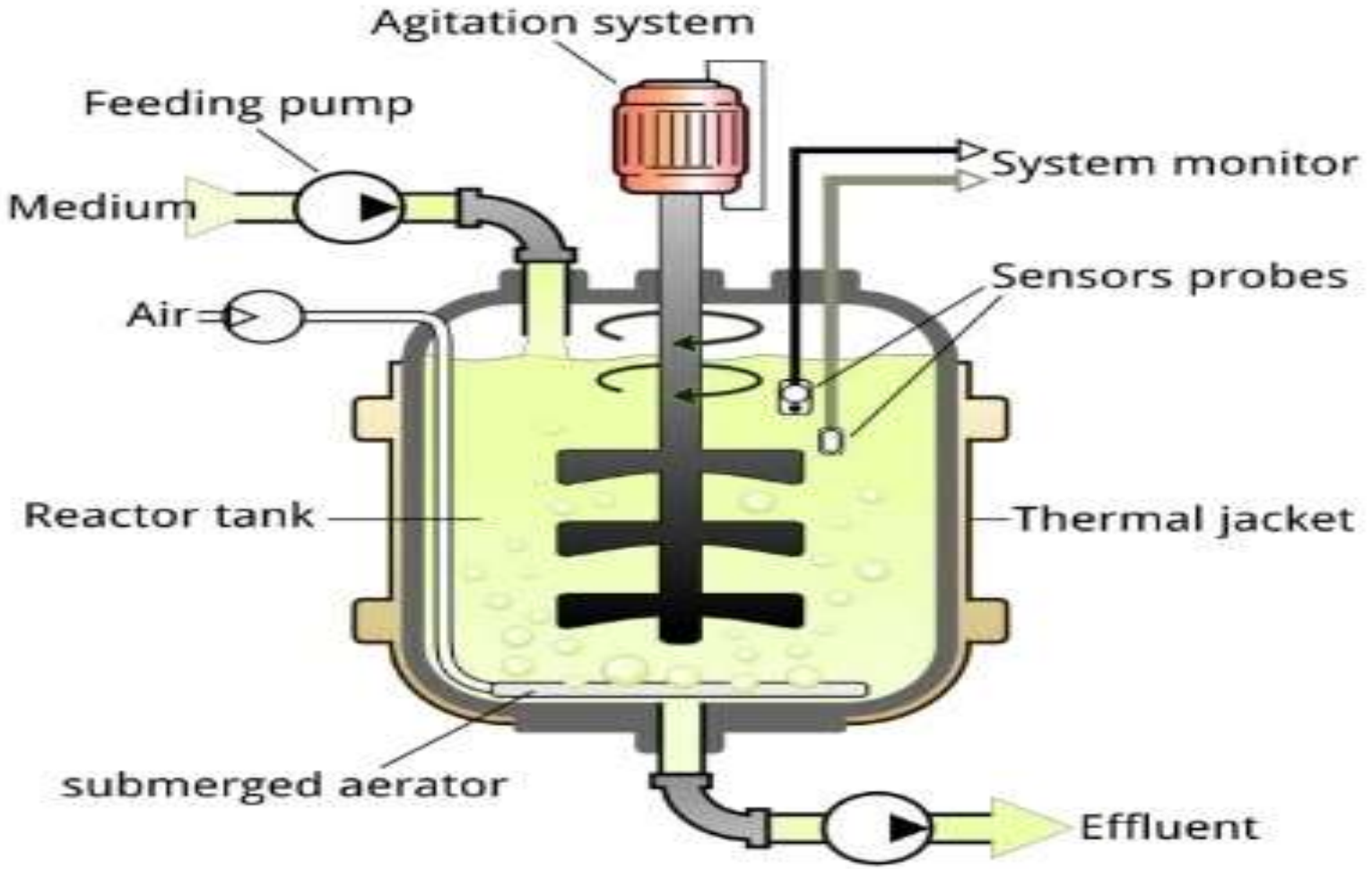


FIG. 40.6. A flow diagram of commercial production method of glutamic acid (glutamate).

