

## **UNIT I: DESIGN OF FERMENTER AND KINETICS**

### **CONTENTS**

#### **1.0 AIMS AND OBJECTIVES**

##### **1.1 INTRODUCTION**

##### **1.2 DESIGN OF FERMENTER**

##### **1.3 TYPES OF FERMENTERS**

##### **1.4 CONTROL AND MONITORING FERMENTATION SYSTEM**

##### **1.5 PID CONTROL SYSTEMS**

##### **1.6 TYPES OF FERMENTATION**

##### **1.7 MICROBIAL KINETICS**

##### **1.8 LET US SUM UP**

##### **1.9 POINTS FOR DISCUSSION**

##### **1.10 LESSON - END ACTIVITIES**

##### **1.11 REFERENCES**

#### **1.0 AIMS AND OBJECTIVES**

This unit imparts knowledge on the various modes of operation of fermentation and important criteria in designing a fermenter.

#### **1.1 INTRODUCTION**

Biotechnology is the culmination of more than 8000 years of human experience using living organisms and the process of fermentation to make products such as bread, cheese, beer and wine. Today biotechnology is applied to manufacturing processes used in health care, food and agriculture, industrial and environmental cleanup, among other applications. A widely accepted definition of Biotechnology is "Application of scientific and engineering principles to processing of materials by biological agents to provide goods and service". Some other definitions replace rather ambiguous word 'biological agents' with more specific words such as microorganisms, cells, plant and animal cells and enzymes. When a biotechnological process is implemented on a commercial scale there is every reason to believe that it will be in some bioreactor or fermenter. The entire process can be divided in three stages.

Stage I : Upstream processing which involves preparation of liquid medium, separation of particulate and inhibitory chemicals from the medium, sterilization, air purification etc.,

Stage II: Fermentation which involves the conversion of substrates to desired product with the help of biological agents such as microorganisms; and

Stage III: Downstream processing which involves separation of cells from the fermentation broth, purification and concentration of desired product and waste disposal or recycle.

A fermentation process requires a fermenter for successful production because it provides the following facilities for the process such as contamination free environment, specific temperature maintenance, maintenance of agitation and aeration, pH control, monitoring Dissolved Oxygen (DO), ports for nutrient and reagent feeding, ports for inoculation and

sampling, fittings and geometry for scale up, minimize liquid loss and growth facility for wide range of organisms.

Aseptic environment or contamination is defined as protection against entry of unwanted organisms. Containment is defined as prevention of escape of viable cells from the process. Both these environment is provided by a fermenter where ever required. Contamination is applicable in all process whereas containment is necessary when pathogenic organism is used for the fermentation process. The containment level varies based on the pathogenicity of the organism used. Some organism are termed **GRAS** ie. Generally Recognized As Safe. Criteria for assessment of hazardous organism are known pathogenicity of organism, virulence level, number of organisms required to initiate infection, routes of infection, known incidence of infection, local existence of vectors and reserves of micro organisms, volume of organisms used in process, techniques used for cultivation and harvesting and prophylaxis and treatment facility. Based on all the criteria if an organism is termed pathogenic the containment of the fermentation process is maintained. Good industrial large scale practice (**GILSP**) involves safe and highly productive organism for the process.

Depending on the type of product, the concentration levels it is produced and the purity desired, the fermentation stage might constitute anywhere between 5-50% of the total fixed and operating costs of the process. Therefore, optimal design and operation of bioreactor frequently dominates the overall technological and economic performance of the process.

In any biological process, the following are unique features.

- (a) The concentrations of starting materials (substrates) and products in the reaction mixture are frequently low; both the substrates and the products may inhibit the process. Cell growth, the structure of intracellular enzymes, and product formation depend on the nutritional needs of the cell (salts, oxygen) and on the maintenance of optimum biological conditions (temperature, concentration of reactants, and pH) with in narrow limits.
- (b) Certain substances inhibitors effectors, precursors, metabolic products influence the rate and the mechanism of the reactions and intracellular regulation.
- (c) Microorganisms can metabolize unconventional or even contaminated raw materials (cellulose, molasses, mineral oil, starch, ores, wastewater, exhaust air, biogenic waste), a process which is frequently carried out in highly viscous, non-Newtonian media.
- (d) In contrast to isolated enzymes or chemical catalysts, microorganisms adapt the structure and activity of their enzymes to the process conditions, whereby selectivity and productivity can change. Mutations of the microorganisms can occur under sub optimal biological conditions.
- (e) Microorganisms are frequently sensitive to strong shear stress and to thermal and chemical influences.
- (f) Reactions generally occur in gas-liquid -solid systems, the liquid phase usually being aqueous.
- (g) The microbial mass can increase as biochemical conversion progresses. Effects such as growth on the walls, flocculation, or autolysis of microorganisms can occur during the reaction.
- (h) Continuous bioreactors often exhibit complicated dynamic behavior.

Due to above mentioned demands made by biological systems on their environment, there is no universal bioreactor. However, the general requirements of the bioreactor are as follows:

- (a) The design and construction of biochemical reactors must preclude foreign contamination (sterility). Furthermore, monoseptic conditions should be maintained during the fermentation and ensure containment.
- (b) Optimal mixing with low, uniform shear achieved by proper designing of agitator and aerator
- (c) Adequate mass transfer (oxygen) achieved by monitoring the speed of agitator and agitator
- (d) Clearly defined flow conditions that can be maintained by proper opening valves and monitoring devices
- (e) Feeding of substrate with prevention of under or overdosing by proper feed ports and monitoring
- (f) Suspension of solids
- (g) Gentle heat transfer
- (h) Compliance with design requirements such as: ability to be sterilized; simple construction; simple measuring, control, regulating techniques; scaleup; flexibility; long term stability; compatibility with up- downstream processes; antifoaming measures.

## 1.2 DESIGN OF FERMENTER

### 1.2.1 Components of fermenter

1. Basic component includes drive motor, heaters, pump, etc.,
2. Vessels and accessories
3. Peripheral equipment (reagent bottles)
4. Instrumentation and sensors

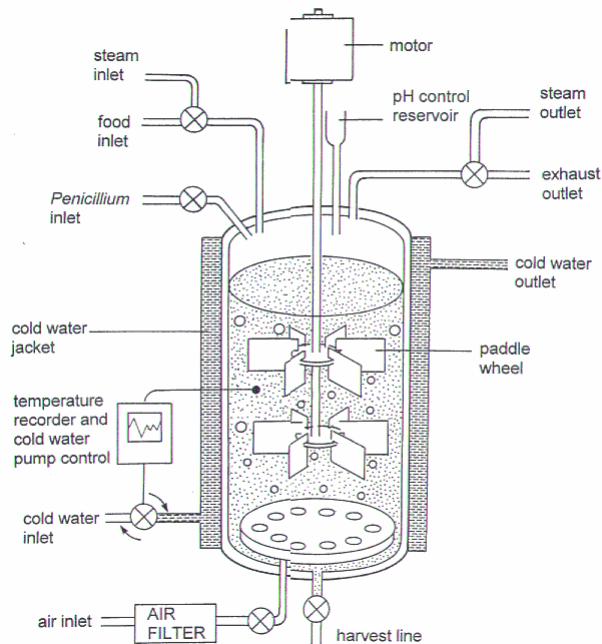
Various components of an ideal fermenter for batch process are:

S.No.	Part	Purpose
1	Top plate	cover (made of steel)
2	Clamp	top plate compressed onto vessel using clamp
3	Seal	separates top plate from vessel (glass) to prevent air leakage
4	Vessel	glass, jacketed, steel with ports for various outputs, inputs, probes etc
5	Drive motor	used to drive mixing shaft
6	Drive shaft	mixes the medium evenly with its impeller
7	Marine impeller	for plant tissue culture
8	Baffles	prevent sedimentation on sides and proper mixing
9	Sparger	air supplier / after filtration via membranes – ensures efficient dispersal – by attached to impeller
10	Exit gas cooler	like condenser remove as much moisture as possible from exhaust
11	Inoculation needle	port to add inoculum
12	Feed pumps	regulates the flow rates of additives (medium, nutrients) variable speed
13	Peristaltic pumps	fixed speed pumps – used for continuous sampling
14	Syringe pump	using a syringe – mostly used in batch

15	Exit gas analysis	CO <sub>2</sub> analyzer, O <sub>2</sub> analyzer, mass spectrometer
16	Sample pipe	through which samples are drawn
17	3 way inlet	to insert different probes

Monitoring and controlling parts of fermenter are:

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



**Fig.1** Ideal fermenter

### 1.2.2 BODY CONSTRUCTION

**1.2.3 Construction materials** differ with small scale, pilot and large scale. In small scale for vessel construction glass or stainless steel may be used. For pilot and large scale process, stainless steel (>4% chromium), mild steel (coated with glass or epoxy material), wood, plastic or concrete may be used as vessel construction material. Any vessel used should not have any corners and smooth surface is essential. The construction material must be non toxic and corrosion proof.

### **Glass vessel (borosilicate glass)**

Type I – glass vessel round or flat bottom with top plate. It can be sterilized by autoclaving and the largest diameter is 60cm.

Type II – glass vessel flat bottom with top and bottom stainless steel plate. This type is used in *in situ* sterilization process and the largest diameter 30cm.

### **Stainless steel**

Stainless steel is used as vessel construction material with the following modifications,

1. >4% chromium (atleast 10-13%) may be added
2. film of thin hydrous oxide - non-porous, continuous, self healing, corrosion resistance
3. inclusion of nickel - improves engineering
4. presence of molybdenum - resistance to halogen salts, brine, sea water
5. tungsten, silicone - improve resistance

Thickness of vessel should be increased with scale. Side plates have lower thickness than top and bottom plates. Top and bottom plate are hemispherical to withstand pressures.

### **1.2.4 SEALING**

Sealing between top plate and vessel is an important criteria to maintain airtight condition, aseptic and containment. Sealing have to be done between three types of surfaces *viz.* between glass-glass, glass- metal and metal-metal. There are three types of sealing. They are gasket, lipseal and 'O' ring. This sealing ensures tight joint in spite of expansion of vessel material during fermentation. The materials used for sealing may be fabric-nitryl or butyl rubbers. The seals should be changed after finite time. There are two way of sealing in O ring type simple sealing and double sealing with steam between two seals.

### **1.2.5 BAFFLES**

Baffles are metal strips that prevent vortex formation around the walls of the vessel. These metal strips attached radially to the wall for every 1/10<sup>th</sup> of vessel diameter. Usually 4 baffles are present but when the vessel diameter is over 3dm<sup>3</sup> around 6-8 baffles are used. There should be enough gap between wall and baffle so that scouring action around vessel is facilitated. This movement minimizes microbial growth on baffles and fermentation walls. If needed cooling coils may be attached to baffles.

### **1.2.6 AERATION SYSTEM (SPARGER)**

Sparger is a device for introducing air into fermenter. Aeration provides sufficient oxygen for organism in the fermenter. Fine bubble aerators must be used. Large bubbles will have less surface area than smaller bubbles which will facilitate oxygen transfer to a greater extent. Agitation is not required when aeration provides enough agitation which is the case Air lift fermenter. But this is possible with only for medium with low viscosity and low total solids. For aeration to provide agitation the vessel height/diameter ratio (aspect ration) should be 5:1. Air supply to sparger should be supplied through filter.

There are three types of sparger *viz.* porous sparger, orifice sparger and nozzle sparger.

1. Porous sparger: made of sintered glass, ceramics or metal. It is used only in lab scale-non agitated vessel. The size of the bubble formed is 10-100 times larger than pore size. There is a pressure drop across the sparger and the holes tend to be blocked by growth which is the limitation of porous sparger.
2. Orifice sparger: used in small stirred fermenter. It is a perforated pipe kept below the impeller in the form of crosses or rings. The size should be ~  $\frac{3}{4}$  of impeller diameter. Air holes drilled on the under surfaces of the tubes and the holes should be atleast 6mm diameter. This type of sparger is used mostly with agitation. It is also used with out agitation in some cases like yeast manufacture, effluent treatment and production of SCP.
3. Nozzle sparger: Mostly used in large scale. It is single open/partially closed pipe positioned centrally below the impeller. When air is passed through this pipe there is lower pressure loss and does not get blocked.
4. Combined sparger agitator: This is air supply via hallow agitator shaft. The air is emitted through holes in the disc or blades of agitator.

### **1.2.7 EXIT GAS COOLER**

Similar to liebig condenser, condenses the moisture from the exhaust gas in the fermenter. This removes as much moisture as possible from the gas leaving the fermenter and prevent excess fluid loss.

### **1.2.8 AGITATION**

Agitation provides uniform suspension of cells in homogenous nutrient medium. This agitation provides bulk fluid and gas phase mixing, air dispersion, facilitates oxygen transfer and heat transfer and uniform environment through out the vessel. There are four classes, namely Disc turbine, Vaned disc, Open turbine of variable pitch and Marine impeller.

Disc turbine prevents flooding by air bubbles. Flooding occurs when the air bubble is not properly dispersed the air pocket is formed one area. Flooded only at 120min/hour of air discharge when disc turbine is used. When open turbine and propeller are used the medium is flooded at 21min per hour of air discharge.

Difference between disc turbine and open turbine is as follows:

Disc turbine	Open turbine
Prevent flood by air bubbles till 120min/hour	Prevent flooding only till 21min/hour
radial flow	Axial flow
Disc forces air to tip of agitator to be dispersed	disc is absent

Rushton disc turbine with 1/3 of fermentor diameter has been optimum for some fermentation process. Now recent designs of agitator have been introduced. Scaba is a new design of agitator that can handle high flow rate before flooding and has Radial flow. But this is not ideal for top to bottom mixing. Prochem maxflow agitator has low power conception with high hydrodynamic thrust. This design has increased downward pumping capacity of blades. In this design agitator/ vessel diameter ratio is 0.4. Appoximately 66% less power requirement even

when viscous and oxygen transfer efficiency improved. Intermig agitator has two units. Unlike the earlier design agitator/ vessel diameter ratio is 0.6-0.7. For this agitator larger air sparger is used and top to bottom mixing not efficient. New turbine designs with dual impeller have been introduced. One for gas disperser and other for aiding circulation with multirod mixing.

### **1.2.9 STIRRER GLANDS AND BEARINGS**

The entry point of stirrer into fermenter may be from top to bottom or sides. Mostly used from bottom so that that leaves more space for entry ports on top. There are four types of stirrer glands and bearings.

- 1) Stuffing box
  - a. sealed by several layers of packing rings of asbestos or cotton yarn-pressed against the shaft by a gland follower
  - b. At high speeds- packing wears – pressure should be applied to ensure tightness
  - c. Difficult to sterilize- satisfactory heat penetration
  - d. Sufficient for GILSP containment
- 2) Mechanical seal
  - a. 2 parts; i) stationary in the bearing housing, ii) other rotates on the shaft.
  - b. Two parts pressed together by springs or expanding bellows
  - c. Steam condensate use to lubricate and cool seals
  - d. safe for containment
  - e. double mechanical seal for level 2
  - f. at level 2 and 3, the condensate is piped to a kill tank
  - g. Disinfectants flushed through the seal
  - h. steam condensate outlet monitoring indicates any seal failure
- 3) Magnetic drives (some animal cell cultures)
  - a. shaft does not pierce the vessel
  - b. two magnets- one driving, held in bearing in housing on outside of head plate and one driven, placed on one end of impeller shaft held in bearing in suitable housing
  - c. ceramic magnets –magnetic power cross 16mm gap
  - d. 300 – 2000 rpm rotation possible
- 4) Simple bush seals
 

Disadvantage of double seals are more difficult to assemble, difficult to detect failure of seal from normal and dead spaces and seals leading to contamination. Hence simple bush seal is preferred in some cases.

### **1.2.10 VALVES AND STEAM TRAPS**

#### **1.2.10.1 Addition valves**

There are four types of addition valves *viz.*(a) Simple ON and OFF, (b) For coarse control, (c) Accurate adjustment and (d) Safety valve-flow in one direction.

There are different models of valves.

1. Opening and closing, raising or lowering of blocking unit
  - a. Gate valve - a sliding disc move in / out of flow path by a turn of the stem

- b. Globe valve - horizontal disc / plug – raised / lowered
  - c. Piston valve - similar to globe valve except a piston controls flow
  - d. Needle valve - similar to globe valve except disc replaced with tapered plug / needle
2. Drilled sphere / plug
- a. Plug valve - parallel / tapered plug with orifice – on 90° turn closes / open the flow path
  - b. Ball valve - similar to plug valve – except a ball (ss) with orifice replaces the plug
3. Disc rotating between bearings  
Butterfly valve - a disc rotates about a shaft – closes against seal to stop flow
4. Rubber diaphragm / tube pinching
- a. Diaphragm valve - similar to pinch valve – except not pinching, but pushing from one side against a diaphragm
  - b. Pinch valve - flexible sleeve closed by a pair of pinch bars (rubber, neoprene etc.)

Based on the four type of applications, the valves are chosen.

ON /OFF application – Globe, Butterfly

Crude flow control – Gate valve

Accurate control – Needle valve

Very sterile operation – Pinch / Diaphragm

#### **1.2.10.2 Check valves**

Valves used to prevent accidental reversal flow of liquid or gas due to break down. There are three types – swing check, lift check, combined stop and check.

#### **1.2.10.3 Pressure control valves**

These types of valves are used for two purposes.

- a) Pressure reduction
- b) Pressure retaining

#### **1.2.10.4 Safety valve**

There are types of safety valve by which the increase in pressure is released. They are,

- a) A spindle lifted from its seating against the pressure – releases pressure
- b) Bursting / rupturing of discs to release pressure

In case of releasing the gas, the escaping gas must be treated before release.

#### **1.2.10.5 STEAM TRAPS**

This steam trap is important to remove any steam condensate. There are two components viz. valve and seat assembly and open / close device. The operation of the component is based on,

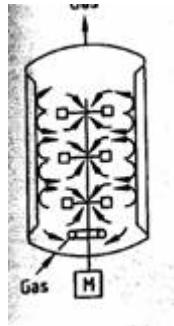
- i) density of fluid : A float (ball / bucket) float in water, sink in steam. When it floats it closes and when it sinks it opens the valve
- ii) temperature of fluid : It has water / alcohol mixture which senses the change in temperature. This mixture expands in hot steam and closes the valve. When it contracts in cool water opens the valve.
- iii) kinetic effect of fluid in motion : if a low density steam is flowing it will be high velocity. Like wise high density will flow with low velocity. The conversion of pressure energy into kinetic energy control the opening and closing

### **1.3 TYPES OF FERMENTERS**

The main function of a fermenter is to provide a controlled environment for the growth of microorganisms or animal cells, to obtain a desired product. Few of the bioreactor types are discussed below:

#### **1.3.1 STIRRED TANK FERMENTER**

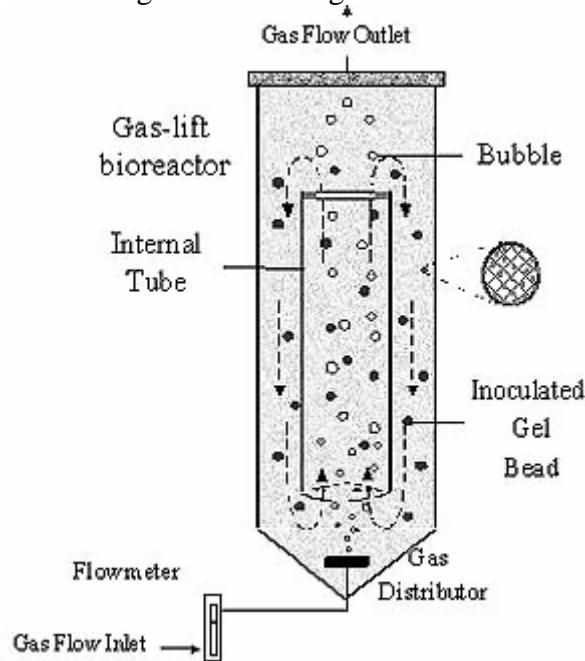
Microbial fermentations received prominence during 1940's namely for the production of life saving antibiotics. Stirred tank reactor is the choice for many (more than 70%) though it is not the best. Stirred tank reactor's have the following functions: homogenization, suspension of solids, dispersion of gas-liquid mixtures, aeration of liquid and heat exchange. The Stirred tank reactor is provided with a baffle and a rotating stirrer is attached either at the top or at the bottom of the bioreactor. The typical decision variables are: type, size, location and the number of impellers; sparger size and location. These determine the hydrodynamic pattern in the reactor, which in turn influence mixing times, mass and heat transfer coefficients, shear rates etc. The conventional fermentation is carried out in a batch mode. Since stirred tank reactors are commonly used for batch processes with slight modifications, these reactors are simple in design and easier to operate. Many of the industrial bioprocesses even today are being carried out in batch reactors though significant developments have taken place in the recent years in reactor design, the industry, still prefers stirred tanks because in case of contamination or any other substandard product formation the loss is minimal. The batch stirred tanks generally suffer due to their low volumetric productivity. The downtimes are quite large and unsteady state fermentation imposes stress to the microbial cultures due to nutritional limitations. The fed batch mode adopted in the recent years eliminates this limitation. The Stirred tank reactor's offer excellent mixing and reasonably good mass transfer rates. The cost of operation is lower and the reactors can be used with a variety of microbial species. Since stirred tank reactor is commonly used in chemical industry the mixing concepts are well developed. Stirred tank reactor with immobilized cells is not favored generally due to attrition problems; however by separating the zone of mixing from the zone of cell culturing one can successfully operate the system.



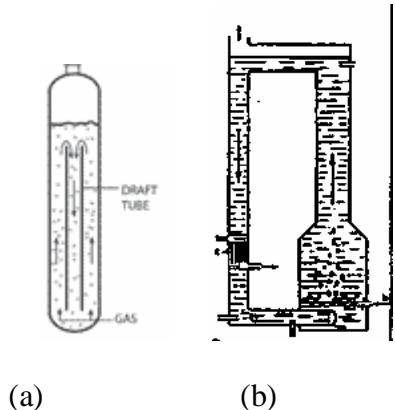
**Fig.2** Stirred tank fermenter

### 1.3.2 AIR-LIFT FERMENTER

Airlift fermenter (ALF) is generally classified as pneumatic reactors without any mechanical stirring arrangements for mixing. The turbulence caused by the fluid flow ensures adequate mixing of the liquid. The draft tube is provided in the central section of the reactor. The introduction of the fluid (air/liquid) causes upward motion and results in circulatory flow in the entire reactor. The air/liquid velocities will be low and hence the energy consumption is also low. ALFs can be used for both free and immobilized cells. There are very few reports on ALFs for metabolite production. The advantages of Airlift reactors are the elimination of attrition effects generally encountered in mechanical agitated reactors. It is ideally suited for aerobic cultures since oxygen mass transfer coefficient are quite high in comparison to stirred tank reactors. This is ideal for SCP production from methanol as carbon substrate. This is used mainly to avoid excess heat produced during mechanical agitation.



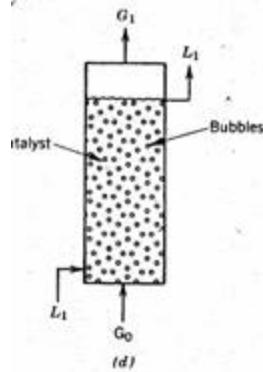
**Fig.3** Air-lift fermenter



**Fig.4** (a) Inner loop air lift fermenter (b) Outer loop air lift fermenter

#### 1.3.4 FLUIDISED BED BIOREACTOR

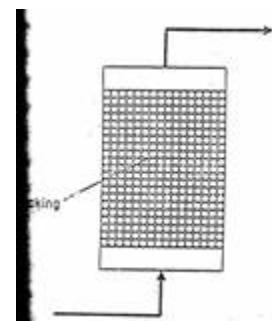
Fluidized bed bioreactors (FBB) have received increased attention in the recent years due to their advantages over other types of reactors. Most of the FBBs developed for biological systems involving cells as biocatalysts are three phase systems (solid, liquid & gas). The fundamentals of three phase fluidization phenomena have been comprehensively covered in chemical engineering literature. The FBBs are generally operated in co-current upflow with liquid as continuous phase and other more unusual configurations like the inverse three phase fluidized bed or gas solid fluidized bed are not of much importance. Usually fluidization is obtained either by external liquid re-circulation or by gas fed to the reactor. In the case of immobilized enzymes the usual situation is of two-phase systems involving solid and liquid but the use of aerobic biocatalyst necessitate introduction of gas (air) as the third phase. A differentiation between the three phase fluidized bed and the airlift bioreactor would be made on the basis that the latter have a physical internal arrangement (draft tube), which provides aerating and non-aerating zones. The circulatory motion of the liquid is induced due to the draft tube. Basically the particles used in FBBs can be of three different types: (i) inert core on which the biomass is created by cell attachment. (ii) Porous particles in which the biocatalyst is entrapped.(iii) Cell aggregates/ flocs (self-immobilization). In comparison to conventional mechanically stirred reactors, FBBs provide a much lower attrition of solid particles. The biocatalyst concentration can significantly be higher and washout limitations of free cell systems can be overcome. In comparison to packed bed reactors FBBs can be operated with smaller size particles without the drawbacks of clogging, high liquid pressure drop, channeling and bed compaction. The smaller particle size facilitates higher mass transfer rates and better mixing. The volumetric productivity attained in FBBs is usually higher than in stirred tank and packed bed bioreactors. There are several successful examples of using FBBs in bioprocess development.



**Fig.5 Fluidised bed bioreactor**

### 1.3.5 PACKED BED BIOREACTOR

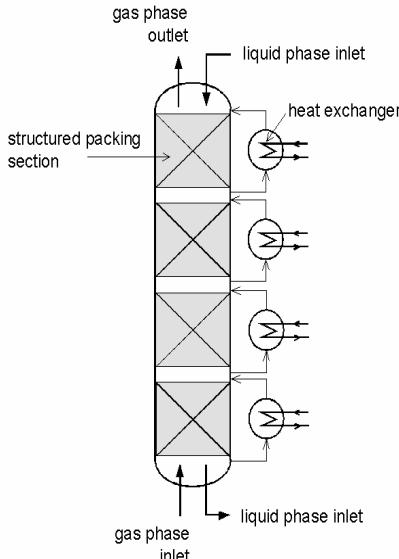
Packed bed or fixed bed bioreactors are commonly used with attached biofilms especially in wastewater engineering. The use of packed bed reactors gained importance after the potential of whole cell immobilization technique has been demonstrated. The immobilized biocatalyst is packed in the column and fed with nutrients either from top or from bottom. One of the disadvantages of packed beds is the changed flow characteristic due to alterations in the bed porosity during operation. While working with soft gels like alginates, carragenan etc the bed compaction which generally occurs during fermentation results in high pressure drop across the bed. In many cases the bed compaction was so severe that the gel integrity was severely hampered. In addition channeling may occur due to turbulence in the bed. Though packed beds belong to the class of plug flow reactors in which backmixing is absent in many of the packed beds slight amount of backmixing occurs which changes the characteristics of fermentation. Packed beds are generally used where substrate inhibition governs the rate of reaction. The packed bed reactors are widely used with immobilized cells. Several modifications such as tapered beds to reduce the pressure drop across the length of the reactor, inclined bed, horizontal bed, rotary horizontal reactors have been tried with limited success.



**Fig.6 Packed bed bioreactor**

### 1.3.6 BUBBLE COLUMN FERMENTER

Bubble column fermenter is a simplest type of tower fermenter consisting of a tube which is air sparged at the base. It is an elongated non-mechanically stirred fermenter with an aspect ratio of 6:1. This type of fermenter was used for citric acid production.



**Fig.7** Bubble column fermenter

## 1.4 CONTROL AND MONITORING FERMENTATION SYSTEM

The integral part of a high-quality bioreactor is a process controller. Such a controller is commonly specially formed for a definite bioreactor brand. This is rather connected with the fact that microorganism cultivation processes have relatively high requirements in respect to precision and sophistication. All this is despite the fact that almost all bioreactors monitor and regulate the same values actually invariably.

There are three types of sensors used in fermenter. They are,  
In-line sensors form integral part of fermenter. The directly measured value controls the process.  
Eg. Antifoam probe

On-line sensors form integral part of fermenter. The measured value must be entered into control system to control process. Eg. Ion specific sensors, mass spectrophotometer.  
Off-line sensors do not form integral part of fermenter. The measured value must be entered into control system for data collection.