PRODUCTION OF CITRIC ACID



PRODUCTION GRISEOFULVIN



FERMENTATION AS A FOOD PRESERVATION TECHNIQUE

Fermented foods are foods that have been prepared in a way so that the bacteria naturally found within them starts to ferment. Fermentation, also known as lacto-fermentation, is a chemical process in which bacteria and other micro-organisms break down starches and sugars within the foods, possibly making them easier to digest, and resulting in a product that is filled with helpful organisms and enzymes.

This process of fermentation is a natural preservative, which means that fermented foods can last a long time.

ADVANTAGES AND DIS ADVANTAGES OF FERMENTATION

- Advantages of fermentation are that lactic acid can be produced and it can produce energy for ATPs.
- Disadvantages of fermentation are that production can be slow, the product is impure and needs to have further treatment and the production carries a high cost and more energy.

IMPORTANCE OF FERMENTATION

Fermentation is important to cells that don't have oxygen or cells that don't use oxygen because:

1.It allows the cells to get 2 ATP gain from one molecule of glucose, even without oxygen.

2.Fermentation takes away the end products of glycolysis so glycolysis can continue ... freeing up the electron carriers, and so on.

3.Fermentation is important to the baking industry because it is the process that yeast uses to produce the bubbles of carbon dioxide that make the dough rise.
4.Fermentation is important in wineries and breweries because yeast uses fermentation to produce alcohol.
5.Fermentation is important in muscles because it allows the muscles to keep getting a little energy from glucose even when the oxygen supply can't keep up with the demand.

Blood Products / Substitutes

Introduction

Blood Products: : Any therapeutic substance prepared from the blood. It consists of	
Blood Components Constituent separated from whole blood	Plasma Derivatives
1. Red cell concentrate 2. Leuko-reduced RBC 3. Plasma 4. Plasma derivatives 5. Granulocyte concentrate 6. Platelet concentrate	1. Fresh frozen plasma 2. Cryoprecipitate 3. Albumin 4. Coagulation factors concentrate 5. Immunoglobulins

Blood Substitutes: It consists of

1. Volume Expanders
2. Synthetic oxygen carriers

Collection

- 350ml{301ml blood+49ml anticoagulant} is taken from previously screened person .

- Tested for- syphilis, HBsAg, HCV, HIV 1&2
- Platelet concentrates for bacterial contamination

- Group determination (ABO & Rh) & presence of any RBC antibody

- Processed into sub-components

Criteria for donor

- Normal body temperature, Blood pressure
- Weight above 50-55 kgs
- Normal Hb levels..
- Free from RTI, skin dz or blood dz
- No h/o drug addiction
- No h/o viral hepatitis
- No HIV infection or risk for it
- No h/o blood transfusion (<=6mo)

Whole Blood

- It is donor blood mixed with an anticoagulant
- Collected by venesection
- Collected in 63 ml of anticoagulant to form 450 ml.

  OR, in 49 ml " " " " " " 350 ml
- Stored at 1-6° C
- Shelf Life up to 5 weeks
- 1 unit{350ml} will increase the Hb level of an

  Adult (60-70Kg) by 0.8 gm/dl

  Pediatric pts by 1gm/dl

- Indication :

- Exchange transfusions.

- Hemorrhage (>= 20%blood loss):
  - -To ↑O_2 carrying capacity
  - -volume replacement
  - -stabilize coagulation factors

Advantages :

- simple and inexpensive
- no special equipment required for processing

Disadvantages :

- Risk of circulatory overload
- Maintaining at ~4°C leads to:
  - -platelet dysfunction
  - -degradation of coagulation factors
  - -decreased 2,3 DPG levels
- Febrile reactions
- Narrow Shelf life- 5wks

Preservation techniques

- Chemical incorporation
- Rejuvenation solutions
- Additive solutions
- Red cell freezing
- Addition of buffers

Anticoagulants

Citrate -chief component of almost all anticoagulants used

chelates Ca^{2+} .·. stops coagulation

Dextrose- energy source.

Phosphate- buffer

1. CG (sod.citrate+glucose)- 1st soln ever used

2. ACD (acid+citrate+dextrose)

3. CPD (citrate+phosphate+dextrose)- Higher pH & 2,3 DPG level is maintained. SHELF-LIFE is 21 days.

4.CPDA (+adenine)- ↑ ADP levels- ↑ glycolysis- ↑ ATP production increases RBC viability to 35 days

5.SAG-M (saline+adenine+glucose+mannitol)-

maintains cell nutrition
increases viability to 42 days,
M-prevents spontaneous hemolysis

Red cell concentrate

Blood to separate under gravity (sedimentation method)

Or,Centrifugation done in special refrigerated centrifuge

Most of the plasma is removed and replaced with a solution of glucose and adenine in saline to maintain viability of red cells.

- Maintained at temp : 2°C to 6°C
- High O_2 carrying capacity
- Optimal target for infusion- 7g/dl
- Indications:
  - replacement of red cells in anemic patients
  - acute massive blood loss

Advantages :

- Easy to prepare
- To avoid volume overload in c/o CCF
- Less chances of infection or alloimmunization
- Less immunosuppressant
- Dec. allergic reaction if plasma is also removed

Disadvantages :

- High Red cells to plasma ratio ⟶ ↑viscosity
  ∴ ↑ time required for passing through cannula & vessels

thus Hct should not exceed 80%

Platelet concentrates

- Platelets separated from plasma obtained after 4-6 donations are pooled

  or,

  from a single donor by plateletapheresis

- Composed of mainly platelets, some nonfunctional WBCs, few RBCs & plasma[maintains pH].

volume= 50 ml contains 5.5x10^9/lt plts

Stored at 20-24^{o}C

Shelf life- 5 days.. Once opened, transfuse within 6 hrs

1 unit of PC increases platelet count by: 5000-10000(adults)

20,000 (children)

75,000-1,00,000(infants)

If single donor- 1 unit of PC should contain >5.5 x 10^9 platelets

If pooled then, > 250x 10^9 platelets

- Indications:
  - Thrombocytopenia
  - Platelet dysfunction
  - Complication of anti-platelet therapy(Clopidogrel)
  - DIC

Fresh Frozen Plasma

- Plasma removed from a unit of whole blood & frozen (by immersing in a solid carbondioxide and ethyl alcohol mixture) at/ below -25° C within 4 hrs. ofcollection.
- Stored at -40 to -50°C
- Composed of plasma, all coagulation factors, albumin & Ig
- 1 unit= 200-250ml
  - Each unit of FFP increases the level of each clotting factor by 2-3 %
- Shelf life: Frozen—1 year(<-30 degree centigrade)

Indications:

-active bleeding in pts with multiple factor deficiencies

-surgery in patient with liver failure(treatment of choice)
-after massive transfusion
-DIC

-rapid reversal of warfarin(Prothrombim complex concentrates,with factors II,IX,X can also be given)
-TTP

Advantage:

it is a acellular component so no chance of transmission of intracellular infection

Cryoprecipitate

- When FFP is allowed to Thaw at 4°C, glutinous precipitate remains and, if the suprenatant plasma is removed, cryoprecipitate
- contains factors VIII (very rich) , fibrinogen as well as factor XIII & vwf.
- Shelf life:2 years , if frozen at -40°C

Used

1. If fibrinogen <1g/dl due to dilution
2. DIC
3. Von Willebrand disease and hemophilia VIII deficiency

Coagulation Factors

Factor VIII concentrate

- Prepared from large pools of donor plasma by fractionation process.
- Commercially prepared, lyophilized powder
- Hemophilia A & von Willebrand's disease.
- Storage : 2 to 6°C

Factor IX

- Commercially prepared, lyophilized powder
- Hemophilia B
- Contains Factor II (prothrombin), VII,IX,X.
- Purified Factor IX contains only IX.
- Refrigerated at 35-45 °F