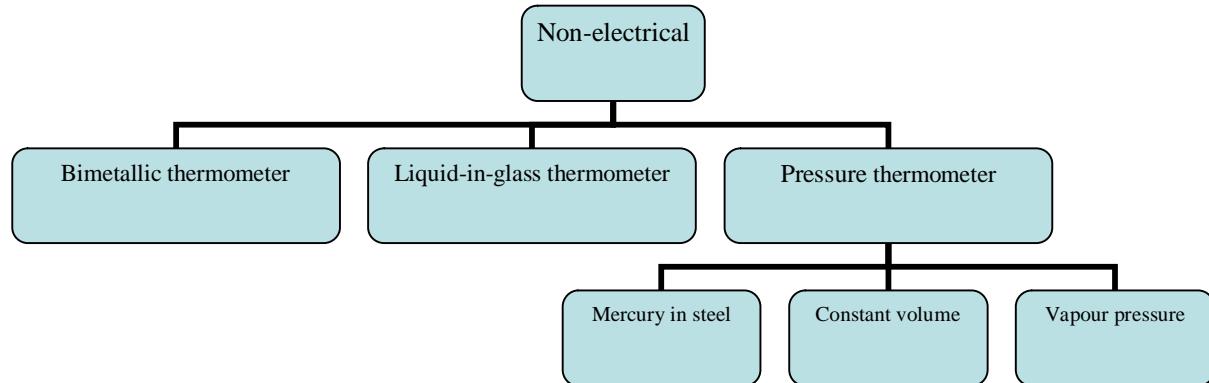
## 1.4 TEMPERATURE

Heat is generated from any fermentation process due to microbial activity and agitation. The heat control in small scale is carried out by thermostatically controlled bath, internal heating coils, heating jacket(water),silicon heating jacket ;large scale: inter coils and cold water circulation. Cooling water is required less for bacteria but more for fungi (due to low optimum temperature for growth).

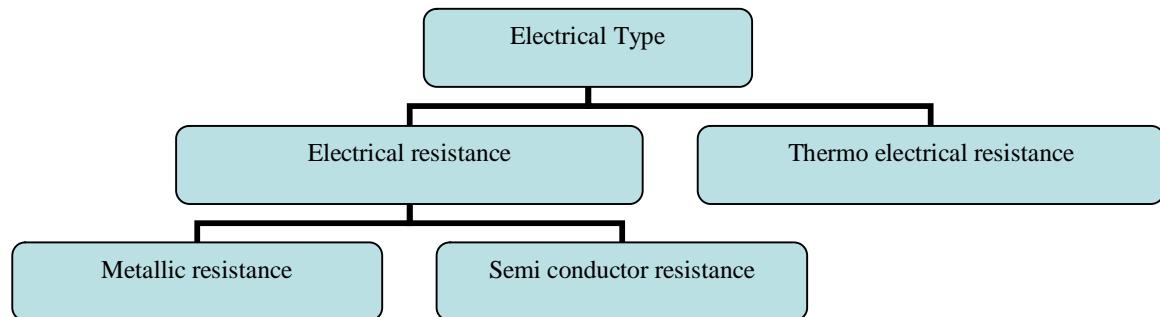
### 1.4.1 TEMPERATURE MEASURING DEVICES

The devices are classified into three major types *viz.* Non-electrical type, Electrical type and Radiation type.

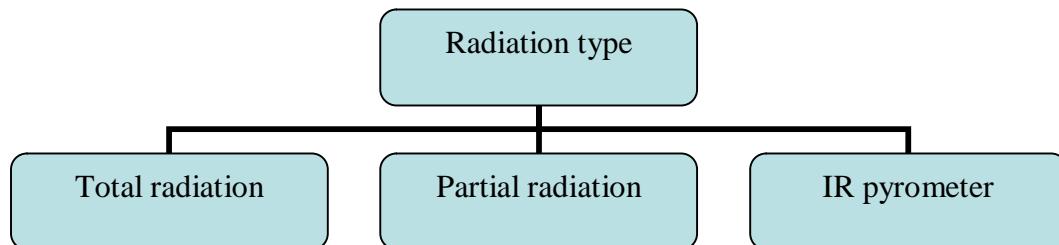
### Non-electrical Type:



### Electrical type:



### Radiation type:



Among the various types listed above three types of temperature measuring devices such as Mercury-in-glass, Electrical resistance and Thermistors are used widely in fermentation process.

**Mercury-in-glass thermometers:** Mercury enclosed in bulb expands with increase in temperature. Expansion is read as measure of temperature and is used only as indication.

**Electrical resistance:** The property of some metals whose resistance changes with change in temperature is used to measure temperature. Bulb with mica is used for accurate measurement and ceramic for less accurate measurement. This is covered by temperature sensing platinum

wire. Leads from bulb connected to measuring device Wheat-stone bridge circuit. It is advantageous in showing fast response to change in temperature.

**Thermistors:** These are semi conductors of pure oxides of iron, nickel or other metals. They exhibit large change in resistance with a small temperature change and hence highly sensitive even little temperature change.

#### 1.4.2 TEMPERATURE CONTROLLING DEVICE

Temperature is an important parameter of fermentation, since, in the cultivation of many microorganisms, the temperature deviation by a couple of degrees can diminish dramatically the growth and biosynthesis productivity. The cultivation temperature is commonly monitored with accuracy not less than  $\pm 0.5^{\circ}\text{C}$ . For temperature measurements, stainless steel Pt100 sensors are normally used. The temperature in laboratory bioreactors is controlled by one of the following ways:

1. A heater is located inside the bioreactor vessel, and cooling is ensured by thin-wall pipes located in the upper cover, which are connected with an electromagnetic valve with the cooling water.
2. Heating and cooling proceed in a thermostat, and this thermostatted water, with the help of a pump, circulates through the bioreactor jacket.

Variant 1 is less complicated, and it ensures a more economic constructive solution. This variant works very well for small bioreactors with the volume up to about 5 litres. Variant 2 ensures a more even distribution of heat throughout the bioreactor volume, which is essential in microorganisms' cultivation.

In the temperature regulation process, the main reason for the regulation inaccuracy is the incorrectly chosen PID parameters. This manifests itself as temperature oscillations. To regulate the temperature precisely, the main obstacle is often the too high minimal portion of the cooling water. In this connection, the valves in the cooling water supply line should be adjusted correspondingly. Another factor for the regulation accuracy is the area and density of the heat transfer surface, since the higher is inertia, the more difficult is to reach a higher accuracy.

Components used for controlling temperature in a fermenter are water inlet, pressure regulator, magnetic inlet valve, heater, circulation pump, jacket, cooling water valve, cold finger, pt-100 sensor, controller, exit gas cooler and drain to remove overflow.

- Manual inlet is used to fill the jacket and closed to allow 30 to 50 drops per minute and compensate evaporation loss.
- Low voltage heating system - when heating is needed. Controlled by controller-regulated by pt-100 sensor
- Pipe line - goes to jacket and re circulated to point before heating system
- Cooling water valve - controlled by controller-regulated by pt-100 sensor. Enters through cold finger which is closed pipeline/coil passes through top plate. Act as heat exchanger with culture
- Exit gas cooler - receives water from point before cooling water valve
- Drain - water from exit gas cooler, overflow from cold finger, overflow from water jacket(drained in to lowest point)

- pt-100 - platinum resistance electrode. Indicate vessel/culture temperature by relating changes in electrical resistance of sensor to temperature
- Novel sparger – impeller design – improves aeration at slow speeds
- Swept back ports – produce negative pressure ideal for animal cell culture

### 1.4.3 GAS FLOW RATE

#### 1.4.3.1 GAS FLOW RATE MEASURING DEVICE

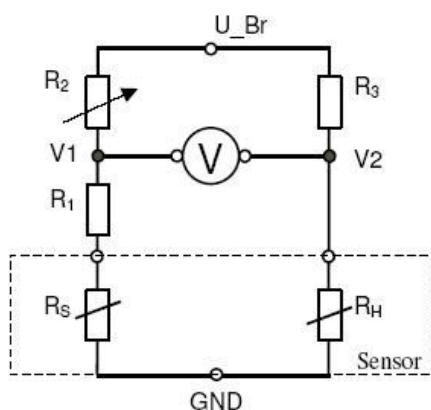
Flow rate can be measured by simple variable area meter.

- a) Rotameter: is a vertically mounted glass tube with an increasing bore size and enclosing a free moving float (a ball or a hollow thimble). The position of float indicates the flow rate. This is less accuracy at low flow rate. Since air coming out of it is non-sterilized, it is placed between inlet and filter. Metal tubes can replace glass tubes. Float position determined by magnetic or electrical techniques. This can be used to measure gas and liquid flow rates.
- b) Thermal mass flowmeter: In this 2 thermistors placed ( $T_1$ ,  $T_2$ ) are placed upstream and downstream of the heat source which may be inside or outside the piping. The mass flow rate  $Q$  can be calculated from the equation,

$$H = QC_p(T_1 - T_2)$$

Where,  $C_p$  – specific flow rate of the gas,  $T_1$ - temperature of gas before heat transfer and  $T_2$  – temperature of gas after heat transfer

This change could induce voltage signal which could be used in data logging.



**Fig.8** Thermal mass flow meter

As shown on the scheme, the heater  $R_H$  and sensor  $R_S$  need to be connected in a bridge circuit. It is essential to determine the correct values of the resistors  $R_1$ ,  $R_2$ , and  $R_3$ . The bridge is in balance as soon as the desired temperature difference between  $R_S$  and  $R_H$  has reached e.g. 10K. At a particular flow velocity the bridge voltage  $V_1-V_2$  needs to be controlled in dependence of the bridge balance  $V_1-V_2$ . The values for  $R_1..R_3$  are depending on the measuring range, the temperature difference  $dT$  and the medium which should be measured. We will provide you with the values of  $R_1..R_3$ , depending on the application. For calibration the  $R_2$  needs to be adjusted within a range of  $\pm 5\%$ . The method of adjustment relies on the application.

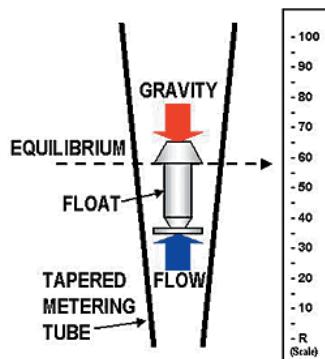
### 1.4.3.2 GAS FLOW RATE CONTROLLING DEVICE

Needle valve is used to control the gas flow rate. Piston movement of the valve is controlled by fluctuations in pressure in flow measuring device. This should be placed upstream of supply when regulated air flow rate is required. This should be placed down stream when fluctuates and back pressure is constant.

### 1.4.4 LIQUID FLOW RATE

#### 1.4.4.1 LIQUID FLOW RATE MEASURING DEVICE

Rotameter can be used to measure liquid flow rate. Alternately electric flow transducer can also be used. In this magnetic coils on the sides of liquid flowing tube supplied with current create magnetic field. Relative velocity of fluid and magnetic field are proportional to voltage induced. Potential difference in fluid is measure by pair of electrodes.



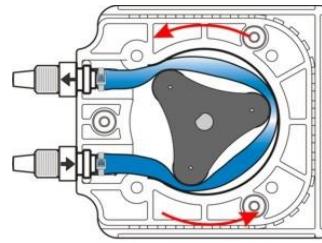
**Fig.9** Rotameter

Load cells, another device can also be used for this purpose. This is an elastic body (solid tubular steel cylinder). The compressive strain by axial load measured by electrical resistance strain gauge fixed to surface of cylinder. This is already calibrated by measuring compressive strain with various loads. The change of resistance is proportional to load which can be determined. This is fitted to either reservoir or fermenter vessel.

#### 1.4.4.2 LIQUID FLOW RATE CONTROLLING DEVICE

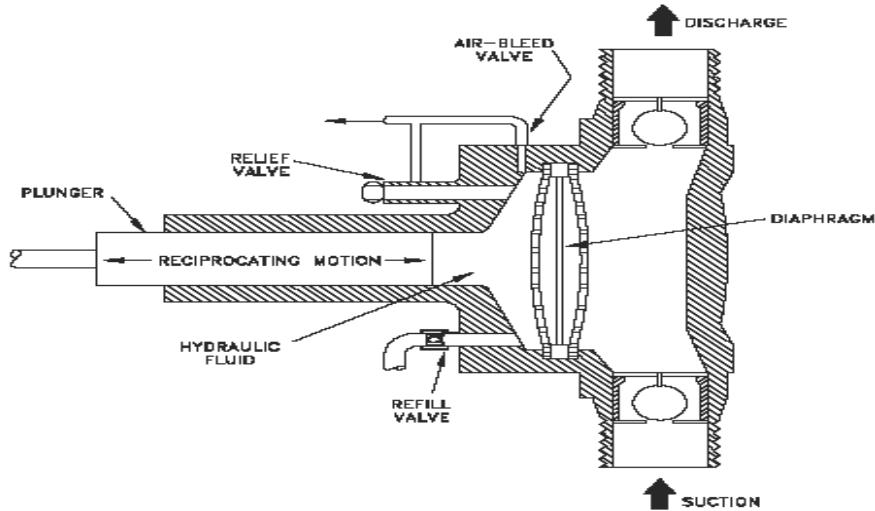
Syringe pump is useful in case of fed batch for controlling the liquid flow rate. A syringe filled with liquid and secured onto the pump. Syringe plunger linked to movable piston and secured firmly to the main body of pump. Regulated piston movement controls the flow rate. This is ideal for low flow rates.

Persitaltic pump are also used to control flow rate. This controls by squeezing and releasing pulse flow. The tubing through which the liquid flows is housed on roller. The circular motion (speed) pinches and released the tubing. More the rollers smoother will the flow. This pump can be controlled by process controller with inputs of respiratory quotient and optical density. Mostly used for addition of acid and base.



**Fig.10** Peristaltic pump

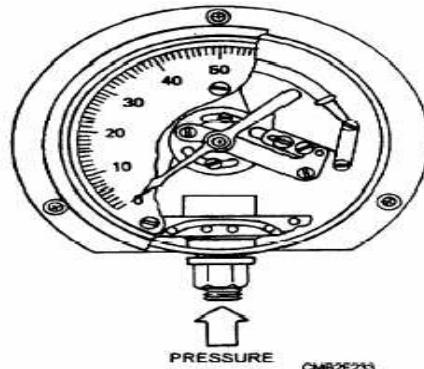
Another type of controlling device is Diaphragm pump. The liquid is allowed to flow through a flexible tube which utilizes a piston and pump controls the flow. The ball valves present on the way of flow prevent leakages and control direction of flow.



**Fig.11** Diaphragm pump

#### 1.4.5 PRESSURE MEASURING AND CONTROLLING DEVICES

Bourbon tube pressure gauge is used to measure the pressure changes. This is a partial coil which has an elliptical cross section. This becomes circular with increasing pressure and the motion straightens out the coil. The process pressure is fixed to one end of coil and other end fixed to geared sector and pinion movement which actuates an indicator pointer. The pointer moves on a calibrated meter which is used to indicate the pressure.



**Fig.12** Bourbon tube pressure gauge

Diaphragm gauge is another device used to measure the pressure under aseptic condition. The change in pressure cause movements in diaphragm fixed to a mechanically levered pointer.

Other devices that can be used for measuring pressure are,

- (i) pressure bellows connected to core of transformer. Movement of core generates voltage output which can be measured
- (ii) strain gauge – a wire subjected to strain result in change of electrical resistance which can be measured
- (iii) piezo electric transducer – a solid crystal (quartz) have asymmetrical charge distribution. Any change in shape due to pressure produce equal external electric charge on the opposite face of crystal (piezo electric effect). This charge can be measured by means of electrodes on both surfaces.

Pressure can be controlled by regulatory valves and safety valves.

#### **1.4.6 AGITATION MEASURING AND CONTROLLING DEVICE**

Agitation speed can be measured by power consumed by agitator shaft. Wattmeter is usually used in large scale process. It is a measure of power consumed for rotation of agitator shaft. This measure is less accurate because power required to rotate against friction in the bearing is taken into consideration.

Torsion dynamometer is used in small scale. This has to be placed outside the vessel and less accurate due to friction. Strain gauges can be mounted on shaft within fermenter from which electric signal is picked up through lead wires passing out of fermenter via an axial hole.

Tachometer can be used to control the agitation speed. The rate of rotation is monitored either by electromagnetic induction or voltage generation or light sensing or magnetic force. Final choice is made by the type of signal required to record or monitor the signal. The agitator speed is also controlled by gear box usage, modifying the size of wheels and drive belts and changing the drive motor.

#### **1.4.7 FOAM SENSING**

The appearance of foam is a very undesirable phenomenon, since, in the course of its appearance, there is a risk to lose an essential part of the fermentation broth. During the

foaming, it is not possible to perform high-quality analyses and measurements. For elimination of foam, 2 methods or their combinations are commonly used:

1. Additional metering of antifoam, based on the information provided by the foam sensor. The given impulses are relatively low, with long pauses and a limited metering time. This additional control is necessary to avoid the possible overdose, since, in this case, the mass exchange parameters can decrease dramatically.
2. Mechanical metering of foam. For this purpose, an upper drive with a special disk-type or other type of the mechanical foam breaking mixer is installed in the bioreactor's upper cover. If an intensive foaming begins, then the mechanical breaking of foam will not help any more.

An optimal solution is the combination of both the parameters. The application of Variant 1 is more widely used in laboratory bioreactors. Foam formation can be sensed by a probe which is a stainless steel rod insulated except at tip and set at a defined level. When foam touches the probe tip current passed through with foam as electrolyte and vessel as earth. This current actuates a vessel/pump to release antifoam into fermenter. Process timer are also included which ensures time gap for antifoam mixing in the medium and reducing foam before next sensing occurs.

#### **1.4.8 DISSOLVED OXYGEN MONITORING**

One of the most specific aspects of the fermentation monitoring is pO<sub>2</sub> measurement and control. pO<sub>2</sub> control is characteristic only for fermentation processes. There are different pO<sub>2</sub> control principles:

1. Varying the mixer's rotational speed n, assuming that pO<sub>2</sub> ~ n.
2. Combining the change of the mixer's rotation speed n and the amount of the inlet compressed air Q. It is assumed that pO<sub>2</sub> ~ n, pO<sub>2</sub> ~ Q. First of all, n is usually regulated until it reaches one of the limiting values - nmin or nmax, and its regulation is realised by varying Q.
3. If n and Q have reached the limiting values, but pO<sub>2</sub> is not within the necessary limits, then the regulating effect does not occur.
4. Feeding up the substrate or its any component. It is assumed that pO<sub>2</sub> is proportional to the feeding up intensity. Feeding up is normally realised with controlled peristaltic pumps. This way is sometimes combined with the regulation of the mixer's rotational speed n and the oxygen or air supply flow Q.

In pO<sub>2</sub> regulation, when adjusting the parameters, the following should be taken into account:

1. pO<sub>2</sub> is commonly adjusted in % from the fixed one. The adjusted pO<sub>2</sub> value has a lower and upper limit. The difference between both these limits is usually 10% - 20%.
2. Important parameters in pO<sub>2</sub> control are the control limits of the mixer's rotational speed n: nmin and nmax. It means that, when controlling pO<sub>2</sub>, n will vary only within this range. These limits are determined in connection with eliminating of different undesirable phenomena:
  1. nmin choice is determined:
    - a. to secure the minimal partly turbulent mixing level;
    - b. by the guaranteed bubble dispersion;

- c. by the prevented sedimentation.
- 2. nmax choice is determined by:
  - a. setting in of the intensive foaming regime;
  - b. irreversible mechanical damages of cells;
  - c. liquid surface fluctuation and evaporation.

Dissolved oxygen can be measured by Galvanic electrode which consists of KCl or KOH or bicarbonate or acetate as electrolyte. Lead is used as anode and silver acts as cathode. The electrodes measure the partial pressure of the dissolved oxygen concentration. The sensing tip of electrode is a membrane made up of Teflon, polyethylene or polystyrene allows gas phase passage so that an equilibrium is established between the gas phases inside and outside the electrode. Due to slow movement of oxygen the changes are read slowly.

Polarographic electrode can also be used which is bulk in size. This has silver as anode, Pt or gold as cathode and KCl as the electrolyte. Membrane needs to be replaced after long use.

Prototypes of a fast response fluorometric sterilizable oxygen sensor are now being developed. This is based on the fact that change in oxygen partial pressure quenches the fluorescence lifetime of a chromophore, tris (4,7-diphenyl – 1,10 – phenanthroline ruthenium II) complex. This is reliable only at low oxygen tension and is autoclavable for inside use.

Direct dissolved oxygen concentrations can be measured by tubing method. A probe has a coil of permeable membrane tubing placed inside the fermenter. Through this membrane helium or nitrogen is passed. Oxygen that diffuses into tubing from the medium is then measured by Paramagnetic gas analyser.

The paramagnetic oxygen analyzer is based on the scientific principle that oxygen is a paramagnetic material, which means that it can be attracted into a magnetic field, or is "magnetically susceptible." Magnetic susceptibility is a measure of the intensity of the magnetization of a substance when it is placed in a magnetic field. Oxygen has an exceptionally high magnetic susceptibility compared to other gases - actually several hundred times greater than that of most other gases. Three types of paramagnetic oxygen analyzers are most generally used in industry: the magnetodynamic, or "dumbbell" type; the thermomagnetic, or "magnetic wind" type; and the magnetopneumatic.

The magnetodynamic oxygen analyzer is the most popular of the three techniques, and consists of a small dumbbell-shaped body made of glass and charged with nitrogen or some other gas of low magnetic susceptibility, a light source, a photocell, a mirror, and a calibrated indicating unit. The dumbbell body is suspended in an enclosed test cell by a quartz or platinum fiber within the magnetic field of a permanent magnet and is free to rotate in the space between the poles of the magnet. Since the dumbbell body is somewhat diamagnetic - i.e., has negative magnetic susceptibility - because of its nitrogen content, the balls of the dumbbell naturally deflect slightly away from the point of maximum magnetic field strength.

When a test sample containing oxygen is introduced into the test cell, the oxygen in the sample is attracted to the point of maximum field strength. the magnitude of dumbbell

displacement is proportional to the amount of oxygen in the sample. The movement of the dumbbell is detected by a light beam from a light source exterior to the test cell. The light beam is reflected from a mirror on the dumbbell body to an exterior photocell. The output of the photocell is amplified and transmitted to an indicating unit that is calibrated to read out the oxygen content in the test sample in percent. Since the difference in magnetic susceptibility between the dumbbell and the gas sample is very subtle for low oxygen concentrations, this method is used only when measuring percent levels of oxygen and not for trace levels. The operation of the magnetodynamic oxygen analyzer is illustrated in the figure below.

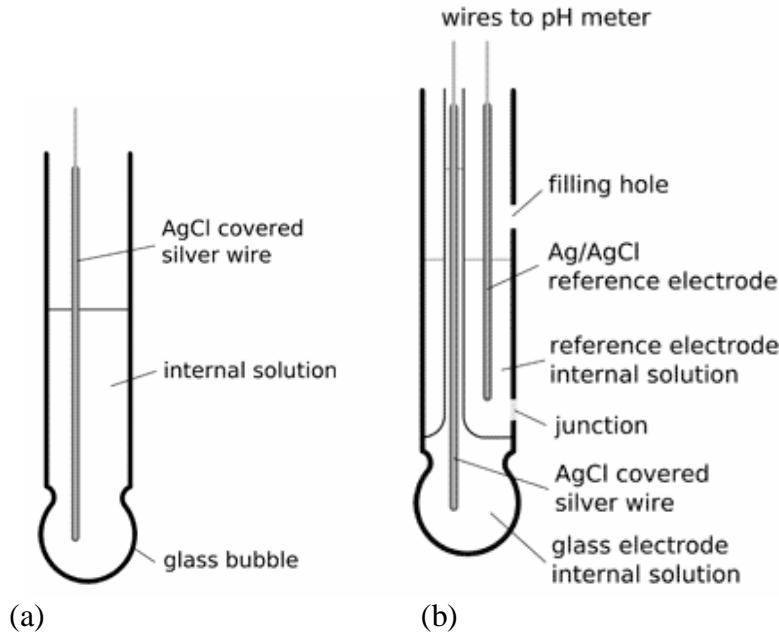
The thermomagnetic oxygen analyzer is based on the principle that the magnetic susceptibility of oxygen decreases inversely with the square of its temperature. It consists of a test chamber containing two tubes for the entry of a test sample. The tubes are connected by a cross tube containing electrical heating filaments at each end of the crossover passage. The two filaments are the arms of a Wheatstone bridge. One end of the cross tube with its heating filament lies in a strong magnetic field created by the poles of a permanent magnet. The test sample is introduced in two equal streams through the two side tubes. Any oxygen in the sample is attracted to the magnetic field because the heating filaments, and the magnetic susceptibility of any oxygen in the sample decreased rapidly as the temperature is increased. The heated sample is displaced by cool oxygen attracted to the magnetic field, and this flow of gas, or "magnetic wind," cools the filament in the magnetic field, causing its resistance to be different from the heating filament at the other (unmagnetized) end of the cross tube. The difference in resistance is measured on a bridge-type instrument, and a signal proportional to the oxygen concentration in the test sample is transmitted to a recording or display instrument.

The magnetpneumatic oxygen analyzer operates on the principle that a test sample containing oxygen molecules, when drawn into a non homogeneous magnetic field and mixed with a reference gas having different oxygen content, will generate a differential pressure. The sample gas is introduced into a test chamber containing a nonhomogeneous magnetic field created by an electromagnet. Oxygen molecules in the sample, because of their paramagnetic properties, flow toward the greatest magnetic field strength. A reference gas with known properties is introduced into the test chamber through two inlets. The reference gas from one inlet mixes with the test sample in the magnetic field and the difference in paramagnetism between the two gases creates a differential pressure, resulting in a balancing flow of reference gas from the other inlet. This balancing flow is measured by a miniature flow sensor and converted into an electrical signal proportional to the pressure differential.

#### **1.4.9 pH MONITORING DEVICES**

A pH measurement is a determination of the activity of hydrogen ions in an aqueous solution. Many important properties of a solution can be determined from an accurate measurement of pH, including the acidity of a solution and the extent of a reaction in the solution. Many chemical processes and properties, such as the speed of a reaction and the solubility of a compound, can also depend greatly on the pH of a solution. In applications ranging from industrial operations to biological processes, it is important to have an accurate and precise measurement of pH.

Most modern pH electrodes consist of a single combination reference and sensing electrode instead of separate electrodes. This type of pH electrode is much easier to use and less expensive than the electrode pair. A combination electrode is functionally the same as an electrode pair.



**Fig.13** (a) Glass electrode (b) Combined electrode

Any pH electrode requires both a sensing electrode and a reference electrode. The sensing electrode consists of a thin hydrogen permeable membrane containing a solution and an electrode. The membrane of the sensing electrode allows hydrogen ions to slowly pass, creating a positive voltage across the membrane. The voltage created in this electrode is then compared to the voltage in the reference electrode. The voltage difference between the two electrodes is then used to determine the pH of the unknown solution using the Nernst equation.

$$E(pH) = E(\text{constant}) + (2.3 * RT/nF) * \log [H^+] \quad (5)$$

Where:

$E(pH)$  = Voltage difference between sensing electrode and Reference electrode (V)

$E(\text{constant})$  = Voltage difference in a solution with  $\text{pH} = 7$  (V)

$R$  = Gas Constant ( $8.314 \text{ J/K*mole}$ )

$T$  = Temperature in Kelvin (K)

$n$  = Number of Valence Electrons per Mole (1 for  $H^+$ )

$F$  = Faraday's Constant ( $96500 \text{ J/V*mole e-}$ )

## 1.5 PID CONTROL SYSTEMS

The process parameters which are measured using probes may be controlled using control loops. A control loop consists of four components: a measuring element, a controller, a final control element and the process to be controlled. A simple type of control is feed back control in which the measuring element senses a process property and generates a corresponding output signal. The controller compares the measurement signal with a predetermined desired value (set

point) and produces an output signal to counteract any differences between the two. The final control element receives the control signal and adjusts the process by changing a valve opening or pump speed and causing the controlled process property to return to the set point.

In Manual control system a plant operative is instructed to monitor the data throughout the time and will take appropriate action in case change from the set point.

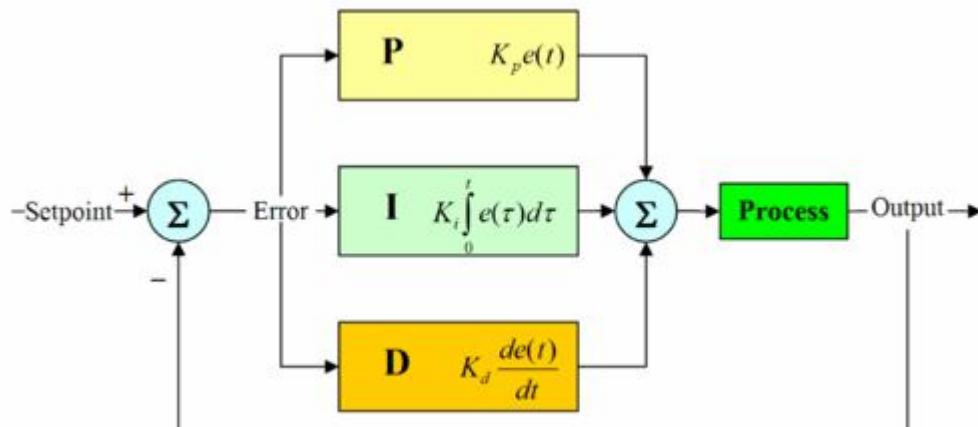
### 1.5.1 PID

A **proportional-integral-derivative controller (PID controller)** is a generic control loop feedback mechanism widely used in industrial control systems. A PID controller attempts to correct the error between a measured process variable and a desired setpoint by calculating and then outputting a corrective action that can adjust the process accordingly.

The PID controller calculation (algorithm) involves three separate parameters; the Proportional, the Integral and Derivative values. The Proportional value determines the reaction to the current error, the Integral determines the reaction based on the sum of recent errors and the Derivative determines the reaction to the rate at which the error has been changing. The weighted sum of these three actions is used to adjust the process via a control element such as the position of a control valve or the power supply of a heating element.

By "tuning" the three constants in the PID controller algorithm the PID can provide control action designed for specific process requirements. The response of the controller can be described in terms of the responsiveness of the controller to an error, the degree to which the controller overshoots the setpoint and the degree of system oscillation. Note that the use of the PID algorithm for control does not guarantee optimal control the system.

Some applications may require using only one or two modes to provide the appropriate system control. This is achieved by setting the gain of undesired control outputs to zero. A PID controller will be called a PI, PD, P or I controller in the absence of the respective control actions. PI controllers are particularly common, since derivative action is very sensitive to measurement noise, and the absence of an integral value prevents the system from reaching its target value due to the control action.



**Fig.14** A block diagram of a PID controller

The PID control scheme is named after its three correcting terms, whose sum constitutes the manipulated variable (MV). Hence:

$$MV(t) = P_{\text{out}} + I_{\text{out}} + D_{\text{out}}$$

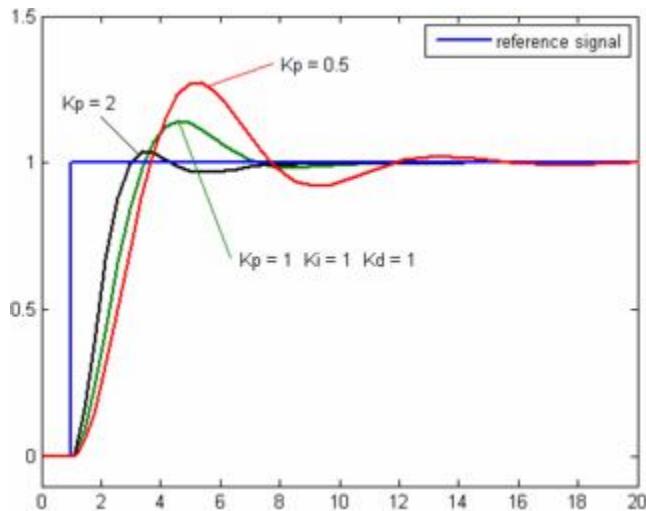
where  $P_{\text{out}}$ ,  $I_{\text{out}}$ , and  $D_{\text{out}}$  are the contributions to the output from the PID controller from each of the three terms, as defined below.

**(i) Proportional term:** The proportional term makes a change to the output that is proportional to the current error value. The proportional response can be adjusted by multiplying the error by a constant  $K_p$ , called the proportional gain.

The proportional term is given by:

$$P_{\text{out}} = K_p e(t)$$

Where,  $P_{\text{out}}$ : Proportional output,  $K_p$ : Proportional Gain, a tuning parameter,  $e$ : Error =  $SP - PV$ ,  $t$ : Time or instantaneous time (the present)



**Fig.15** Change of response for varying  $K_p$

A high proportional gain results in a large change in the output for a given change in the error. If the proportional gain is too high, the system can become unstable (See the section on Loop Tuning). In contrast, a small gain results in a small output response to a large input error, and a less responsive (or sensitive) controller. If the proportional gain is too low, the control action may be too small when responding to system disturbances.

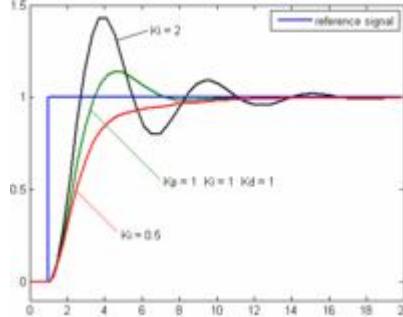
In the absence of disturbances pure proportional control will not settle at its target value, but will retain a steady state error that is a function of the proportional gain and the process gain. Despite the steady-state offset, both tuning theory and industrial practice indicate that it is the proportional term that should contribute the bulk of the output change.

**(ii) Integral term :** The contribution from the integral term is proportional to both the magnitude of the error and the duration of the error. Summing the instantaneous error over time

(integrating the error) gives the accumulated offset that should have been corrected previously. The accumulated error is then multiplied by the integral gain and added to the controller output. The magnitude of the contribution of the integral term to the overall control action is determined by the integral gain,  $K_i$ .

The integral term is given by:

$$I_{\text{out}} = K_i \int_0^t e(\tau) d\tau$$



**Fig.16** Change of response for varying  $K_i$

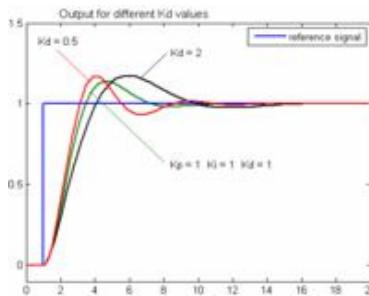
Where,  $I_{\text{out}}$ : Integral output,  $K_i$ : Integral Gain, a tuning parameter,  $e$ : Error =  $SP - PV$ ,  $\tau$ : Time in the past contributing to the integral response

The integral term (when added to the proportional term) accelerates the movement of the process towards setpoint and eliminates the residual steady-state error that occurs with a proportional only controller. However, since the integral term is responding to accumulated errors from the past, it can cause the present value to **overshoot** the setpoint value (cross over the setpoint and then create a deviation in the other direction). For further notes regarding integral gain tuning and controller stability, see the section on Loop Tuning.

**(iii) Derivative term:** The rate of change of the process error is calculated by determining the slope of the error over time (i.e. its first derivative with respect to time) and multiplying this rate of change by the derivative gain  $K_d$ . The magnitude of the contribution of the derivative term to the overall control action is termed the derivative gain,  $K_d$ .

The derivative term is given by:

$$D_{\text{out}} = K_d \frac{de}{dt}$$



**Fig. 17** Change of response for varying  $K_d$

Where,  $D_{\text{out}}$ : Derivative output,  $K_d$ : Derivative Gain, a tuning parameter,  $e$ : Error =  $SP - PV$ ,  $t$ : Time or instantaneous time (the present)

The derivative term slows the rate of change of the controller output and this effect is most noticeable close to the controller setpoint. Hence, derivative control is used to reduce the magnitude of the overshoot produced by the integral component and improve the combined controller-process stability. However, differentiation of a signal amplifies noise in the signal and thus this term in the controller is highly sensitive to noise in the error term, and can cause a process to become unstable if the noise and the derivative gain are sufficiently large. The output from the three terms, the proportional, the integral and the derivative terms are summed to calculate the output of the PID controller. Defining  $u(t)$  as the controller output, the final form of the PID algorithm is:

$$MV(t) = K_p e(t) + K_i \int_0^t e(\tau) d\tau + K_d \frac{de}{dt}$$

and the tuning parameters are

1.  $K_p$ : **Proportional Gain** - Larger  $K_p$  typically means faster response since the larger the error, the larger the feedback to compensate. An excessively large proportional gain will lead to process instability.
2.  $K_i$ : **Integral Gain** - Larger  $K_i$  implies steady state errors are eliminated quicker. The trade-off is larger overshoot: any negative error integrated during transient response must be integrated away by positive error before we reach steady state.
3.  $K_d$ : **Derivative Gain** - Larger  $K_d$  decreases overshoot, but slows down transient response and may lead to instability.

## 1.6 TYPES OF FERMENTATION

Any fermentation process can be carried out as Batch, Continuous and Fed Batch.

1. Batch: fermentation process allowed proceeding for limited time with required controlled conditions. After the stipulated incubation period the process stopped and yield recovered
2. Continuous: fermentation process allowed to proceed continuously with continuous supply of required nutrients and sample withdrawn regularly
3. Fed batch: fermentation process allowed to proceed continuously and sample withdrawn discontinuously with intermittently addition of medium

### 1.6.1 BATCH CULTURE

Batch culture is a closed culture system which contains initial, limited amount of medium. As the growth of microorganism proceeds, the medium availability changes and hence the organism goes through a number of phases as illustrated next section (2.7).

### 1.6.2 CONTINUOUS CULTURE

In continuous culture, the exponential growth phase of organism may be prolonged by the addition of fresh medium to the vessel. The vessel should be designed in such a way that the added volume displaced an equal volume of culture from the vessel. If medium is fed

continuously to such vessel at a suitable rate, a steady state is achieved eventually. Steady state is formation of new biomass in the vessel is equivalent to the loss of cells from the vessel. The medium flow into the vessel is related to the total volume of the medium in the vessel expressed as dilution rate, D, which can be expressed in the form of mathematical equation,

$$D = F/V$$

Where, F is the flow rate ( $\text{dm}^3\text{h}^{-1}$ ) and V is the total volume. The net change in cell concentration over a time period may be expressed as,

$$\frac{dx}{dt} = \text{growth} - \text{output or } \mu x - Dx$$

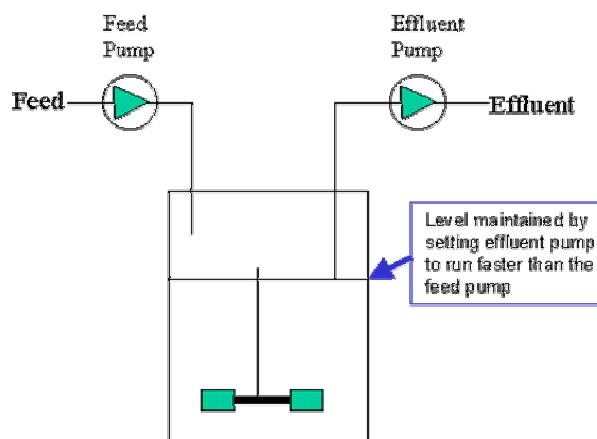
Under steady state conditions the cell concentration remains constant, thus  $\frac{dx}{dt} = 0$ . and the equation becomes,

$$\mu x = Dx$$

and

$$\mu = D$$

Thus, under steady state conditions the specific growth rate is controlled by the dilution rate which is a controllable variable. An important objective of continuous culture operation is to control cell growth at a level at which productivity is optimum. There are several ways in which this can be achieved. One is to maintain a constant fermenter volume and to use a flow rate that gives an appropriate productivity. In this mode of operation, the fermenter system is known as a **chemostat**. The chemostat is by far the simplest and most common mode of operation of a continuous culture. The cell growth in chemostat cultures is maintained steady by a constant inflow of fresh medium consisting of nutrients (nitrogen, phosphorous, glucose) at a concentration so as to be growth limiting. Other constituents of such a medium are present at concentrations higher than required. Increase or decrease in the concentration of the growth limiting factor is correspondingly expressed by increase or decrease in the growth rate of cells. The volume of the chemostat can be controlled either by using a pump. Where contamination can be a significant problem, a pump based control system is preferred. This setup is commonly used in laboratory investigations and animal cell culture systems. An overflow system has the advantage in that only one pump is required. However as the effluent flow rate is determined by gravity alone, there is a greater possibility of contaminants moving up the effluent tube into the reactor. Overflow systems are however widely used in wastewater treatment and have been used in the large scale continuous culture of bacteria.



**Fig.18 CONTINUOUS CULTURE**

In other techniques, a fermenter variable, eg. turbidity or pH, will be monitored using an appropriate detector and the liquid flow rate will be automatically adjusted so as to maintain the variable at a constant level. Examples of these types of continuous fermenters are the pH-stat, turbidostat and nutristat. Apart from the pH-stat, these reactors are however rarely used as the necessary measurement-control systems are generally unreliable over long periods of time.

A turbidostat is a continuous culturing method where the turbidity of the culture is held constant by manipulating the rate at which medium is fed. If the turbidity tends to increase, the feed rate is increased to dilute the turbidity back to its setpoint. When the turbidity tends to fall, the feed rate is lowered so that growth can restore the turbidity to its set point.

The most widespread large scale application of continuous culture reactors is in wastewater treatment. Activated sludge plants, trickle bed filters, anaerobic digester and ponds all operate in an continuous manner. Cell immobilization is also often employed to improve the efficiency of the process. Continuous cultures are well established in the wastewater industry for several reasons:

- Unlike pure culture microbial and animal cell systems, contamination is not a consideration, as the wastewater feed will always contain microorganisms.
- Continuous reactors have long been used in waste treatment and their use is not considered a risk.
- Finally using batch cultures is simply not economically feasible. Wastewater flows are often measured in mega litres per hour and batch reactors simply could not cope with the load.

### **1.6.3 FED BATCH CULTURE**

Two basic approaches to the fed-batch fermentation can be used: the constant volume fed-batch culture or Fixed Volume Fed-Batch - and the Variable Volume Fed-Batch.

#### **1.6.3.1 FIXED VOLUME FED-BATCH**

In this type of fed-batch, the limiting substrate is fed without diluting the culture. The culture volume can also be maintained practically constant by feeding the growth limiting substrate in undiluted form, for example, as a very concentrated liquid or gas (ex. oxygen). Alternatively, the substrate can be added by dialysis or, in a photosynthetic culture, radiation can be the growth limiting factor without affecting the culture volume.

A certain type of extended fed-batch - *the cyclic fed-batch culture for fixed volume systems* - refers to a periodic withdrawal of a portion of the culture and use of the residual culture as the starting point for a further fed-batch process. Basically, once the fermentation reaches a certain stage, (for example, when aerobic conditions cannot be maintained anymore) the culture is removed and the biomass is diluted to the original volume with sterile water or medium containing the feed substrate. The dilution decreases the biomass concentration and result in an increase in the specific growth rate. Subsequently, as feeding continues, the growth rate will

decline gradually as biomass increases and approaches the maximum sustainable in the vessel once more, at which point the culture may be diluted again.

### **1.6.3.2 VARIABLE VOLUME FED-BATCH**

As the name implies, a variable volume fed-batch is one in which the volume changes with the fermentation time due to the substrate feed. The way this volume changes it is dependent on the requirements, limitations and objectives of the operator.

The feed can be provided according to one of the following options:

- (i) the same medium used in the batch mode is added;
- (ii) a solution of the limiting substrate at the same concentration as that in the initial medium is added; and
- (iii) a very concentrated solution of the limiting substrate is added at a rate less than (i), (ii) and (iii).

This type of fed-batch can still be further classified as *repeated fed-batch process* or *cyclic fed-batch culture*, and *single fed-batch process*. The former means that once the fermentation reached a certain stage after which is not effective anymore, a quantity of culture is removed from the vessel and replaced by fresh nutrient medium. The decrease in volume results in a increase in the specific growth rate, followed by a gradual decrease as the quasi-steady state is established.

The latter type refers to a type of fed-batch in which supplementary growth medium is added during the fermentation, but no culture is removed until the end of the batch. This system presents a disadvantage over the fixed volume fed-batch and the repeated fed-batch process: much of the fermenter volume is not utilized until the end of the batch and consequently, the duration of the batch is limited by the fermenter volume.

### **1.6.3.3 ADVANTAGES AND DISADVANTAGES OF THE FED-BATCH REACTORS**

Fed-batch fermentation is a production technique in between batch and continuous fermentation. A proper feed rate, with the right component constitution is required during the process.

Fed-batch offers many advantages over batch and continuous cultures. From the concept of its implementation it can be easily concluded that under controllable conditions and with the required knowledge of the microorganism involved in the fermentation, the feed of the required components for growth and/or other substrates required for the production of the product can never be depleted and the nutritional environment can be maintained approximately constant during the course of the batch. The production of by-products that are generally related to the presence of high concentrations of substrate can also be avoided by limiting its quantity to the amounts that are required solely for the production of the biochemical. When high concentrations of substrate are present, the cells get "overloaded", this is, the oxidative capacity of the cells is exceeded, and due to the Crabtree effect, products other than the one of interest are produced, reducing the efficacy of the carbon flux. Moreover, these by-products prove to even

"contaminate" the product of interest, such as ethanol production in baker's yeast production, and to impair the cell growth reducing the fermentation time and its related productivity.

Sometimes, controlling the substrate is also important due to catabolic repression. Since this method usually permits the extension of the operating time, high cell concentrations can be achieved and thereby, improved productivity [mass of product/(volume.time)]. This aspect is greatly favored in the production of growth-associated products. Additionally, this method allows the replacement of water loss by evaporation and decrease of the viscosity of the broth such as in the production of dextran and xanthan gum, by addition of a water-based feed. As previously mentioned, fed-batch might be the only option for fermentations dealing with toxic or low solubility substrates.

When dealing with recombinant strains, fed-batch mode can guarantee the presence of an antibiotic throughout the course of the fermentation, with the intent of keeping the presence of an antibiotic-marked plasmid. Since the growth can be regulated by the feed, and knowing that in many cases a high growth rate can decrease the expression of encoded products in recombinant products, the possibility of having different feeds and feed modes makes fed-batch an extremely flexible tool for control in these cases. Because the feed can also be multisubstrate, the fermentation environment can still be provided with required protease inhibitors that might degrade the product of interest, metabolites and precursors that increase the productivity of the fermentation.

Finally, in a fed-batch fermentation, no special piece of equipment is required in addition to that one required by a batch fermentation, even considering the operating procedures for sterilization and the preventing of contamination. A cyclic fed-batch culture has an additional advantage: the productive phase of a process may be extended under controlled conditions. The controlled periodic shifts in growth rate provide an opportunity to optimize product synthesis, particularly if the product of interest is a secondary metabolite whose maximum production takes place during the deceleration in growth.

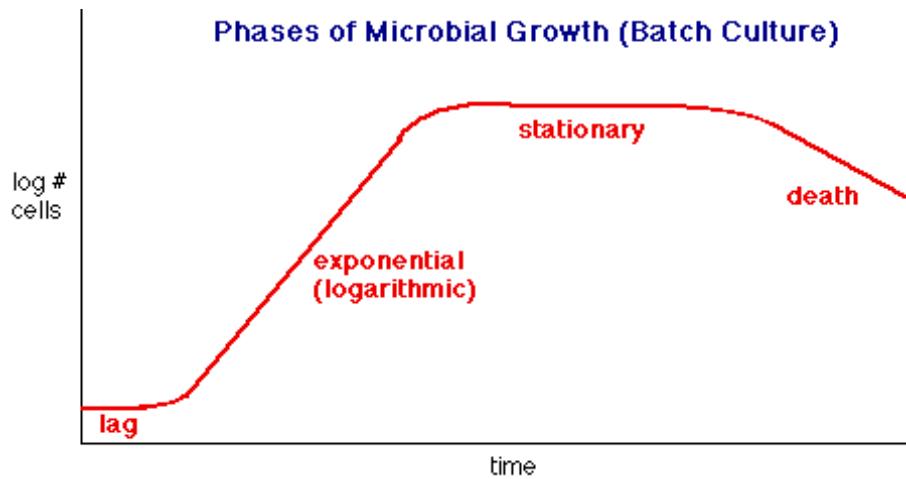
## 1.7 MICROBIAL KINETICS

Growth is an increase in cellular constituents that may result in an increase in cell size, an increase in cell number, or both. When a microbiologist speaks of microbial growth it is usually increase in **cell number** that he/she is after. Consequently, there is a tendency for microbiologists to follow microbial growth as **populations** rather than following the growth of individual cells. Microbiologists tend to be more interested in **population sizes** than the size (mass) of any individual cell. Typical measurement of **microbial growth** will be done over the span of more than one **microbial generation**. An **increase in cell number** is an immediate consequence of cell division. Increase in cell numbers occurs when microorganisms reproduce by a process like **budding** or **binary fission**. **Budding** is a form of reproduction in which a new cell is formed as an outgrowth from the parent cell, as in the case of yeast and some bacteria. The majority of bacteria reproduce by a mechanism termed **binary fission**.

### 1.7.1 GROWTH CURVE

The population growth is studied by analyzing the **growth curve** of a microbial culture. The **standard bacterial growth curve** describes various stages of growth a pure culture of bacteria will go through, beginning with the addition of cells to sterile media and ending with the death of all of the cells present. The **standard bacterial growth curve** describes various stages of growth a pure culture of bacteria will go through, beginning with the addition of cells to sterile media and ending with the death of all of the cells present

Bacteria added to fresh media typically go through **four** more-or-less distinct **phases** of growth: **lag**, **exponential**, **stationary**, and **death**.



**Lag phase:** The period of apparent inactivity in which the cells are adapting to a new environment and preparing for reproductive growth. Cells are usually synthesizing new components. In practice, bacteria from one medium to another, where there are chemical differences between the two media, typically results in a **lag** in cell division. This lag in division is associated with a **physiological adaptation** to the new environment. Cells may **increase in size** during this time, but simply **do not divide** (by binary fission). Lag phase varies considerably in **length** depending upon the **condition of the microorganisms** and the nature of the **medium**.

**Log (exponential) phase:** The period in which the organisms are growing at the maximal rate possible given their genetic potential, the nature of the medium, and the conditions under which they are growing. Cells in optimum growth state, divide repeatedly by binary fission at maximal rate; the population doubles in every **generation time**. Generation time, also called **Doubling time**, is the time it takes a bacterium to do one binary fission starting from having just divided. Generation times vary markedly with the species of microorganism and environmental conditions; they can range from 10 minutes for a few bacteria to several days with some eukaryotic microorganisms. The population is most uniform in terms of chemical and physical properties during this period.

**Stationary phase:** Eventually population growth ceases, and the growth curve becomes horizontal. **Increase** in cell number due to **cell divisions** exactly **balanced** by a **decrease** in cell number due to **death**. **Cell death** may result from **Nutrient limitation & Toxic waste**

**accumulation** (e.g. acid buildup from fermentation); as well as O<sub>2</sub> depletion, critical population level reached.

**Death phase:** Stationary phase, in a standard bacterial growth curve, is followed by a die-off of cells, called Death phase. It is the period in which the cells are dying at an exponential rate. Some of the reasons are: continued accumulation of **wastes**, loss of cell's ability to detoxify **toxins**, etc.

## 1.7.2 ALGEBRA OF EXPONENTIAL GROWTH

Microbial growth can be described by certain mathematical terms: **Mean Generation Time** and **Mean Growth Rate**.

**Mean generation (doubling) time** is the time required for the population to double.

**Mean growth rate** constant is the number of generations per unit time, often expressed as generations per hour.

**Growth equation:**

$$n = \frac{\log_{10} N_f - \log_{10} N_0}{.301}$$

**n = number of generations.**

N<sub>f</sub> = final conc. of cells (e.g. 10<sup>9</sup>/ml).

N<sub>0</sub> = initial conc. of cells (e.g. 10<sup>3</sup>/ml)

$$N_f = 2^n N_0$$

## 1.7.2 Measurement of Microbial Growth

There are many ways to measure microbial growth (growth rates & generation times). Population mass or number may be followed; growth increases both.

### 1.7.2.2 Direct cell count methods

This may be accomplished by **direct microscopic observation** on specially etched slides (such as Petroff-Hausser chambers or hemocytometers) or by using **electronic counters**. Electronic counters, such as Coulter Counters, count microorganisms as they flow through a small hole or orifice. Direct cell count methods do not distinguish between living and dead cells.

### 1.7.2.3 Viable cell counts

This method involves plating diluted samples (using a **pour plate** or **spread plate**) onto suitable growth media and monitoring colony formation; this type of method counts only those cells that are reproductively active. It is typically carried out by **Colony Forming Units (CFU) assay**.

### 1.7.3 KINETIC MODEL OF GROWTH (MONOD)

The concentration of biomass,  $X$  (mg/L), increases as a function of time due to conversion of food to biomass:

$$\frac{dX}{dt} = \mu X$$

Where  $\mu$  is the specific growth rate constant ( $d^{-1}$ ). This represents the mass of cells produced/mass of cells per unit of time.

Balanced (exponential) growth occurs when all cellular components are synthesized at constant rates relative to one another. Unbalanced growth occurs when the rates of synthesis of some components change relative to the rates of synthesis of other components. This usually occurs when the environmental conditions or nutrient levels change. Growth has been often represented by mathematical models. In the case of media limitation, the equation of Monod is most often used.

$$\mu = \mu_m \frac{S}{K_s + S}$$

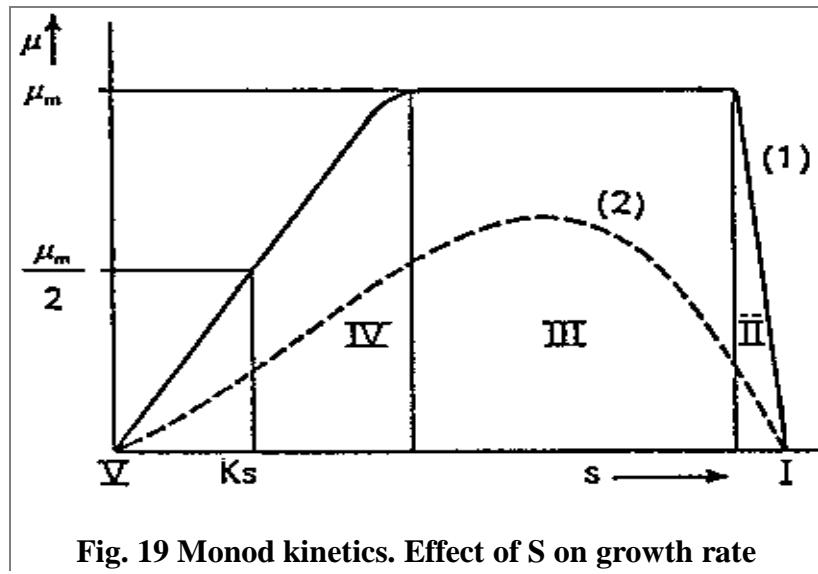
Where,  $\mu_m$  =  $\mu$  maximum specific growth rate,  $K_s$  = Saturation constant

If  $K_s \ll S$ ,  $\frac{S}{K_s + S} \sim 1$  and the growth is exponential;

Two constants are used to describe the growth rate

- $\mu_m$  (mg/L) is the maximum growth rate constant (the rate at which the substrate concentration is not limiting)
- if the value of  $K_s$  is relatively high in comparison to  $S$ , when  $S$  decrease, a decelerating growth phase is reached. Values of  $\mu$  are dependent on the combination media-organism but are most often between 0.1 and 0.4  $h^{-1}$ .
- $K_s$  is the half-saturation constant ( $d^{-1}$ ) (i.e., concentration of  $S$  when  $\mu_m = \mu_m / 2$ )

Values  $K_s$  can be determined by plotting  $1/\mu_m$  against  $1/S$ . The equation becoming  $1/\mu_m = [K_s/\mu_m \times 1/S] + 1/\mu_m$



In the case of non exponential growth the  $K_s$  value can be approximated from the curve  $\mu_m = f(s)$  (Fig.). In batch culture, it is difficult to observe exponential increases in biomass for more than five doubling times. After a certain growth time, the organism will eventually modify the physicochemical condition of its environment and corresponding growth slow down will occur due to limitation in nutrient concentration or oxygen transfer or accumulation of staling products. Morphologically, linear growth can be correlated with a blockage of the branching whereas branching of the mycelium or the fermentation of blastospores "yeast-like cells" induce an exponential increase of the biomass in case of fungus.

Biomass productivity can be calculated by the following equation,

$$\frac{dX}{dt} = \text{growth rate} - \text{death rate} = \mu X - k_d X$$

Where  $k_d$  represents the endogenous decay rate ( $d^{-1}$ ) (i.e., microorganism death rate).

Substituting the growth rate constant:

$$\frac{dX}{dt} = \left( \frac{\mu_m S}{K_s + S} \right) X - k_d X$$

Substrate utilized may be used to calculate the yield factor by the following equation.

$$\frac{dS}{dt} = \frac{1}{Y} \frac{dX}{dt}$$

Where Y is the yield factor (mg of biomass produced/mg of food consumed)

Y range:

- Aerobic: 0.4 - 0.8 mg/mg
- Anaerobic: 0.08 - 0.2 mg/mg

$$\frac{dS}{dt} = \frac{1}{Y} \frac{dX_{\text{growth}}}{dt} = \frac{1}{Y} \left( \frac{\mu_m S X}{K_s + S} \right)$$

### 1.7.3.1 KINETICS OF FIXED VOLUME FED BATCH

The mathematical development that is going to be presented here has the following assumptions:

- The feed is provided at a constant rate
- The production of mass of biomass per mass of substrate is constant during the fermentation time and
- A very concentrated feed is being provided to the fermenter in such a way that the change in volume is negligible.