Immunoglobulin

- It is a concentrated solution of IgG antibody component of plasma
- Prepared from large pools of donors
- Uses
  1. To reduce infective complications in patients with Ab deficiencies
  2. Immunological d/o- Immune thrombocytopenia, GBS
  3. Anti-zoster Ig in varicella zoster prophylaxis
  4. Anti-Rhesus D Ig in pregnancy to prevent hemolytic dsz in newborn
- Can Cause
  - Acute renal failure (in elderly)
  - Acute reactions

Fibrinogen

- Prepared by organic liquid fractionation of plasma
- Stored in dried form
- Can be reconstituted with distilled water
- Used in cases of severe fibrinogen depletion

  eg DIC, congenital afibrinogenaemia
- Carry high risk of hepatitis

Blood Substitutes

1. volume expanders
2. synthetic oxygen carriers

1. Volume expanders: inert compounds

• crystalloid-based	• colloid-based
▪Ringer's lactate ▪Normal saline ▪D5W (dextrose 5% in water) ▪Dextrose with normal saline ▪Hypertonic saline	• Dextrans • Albumin • Gelofusin • Hydroxyethyl starch

Crystalloids

1)) Ringer's Lactate Solution(Hartman Solution)

a) Consists of Na, Cl, K, Ca, Lactate. pH=6.5, Osmolarity=273mmol/lt (slightly hypotonic)

b) Blood should not be given through the same drip set as it contains Ca.

c) Crystalloid of choice for blood loss replacement.

2)) Normal Saline {0.9% NaCl (isotonic)}

a) Preferred over RL for treating

- Hypochloremic metabolic alkalosis
- Brain injury (Ca^{2+} and lactate can increase the neuronal injury)
- Hyponatremia

- ***3)5% Dextose***
  a) Is isotonic, but with the metabolism of glucose
  - inside the body, becomes hypotonic.
  b) Blood cannot be given through the same drip set otherwise rouleaux formation will cause clumping of RBCs

4) Dextrose Normal Saline (DNS)

a) Is hypertonic

b) But 1/5 NS +4.3% dextose and ¼ NS +5% dextrose are isotonic and are best used as maintenance fluids.

Colloids

- ***1) Dextrans (Lomodex)***
  - Polysaccharides, can be stored for 10 years, half life=2-8 hours
  - Advantages
    a) Non toxic, neutral and chemically inert
    b) Low molecular wt dextran improves microcirculation
  - Drawbacks
    a) Interfere with blood grouping and cross matching (by causing RBC aggregation, so a blood sample should be taken before-hand)
    b) Interferes with platelet function and is a/w abnormal bleeding (so total volume given should not exceed 1000ml)
    c) Can cause severe anaphylaxis
    d) Large molecular weight dextrans can block renal tubules
    e) ARDS (rarely) because of direct toxic effect on pulmonary capillaries

- ***2) Albumin(available as 5% and 20% solution)***
  - Very expensive, intravascular half-life= 10-15days
  - Used in protein loss like, peritonitis, liver failure, burns, protein losing enterepathies.
  - 20% is hypertonic and expands plasma volume by more than the amount infused.
  - Stored for several months in liquid form at 4degree
- ***3)Gelatins (Haemaccel)(available as 3.5% solution)***
  - Consists of Gelatin, Na, Cl, K, Ca
  - Expand plasma effectively for 2 hours
  - At clinically used doses, these do not interfere with blood grouping, plt fxn and clotting but at high doses can interfere clotting.
  - As it contains high Calcium, citrated blood should not be mixed.

Synthetic oxygen carriers:

mimic blood's O_2 transport ability

Types

1. Abiotic - perfluorocarbons, perfluoroctyl bromide
2. Biomimetic - HBOC(Hb based oxygen carriers)

- recombinant erythropoietin, hemoglobin under clinical trials

some available products:

Hemopure
Oxygent
PolyHeme

Perfluorocarbons

- High O_2 carrying capacity
- Emulsified and transfused
- Short survival in the circulation
- Adverse effects : Flu like symptoms.
  Immunologic effects.

HBOC

- Hb synthesised by controlled lysis of Red Cells
- Short half life as compared to RBCs
- Side effects :
  GI distress, neurotoxicity,
  vasoconstriction,
  interfere with macrophage system.

THANKS