The equations that describe the system in terms of specific growth rate, biomass and product concentration (for both growth and non-growth associated products) with time are the following:

**Table 3.6.1.1. Mathematical modelling of fixed volume fed-batch.**

Parameter	Equation
Specific Growth Rate	$\mu = (F \cdot Y_{x/s}) / x$
Biomass (as a function of time)	$x_t = x_0 + F \cdot Y_{x/s} \cdot t$
Product Concentration (non-growth associated)	$P = P_i + q_p \cdot x_0 \cdot t + q_p \cdot F \cdot Y_{x/s} \cdot t^2 / 2$
Product Concentration (non-growth associated)	$P = P_i + r_p \cdot t$

Where,

- $x$  is the biomass [mass biomass/volume]
- $x_0$  is the biomass in the beginning of the fermentation [mass biomass/volume]
- $t$  is time
- $F$  is the substrate feed rate [mass substrate/(volume.time)] and
- $Y_{x/s}$  is the yield factor [mass biomass/mass substrate]
- $\mu$  is the specific growth rate [ $\text{time}^{-1}$ ]
- $P$  is the product concentration {mass product/volume} and
- $q_p$  is the specific production rate of product [mass product/(mass biomass . time)]
- $r_p$  is the product formation rate [mass product/(volume . time)]

From equations, it can be observed that

- (i) the specific growth rate decreases with time because the biomass (in the denominator) is increasing with time and
- (ii) the biomass increases linearly with time.

The product variation with time will depend on its being growth or non-growth associated, this is, will depend on  $q_p$  (specific product formation defined as the product formation rate divided by the biomass) being dependent on the specific growth rate or not, respectively.

### 1.7.3.2 VARIABLE VOLUME FED-BATCH

In a variable volume fed-batch fermentation, an additional element should be considered: the feed. Consequently, the volume of the medium in the fermenter varies because there is an inflow and no outflow. Again, it is going to be considered that the growth of the microorganism is limited by the concentration of one substrate.

For the mathematical developments that will be presented, the assumptions are

- Specific growth rate is uniquely dependent on the concentration of the limiting substrate
- The concentration of the limiting substrate in the feed is constant
- The feed is sterile
- The yields are constant during the fermentation time

Table 3.6.2.1. summarizes the equations that apply to this situation. These relations are the base for all further calculations and specific cases of variable volume fed-batch fermentation.

**Table 3.6.2.1. Mass balances for the main components for a fed-batch reaction.**

Component	Mass Balance Equation
Overall	$F = dV/dt$
Biomass	$dx/dt = x \cdot (\mu \cdot V - K_d \cdot V - F) / V$
Substrate	$ds/dt = F \cdot (s_0 - s)/V - \mu \cdot x / Y_{x/s}$
Product	$dP/dt = q_p \cdot x - P \cdot F / V$

- $V$  is the volume of the fermenter
- $t$  is the time
- $F$  is the feed rate [volume/time].
- $x$  is the biomass concentration [mass biomass/volume]
- $\mu$  is the specific growth rate [ $\text{time}^{-1}$ ]
- $K_d$  is the specific death rate [ $\text{time}^{-1}$ ]
- $s$  is the substrate concentration in the fermenter [mass substrate/volume]
- $s_0$  is the substrate concentration in the feed [mass substrate/volume]
- $Y_{x/s}$  is the yield factor [mass biomass/mass substrate]
- $P$  is the product concentration { mass product/volume} and
- $q_p$  is the specific production rate of product [mass product/(mass biomass . time)]

## 1.8 LET US SUM UP

- Various designs of fermenters are discussed which can be used for various types of fermentation processes
- Basic components of fermenter are explained in detail
- Monitoring and controlling devices to maintain a controlled condition in a fermenter have also been discussed
- Different types of fermentation processes such as batch, continuous and fed-batch
- Finally the kinetics involving various factors influencing the growth conditions in various processes have also explained

### **1.9 POINTS FOR DISCUSSION**

1. Evaluate the use of batch and continuous process.
2. Justify the need for controlled conditions in a fermenter.
3. Analyze the growth kinetics of a bacteria and mathematical modeling.

### **1.10 LESSON - END ACTIVITIES**

1. Explain about the various components of an ideal fermenter.
2. List out the essential parts of fermenter and write short note on each part Describe the various types of fermenter.
3. Write about stirred tank, airlift, fluidized bed, bubble column fermenter.
4. Explain how the various process parameters are monitored in a fermenter.
5. Write about the measuring and controlling devices for monitoring temperature, pressure, agitation, pH, dissolved oxygen and flow rate
6. Elaborate on different types of fermentation process.
7. Write about Batch, Continuous and Fed-batch processes
8. Discuss in detail the growth kinetics of microbes.
9. Write an account on four phases of growth and kinetics involved in all types of fermentation processes.

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## UNIT II DOWN STREAM PROCESSING

### **CONTENTS**

- 2.0 AIMS AND OBJECTIVES**
- 2.1 INTRODUCTION**
- 2.2 FOAM SEPARATION**
- 2.3 SEPARATION OF CELLS AND INSOLUBLE PRODUCTS**
- 2.4 CELL DISRUPTION**
- 2.5 SEPARATION OF SOLUBLE PRODUCTS**
- 2.6 PURIFICATION TECHNIQUES**
- 2.7 PRODUCT POLISHING**
- 2.8 LET US SUM UP**
- 2.9 POINTS FOR DISCUSSION**
- 2.10 LESSON - END ACTIVITIES**
- 2.11 REFERENCES**

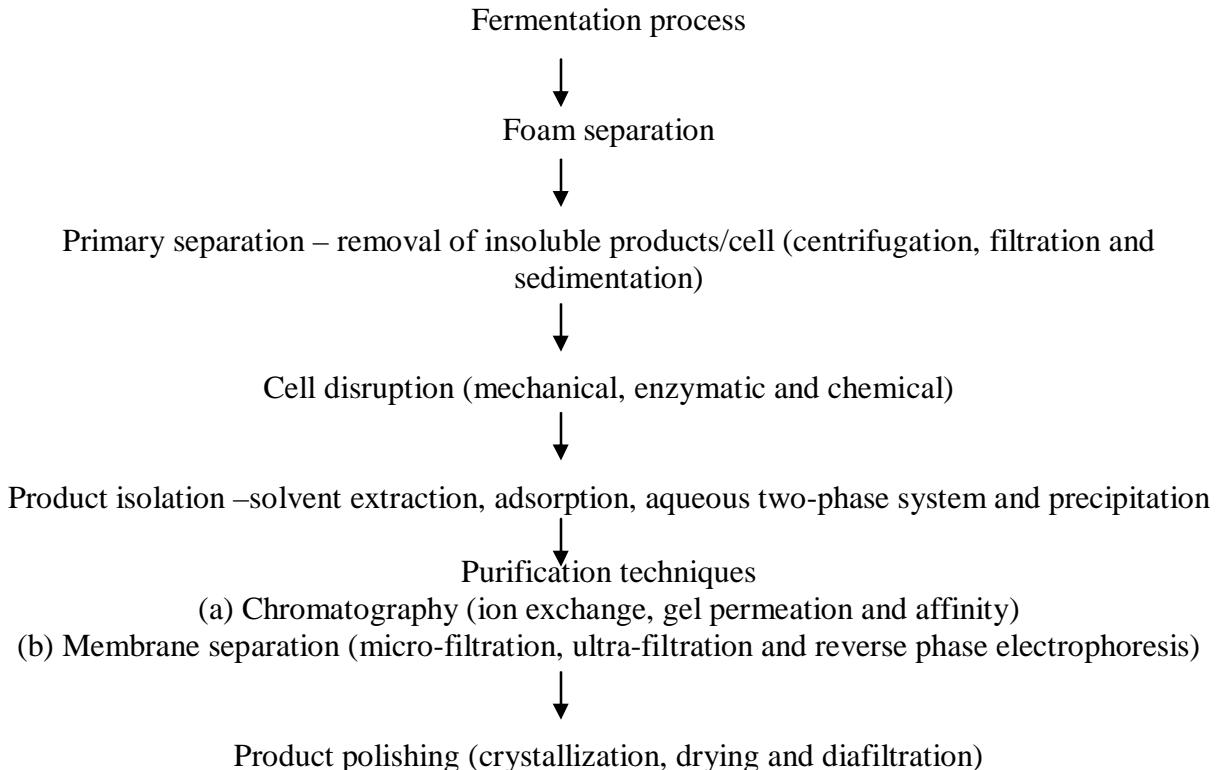
### **2.0 AIMS AND OBJECTIVES**

This unit explains the processes and techniques used for purification of a product from culture medium after fermentation process.

### **2.1 INTRODUCTION**

Downstream processing is an essential part of bioprocess technology in that the desired product needs to be isolated, purified and for different end uses. A variety of microorganisms including genetically engineered species are used for the production of desired products. The products formed may be secreted into the broth or may be retained within the cell introducing complexity in the recovery of the product. In view of the complexity, downstream processing involves various techniques and methodologies. Bioproducts differ greatly in their nature hence different separation principles and mechanisms depending on molecular mass, charge distribution, hydrophobicity, distribution coefficient, structure and immunogenic structure and specific affinity towards other biomolecules becomes necessary for their isolation and purification. The choice of the separation methodology depends to a large extent on the nature of the product, its quantity and the extent of purity required.

Down stream processing involves the following primary steps:



**Fig. 1** Flowchart of downstream processing

## 2.2 FOAM SEPARATION

Initial step in the down stream processing is the removal of foam without loss of cells and products. Whole cells or proteins gets attached to the surface of the air bubbles rising through liquid forming foam and when separated will be lost. To minimize the loss two ways of foam separation has been adapted.

1. Excess foam collected in a separate outlet and the foam is mechanically broken. The cells and protein from the foam is collected for further usage.
2. Materials are made surface active and collected termed as colligands and the Surfactants used for that purpose are termed collectors.

Addition of surfactants is found to improve the percentage of removal of cells.

## 2.3 SEPARATION OF CELLS AND INSOLUBLE PRODUCTS

### 2.3.1 SEDIMENTATION

Following any industrial fermentation it is essential to sediment suspended particles which involves coagulation and flocculation. Sedimentation process may be achieved naturally with selected strains of brewing yeasts if chilled at the end of fermentation. Apart from temperature, other mechanisms that induce flocculation are neutralization of opposing charges,

reduction in surface hydrophilicity and bridging with high molecular weight polymers. Addition of chemicals such as acids and bases change the pH and hence the charge on the particles facilitating coagulation. Simple electrolytes such as ferric chloride and alum screen the electrostatic repulsion between the colloidal particles thereby permitting van der Waals' force to operate and leads to coagulation. Sometimes the polymers bridge between the particles leading to agglomeration of particles. The majority of flocculating agents currently in use are poly electrolytes and polyamines. Other agents are alum, calcium salts and ferric salts. After flocculation the particles are separated by filtration and centrifugation.

### 2.3.2 FILTRATION

Filtration is defined as the separation of solid in a slurry consisting of the solid and fluid by passing the slurry through a septum called filter medium. For filtration in some cases filter aids (diatomaceous earth) are used to improve porosity and faster flow rate. Flow of slurry through a uniform and constant depth porous bed is ruled by Darcy equation,

$$\text{Rate of flow} = dV/dt = kA\Delta P/\mu L$$

Where,

$\mu$  - liquid viscosity

L – depth of filter bed

$\Delta P$  – pressure differential across the filter bed

A – area of filter exposed

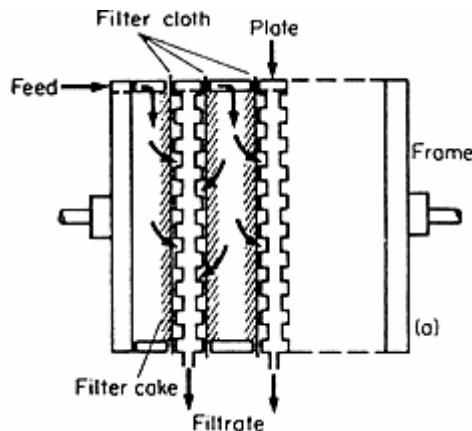
K – constant (depends on surface area of filter bed and voidage volume)

L, the depth of filter bed can be defined as volume of filtrate passed in time 't' and varies with volume of cake deposited per unit volume of filtrate. By carrying out small scale filtration trials, it is possible to determine K, and then the solved equation may be applied for large scale filtration calculation.

#### 2.3.2.1 Types of filters

##### 2.3.2.1 Batch filters

- (i) **Plate and frame filters:** In this plates and frames are arranged alternately (Fig.2) assembled on a horizontal framework. Plates are covered with filter clothes and held together by hand screw to prevent leakage between frames. The slurry is fed through the continuous channel by the holes in the corners of the plates and frames. The filtrate passes through the filter cloth or pad, runs down grooves in the filter plates and is then discharged through outlet taps to a channel.

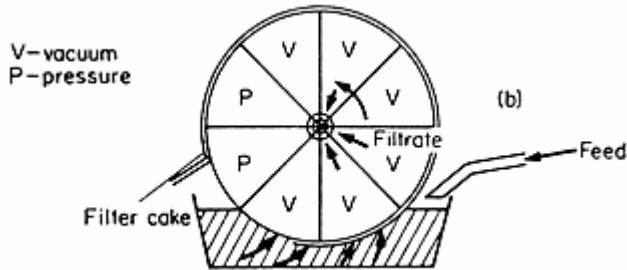


**Fig. 2** Plate and Frame filters

- (ii) **Pressure leaf filters:** These filters incorporate a number of leaves, each consisting of a metal framework of grooved plates which is covered with a fine wire mesh, or occasionally a filter cloth and often precoated with a layer of cellulose fibers. The slurry is fed into the filter which operates under pressure or by suction with a vacuum pump. There are three types based on the arrangement of filters. (a) Vertical metal-leaf filter – consists of a number of vertical porous metal leaves mounted on a hollow shaft in a cylindrical pressure vessel. The solids from the slurry gradually build up on the surfaces of the leaves and the filtrate is removed from the plates via the horizontal hollow shaft (b) Horizontal metal-leaf filter – consists of metal leaves mounted on a vertical hollow shaft within a pressure vessel. Filtration is continued until the cake fills the space between the disc shaped leaves or when the operational pressure has become excessive (c) stacked disc filter – this metafilter consists of a number of precision made rings which are stacked on a fluted rod. The filtrate passes between the discs and is removed through the grooves of the fluted rods, while solids are deposited on the filter coating.

### 2.3.2.2 Continuous filters

- (i) **Rotary vacuum filtration :** Drum covered with diatomaceous earth matter and allowed to rotate under vacuum with half immersed in the slurry tank (Fig.). 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 form 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. The mechanism of cake discharge is achieved by three ways. (a) string discharge – Long lengths of string 1.5cm apart are threaded over the drum and round two rollers. The cake is lifted free from the upper part of the drum when the vacuum pressure is released and carried to the small rollers where it falls free (b) scraper discharge – by using a knife or scraper positioned accurately to slice off the cake (c) scraper discharge with precoating – to avoid blockage of filter cloth in the drum by cells a scraper which is coated with a layer of filter-aid 2 to 10 cm thick

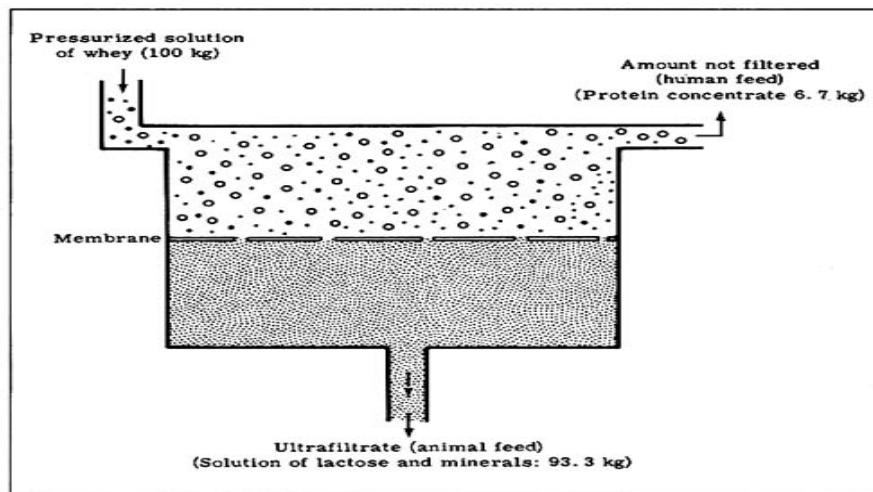


**Fig. 3** Rotary vacuum filter

(ii) **Micro or Ultra Filtration:** Filtration of suspended particles can be achieved by either dead end filtration or cross flow filtration.

(a) Dead end filtration – Solution poured over the membrane and the filtrate is collected at the bottom. On prolonged filtration pores become blocked which reduce the filtering capacity.

(b) Cross flow filtration – To prevent the blockage the solution is passed over the membrane. Cell suspension enters laterally and flows over the membrane. The filtrate gets collected at the bottom whereas the cells are pushed to the opposite end by the continuous flow of suspension which is sent out via an outlet at the opposite end. The liquid is again passed through a tube which recycles back to the flow. Since the cells do not block the pores the filtration process can be performed continuously.



**Fig. 4** Cross flow filtration

### 2.3.2.3 Adsorption on filter aids:

Filter aids, are inert incompressible discrete particles of high permeability. Solids such as wood pulp, starch powder, cellulose, inactive carbon, when added as filter aid enhances their filterability. Filter aids absorb small particles, which otherwise clog the filter pores. Filter aids also reduce the compressibility of the accumulated biomass by adsorbing the colloidal particles.

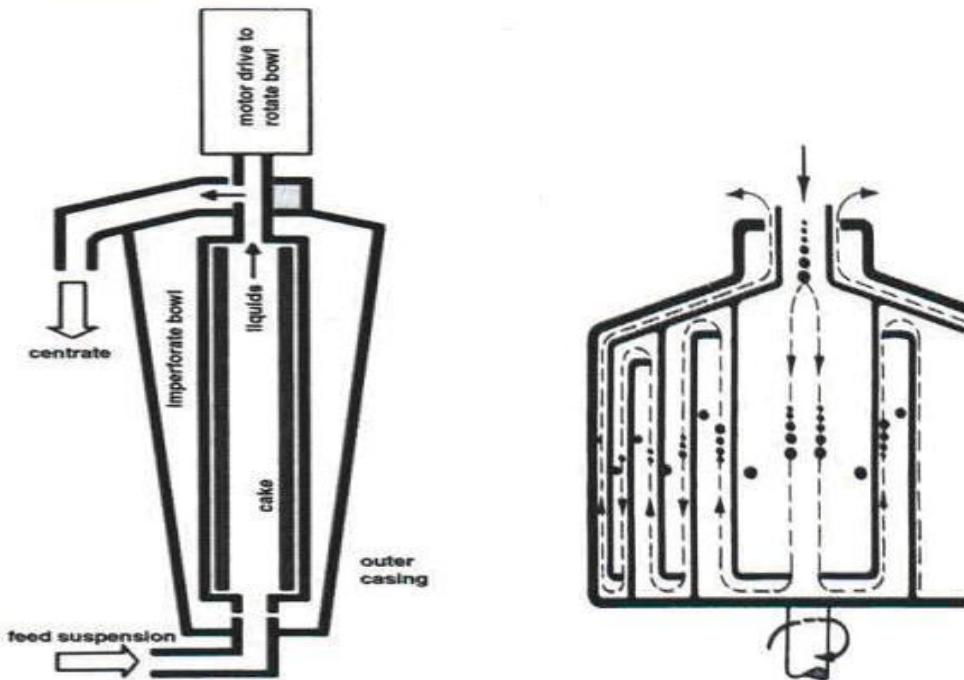
### 2.3.3 CENTRIFUGATION

Centrifugation is a common method used to separate cells from cultured broth. It employs centrifugal force to promote accelerated settling of particles in a solid-liquid mixture. Separation is achieved by means of accelerated gravitational force by rapid rotation. Microorganisms and other cells from the fermented slurry can be removed by using centrifuge when filtration is not a satisfactory separation method. The particle size that can be separated range from  $0.1\mu\text{m}$  to  $100\mu\text{m}$ . Separation is based on Stoke's law, which states that the rate of Newtonian viscosity characteristics is proportional to the square of the diameter of the particles which is expressed as,

$$Vg = d^2g (\rho_p - \rho_L) / 18\mu$$

Where,  $Vg$  – rate of sedimentation,  $d$  – particle diameter,  $g$  – gravitational force,  $\rho_p$  – liquid density,  $\rho_L$  – particle density,  $\mu$  - viscosity

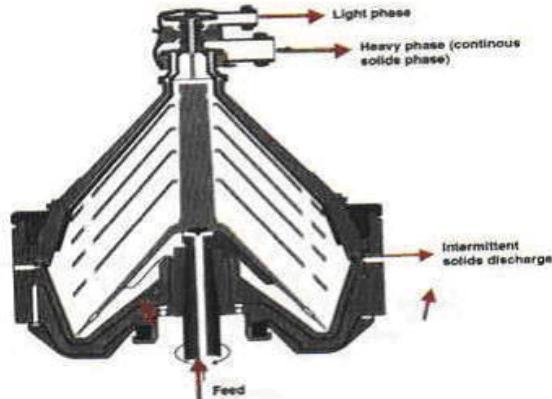
- (1) Tubular centrifuges:** This is used of separate particle size of  $0.1 - 200\mu\text{m}$ . This is simple machine made of a tube rotating between bearings at each end. The suspension enters at the bottom of the centrifuge and high centrifugal forces act to separate the solids and liquids. The bulk of solids will adhere to the walls of the bowl, while the liquids exit at the top of the centrifuge.



**Fig. 5 (a)** Tubular centrifuge and (b) Chamber bowl centrifuge

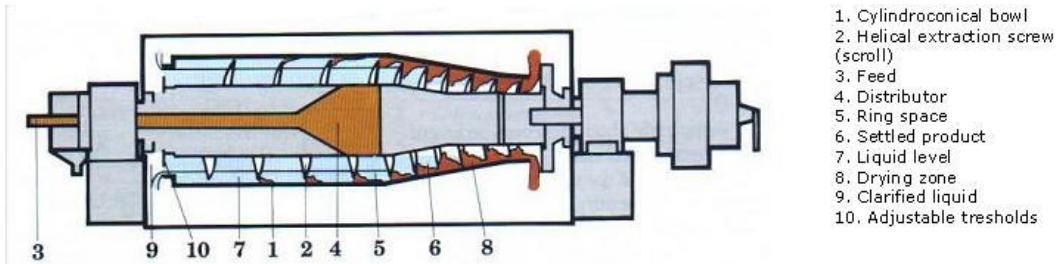
- (2) Chamber bowl centrifuge:** This centrifuge has a number of tubular bowl arranged coaxially. The main bowl contains cylindrical inserts that divide the volume of the bowl into a series of annular chambers, which operate in sequence. The feed enters the centre of the bowl and passes through each chamber. The solids settle on the outer walls of each chamber and the clarified liquid overflows from the largest diameter chamber.

**(3) Disc centrifuge:** This is a simplest design of disc stack separator is a closed bowl, containing the disc stack, with an arrangement to collect residual solids at the outer part of the bowl, from where they have to be removed manually after stopping rotation. The solids are discharged from the bowl through nozzles, which are always open.



**Fig. 6** Disc centrifuge

**(4) Decanter centrifuge:** This used for continuous handling of slurry. This sedimentation centrifuge is designed to handle significant solid concentration in feed suspension. It consists of horizontal cylindrical bowl rotating at a high speed, with a helical extraction screw placed co-axially. The screw perfectly fits the internal contour of the bowl, only allowing clearance between bowl and scroll. The differential speed between the screw and scroll provides the conveying motion to collect and remove solids that accumulate at the bowl wall.



**Fig. 7** Decanter centrifuge

## 2.4 CELL DISRUPTION

### 2.4.1 Chemical methods

Chemical treatment for disrupting cell include alkali, organic solvent, detergents and chaotropic agents.

**2.4.1.1** Alkali acts on the cell wall and results in saponification of membrane lipids. Alkali such as NaOH addition alters the pH and affect the integrity of the cell membrane. It is carried out at pH range of 11 to 12 for about 20 to 30 min.

**2.4.1.2** Organic solvents results in disruption of cell wall. Cell wall absorbs the solvent resulting in swelling and rupture of cell wall. At low concentration the cell wall is not ruptured but the permeability is increased. Product stability should be considered when choosing the solvent. Eg. Toluene is used for *Agrobacterium radiobacter*, Ethyl acetate used for yeast and Dimethyl sulfoxide (DMSO) for plant cell wall. Other organic solvents used are benzene, chlorobenzene, xylene, cumene, octanol, etc.

**2.4.1.3** Detergents permeabilize cells by solubilizing cell membranes. They are amphipathic capable of interacting with both water and lipids and solubilize the cell wall. Eg. Anionic detergents such as Sodium dodecyl sulphate (SDS), sodium squalonate, Cationic detergents such as cetyltrimethyl ammonium bromide (CTAB) and Non-ionic detergents such Triton X100.

**2.4.1.4** Chaotropic agents disrupt the structure of water making it less hydrophilic and weaken the hydrophobic interactions and increase permeability

## 2.4.2 Biological methods

Enzymatic digestion is involved in two stages (i) cell wall disruption resulting in the release of cell wall proteins leaving the protoplast intact and (ii) digestion of organelle membrane to release the organelle proteins. Digestion may be achieved by hydrolyzing cell walls by specific enzymes such as Lysozyme. Hydrolyzing cell wall by combination of enzymes ( $\beta$  - 1,3 – glucanase,  $\beta$ - 1,6 – glucanase, mannanase, chitinase etc) may be also used for digestion in cases like plants cell wall.

## 2.4.3 Physical methods

### 2.4.3.1 Mechanical methods

In Ultrasonic vibrators (sonicators) the ultrasound waves of frequencies greater than 20 kHz ruptures the cell wall by a phenomenon known as cavitation. The passage of ultrasound waves creates alternating areas of compression and rarefaction which change rapidly. The cavities formed in the areas of rarefaction rapidly collapses as the area changes to one of compression. The bubbles produced in the cavities collapse creating shock waves which disrupt cell walls.

In laboratory grinding with a ball mill or a waring blender may be used. Waring blender is particularly effective with animal cells and tissues as well as with mycelial organisms. In industrial scale, cell disruption is carried out using a bead mill or high pressure homogenizer. Vertical or horizontal bead mill consists of a grinding cylinder with a central shaft fitted with a number of impellers and driven by motor. The cell suspension is pumped into the cylinder and cell disruption occurs due to shear forces produced between velocity gradients because of the rotary motion of cells and beads. In addition, collision between beads and cells and grinding of cells between rolling beads also contribute to the disruptive forces.

High pressure homogenization consists of a high pressure positive displacement pump couple to an adjustable discharge valve with a restricted orifice. The cell suspension is pumped

through the homogenizing valve at 200 – 1000 atmospheric pressure depending on microbes and cell concentration. Cell disruption occurs due to stress due to impingement, normal stress during passage through narrow channel and shear stress due to pressure drop.

#### **2.4.3.2 Non mechanical methods**

Osmotic shock provided by adding cells into twice the volume of pure water. The cells swell due to osmotic flow of water ultimately bursting. Freezing thawing cycles cause loss of membrane integrity and cell wall is ruptured. In Thermolysis the heat inactivates the organism by disrupting the cell walls without affecting the products. The effect of heat shock depends on pH, ionic strength, presence of chelating or sequestering agents such as EDTA. Combination of mechanical and non mechanical gives best results

### **2.5 SEPARATION OF SOLUBLE PRODUCTS**

#### **2.5.1 Liquid – liquid extraction**

It is a classical method for recovery as well as concentration of various products. Solvent extraction has several advantages such as selectivity of extraction directly from broth or reaction medium, reduction in product loss as the product is just transferred to a second phase and easy scale up. Solvent extraction involves extraction of compound in a liquid phase to another liquid. The solute originally present in aqueous phase gets partitioned in both the phases. The distribution between the two immiscible liquids and solubility in two liquids decide the efficacy of extraction. The choice of solvent selection was based on dielectric point. The dielectric constant is a measure of the degree of molar polarization of a compound. An increase dielectric pole increases the polarity of the solute.

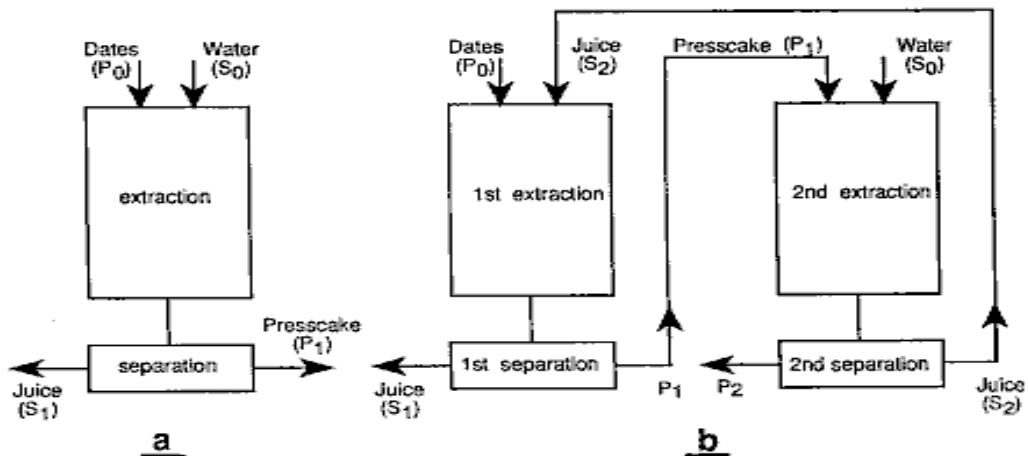
$$D = C/C_0$$

Where, D – dielectric constant, C – electrostatic capacity of a condenser containing the substance between the plates,  $C_0$  – electrostatic capacity of the same condenser when completely evacuated. The final choice of solvent is influenced by partition coefficient (k).

$k$  = concentration of solute in extract / concentration of solute in raffinate

High value of  $k$  required single stage extraction and low value  $k$  requires multistage extraction.

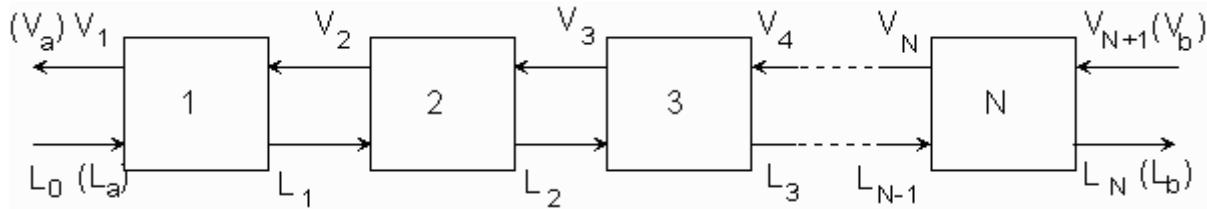
In single stage batch extraction, the aqueous feed is mixed with the organic solvent and after equilibration; the extract phase containing the desired solute is separated out for further processing. In some cases, a single stage extraction may not be enough and multi stage process is required wherein fresh volume of solvent is contacted with the raffinate.



**Fig. 8** (a) Single stage and (b) double stage extraction process

Continuous extraction can be carried out by co-current or counter current methods. In Co-current extraction there are  $n$  mixer vessels in line and the raffinate goes from vessel 1 to vessel  $n$ . Fresh solvent is added to each stage and the extracting solvent pass through the cascade in the same direction. At every stage the extract is recovered.

In Counter current extraction the extracted raffinate passes from vessel 1 to vessel  $n$  while the product-enriched solvent is flowing from vessel  $n$  to vessel 1. This is the most efficient method of extraction.



**Fig. 9** Counter current extraction process

Extraction is achieved by three mechanisms *viz.*, physical extraction, dissociative extraction and selective extraction. Physical extraction involves preferential dissolution of the desired solute in a chosen organic solvent. Dissociative extraction involves the modification of the physical property of the solute to increase the solubility in organic phase. For example for extraction of organic acids pH is adjusted below pK value enhancing dissociation and thus extraction. Selective extraction involves modifying the solute solubility through ion pair or complex or adduct formation. For example long chain aliphatic amines are used for citric acid extraction.

Solvent recovery after extraction process is essential one which is usually done by distillation. The distillation is performed in three stages (i) evaporation of solvent into vapour phase, (ii) vapour-liquid separation and (iii) condensation to collect solvent.

## 2.5.2 Aqueous two phase extraction

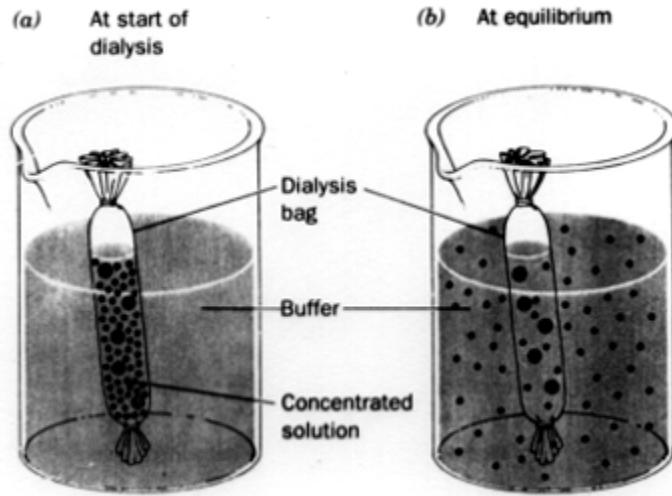
The basic principle involves differential partitioning of solute in two immiscible phases. Phase separation occurs when hydrophilic polymers are added to an aqueous solution. At low concentration of polymers, homogenous solution is formed but at discrete concentration rise, two immiscible phases are formed using two aqueous phases with incompatible polymers such as PEG and dextran. Eg. PEG water / dextran water and PEG water/K-phosphate water, PEG phosphates. Homogenates are prepared with the two incompatible polymers after which mixer – phase separation is done by keeping idle. The bottom phase and top phase separated. Then the soluble and nonsoluble substances are separated by ultra filtration and product recovered. The retentate may be recycled for further recovery.

**2.5.2.1 Precipitation** - by decreasing the solubility of the solutes the solute can be separated by precipitation Solubility of the particle can be changed by,

1. Salting out – by increasing ionic strength by adding salts as ammonium sulphate, disodium sulphate
2. Solubility reduction at low temperature – by adding organic solvents at low temperature
3. Solvent precipitation – adding salt, pH adjustment and low temperature
4. Isoelectric precipitation – by changing the pH to isoelectric pH (no charge in proteins)
5. Use of electrolytes – ionic polymers (ionic polysaccharides), non ionic polymer (dextrans)

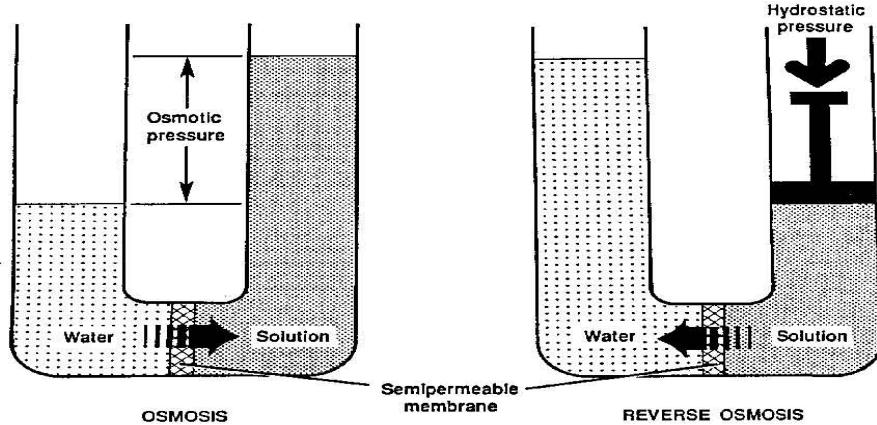
**2.5.2.2 Adsorption** - This refers to the binding of molecules or particles to a surface or adsorption of solutes from liquid media onto solids. Adsorption occurs due to van der Waals' force, strong ionic bonds. There are different types of solid – liquid contactors to facilitate adsorption viz., packed bed, moving bed, fluidized bed, and agitated vessel contactors. Packed beds are adsorbent bed packed and liquid sent through it. In Moving bed, adsorbent solid is continuously supplied, after adsorbing get removed from vessel. Fluidized bed is in which adsorbent solid is suspended in liquid. Packed and moving beds are widely used.

**2.5.2.3 Dialysis** is a membrane separation used to remove low molecular weight solutes (organic acids, inorganic ions). Dialysis is the movement of molecules by diffusion from high concentration to low concentration through a semi-permeable membrane. Only those molecules that are small enough pass through the membrane pores and reach equilibrium with the entire volume of solution in the system. Once equilibrium is reached, there is no further net movement of the substance because molecules keep moving through the pores in and out of the dialysis unit at the same rate. Factors affecting dialysis: dialysis buffer volume, buffer composition, number of buffer changes, time, temperature and particle size. Membranes containing convoluted pores, not the tube-like pores often found in traditional dialysis tubing.



**Fig. 10** Dialysis process

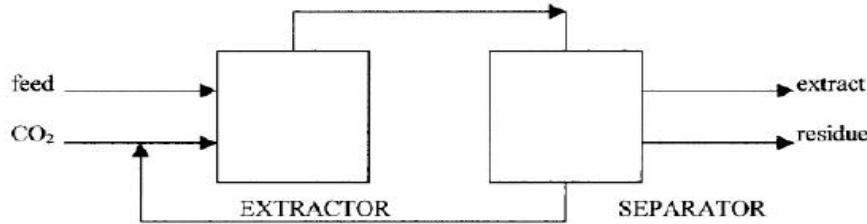
**2.5.3.4 Reverse osmosis** is a process where the solvent molecules are forced by an applied pressure to flow through a semi permeable membrane in the opposite direction. Reverse osmosis from low to high by applying pressure uses membrane (pore size 0.0001 – 0.001  $\mu\text{m}$ ) permeable to water but not dissolved salts of low molecular weight. This method is applicable to concentrate smaller molecules.



**Fig. 11** Reverse osmosis process

## 2.5.4 Supercritical fluid extraction

Supercritical fluid extraction involves the dissolution power of super critical fluids *i.e.* fluids above their critical temperature and pressure. Critical temperature is defined as the temperature above which a distinct liquid phase cannot exist regardless of pressure. The vapour pressure of the substance at its critical temperature is called the critical pressure. Alternately, pressure and temperature required to liquefy a gas are critical temperature and pressure. At temperature and pressure above but close to the critical point a substance exists as a supercritical fluid. For example Carbon di oxide, NO, SO<sub>2</sub> are used in extraction of  $\beta$ -carotene, vanilla, vegetable oil, etc.

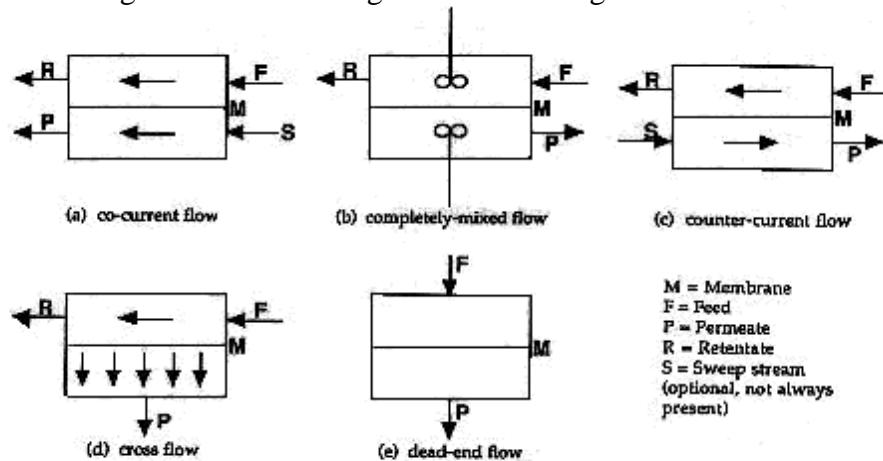


**Fig. 12** Supercritical fluid extraction process. CO<sub>2</sub> is fluidized and used for extraction

## 2.6 PURIFICATION TECHNIQUES

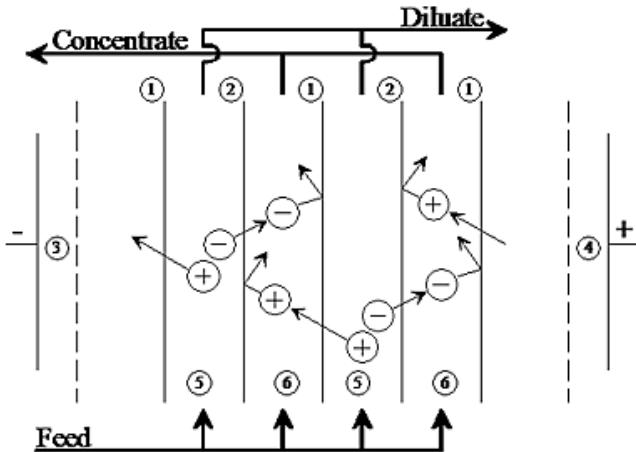
### 2.6.1 Membrane filtration

Membrane filtration is performed by either microfiltration or ultrafiltration. Microfiltration utilises microscope filters which is used to separate, 0.1 - 10µM particles. Ultra filtration has a thin skin with small pores over a thick highly porous support to prevent clogging and is used to separate very small particles. Both are pressure driven filters. Low molecular weight solutes passes through the filters and high molecular weight solute retain on membrane.



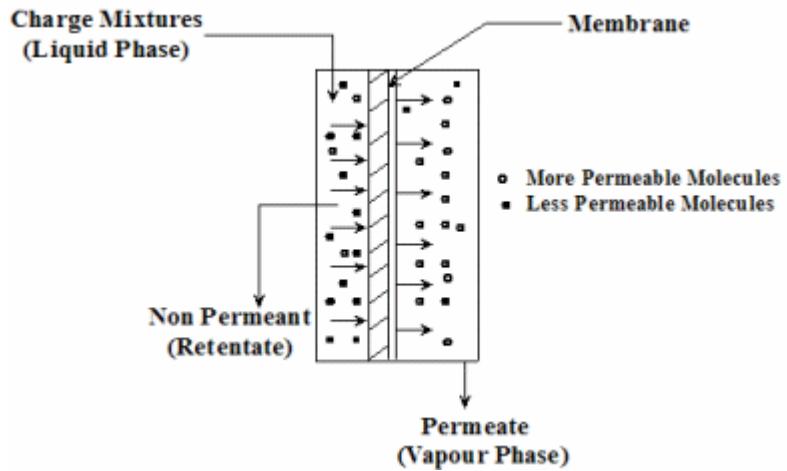
**Figure 1:** Types of ideal continuous flows used in membrane-based separations

Electrodialysis is separation of ions occurs due to the imposed potential difference across the ion selective cationic and anionic exchange membranes. The unit consists of a stack of compartment formed by alternate cationic and anionic exchange membranes. Electrokinetic transport of positively charged species through the cationic membranes and negatively charged through anionic membrane occurs and selective separation of mixtures of ionic species depending on the different ionic mobilities of species is facilitated.



**Fig. 13** Electrodialysis (1) cation selective membrane (2) anion selective membrane (3) cathode (4) anode (5,6) spacers

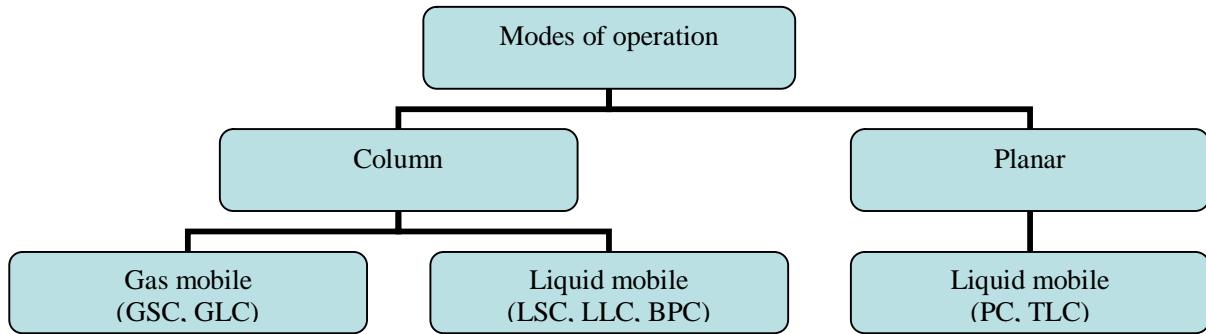
Pervaporation is also a membrane separation technique accompanied by a change of phase of the species transported across the membrane, usually from liquid to vapour. Pervaporation involves the separation of two or more components across a membrane by differing rates of diffusion through a thin polymer and an evaporative phase change comparable to a simple flash step. This is useful in separation of liquid component of a mixture. Either vacuum is applied to remove the vapour phase or an inert carrier gas takes away the vapour.



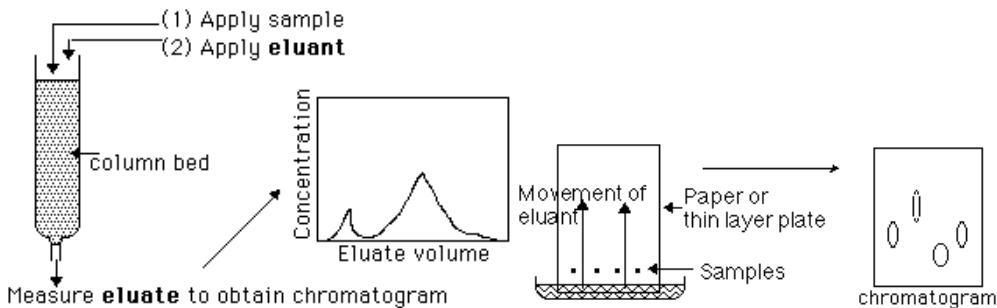
**Fig. 14** Pervaporation process

## 2.6.2 Chromatography

In any chemical or bioprocessing industry, the need to separate and purify a product from complex mixture is a necessary step. Today there exist a number of separation methods. Chromatography is a special separation process by passing through a bed or adsorbent material.



**Fig. 15** Various modes of operation of chromatography



**Fig. 16 (a)** Column chromatography

**(b)** Planar chromatography

Mechanism of separation in chromatography is based on different properties of the separating particle. Based on this, a broad range of physical methods are used to separate the product.

1. Adsorption – based on adsorption of solute onto solid particles eg alumina, silica gel
2. Liquid – liquid partition chromatography – based on different partition coefficient (solubility) of solute between adsorbed liquid phase and passing solution
3. Ion exchange – based on the adsorption of ions on ion exchange resins
4. Gel filtration – based on penetration of solute molecules into pores of packed gel
5. Affinity – based on specific chemical interaction between solute and added ligand on supporting matrix
6. Hydrophobic – based on hydrophobic interactions between solute and support particles
7. Reverse phase is a powerful analytical tool and involves a hydrophobic, low polarity stationary phase which is chemically bonded to an inert solid such as silica

Each of the separation mechanism can be performed in different ways utilizing various mobile and stationary phase. They are

#### Adsorption

- solid stationary and liquid mobile phase (LST, TLC)
- solid stationary and gas mobile phase (GSC)

### Partition

- mobile liquid + stationary liquid, PC, LLC, normal phase, HPLC
- mobile Super Critical fluid, stationary organic bonded phase (SCFC)
- mobile Liquid + stationary organic bonded phase BPC
- mobile Gas, stationary Liquid/organic BP, GC/GLC

### Ion exchange

- mobile Liquid + stationary resin IEC

### Size exclusion

- mobile Liquid + stationary poly bead GPC Gel filtration

Aims of chromatographic techniques are,

1. elution analysis – to separate individual component ie qualitative or quantitative
2. frontal analysis – to concentrate the component on the stationary phase for recovery at a later stage in a concentrated form
3. displacement analysis – to regenerate the column after its use/to remove a particular component in the column

#### **2.6.2.1 Factors / parameters affecting chromatography**

**2.6.2.1.1 Partition coefficient  $k = C_s/C_m$**  where, molar concentration of solute in stationary ( $C_s$ ) and mobile phase ( $C_m$ ). If  $k$  is 1 no separation occurs

**2.6.2.1.2 Retention time** is the time of solute to reach the detector from the moment of its injection into the column

$$F = V \times P/t_m$$

where,  $F$  - flow rate,  $V$  – volume of column cross sectional area ( $\pi d^2/4 \times L$ ),  $P$  – porosity of stationary phase,  $t_m$  – time to pass through the column ( $t_m = L/\mu$  where  $L$  – length,  $\mu$  - velocity of mobile phase).

Retention time is related to partition coefficient,

$$t_R = t_m [ 1 + k (V_s/V_m) ]$$

where  $V_m$  – mobile phase volume,  $V_s$  – stationary phase volume,  $t_R$  – retention time

Factors influencing retention time are nature of stationary phase, composition of mobile phase, column dimension and mobile phase flow rate. Retention time is based on nature of substance and it is an important qualitative analysis.

**2.6.2.1.3 Retention volume** is defined as volume of the mobile phase required to transport a solute from the point of its injection, passage through column to the detector

$$V_R = t_R \times F$$

Where,  $V_R$  – retention volume,  $t_R$  – retention time,  $F$  – flow rate of mobile phase.

The relation retention volume to partition coefficient is expressed as,

$$V_R = V_m + kV_s$$

Where,  $V_m$  – mobile phase volume contained within the column

**2.6.2.1.4 Capacity factor and retention ratio** is a measure of retention of solute component and is also called solute partition ratio/mass distribution ratio  $k'$ . It is a ratio of total amount of solute in stationary phase to that in the mobile phase *viz.*

$$k' = C_s V_s / C_m V_m \text{ or } k' = K/\beta$$

where,  $\beta$  - phase ratio,  $K$  – partition coefficient. In paper and thin layer, retardation factor ( $R_f$ ) decides the mobility of solute relative the mobility of the solvent / mobile phase

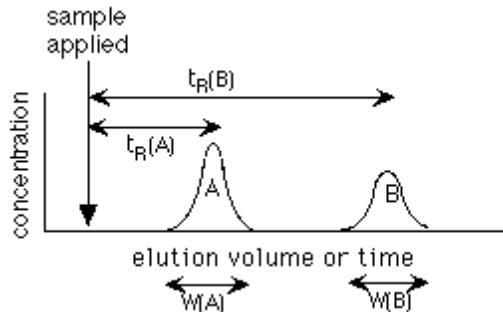
$$k' = 1 - R_f / R_f$$

**2.6.2.1.5 Relative retention**  $\alpha$  is the ability of the chromatographic system to separate peaks for 2 solutes. This describes the differential migration rate of the solutes  $\alpha = k_2/k_1$ . Relative retention depends on the nature of the two phases and column temperature. The choice of stationary phase important where  $\alpha$  should be equal to 1.05 or greater up to 2. If the relative retention is more the time required for completing the run is increased.

**2.6.2.1.6 Column efficiency** is related to the width of chromatography peak. An ideal peak has Gaussian shape. Ideal Gaussian peak has peak widths at the inflection point at half height and at the base of peak are related in terms of standard deviation

$$y = y_o e^{-x^2/2\sigma^2}$$

where  $y$  – height at any point on the curve,  $y_o$  – height at peak maximum,  $x$  – distance more or less point on curve and the ordinate passing through the peak maximum,  $\sigma$  - standard deviation and  $\sigma^2$  – variance. Gaussian peak is symmetrical when the peak width  $w$  at any point is  $2x$ . Inflection points ( $x = \sigma$ ) of the peak occur at a height of 0.607  $y_o$ .



**Fig. 17** Gaussian peak

Efficiency of column described in terms of number of theoretical plates ( $n$ ) and the plate height. Plate height is actually variance divided by the length of the column ( $h = \sigma^2/L$ ) and length of the column occupied by one theoretical plate or one effective plate is determined by the equation,  $h=L/n$ . The number of effective plates ( $N$ ) of the actual retention of the solute is given by,

$$N = 16 (t_{R'} / \omega_b)^2$$

where,  $t_{R'}$  – adjusted retention time and  $\omega_b$  – peak width at the base.

**2.6.2.1.7 Resolution** of two chromatography peak  $R_s$  is a measure of their separation defined as the distance between peak maxima compared with the average base widths of the two peaks.

$$R_s = 2(t_{R2} - t_{R1}) / (\omega_{b1} + \omega_{b2})$$

Where,  $t_R$  and  $w$  – measured in same width. This depends on 3 independent factors *viz.* column selectivity or separation factor ( $\alpha$ ), Retention factor or capacity factor ( $k'$ ) and Number of

theoretical plates ( $n$ ) (efficiency), If  $R_s = 1$ , 2 peaks just touch each other.  $R_s < 1$  they are incompletely separated. When  $R_s$  is equal or more than 1 complete separation is achieved.

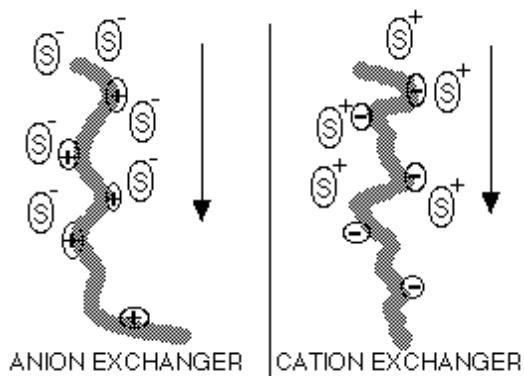
**2.6.2.1.8 Peak asymmetry** is more prevalent at low concentration, due to higher  $k'$  values, due to poorly packed columns and sample injection problems.

### 2.6.3 Ion exchange chromatography

Ion exchange chromatography separation and purification is based on the basis of charge. Basic principle involves reversible competitive binding of different ions of one kind to immobilized ion exchange groups of opposite charge which is bound to the chromatographic matrix called ion exchanger.

In a column cationic matrix (negatively charged) when positively charged components are loaded, more positively charged components bind strongly to the matrix whereas negative charged and neutral components eluted without retention. The factors influencing the binding are net charge, anisotropy (charge distribution on protein surface), ionic strength, pH of solvent, nature of ions and other additives. pH of the medium and solvent are important since they determine the effective charge on both proteins and matrix.

There are two types of ion exchangers. Cation exchangers have acidic groups with net negative charge and positively charged exchangeable ions. Anion exchangers have basic groups with net positive charge and negatively charged exchangeable ions. Charges on ion exchanger based pH of solvent.

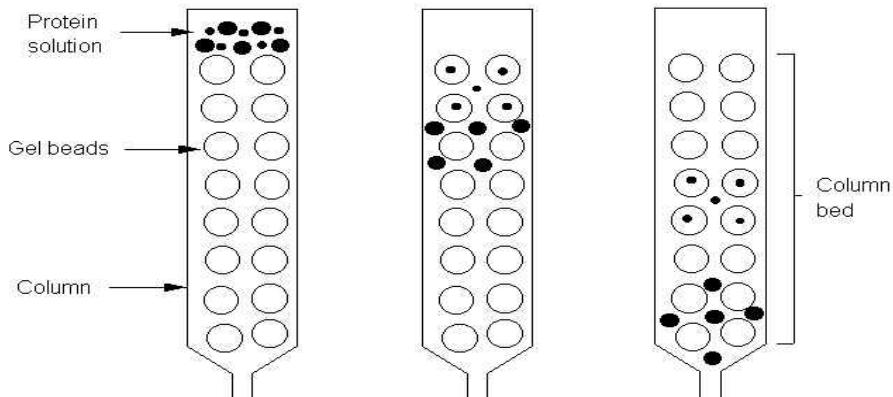


**Fig. 18** Ion exchange column

### 2.6.4 Gel permeation chromatography

Gel permeation chromatography separation is based on the difference in size of the particle. The gel column consists of polysaccharide dextran cross-linked to form a three dimensional network in the shape of bead. The extent of cross-linking during manufacture of gels controls the pore size within the gel beads. Small molecules can enter the pores of the gel but larger molecules are excluded. Elution of molecules is in decreasing order of size since eluting solvent has lesser access to the smaller molecules than the larger molecules. Hence the larger molecules are eluted first followed by smaller ones.

Cross-linked dextrans (sephadex) and agarose (sepharose), polyacrylamide and porous glass gels are commercially available. The degree of cross-linking is carefully controlled to give a range of products capable of fractionating molecules of a limited size range. The factors affecting the resolution are void volume, size of the molecule, molecular weight of the molecule pore size of the gel bead, elution volume and number of theoretical plates.

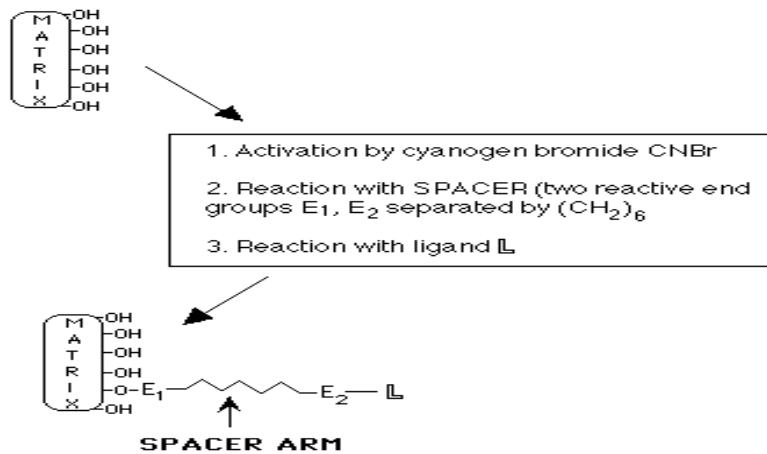


**Fig. 19** Gel permeation column chromatography

## 2.6.5 Affinity column chromatography

This chromatography includes a group of closely related techniques such as bioaffinity, dye-ligand and immobilized metal ion affinity chromatographic techniques. The stationary phase matrix is specially prepared to isolate or purify biomolecules based on their specific interactions between the matrix immobilized functional groups and functional groups on the surface of the biomolecules. The nature of these interactions may be simple or complex. A ligand which has a functional group with affinity towards the biomolecules is usually attached covalently to the support matrix and packed in a column. When the biomolecules are loaded, only that recognize the ligand are retained whereas the remaining mixtures are eluted. Then washed to removed any non specific molecules. The adsorbed component can then be eluted out in pure form by using a suitable solvent or eluent.

Some specific ligands are lectins such as con A for glycoprotein isolation. The choice of the ligand depends mainly on two factors namely, (i) availability of chemically modified groups on the ligand to facilitate its attachment to the matrix while retaining binding capacity with the biomolecule (ii) affinity towards the counter-ligand with suitable K values to ensure maximum binding and desorption. Spacer arm usage helps in proper access of the binding site to the molecule. Commonly used spacer arm are linear aliphatic hydrocarbons with terminal functional groups to facilitate the binding of the spacer molecule to the matrix as well as the ligand. For coupling the ligand to the matrix the following steps have to be adopted. (i) chemical activation of the matrix, (ii) immobilization of the ligand via the chosen functional group and (iii) blocking or deactivating the residual active groups. After coupling and blocking the matrix can be packed and used for separation. In the economic point of view, the column is regenerated and reused.



**Fig. 20** Affinity chromatography matrix preparation

## 2.7 PRODUCT POLISHING

### 2.7.1 Crystallization

It is a process where solid particles of specified size and shape are formed from a homogenous phase. It is the most common method of final purification of a desired product because crystals obtained are usually of exceptional purity and free from even closely related impurities. Crystallization can be brought about by (a) cooling the solution with negligible evaporation (b) evaporation of the solvent with little or no cooling as in evaporator crystallizer or (c) combined cooling and evaporation as in adiabatic or vacuum crystallizers.

#### 2.7.1.1 Drying by conduction

Drying with evaporators removes the solvent, mainly water, from the desired product by heat conduction. A typical evaporator has two principal functions to exchange heat and to separate vapour that is formed from liquid. It has got three principal functional sections viz., the heat exchanger, the evaporating sections where the liquid boils and evaporates and the separator in which the vapour leaves the liquid and passes to the condenser.

Types of evaporator are

Direct contact drying involves bringing the material to be dried into contact with a heated surface and heat is supplied to the product mainly by conduction.

1. Open pans involves boiling of the liquid in open pans for evaporation. This is restricted to the heat stable product
2. Horizontal tube evaporator is an improved open pan method in which pan is fixed in a vertical cylinder
3. Plate evaporator involves plate heat exchanger may be adapted for use as an evaporator
4. Long tube evaporator is tall slender, vertical tubes with length to diameter ratio of 100:1. The liquid pass down the heated tubes
5. In Forced circulation evaporator, a pump circulation is included such that the heat exchange surface can be divorced form the boiling and vapour separating sections

6. In Batch vacuum dryers the tray with materials is enclosed in a large cabinet which is evacuated.
7. Drum drying involves spreading the material over the surface of a heated drum

### **2.7.1.2 Drying by convection**

Hot air drying uses a moving stream of hot air in contact with the product to be dried. Heat is supplied to the product mainly by convection. Examples of such drying are Kiln driers, tray or compartment driers, etc.

1. Tray dryers involves the spreading out the materials as a thin layer on trays and heat is applied by air current over the trays
2. Tunnel dryers are a developed tray dryers. The tray with the materials move through a tunnel on trolleys where heat is applied and vapours removed
3. In Pneumatic dryers or Conveyor driers the materials are conveyed rapidly in an air stream. Heated air sweeps away the heat
4. In Fluidized bed driers the materials is suspended against gravity in an upward-flowing air stream
5. Rotary driers is in which the materials are filled in a horizontally inclined cylinder and heated either by air flow through the cylinder
6. Trough dryers involves a trough shaped conveyor belt
7. In Bin dryers the container is a bin with perforated bottom through which hot air is blown upwards
8. In Belt dryers the materials to be dried is spread as a thin layer over a mesh or solid belt and air passed through the material
9. Spray driers involves the slurry is broken down into small droplets and sprayed into a current of heated air

### **2.7.2 Lyophilization**

Freeze drying technically known as Lyophilization is a process of sublimation in which the water molecules in a solid phase specimen are directly converted to free water molecules in vapour phase. It is the most complex and expensive form of drying, its use is restricted to delicate, heat sensitive materials. In the freeze drying process, material is frozen by exposure to cold air followed by sublimation of ice in vacuum from the frozen state to produce a dried product.

In batch freeze driers, a vacuum cabinet, a vacuum system and a heating system are present. Refrigerated condensers backed by a mechanical pumping system are commonly used, commercially. The pumping system is essential to pump the frozen materials down the cabinet pressure initially in a short time to prevent melting of the frozen products. Heat may be supplied by conduction or radiation or from a microwave radiator. The removal of the water by sublimation results in a porous structured product which retains shape and size.

### 2.7.3 Diafiltration

In this diafiltration process a hydrophobic membrane is used. The solvent with solute fill the pores and the products do not pass across due to pressure on other side. The solute immobilized on membrane with phosphate that can be extracted afterward.

### 2.8 LET US SUM UP

- Down stream processing is a sequential process of purifying a product from the culture medium after fermentation
- Initial purification step involves removal of cells from the culture medium by filtration and centrifugation. Sedimentation is mostly preferred to separate suspended solids in the medium
- If the product is intracellular the cells have to be disrupted to release the products into the exterior and further purification steps have to be adopted. The cell disruption can be achieved by chemical, physical and biological methods.
- After the cellular disruption, both the purification from intracellular and extracellular matter involves similar methodologies
- Removal of excess water from the products can be achieved by extraction process. Extraction process may be of different types such as liquid-liquid extraction, aqueous two phase extraction and supercritical fluid extraction.
- After concentration of product by extraction further purification can be attained by adopting various chromatographic techniques and membrane filtration methods
- Finally the purified product can be crystallized by heat process, lyophilization and diafiltration

### 2.9 POINTS FOR DISCUSSION

1. Analyze the various all disruption methods.
2. Discuss the factors affecting chromatography.

### 2.10 LESSON - END ACTIVITIES

1. What are the various steps involved in down stream processing?
2. Give a detailed account of techniques used for cell separation.
3. When and how cell disruption is carried out in down stream processing?
4. Write a brief account on various types of centrifugation.
5. Give a detailed account on liquid-liquid extraction.
6. How is aqueous phase extraction performed?
7. Describe the various types of chromatographic techniques.
8. Explain the various factors influencing product separation by chromatography.
9. Write a short note on (i) reverse osmosis (ii) pervaporation (iii) diafiltration.
10. Discuss in detail the process of product polishing.

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## UNIT III: FERMENTED FOOD PRODUCTS

### **CONTENTS**

#### **3.0. AIMS AND OBJECTIVES**

##### **3.1. INTRODUCTION**

##### **3.2. FERMENTATION AND DAIRY PRODUCTS**

##### **3.3. PRODUCTION OF DISTILLED BEVERAGES**

##### **3.4. FOOD ADDITIVES**

##### **3.5. PIGMENTS**

##### **3.6 LET US SUM UP**

##### **3.7 POINTS FOR DISCUSSION**

##### **3.8 LESSON END ACTIVITIES**

##### **3.9 REFERENCES**

#### **3.0. AIMS AND OBJECTIVES**

This unit imparts knowledge on the various fermented products as a result of fermentation also dealt with food additives and pigments.

#### **3.1. INTRODUCTION**

Fermentation typically refers to the conversion of sugar to alcohol using yeast under anaerobic conditions. A more general example of fermentation is the chemical conversion of carbohydrates into alcohols or acids. When fermentation stops prior to complete conversion of sugar to alcohol, a stuck fermentation is said to have occurred. The science of fermentation is known as zymology.

French chemist Louis Pasteur was the first known *zymologist*, when in 1857 he connected yeast to fermentation. Pasteur originally defined fermentation as "respiration without air". Pasteur performed careful research and concluded;

"I am of the opinion that alcoholic fermentation never occurs without simultaneous organization, development and multiplication of cells.... If asked, in what consists the chemical act whereby the sugar is decomposed ... I am completely ignorant of it."

The German Eduard Buchner, winner of the 1907 Nobel Prize in chemistry, later determined that fermentation was actually caused by a yeast secretion that he termed *zymase*.

Fermented foods provide and preserve vast quantities of nutritious foods in a wide diversity of flavors, aromas, and textures which enrich the human diet. Fermented foods have been with us since humans arrived on earth. They will be with us far into the future, as they are the source of alcoholic foods, beverages, vinegar, pickled vegetables, sausages, cheeses, yogurts, vegetable protein amino acid/peptide sauces and pastes with meat-like flavors, and leavened and sourdough breads. All consumers today have a considerable portion of their nutritional needs met through fermented foods and beverages.

Classification of Food Fermentations (Steinkraus 1995, 1997) Food fermentations can be classified in a number of ways (Dirar 1993): by categories (Yokotsuka 1982)— 1) alcoholic beverages fermented by yeasts; (2) vinegars fermented with Acetobacter; (3) milks fermented with lactobacilli; (4) pickles fermented with lactobacilli; (5) fish or meat fermented with lactobacilli; and, (6) plant proteins fermented with molds with or without lactobacilli and yeasts; by classes (Campbell-Platt 1987) (1) beverages; (2) cereal products; (3) dairy products; (4) fish products; (5) fruit and vegetable products; (6) legumes; and, (7) meat products; by commodity(Odunfa 1988) (1) fermented starchy roots; (2) fermented cereals; (3) alcoholic beverages; (4) fermented vegetable proteins; and, (5) fermented animal protein; by commodity (Kuboye 1985) (1) cassava based; (2) cereal; (3) legumes; and, (4) beverages. Dirar (1993) states that the Sudanese traditionally classify their foods, not on the basis of microorganisms or commodity but on a functional basis: (1) Kissar (staples)-porridges and breads such as aceda and kissra; (2) Milhat (sauces and relishes for the staples); (3) marayiss (30 types of opaque beer, clear beer, date wines and meads and other alcoholic drinks); and, (4) Akil-munasabat (food for special occasions). Steinkraus (1983a; 1996) classified fermentations according to the following categories that will serve as the basis for this paper:

1. Fermentations producing textured vegetable protein meat substitutes in legume/cereal mixtures. Examples are Indonesian tempe and ontjom.

2. High salt/savory meat-flavored/amino acid/peptide sauce and paste fermentations. Examples are Chinese soy sauce, Japanese shoyu and Japanese miso, Indonesian kecap, Malaysian kicap, Korean kanjang, Taiwanese inyu, Philippine taosi, Indonesian tauco, Korean doenjang/kochujang, fish sauces: Vietnamese nuocmam, Philippine patis, Malaysian budu, fish pastes: Philippine bagoong, Malaysian belachan, Vietnamese mam, Cambodian prahoc, Indonesian trassi and Korean jeotkal. These are predominately Oriental fermentations but the use of these products is becoming established in the United States.

3. Lactic acid fermentations. Examples of vegetable lactic acid fermentations are: sauerkraut, cucumber pickles, olives in the Western world; Egyptian pickled vegetables in the middle East; Indian pickled vegetables and Korean kim-chi, Thai pak-sian -dong, Chinese hum-choy, Malaysian pickled vegetables and Malaysian tempoyak. Lactic acid fermented milks include: yogurts in the Western world, Russian kefir, Middle-East yogurts, liban (Iraq), Indian dahi, Egyptian laban rayab, laban zeer, Malaysian tairu (soybean milk). Lactic acid fermented cheeses in the Western world and Chinese sufu/tofu-ru. Lactic acid fermented yogurt/wheat mixtures: Egyptian kishk, Greek trahanas, Turkish tarhanas. Lactic acid fermented cereals and tubers (cassava): Mexican pozol, Ghanian kenkey, Nigerian gari; boiled rice/raw shrimp/raw fish mixtures: Philippine balao balao, burong dalag; lactic fermented/leavened breads: sourdough breads in the Western world; Indian idli, dhokla, khaman, Sri-lankan hoppers; Ethiopian enjera, Sudanese kisra and Philippine puto; Western fermented sausages and Thai nham (fermented fresh pork).

4. Alcoholic fermentations. Examples are grape wines, Mexican pulque, honey wines, South American Indian chicha and beers in the Western World; wines and Egyptian bouza in the Middle East; Palm and Jackfruit wines in India, Indian rice beer, Indian madhu, Indian ruhi; in Africa, Ethiopian tej, Kenyan muratina, palm wines, Kenyan urwaga, Kaffir/bantu beers,

Nigerian pito, Ethiopian talla, Kenyan busaa, Zambian maize beer; in the Far East, sugar cane wines, palm wines, Japanese sake, Indonesian tape, Malaysian tapuy, Chinese lao-chao, Thai rice wine, Indonesian brem, Philippine tapuy.

5. Acetic acid/vinegar fermentations. Examples are apple cider and wine vinegars in the West; palm wine vinegars in Africa and the Far East, coconut water vinegar in the Philippines; tea fungus/ Kombucha in Europe, Manchuria, Indonesia, Japan and recently in the United States; Philippine nata de pina and nata de coco.

6. Alkaline fermentations. Examples are Nigerian dawadawa, Ivory Coast soumbara, African iru, ogiri, Indian kenima, Japanese natto, Thai thua-nao.

7. Leavened breads. Examples are Western yeast and sourdough breads; Middle East breads.

8. Flat unleavened breads. The above classes of fermented foods are found around the world. The lines between the various classifications are not always distinct. Tempe in class 1 involves a lactic acid fermentation during soaking of the soybeans. Yeast (alcoholic)/ lactobacilli (lactic acid) interactions are rather frequent for example in sourdough breads, in primitive beers and wines and in Chinese soy sauce/Japanese shoyu/Japanese miso fermentations (Wood 1985). Nevertheless, Steinkraus (1983a, 1996) has found the above classification useful and a way of predicting what microorganisms may be involved and what chemical, physical and nutritive changes may occur in new unfamiliar fermented foods. The classification also relates well to safety factors found in fermented foods. Fermented foods were originally household and expanded to cottage industry as consumer demand required. Some food fermentations such as Japanese shoyu, miso and sake, South African maize/sorghum beers, South African mageu/mahewu Nigerian ogi and gari have been industrialized (Steinkaus 1989).

9. Alkaline fermentations. Examples are Nigerian dawadawa, Ivory Coast soumbara, African iru, ogiri, Indian kenima, Japanese natto, Thai thua-nao.

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11. Flat unleavened breads. The above classes of fermented foods are found around the world. The lines between the various classifications are not always distinct. Tempe in class 1 involves lactic acid fermentation during soaking of the soybeans. Yeast (alcoholic)/ lactobacilli (lactic acid) interactions are rather frequent for example in sourdough breads, in primitive beers and wines and in Chinese soy sauce/Japanese shoyu/Japanese miso fermentations (Wood 1985). Nevertheless, Steinkraus (1983a, 1996) has found the above classification useful and a way of predicting what microorganisms may be involved and what chemical, physical and nutritive changes may occur in new unfamiliar fermented foods. The classification also relates well to safety factors found in fermented foods. Fermented foods were originally household and expanded to cottage industry as consumer demand required. Some food fermentations such as Japanese shoyu, miso and sake, South African maize/sorghum beers, South African mageu/mahewu Nigerian ogi and gari have been industrialized (Steinkaus 1989).



## 3.2 DAIRY PRODUCTS

A dairy is a facility for the extraction and processing of animal milk -- mostly from cows, sometimes from buffalo, sheep, horses or goats -- for human consumption.

In the past, people in agricultural societies owned dairy animals that they milked for domestic or village consumption, a typical example of a cottage industry. The animals might serve multiple purposes (e.g. first for pulling a plow and at the end of its useful life as meat). With industrialisation and urbanisation, the supply of milk became a commercial industry, with specialised breeds of cow being developed for dairy, as distinct from beef or draft animals. Historically, the milking and the processing took place close together in space and time: on a dairy farm.

### 3.2.1 MILK PROCESSING

#### 3.2.1.1. CHEESE - PRINCIPLES OF CHEESE PROCESSING

Cheese is another product made from milk. Whole milk is reacted to form curds that can be compressed, processed and stored to form cheese. In countries where milk is legally allowed to be processed without pasteurization a wide range of cheeses can be made using the bacteria naturally in the milk. In most other countries, the range of cheeses is smaller and the use of artificial cheese curing is greater. Whey is also the byproduct of this process.

Cheese has historically been an important way of "storing" milk over the year, and carrying over its nutritional value between prosperous years and fallow ones. It is a food product that, with bread and beer, dates back to prehistory in Middle Eastern and European cultures, and like them is subject to innumerable variety and local specificity. Although nowhere near as big as the market for cow's milk cheese, a considerable amount of cheese is made commercially from other milks, especially goat and sheep.

Cheese is a solid food made from the milk of cows, goats, sheep and other mammals. Cheese is made by coagulating milk. This is accomplished by first acidification with a bacterial culture and then employing an enzyme, rennet (or rennet substitutes) to coagulate the milk to "curds and whey." The precise bacteria and processing of the curds play a role in defining the texture and flavor of most cheeses. Some cheeses also feature molds, either on the outer rind or throughout.

There are hundreds of types of cheese produced all over the world. Different styles and flavors of cheese are the result of using milk from various mammals or with different butterfat contents, employing particular species of bacteria and molds, and varying the length of aging and other processing treatments. Other factors include animal diet and the addition of flavoring agents such as herbs, spices, or wood smoke. Whether the milk is pasteurized may also affect the flavor. The yellow to red coloring of many cheeses is a result of adding annatto. Cheeses are eaten both on their own and cooked as part of various dishes; most cheeses melt when heated.

For a few cheeses, the milk is curdled by adding acids such as vinegar or lemon juice. Most cheeses, however, are acidified to a lesser degree by bacteria, which turn milk sugars into lactic acid, followed by the addition of rennet to complete the curdling. Rennet is an enzyme

mixture traditionally obtained from the stomach lining of young cattle, but now also laboratory produced. Vegetarian alternatives to rennet are available; most are produced by fermentation of the fungus *Mucor miehei*, but others have been extracted from various species of the *Cynara* thistle family.

Cheese has served as a hedge against famine and is a good travel food. It is valuable for its portability, long life, and high content of fat, protein, calcium, and phosphorus. Cheese is a more compact form of nutrition and has a longer shelf life than the milk from which it is made. Cheesemakers can place themselves near the center of a dairy region and benefit from fresher milk, lower milk prices, and lower shipping costs. The substantial storage life of cheese lets a cheesemaker sell when prices are high or when money is needed.

### **3.2.1.2. ORIGINS**

A piece of soft curd cheese, oven baked to increase its life. Cheese is an ancient food whose origins predate recorded history. There is no conclusive evidence indicating where cheese making originated, either in Europe, Central Asia or the Middle East, but the practice had spread within Europe prior to Roman times and, according to Pliny, had become a sophisticated enterprise by the time the Roman Empire came into being.

Proposed dates for the origin of cheese making range from around 8000 BCE (when sheep were first domesticated) to around 3000 BCE. The first cheese may have been made by people in the Middle East or by nomadic Turkic tribes in Central Asia. Since animal skins and inflated internal organs have, since ancient times, provided storage vessels for a range of foodstuffs, it is likely that the process of cheese making was discovered accidentally by storing milk in a container made from the stomach of an animal, resulting in the milk being turned to curd and whey by the rennet from the stomach. There is a legend to this effect, about Arab nomad carrying milk across the desert.

Cheese making may also have begun independent of this by the pressing and salting of curdled milk in order to preserve it. Observation that the effect of making milk in an animal stomach gave more solid and better-textured curds, may have led to the deliberate addition of rennet.

The earliest archaeological evidence of cheese making has been found in Egyptian tomb murals, dating to about 2000 BCE. The earliest cheeses were likely to have been quite sour and salty, similar in texture to rustic cottage cheese or feta, a crumbly, flavorful Greek cheese.

Cheese produced in Europe, where climates are cooler than the Middle East, required less aggressive salting for preservation. In conditions of less salt and acidity, the cheese became a suitable environment for a variety of beneficial microbes and molds, which are what give aged cheeses their pronounced and interesting flavors.

Then, bacteria in cheese making had come from the environment or from recycling an earlier batch's whey; the pure cultures meant a more standardized cheese could be produced.

Factory-made cheese overtook traditional cheese making in the World War II era, and factories have been the source of most cheese in America and Europe ever since. Today, Americans buy more processed cheese than "real", factory-made or not.

### **3.2.1.3. MAKING CHEESE**

#### **3.2.1.3.1. Curdling**

The only strictly required step in making any sort of cheese is separating the milk into solid curds and liquid whey. Usually this is done by acidifying the milk and adding rennet. The acidification is accomplished directly by the addition of an acid like vinegar in a few cases (paneer, queso fresco), but usually starter bacteria are employed instead. These starter bacteria convert milk sugars into lactic acid. The same bacteria (and the enzymes they produce) also play a large role in the eventual flavor of aged cheeses. Most cheeses are made with starter bacteria from the Lactococci, Lactobacilli, or Streptococci families. Swiss starter cultures also include Propionibacter shermani, which produces carbon dioxide gas bubbles during aging, giving Swiss cheese or Emmental its holes.

Some fresh cheeses are curdled only by acidity, but most cheeses also use rennet. Rennet sets the cheese into a strong and rubbery gel compared to the fragile curds produced by acidic coagulation alone. It also allows curdling at a lower acidity—important because flavor-making bacteria are inhibited in high-acidity environments. In general, softer, smaller, fresher cheeses are curdled with a greater proportion of acid to rennet than harder, larger, longer-aged varieties.

#### **3.2.1.3.2. Curd processing**

Ancient Swiss way of making cheese (heating stage). If needed, the wooden holder can be turned, moving the pot away from fire

During industrial production of Emmental cheese, the as-yet-un drained curd is broken up by rotating mixers. At this point, the cheese has set into a very moist gel. Some soft cheeses are now essentially complete: they are drained, salted, and packaged. For most of the rest, the curd is cut into small cubes. This allows water to drain from the individual pieces of curd.

Some hard cheeses are then heated to temperatures in the range of 35 °C–55 °C (100 °F–130 °F). This forces more whey from the cut curd. It also changes the taste of the finished cheese, affecting both the bacterial culture and the milk chemistry. Cheeses that are heated to the higher temperatures are usually made with thermophilic starter bacteria which survive this step—either lactobacilli or streptococci.

Salt has a number of roles in cheese besides adding a salty flavor. It preserves cheese from spoiling, draws moisture from the curd, and firms up a cheese's texture in an interaction with its proteins. Some cheeses are salted from the outside with dry salt or brine washes. Most cheeses have the salt mixed directly into the curds.

A number of other techniques can be employed to influence the cheese's final texture and flavor. Some examples:

**3.2.1.3.3. Stretching** (Mozzarella, Provolone). The curd is stretched and kneaded in hot water, developing a stringy, fibrous body.

**3.2.1.3.4. Cheddaring** (Cheddar, other English cheeses). The cut curd is repeatedly piled up, pushing more moisture away. The curd is also mixed (or milled) for a long period of time, taking the sharp edges off the cut curd pieces and influencing the final product's texture.

**3.2.1.3.5. Washing** (Edam, Gouda, Colby) The curd is washed in warm water, lowering its acidity and making for a milder-tasting cheese. Most cheeses achieve their final shape when the curds are pressed into a mold or form. The harder the cheese, the more pressure is applied. The pressure drives out moisture — the molds are designed to allow water to escape — and unifies the curds into a single solid body.

#### 3.2.1.3.6. Aging

A newborn cheese is usually salty yet bland in flavor and, for harder varieties, rubbery in texture. These qualities are sometimes enjoyed—cheese curds are eaten on their own—but usually cheeses are left to rest under carefully controlled conditions. This aging period (also called ripening, or, from the French, affinage) can last from a few days to several years. As a cheese ages, microbes and enzymes transform its texture and intensify its flavor. This transformation is largely a result of the breakdown of casein proteins and milk fat into a complex mix of amino acids, amines, and fatty acids.

Some cheeses have additional bacteria or molds intentionally introduced to them before or during aging. In traditional cheese making, these microbes might be already present in the air of the aging room; they are simply allowed to settle and grow on the stored cheeses. More often today, prepared cultures are used, giving more consistent results and putting fewer constraints on the environment where the cheese ages. These cheeses include soft ripened cheeses such as Brie and Camembert, blue cheeses such as Roquefort, Stilton, Gorgonzola, and rind-washed cheeses such as Limburger.

#### 3.2.1.4. Types of cheese

No one categorization scheme can capture all the diversity of the world's cheeses. In practice, no single system is employed and different factors are emphasised in describing different classes of cheeses. This typical list of cheese categories is from foodwriter, Barbara Ensrud.

Variety of cheeses in a supermarket Fresh, Whey , Pasta Filata, Semi-soft, Semi-firm , Hard , Double and triple cream , Soft-ripened, Blue vein, Goat or sheep, Strong-smelling Processed, Fresh, whey and stretched curd cheeses.

The main factor in the categorization of these cheese is their age. Fresh cheeses without additional preservatives can spoil in a matter of days. For these simplest cheeses, milk is curdled and drained, with little other processing. Examples include cottage cheese, Romanian Caș,

Neufchâtel (the model for American-style cream cheese), and fresh goat's milk chèvre. Such cheeses are soft and spreadable, with a mild taste. Whey cheeses are fresh cheeses made from the whey discarded while producing other cheeses. Provencal Brousse, Corsican Brocciu, Italian Ricotta, Romanian Urda, Greek Mizithra, and Norwegian Geitost are examples. Brocciu is mostly eaten fresh, and is as such a major ingredient in Corsican cuisine, but it can be aged too. Traditional Pasta Filata cheeses such as Mozzarella also falls into the fresh cheese category. Fresh curds are stretched and kneaded in hot water to form a ball of Mozzarella, which in southern Italy is usually eaten within a few hours of being made. Stored in brine, it can be shipped, and is known world-wide for its use on pizzas. Other firm fresh cheeses include paneer and queso fresco.

#### **3.2.1.4.1. Cheeses classed by texture**

Emmentaler Parmigiano reggiano. Categorizing cheeses by firmness is a common but inexact practice. The lines between "soft", "semi-soft", "semi-hard", and "hard" are arbitrary, and many types of cheese are made in softer or firmer variations. The factor controlling the hardness of a cheese is its moisture content which is dependent on the pressure with which it is packed into molds and the length of time it is aged. Semi-soft cheeses and the sub-group, Monastery cheeses have a high moisture content and tend to be bland in flavor. Some well-known varieties include Harvati, Munster and Port Salut. Cheeses that range in texture from semi-soft to firm include Swiss-style cheeses like Emmental and Gruyère. The same bacteria that give such cheeses their holes also contribute to their aromatic and sharp flavors. Other semi-soft to firm cheeses include Gouda, Edam, Jarlsberg and Cantal. Cheeses of this type are ideal for melting and are used on toast for quick snacks. Harder cheeses have a lower moisture content than softer cheeses. They are generally packed into molds under more pressure and aged for a longer time. Cheeses that are semi-hard to hard include the familiar cheddar, originating in the Cheddar Gorge of England but now used as a generic term for this style of cheese, of which varieties are imitated world-wide and are marketed by the length of time they have been aged. Cheddar is one of a family of semi-hard or hard cheeses (including Cheshire and Gloucester) whose curd is cut, gently heated, piled, and stirred before being pressed into forms. Colby and Monterey Jack are similar but milder cheeses; their curd is rinsed before it is pressed, washing away some acidity and calcium. A similar curd-washing takes place when making the Dutch cheeses Edam and Gouda. Hard cheeses — "grating cheeses" such as Parmesan and Pecorino Romano — are quite firmly packed into large forms and aged for months or years.

#### **3.2.1.4.2. Cheeses classed by content**

Some cheeses are categorized by the source of the milk used to produce them or by the added fat content of the milk from which they are produced. While most of the world's commercially available cheese is made from cows' milk, many parts of the world also produce cheese from goats and sheep, well-known examples being Roquefort, produced in France, and Pecorino Romano, produced in Italy, from ewes's milk. One farm in Sweden also produces cheese from moose's milk.[10] Sometimes cheeses of a similar style may be available made from milk of different sources, Fetta style cheeses, for example, being made from goats' milk in Greece and of sheep and cows milk elsewhere.

Double cream cheeses: are soft cheeses of cows' milk which are enriched with cream so that their fat content is 60% or, in the case of triple creams, 75%.

Moldy cheeses: There are three main categories of cheese in which the presence of mold is a significant feature: soft ripened cheeses, washed rind cheeses and blue cheeses.

Soft-ripened cheeses: are those which begin firm and rather chalky in texture but are aged from the exterior inwards by exposing them to mold. The mold may be a velvety bloom of *Penicillium candida* or *P. camemberti* that forms a flexible white crust and contributes to the smooth, runny, or gooey textures and more intense flavors of these aged cheeses. Brie and Camembert, the most famous of these cheeses, are made by allowing white mold to grow on the outside of a soft cheese for a few days or weeks. Goats' milk cheeses are often treated in a similar manner, sometimes with white molds (*Chèvre-Boîte*) and sometimes with blue.

Washed-rind cheeses: are soft in character and ripen inwards like those with white molds; however, they are treated differently. Washed rind cheeses are periodically cured in a solution of saltwater brine and other mold-bearing agents which may include beer, wine, brandy and spices, making their surfaces amenable to a class of bacteria *Brevibacterium linens* (the reddish-orange "smear bacteria") which impart pungent odors and distinctive flavors. Washed-rind cheeses can be soft (Limburger), semi-hard (Munster), or hard (Appenzeller). The same bacteria can also have some impact on cheeses that are simply ripened in humid conditions, like Camembert.

Blue cheese: is created by inoculating a cheese with *Penicillium roqueforti* or *Penicillium glaucum*. This is done while the cheese is still in the form of loosely pressed curds, and may be further enhanced by piercing a ripening block of cheese with skewers in an atmosphere in which the mold is prevalent. The mold grows within the cheese as it ages. These cheeses have distinct blue veins which give them their name, and, often, assertive flavors. The molds may range from pale green to dark blue, and may be accompanied by white and crusty brown molds. Their texture can be soft or firm.

Some of the most renowned cheeses are of this type, each with its own distinctive color, flavor, texture and smell. They include Roquefort, Gorgonzola, and Stilton.

### **3.2.1.5. Processed cheese**

Processed cheese is made from traditional cheese and emulsifying salts, often with the addition of milk, more salt, preservatives, and food coloring. It is inexpensive, consistent, and melts smoothly. It is sold packaged and either pre-sliced or unsliced, in a number of varieties. It is also available in spraycans. Processed cheese is the most-consumed category of cheese in the United States.

Paner (sometimes spelled Panir or Paner), is the most common Indian form of cheese. It is an unaged, acid-set, non-melting farmer cheese that is similar to acid-set fresh mozzarella and queso blanco, except that it does not have salt added, much like hoop cheese. Another significant difference between mozzarella and paneer is the fact that mozzarella melts like many other cheeses whereas paneer does not melt while cooking.

Most paneer is simply pressed into a cube and then sliced or chopped, although Bengali paneer is beaten or kneaded like mozzarella. Paneer is one of the few types of cheese indigenous to the Indian subcontinent, and is widely used in Indian cuisine and even some Middle Eastern and Southeast Asian cuisine. Unlike most cheeses in the world, the making of paneer does not involve rennet; it is therefore completely vegetarian. Paneer is a primary source of protein for Buddhists (typically those of Southeast Asian origin) who adhere to vegetarian as opposed to vegan diets. Paneer is known in North India and Pakistan by the same name; however, in Orissa and Bengal it is known by the name Chhana and in South India, by names derived from Paneer and Channa (not to be confused with Chana, the Indian name for the chick pea). The Eastern variety (Chhana, pronounced crumbles more easily than the North and South Indian variants of paneer.

### **3.2.1.6. PASTEURISATION**

Is the process of heating liquids for the purpose of destroying viruses and harmful organisms such as bacteria, protozoa, molds, and feces. The process was named after its inventor, French scientist Louis Pasteur.

Unlike sterilization, pasteurization is not intended to kill all micro-organisms (pathogenic) in the food. Instead, pasteurization aims to achieve a "logarithmic reduction" in the number of viable organisms, reducing their number so they are unlikely to cause disease (assuming the pasteurized product is refrigerated and consumed before its expiration date). Commercial-scale sterilization of food is not common, because it adversely affects the taste and quality of the product.

#### **3.2.1.6.1. Pasteurization processes**

Pasteurization typically uses temperatures below boiling since at temperatures above the boiling point for milk casein micelles will irreversibly aggregate (or "curdle"). There are two types of pasteurization used today: high temperature/short time (HTST) and ultra-high temperature (UHT). There are two methods for HTST pasteurization: batch and continuous flow. In the batch process, a large quantity of milk is held in a heated vat at 63 °C (145 °F) for 30 minutes, followed by quick cooling to about 4 °C (39 °F). In the continuous flow process, milk is forced between metal plates or through pipes heated on the outside by hot water. UHT processing holds the milk at a temperature of 138 °C (250 °F) for a fraction of a second. Milk simply labeled "pasteurized" is usually treated with the HTST method, whereas milk labeled "ultra-pasteurized" or simply "UHT" must be treated with the UHT method.

The HTST pasteurization standard was designed to achieve a 5-log reduction (0.00001 times the original) in the number of viable microorganisms in milk. This is considered adequate for destroying almost all yeasts, mold, and common spoilage bacteria and also to ensure adequate destruction of common pathogenic heat-resistant organisms (including particularly *Mycobacterium tuberculosis*, which causes tuberculosis and *Coxiella burnetii*, which causes Q fever). HTST pasteurization processes must be designed so that the milk is heated evenly, and no part of the milk is subject to a shorter time or a lower temperature. The milk is heated to 72 °C (161 °F) for 15-20 seconds for the HTST process.

### 3.3. PRODUCTION OF DISTILLED BEVERAGES

#### 3.3.1. ALCOHOL

An alcoholic beverage is a drink containing ethanol, commonly known as alcohol, although in chemistry the definition of alcohol includes many other compounds.

Ethanol is a centrally-acting drug with a depressant effect, and many societies regulate or restrict its sale and consumption. Countries place various legal restrictions on the sale of alcoholic drinks to young people. The manufacture and consumption of alcohol is found to some degree in most cultures and societies around the world, from hunter-gatherer tribes to organized nation-states. The consumption of alcohol is often important at social events in such societies and may be an important aspect of a community's culture.

Ethanol is only slightly toxic compared to other alcohols, but has significant psychoactive effects at sub lethal doses. Significant blood alcohol content is considered legal drunkenness as it reduces attention, lengthens reaction time and lowers inhibitions. Alcoholic beverages are addictive when consumed repeatedly or in high doses and the state of addiction to ethanol is known as alcoholism

Ethanol ( $\text{CH}_3\text{CH}_2\text{OH}$ ), the active ingredient in alcoholic drinks, for consumption purposes is always produced by fermentation—the metabolism of carbohydrates by certain species of yeast in the absence of oxygen. The process of culturing yeast under alcohol-producing conditions is referred to as brewing. The same process produces carbon dioxide in situ, and may be used to carbonate the drink in home brewing. However, this method leaves yeast residues and on the industrial scale, carbonation is done separately.

Drinks with a concentration of more than 50% ethanol by volume (100 US proof) are flammable liquids and easily ignited. Some exotic ones gain their distinctive flavors through intentional ignition of the drink, such as the Flaming Dr. Pepper. Spirits with a higher proof can be ignited with ease by heating slightly, e.g. adding the spirit to a warmed shot glass.

In chemistry, alcohol is a general term for any organic compound in which a hydroxyl group (-OH) is bound to a carbon atom, which in turn may be bound to other carbon atoms and further hydrogens. Other alcohols such as propylene glycol and the sugar alcohols may appear in food or beverages regularly, but these alcohols do not make them "alcoholic". Methanol (one carbon), the propanols (three carbons giving two isomers), and the butanols (four carbons, three isomers) are all commonly found alcohols, and none of these three should ever be consumed in any form. Alcohols are oxidized into the corresponding aldehydes and then into the corresponding carboxylic acids. These metabolic products cause a poisoning and acidosis. In the case of other alcohols than ethanol, the aldehydes and carboxylic acids are poisonous and the acidosis can be lethal. In contrast, fatalities from ethanol are mainly found in extreme doses and related to induction of unconsciousness or chronic addiction (alcoholism).

When compared to other alcohols, ethanol is only slightly toxic, with a lowest known lethal dose in humans of 1400 mg/kg, and a LD<sub>50</sub> of 9000 mg/kg (oral, rat). Nevertheless, accidental overdosing of alcoholic drinks, especially those of concentrated variety, is a risk for

women, lightweight persons and children. These people have a smaller quantity of water in their body, so that alcohol is diluted less. A blood alcohol concentration of 50 to 100 mg/dL is considered legal drunkenness. The threshold of effects is at 22 mg/dL.

Low-alcohol-content drinks are produced by fermentation of sugar- or starch-containing products, and high-alcohol ones are produced by distillation of these. Sometimes, the alcohol content is increased by adding distilled products, particularly in the case of wines. Such fortified wines include Port and Sherry.

The process involved (as well as the resulting alcohol content) defines the finished product. Beer involves a relatively short (incomplete) fermentation process and an equally short aging process (a week or two) resulting in an alcohol content generally between 3-8%, as well as natural carbonation. Wine involves a longer (complete) fermentation process, and a relatively long aging process (months or years -- sometimes decades) resulting in an alcohol content between 7-18%. Sparkling wine is generally made by adding a small amount of sugar before bottling, which causes a secondary fermentation to continue in the bottle. Distilled products are generally not made from a "beer" that would normally be palatable as fermentation is normally completed, but no aging is involved until after distillation. Most are 30% or greater alcohol by volume. Liqueurs are characterized by the way in which their flavors are infused and typically have high sugar content. Spirits typically contain 37.5% alcohol or greater and are not infused with flavors during the distilling process, however some modern spirits are infused with flavors after distilling (the Swedish vodka Absolute, for instance).

### **3.3.2. WINE**

Wine is an alcoholic beverage made from the fermentation of unmodified grape juice. The natural chemical balance of grapes is such that they ferment without the addition of sugars, acids, enzymes or other nutrients. Although other fruits like apples and berries can also be fermented, the resultant "wines" are normally named after the fruit (for example, apple wine or elderberry wine) and are generically known as fruit or country wine. Others, such as barley wine and rice wine (e.g. sake) are made from starch-based materials and resemble beer more than wine, while ginger wine is fortified with brandy. In these cases, the use of the term "wine" is a reference to the higher alcohol content, rather than production process. The commercial use of the English word "wine" (and its equivalent in other languages) is protected by law in many jurisdictions.

The word "wine" derives from the Proto-Germanic \*winam, an early borrowing from the Latin vinum, "wine" or "(grape) vine", itself derived from the Proto-Indo-European stem \*win-o-(cf. Ancient Greek οἶνος oînos). Similar words for wine or grapes are found in the Semitic languages and in Georgian, and the term is considered an ancient wanderwort.

#### **3.3.2.1. Hops**

Crushed hops: The flower of the hop vine is used as a flavoring and preservative agent in nearly all beer made today. The flowers themselves are often called "hops". Hops were used in beer by Jews in Babylon around 400 BCE, and by monastery breweries, such as Corvey in

Westphalia, Germany, from 822 CE, though the date normally given for widespread cultivation of hops for use in beer is the thirteenth century.

Hops contain several characteristics that brewers desire in beer: hops contribute a bitterness that balances the sweetness of the malt; hops also contribute floral, citrus, and herbal aromas and flavors to beer; hops have an antibiotic effect that favors the activity of brewer's yeast over less desirable microorganisms; and the use of hops aids in "head retention", the length of time that a foamy head created by carbonation will last. The bitterness of beers is measured on the International Bitterness Units scale. Beer is the sole major commercial use of hops.

In the past, other plants have been used for similar purposes; for instance, *Glechoma hederacea*. Combinations of various aromatic herbs, berries, and even ingredients like wormwood would be combined into a mixture known as gruit and used as hops are now used.

### **3.3.2.2. Yeast**

Main articles: Brewer's yeast, *Saccharomyces cerevisiae*, and *Saccharomyces uvarum*. Yeast is the microorganism that is responsible for fermentation in beer. Yeast metabolizes the sugars extracted from grains, which produces alcohol and carbon dioxide, and thereby turns wort into beer. In addition to fermenting the beer, yeast influences the character and flavour. The dominant types of yeast used to make beer are ale yeast (*Saccharomyces cerevisiae*) and lager yeast (*Saccharomyces uvarum*); their use distinguishes ale and lager. *Brettanomyces* ferments lambics, and *Torulaspora delbrueckii* ferments Bavarian weissbier. Before the role of yeast in fermentation was understood, fermentation involved wild or airborne yeasts. A few styles such as lambics rely on this method today, but most modern fermentation adds pure yeast cultures directly to wort.

### **3.3.2.3. Clarifying Agent**

Some brewers add one or more clarifying agents to beer. Common examples of these include isinglass finings, obtained from swimbladders of fish; kappa carrageenan, derived from seaweed; Irish moss, a type of red alga; polyclar (artificial), and gelatin. Clarifying agents typically precipitate out of the beer along with protein solids, and are found only in trace amounts in the finished product.

## **3.3.3. BRANDY**

A bottle of calvados Pays D'AugeBrandy (short for brandywines, from Dutch brandewijn—"burnt wine") is a general term for distilled wine, usually 40–60% ethyl alcohol by volume. In addition to wine, this spirit can also be made from grape pomace or fermented fruit juice. Unless specified otherwise, brandy is made from grape wine. It is normally consumed as an after-dinner drink. Brandy made from wine is generally colored with caramel coloring to imitate the effect of long aging in wooden casks; pomace and fruit brandies are generally drunk unaged, and are not usually colored.

### **3.3.4. BEER**

A great many beers are brewed across the globe. Local traditions will give beers different names, giving the impression of a multitude of different styles. However, the basics of brewing beer are shared across national and cultural boundaries.

#### **3.3.4.1. Grains**

Barley malt is the most common grain used to make beer. Wheat, corn and rice are the secondary grains — used as an adjunct to the barley. Rye, sorghum, and oats are occasionally used as an adjunct. Alcoholic strength

Beer ranges from less than 3% alcohol by volume (abv) to almost 30% abv. The alcohol content of beer varies by local practice or beer style. The pale lagers that most consumers are familiar with fall in the range of 4–6%, with a typical abv of 5%. The customary strength of British ales is quite low, with many session beers around 4% abv. Some beers, such as tafelbier (table beer) are of such low alcohol content (1%~4%) that they are served instead of soft drinks in some schools. Beer sold at baseball games in the United States is generally about 3% abv. to avoid undue rowdiness in the stands.

The alcohol in beer comes primarily from the fermentation of sugars that are produced during mashing. The quantity of fermentable sugars in the wort and the variety of yeast used to ferment the wort are the primary factors that determine the amount of alcohol in the final beer. Additional fermentable sugars are sometimes added to increase alcohol content, and enzymes are often added to the wort for certain styles of beer (primarily 'light' beers) to convert more complex carbohydrates (starches) to fermentable sugars. Alcohol is a waste product of yeast metabolism and is toxic to the yeast; typical brewing yeast cannot survive at alcohol concentrations above 12% by volume. Low temperatures and too little fermentation time decrease the effectiveness of yeasts, and consequently decrease the alcohol content.

## **3.4. FOOD ADDITIVES**

### **3.4.1. Introduction**

Food additives are substances added to food to preserve flavor or improve its taste and appearance. Some additives have been used for centuries; for example, preserving food by pickling (with vinegar), salting, as with bacon, preserving sweets or using sulfur dioxide as in some wines. With the advent of processed foods in the second half of the 20th century, many more additives have been introduced, of both natural and artificial origin.

Food additives have been used for centuries. Salt, sugar and vinegar were among the first and used to preserve foods. In the past 30 years, however, with the advent of processed foods, there has been a massive explosion in the chemical adulteration of foods with additives. Considerable controversy has been associated with the potential threats and possible benefits of food additives.

Most food additives are considered safe. However, some are known to be carcinogenic or toxic. Hyperactivity in children, allergies, asthma, and migraines are often associated with adverse reactions to food additives.

Since 1987 Australia has had an approved system of labeling for additives in packaged foods. Each food additive has to be named or numbered. The numbers are the same as in Europe, but without the prefix 'E'.

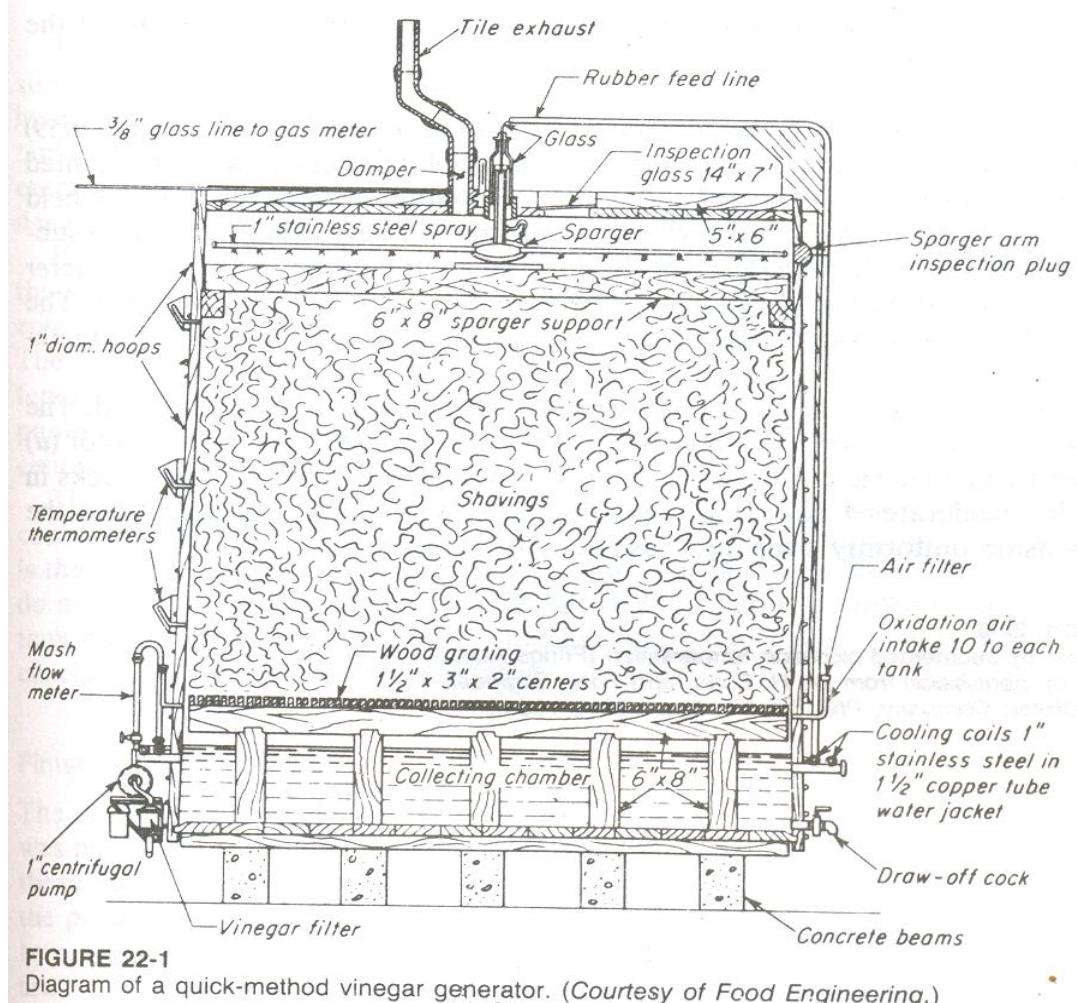
Food acids are added to make flavors "sharper", and also act as preservatives and antioxidants. Common food acids include vinegar, citric acid, tartaric acid, malic acid, fumaric acid, lactic acid.

### **3.4.2. ORGANIC ACIDS**

#### **3.4.2.1. Acetic acid**

Acetic acid, also known as ethanoic acid, is an organic chemical compound with the formula  $\text{CH}_3\text{COOH}$  best recognized for giving vinegar its sour taste and pungent smell. Pure, water-free acetic acid (glacial acetic acid) is a colorless liquid that attracts water from the environment (hygroscopy), and freezes below  $16.7^\circ\text{C}$  ( $62^\circ\text{F}$ ) to a colorless crystalline solid. Acetic acid is corrosive, and its vapor causes irritation to the eyes, a dry and burning nose, sore throat and congestion to the lungs, however, it is considered a weak acid due to the fact that at standard temperature and pressure the dissociated acid exists in equilibrium with the undissociated form in aqueous solutions, in contrast to strong acids, which are fully dissociated.

Acetic acid is one of the simplest carboxylic acids (the second-simplest, next to formic acid). It is an important chemical reagent and industrial chemical that is used in the production of polyethylene terephthalate mainly used in soft drink bottles; cellulose acetate, mainly for photographic film; and polyvinyl acetate for wood glue, as well as many synthetic fibres and fabrics.



**FIGURE 22-1**

Diagram of a quick-method vinegar generator. (Courtesy of Food Engineering.)

### Fig. 1. ACETIC ACID PRODUCTION

**Lactic Acid** Lactic acid (IUPAC systematic name: 2-hydroxypropanoic acid), also known as milk acid, is a chemical compound that plays a role in several biochemical processes. It was first isolated in 1780 by a Swedish chemist, Carl Wilhelm Scheele, and is a carboxylic acid with a chemical formula of  $C_3H_6O_3$ . It has a hydroxyl group adjacent to the carboxyl group, making it an alpha hydroxy acid (AHA). In solution, it can lose a proton from the acidic group, producing the lactate ion  $CH_3CH(OH)COO^-$ . It is miscible with water or ethanol, and is hygroscopic.

Lactic acid is chiral and has two optical isomers. One is known as L-(+)-lactic acid or (S)-lactic acid and the other, its mirror image, is D-(-)-lactic acid or (R)-lactic acid. L-(+)-Lactic acid is the biologically important isomer.

In animals, L-lactate is constantly produced from pyruvate via the enzyme lactate dehydrogenase (LDH) in a process of fermentation during normal metabolism and exercise. It does not increase in concentration until the rate of lactate production exceeds the rate of lactate removal which is governed by a number of factors including: monocarboxylate transporters,

concentration and isoform of LDH and oxidative capacity of tissues. The concentration of blood lactate is usually 1-2 mmol/L at rest, but can rise to over 20 mmol/L during intense exertion.

Lactic acid fermentation is also performed by *Lactobacillus* bacteria. These bacteria can operate in the mouth; the acid they produce is responsible for the tooth decay known as caries.

In medicine, lactate is one of the main components of Ringer's lactate or lactated Ringer's solution (Compound Sodium Lactate or Hartmann's Solution in the UK). This intravenous fluid consists of sodium and potassium cations, with lactate and chloride anions, in solution with distilled water in concentration so as to be isotonic compared to human blood. It is most commonly used for fluid resuscitation after blood loss due to trauma, surgery or a burn injury.

### **3.4.2.2. Lactic Acid**

Lactic acid is primarily found in sour milk products, such as: koumiss, leban, yogurt, kefir and some cottage cheeses. The casein in fermented milk is coagulated (curdled) by lactic acid.

Although it can be fermented from lactose (milk sugar), most commercially used lactic acid is derived by using bacteria such as *Bacillus acidilacti*, *Lactobacillus delbueckii* or *Lactobacillus bulgaricus* to ferment carbohydrates from nondairy sources such as cornstarch, potatoes and molasses. Thus, although it is commonly known as "milk acid", products claiming to be vegan do sometimes feature lactic acid as an ingredient.

Lactic acid may also be found in various processed foods, usually either as a pH adjusting ingredient, or as a preservative (either as antioxidant or for control of pathogenic micro-organisms). It may also be used as a fermentation booster in rye and sourdough breads

### **3.4.2.3. Citric acid**

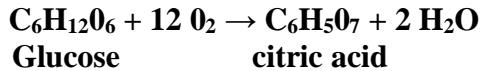
Citric acid is a weak organic acid found in citrus fruits. It is a natural preservative and is also used to add an acidic (sour) taste to foods and soft drinks. In biochemistry, it is important as an intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all living things. It also serves as an environmentally benign cleaning agent and acts as an antioxidant.

Citric acid exists in a variety of fruits and vegetables, but it is most concentrated in lemons and limes, where it can comprise as much as 8% of the dry weight of the fruit. Citric acid also is made from corn. Citric acid made from corn is being used in increasing frequency as reported in Michael Pollan's book *The Omnivore's Dilemma*.

#### **3.4.2.3.1. Organisms Used**

*Aspergillus niger* is the principal mold used in citric acid production, although various other molds are known to be able to make the acid and many have been tried experimentally: molds such as *A. clavatus*, *A. wentii*, *Penicillium luteum*, *P. citrinum*, *Mucor pyriformis*, and others. Apparently different strains of *A. niger* are preferred for surface methods of citric acid

production than for methods involving submerged growth. A simplified theoretical equation for the production of citric acid from glucose is



A series of intermediate steps is involved, and other products are produced. Raw Materials most citric acid is made from molasses, with beet molasses preferred over cane. Cane blackstrap and cane invert molasses also have been tried, as well as solutions of glucose or sucrose, plus sources of nitrogen and minerals. The source of nitrogen usually is some simple compound such as ammonium salts or urea. The concentration of mineral ions is very important in obtaining good yields, especially levels of iron, zinc, and manganese. A slight deficiency in nitrogenous food and phosphate also favors production. Factors that tend to reduce the formation of mycelium below the maximum therefore appear to increase the yield of citric acid. Conditions favoring citric acid production-tend to suppress the formation of oxalic acid.

### **3.4.2.3.2. Production of Citric Acid**

The older method of citric acid manufacture was by means of surface growth; the mold mycelium was cultured on shallow layers of medium in trays or similar containers. Modern methods involve the submerged growth of the mycelium. It should be noted again that the strain of mold selected and the medium for production differ with the method of cultivation of the ~old. Most methods utilize a sugar concentration between 14 and 20 percent, a fairly low pH that is more acid for the surface method, and a temperature of about 25 to 30 C and a fermentation period of 7 to 10 days for the surface method.

### **3.4.2.3.3. Uses of Citric Acid**

In the food industries, citric acid is added to flavoring extracts, soft drinks, and candies. It has been added to fish to adjust the pH to about 5.0 to aid in its preservation, to artichokes to enable the processor to utilize a mild heat treatment in their canning than would otherwise be possible, to crabmeat to prevent discoloration, as a synergist with antioxidants for oils, and as a dip for sliced peaches to delay browning. A large portion of the citric acid produced is used for medicinal purposes. Shorter time for the submerged method. As a food additive, citric acid is used as a flavoring and preservative in food and beverages, especially soft drinks. It is denoted by E number E330. Citrate salts of various metals are used to deliver those minerals in a biologically available form in many dietary supplements. The buffering properties of citrates are used to control pH in household cleaners and pharmaceuticals.

## **3.4.3. AMINO ACIDS**

### **3.4.3.1. Glutamic Acid**

Glutamic acid (Glu, E), is the protonated form of glutamate (the anion). Glutamate is one of the 20 proteinogenic amino acids. It is not among the essential amino acids.

Glutamic acid is present in a wide variety of foods and is responsible for one of the five basic tastes of the human sense of taste, especially in its physiological form, the sodium salt of glutamate in a neutral pH. Ninety-five percent of the dietary glutamate is metabolized by intestinal cells in a first pass.

Overall, glutamic acid is the single largest contributor to intestinal energy. As a source for umami, the sodium salt of glutamic acid, monosodium glutamate (MSG) is used as a food additive to enhance the flavor of foods, although an identical effect can be achieved by mixing and cooking together different ingredients rich in this amino acid and other umami substances as well.

#### **3.4.3.2. Lysine**

Lysine is a  $\alpha$ -amino acid with the chemical formula HOOCCH(NH<sub>2</sub>)(CH<sub>2</sub>)<sub>4</sub>NH<sub>2</sub>. Its three letter code is Lys, its one letter code is K, and its codons are AAA and AAG. This amino acid is an essential amino acid, which means that humans cannot synthesize it. Lysine is a base, as are arginine and histidine. The  $\epsilon$ -amino group often participates in hydrogen bonding and as a general base in catalysis.

#### **3.4.3.3. Threonine**

Threonine is an  $\alpha$ -amino acid with the chemical formula HO<sub>2</sub>CCH(NH<sub>2</sub>)CH(OH)CH<sub>3</sub>. Its three letter code is thr, its one letter code is T, and its codons are ACU and ACA. This essential amino acid is classified as polar. Together with serine and tyrosine, threonine is one of three proteinogenic amino acids bearing an alcohol group.

Foods high in threonine include cottage cheese, poultry, fish, meat, lentils, and sesame seeds. Histidine (His, H) is one of the 20 most common natural amino acids present in proteins. In the nutritional sense, in humans, histidine is considered an essential amino acid, but mostly only in children.

#### **3.4.3.4. Arginine**

Arginine is found in chocolate, wheat germ and flour, buckwheat, granola, oatmeal, dairy products (cottage cheese, ricotta, nonfat dry milk, skim yogurt), beef (roasts, steaks), pork (Canadian bacon, ham), nuts (coconut, pecans, cashews, walnuts, almonds, Brazil nuts, hazel nuts, peanuts), seeds (pumpkin, sesame, sunflower), poultry (chicken and turkey light meat), wild game (pheasant, quail), seafood (halibut, lobster, salmon, shrimp, snails, tuna in water), chick peas, cooked soybeans, and some energy drinks.

## 3.5. PIGMENTS

### 3.5.1. FLAVORANTS

Flavors are additives that give food a particular taste or smell, and may be derived from natural ingredients or created artificially.

**Flavor enhancers:** Flavor enhancers enhance a food's existing flavors. They maybe extracted from natural sources (through distillation, solvent extraction, maceration, among other methods) or created artificially.

### 3.5.2. PIGMENTS

A pigment is a material that changes the color of light it reflects as the result of selective color absorption. Materials that humans have chosen and developed for use as pigments usually have special properties that make them ideal for coloring other materials. A pigment must have a high tinting strength relative to the materials it colors. It must be stable in solid form at ambient temperatures.

For industrial applications, as well as in the arts, permanence and stability are desirable properties. Pigments that are not permanent are called fugitive. Fugitive pigments fade over time, or with exposure to light, while some eventually blacken. A food coloring is any substance that is added to food to change its color. It is sometimes used in cooking.

### 3.5.3. NISIN

Nisin is a polycyclic peptide antibacterial with 34 amino acid residues used as a food preservative. It contains the uncommon amino acids lanthionine (Lan), methyllanthionine (MeLan), didehydroalanine (Dha) and didehydroaminobutyric acid (Dhb). These unusual amino acids are introduced by posttranslational modification of the precursor peptide. In these reactions a ribosomally synthesized 57-mer is converted to the final peptide. The unsaturated amino acids originate from serine and threonine, and the enzyme-catalysed addition of cysteine residues to the didehydro amino acids result in the multiple thioether bridges.

Nisin and epidermin are members of a family of lantibiotics that bind to a cell wall precursor lipid component of target bacteria and disrupt cell wall production.

Nisin is produced by fermentation using the bacterium *Lactococcus lactis*. Commercially it is obtained from natural substrates including milk and is not chemically synthesized. It is used in processed cheese production to extend shelf life by suppressing gram-positive spoilage and pathogenic bacteria. There are many other applications of this preservative in food and beverage production. Due to its highly selective spectrum of activity it is also employed as a selective agent in microbiological media for the isolation of gram-negative bacteria, yeast and moulds. Subtilin and Epidermin are related to Nisin, all members of a class of molecules called lantibiotics.

### **3.5.4. BACTERIOCIN**

Bacteriocins are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). They are phenomenologically analogous to yeast and paramecium killing factors, and are structurally, functionally, and ecologically diverse. Bacteriocins were first discovered by A. Gratia in 1925.

Bacteriocins are categorized in several ways, including producing strain, common resistance mechanisms, and mechanism of killing. There are several large categories of bacteriocin which are only phenomenologically related. These include the bacteriocins from gram-positive bacteria, the colicins, the microcins, and the bacteriocins from Archaea. The bacteriocins from *E. coli* are called colicins (formerly called 'colicines'). They are the longest studied bacteriocins. They are a diverse group of bacteriocins and do not include all the bacteriocins produced by *E. coli*. In fact, one of the oldest known so-called colicins was called colicin V and is now known as microcin V. It is much smaller and produced and secreted in a different manner than the classic colicins.

The bacteriocins of lactic acid-fermenting bacteria are well studied because of the commercial use of these bacteria in the food industry for making dairy products such as cheese. Bacteriocins are classified according to their extent of posttranslational modification. The lantibiotics are a class of more extensively modified bacteriocins, also called Class I. Bacteriocins for which disulfide bonds are the only modification to the peptide are Class II bacteriocins. Most bacteriocins are biologically active single-chain peptides.

### **3.6. LET US SUM UP**

- The importance of dairy products with their production is discussed.
- The production processes of alcoholic beverages are discussed.
- Organic compounds and amino acid productions are discussed.
- Production of various economic important commodities like food additives and pigments are discussed.

### **3.7 OINTS FOR DISCUSSION**

1. Discuss PASTEURIZATION.
2. Analyze the differences in the production of distilled beverages.

### **3.8 LESSON - END ACTIVITIES**

1. Give a brief about the history of fermentation.
2. Describe about wine preparation.
3. "Organic acids are most used in food industry" – elaborate the idea by giving any two examples.
4. Which organisms are used for the production of citric acid? Give details of the production process.
5. What you understand by bacteriocins?

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## UNIT IV PHARMACEUTICAL PRODUCTS

### **CONTENTS**

- 4.0. AIMS AND OBJECTIVES**
- 4.1. INTRODUCTION**
- 4.2. ANTIBIOTICS**
- 4.3. VITAMINS**
- 4.4. PROBIOTICS**
- 4.5. THERAPEUTIC PROTEINS**
- 4.6. LET US SUM UP**
- 4.7 POINTS FOR DISCUSSION**
- 4.8. LESSON - END ACTIVITIES**
- 4.9 REFERENCES**

### **4.0. AIMS AND OBJECTIVES**

This unit explains the industrial production of various important pharmaceutical products.

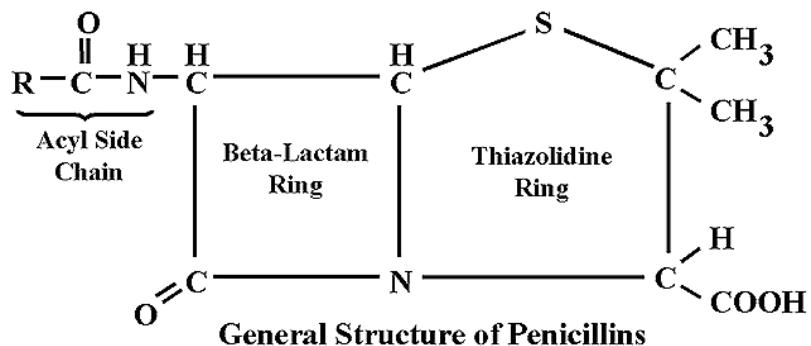
### **4.1. INTRODUCTION**

Advances in biochemistry and molecular biology have helped to understand the genetic basis of inherited diseases. It was a dream of the researchers to replace the defective genes with good ones, and cure the disorders. In this context, the development of recombinant DNA technology helped the researchers to have light on their research in search of new therapeutics. The commercial exploitation of recombinant DNA technology began in late 1970's by a few biotechnological companies to produce proteins. Now there are at least 400 different proteins produced by the technique. In this chapter, few such proteins, vitamins and other therapeutics which are of therapeutic value and economic importance for human are discussed briefly.

### **4.2. ANTIBIOTICS**

#### **4.2.1. PENICILLIN**

Penicillins are group of  $\beta$ - lactam containing bactericidal antibiotics. Being the first among the antibiotics to be discovered, penicillins are historically important. The basic structure of penicillin consists of a lactam ring and a thiazolidine ring fused together to form 6-aminopenicillanic acid.



**Fig 1 STRUCTURE OF PENICILLIN**

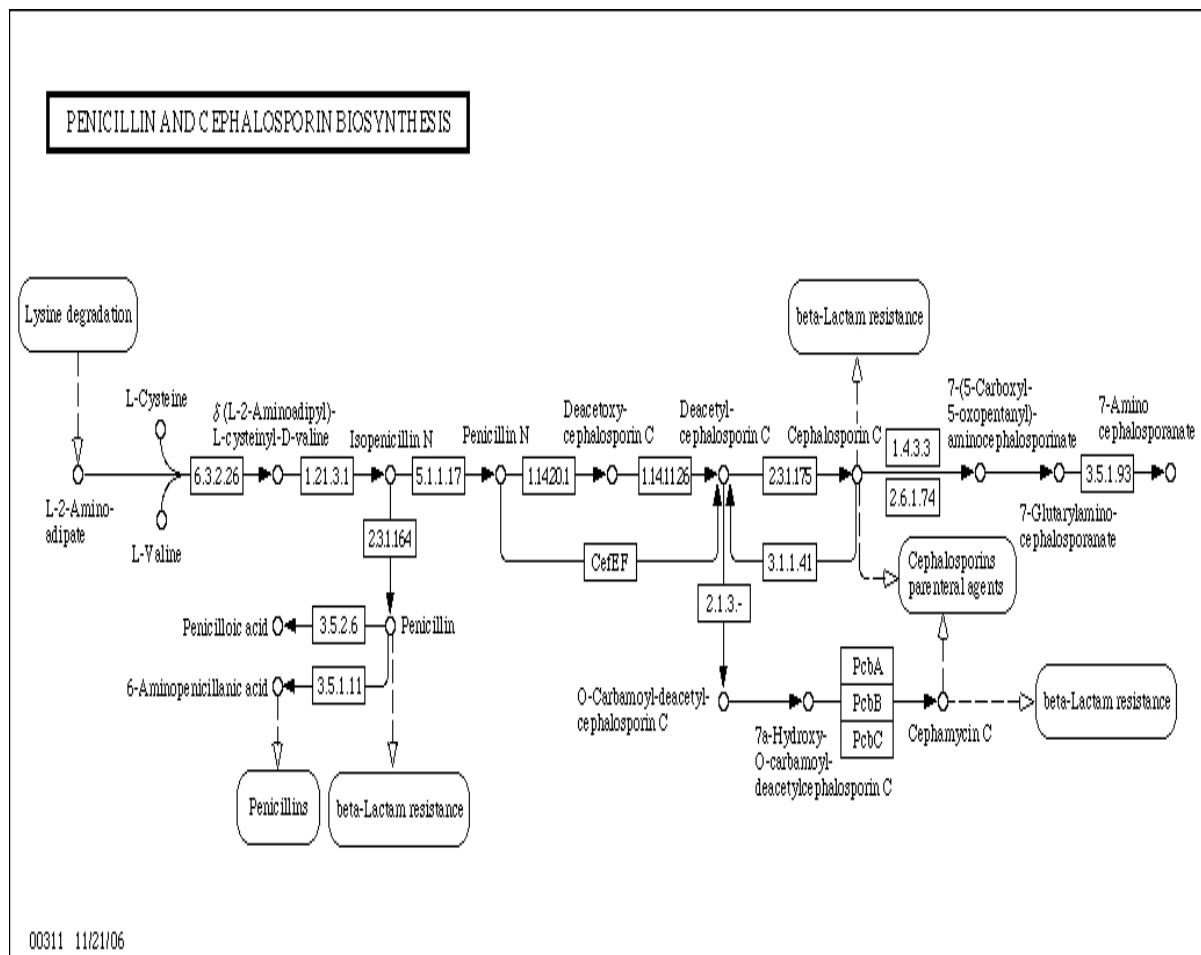
#### 4.2.1.1. MECHANISM OF PENICILLIN

Natural penicillins – V and G are effective against several Gram positive bacteria. They inhibit the bacterial cell wall synthesis and cause cell death. Natural penicillins are inefficient against microorganisms that produce  $\beta$ - lactamase (can hydrolyze penicillin).

Semi - synthetic penicillin (Cloxacillin, ampicillin, floxacillin and azlocillin) that are resistant to  $\beta$ - lactamase have been developed and successfully used against Gram negative bacteria.

#### 4.2.1.2. BIOSYNTHESIS OF PENICILLIN

L- $\alpha$ -Aminoadipic acid combines with L-cysteine, and then with L-valine to form a tripeptide namely  $\alpha$ -L-aminoadipylcysteinylvaline. This compound undergoes cyclization to form isopenicillin which reacts with phenylacetyl CoA to produce Penicillin G. In this reaction, aminoadipic acid gets exchanged with phenylacetic acid.



**Fig 2 PENICILLIN BIOSYNTHESIS**

#### **4.2.1.3. REGULATION OF BIOSYNTHESIS**

Some of the biochemical reactions for the synthesis of penicillin and lysine are common. Thus, L-  $\alpha$ -amino adipic acid is a common intermediate for the synthesis of penicillin and lysine. The availability of amino adipic acid plays a significant role in regulating the synthesis of penicillin.

Penicillin biosynthesis is inhibited by glucose through catabolite repression. For this reason, penicillin was produced by a slowly degraded sugar like lactose. The concentrations of phosphate and ammonia also influence penicillin synthesis.

#### **4.2.1.4. PRODUCTION PROCESS OF PENICILLIN**

The lyophilized culture of spores is cultivated for inoculum development which is transferred to prefermenter, and then to fermenter. Penicillin production is an aerobic process and therefore, a continuous supply of oxygen to the growing culture is very essential. The required aeration rate is 0.5-1 vvm.pH -6.5, optimal temperature – 25 to 27°C and is carried out by submerged processes.

The medium used for fermentation comprises of corn steep liquor (4-5% dry weight) and carbon source usually lactose. An addition of yeast extract, soy meal or whey is done for a good nitrogen supply. Phenylacetic acid which serves as a precursor for penicillin biosynthesis is continuously fed along with sugar for good yield of penicillin. For efficient synthesis of penicillin, the growth of the organism from spores must be loose form and not as pellets. The growth phase is around 40 hours with a doubling time of 6-8 hours. After the growth phase is stabilized, the penicillin production exponentially increases with appropriate culture conditions. The penicillin production phase can be extended to 150-180 hours.

#### **4.2.1.5. RECOVERY OF PENICILLIN**

As the fermentation is complete, the broth containing about 1% penicillin is processed for extraction. The mycelium is removed by filtration. Penicillin is recovered by solvent (n-butylacetate or methylketone) extraction at low temperature (<10°C) and acidic pH (<3). By this way, the chemical and enzymatic degradation of penicillin can be minimized.

The penicillin containing solvent is treated with activated carbon to remove impurities and pigments. Penicillin can be recovered by adding potassium or sodium acetate. The potassium or sodium salts of penicillin can be further processed (in dry solvents such as n-butanol or isopropanol) to remove impurities. The yield of penicillin is around 90%.

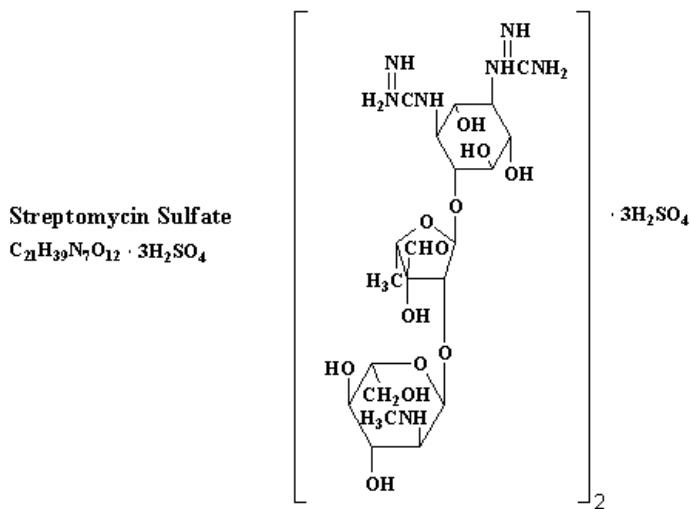
As the water is totally removed penicillin salts can be crystallized and dried under required pressure. This can be then processed to finally produce the pharmaceutical dosage forms. Penicillins G and H are fermented products obtained from fungus *Penicillium chrysogenum*.

#### 4.2.1.6. PRODUCTION OF 6-AMINO PENICILLANIC ACID

The penicillins G and H are mostly used as the starting material for the production of several synthetic penicillins containing the basic nucleus namely 6-amino penicillanic acid (6-APA). Immobilized penicillin amidases enzymes have been developed for specific hydrolysis of penicillin G and V. penicillin salt of either G or V can be used for hydrolysis by immobilized enzyme system. The pH during hydrolysis is kept around 7-8, and the product 6-APA can be removed by bringing down pH to 4 by precipitation in the presence of water immiscible solvent. In general, the enzymatic hydrolysis is more efficient for penicillin V than for G. However, penicillin G is more versatile compound, as it required for ring expansions.

#### 4.2.2. STREPTOMYCIN

Streptomycin was the first aminoglycoside that was successfully used to treat tuberculosis. It is isolated from the organism *Streptomyces griseus*. Usually aminoglycosides are regarded as reverse antibiotics as resistance may develop easily. These are very potent against Gram positive and negative bacteria besides mycobacteria. At the molecular level, aminoglycosides bind to 30S ribosome and block protein synthesis. Prolonged use of these antibiotics causes damage to kidneys, and hearing impairment.



**Fig 3 STRUCTURE OF STREPTOMYSIN SULPHATE**

#### 4.2.2.1. BIOSYNTHESIS OF STREPTOMYCIN

Glucose 6-phosphate obtained from glucose takes three independent routes to respectively produce streptidine 6-phosphate, L-dehydrostreptose and N-methylglucosamine. The former two compounds condense to form an intermediate which later combines with methylglucosamine to produce dihydrostreptomycin 6-phosphate. This compound in the next of couple reactions gets converted to streptomycin.

#### **4.2.2.2. REGULATION OF BIOSYNTHESIS**

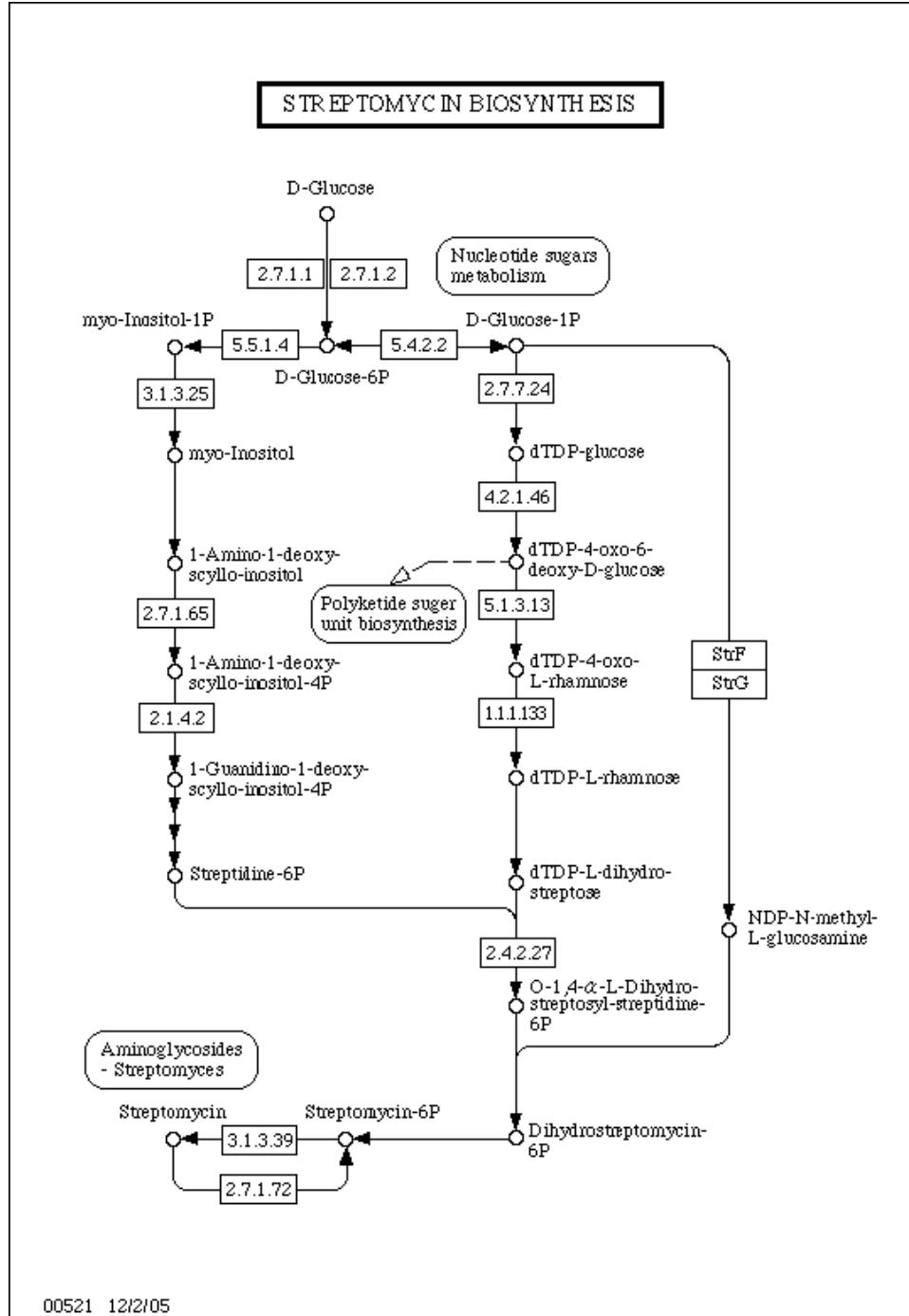
Very little is known about the regulation of streptomycin synthesis. A compound named as factor A (chemically isocapryloyl hydroxymethyl- $\gamma$ -butyrate) has been isolated from streptomycin producing strains of *S. griseus*. Factor A promotes streptomycin production. In fact, factor A<sup>-</sup> mutants that cannot synthesize streptomycin have been isolated. They can synthesize streptomycin on adding factor A. the nutrient sources carbohydrates (glucose), ammonia and phosphate also regulate (by feed back mechanism) streptomycin production.

#### **4.2.2.3. PRODUCTION PROCESS OF STREPTOMYCIN**

The medium used for streptomycin usually consists of soy meal or soy flour or corn syrup that can supply glucose at a slow rate (amylase activity is poor in *streptomyces sp.*). The initial supply of nitrogen and phosphate is also obtained from soy meal. This is required since glucose, ammonia and phosphate in high quantities inhibit streptomycin synthesis. The fermentation conditions are –temperature 27-30°C, pH 6.5-7.5, and aeration rate 0.5-1vvm. The duration of fermentation process depends on the strain used, and is between 6-8 days.

#### **4.2.2.4. RECOVERY OF STREPTOMYCIN**

Streptomycin or other aminoglycosides are basic in nature. They can be recovered by weak cationic exchange resins in an ion exchange column. Treatment with activated carbon is often necessary to remove impurities. Streptomycin can be precipitated in the form of sulphate salt.

**Fig 4 STREPTOMYCIN BIOSYNTHESIS**

#### 4.2.3. TETRACYCLINE

Tetracyclines are broad spectrum antibiotics with widespread medical use. They are effective against Gram positive and negative bacteria, besides other organisms (mycoplasma, Chlamydia rickettsias). These are used to combat stomach ulcers. They inhibit protein biosynthesis by blocking the binding of aminoacyl tRNA to ribosomes (A site). Organisms that

produce tetracycline are *Streptomyces aureofaciens*, *Streptomyces rimosus*. The basic structure of tetracycline composed of a naphthacene ring (a four ring structure).

#### **4.2.3.1. BIOSYNTHESIS OF TETRACYCLINE**

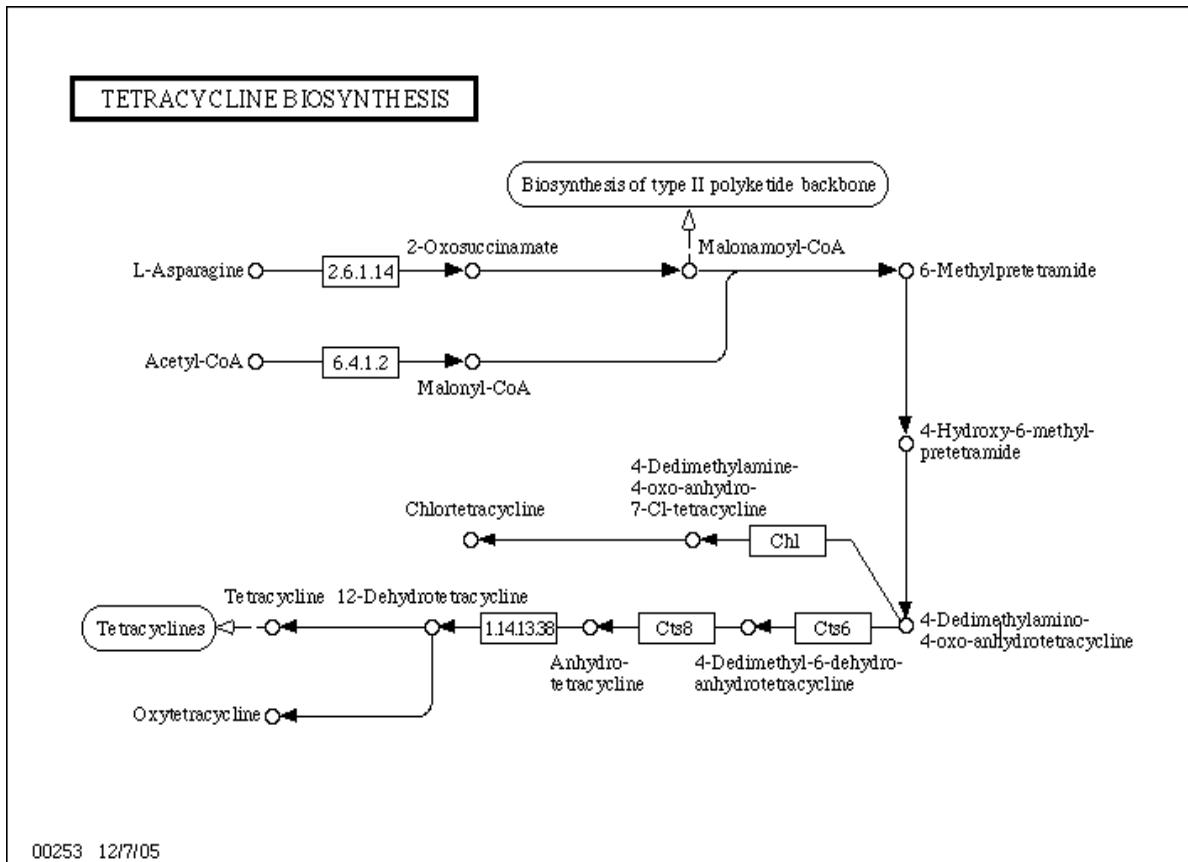
The pathway for the biosynthesis of tetracyclines is very complex. When glucose gets oxidized, it forms acetyl CoA and then malonyl CoA. On transamination, the later gives malonomoyl CoA. The enzyme anthracene synthase complex binds to malonomoyl CoA and brings out the condensation of 8 molecules of malonyl CoA to form a polyketide intermediates. These intermediates undergo a series of reactions to finally produce chlortetracycline.

#### **4.2.3.2. REGULATION OF BIOSYNTHESIS**

Carbohydrate metabolism controls chlortetracycline synthesis. For more efficient synthesis of the antibiotic, glycolysis has to be substantially low. The addition of phosphate reduces chlortetracycline production.

#### **4.2.3.3. PRODUCTION PROCESS OF CHLORTETRACYCLINE**

The fermentation medium consists of corn steep liquor, soy flour or peanut meal for the supply of nitrogen and carbon sources. Continuous feeding of carbohydrate is desirable for good growth of the organism and production of the antibiotic. This can be done either by addition of crude carbon sources or by supplying glucose or starch. For more efficient production of chlortetracycline, the supply of ammonium and phosphate has to be maintained at a low concentration. Ideal conditions are – temperature 27-30°C, pH 6.5-7.5, and aeration 0.8-1vvm. The duration of fermentation is around 4 days.



**Fig 5 TETRACYCLINE BIOSYNTHESIS**

#### 4.2.3.4. RECOVERY OF CHLORTETRACYCLINE

At the end of the fermentation, the culture broth is filtered to remove the mycelium. The filtrate is treated with n-butanol or methylisobutylketone in acidic or alkaline condition for extracting the antibiotic. It is then absorbed to activated charcoal to remove other impurities. Chlortetracycline is eluted and crystallized.

#### 4.2.3.5. PRODUCTION OF TETRACYCLINE – DIFFERENT PROCESS

The production of tetracycline can be achieved by one or more of the following ways.

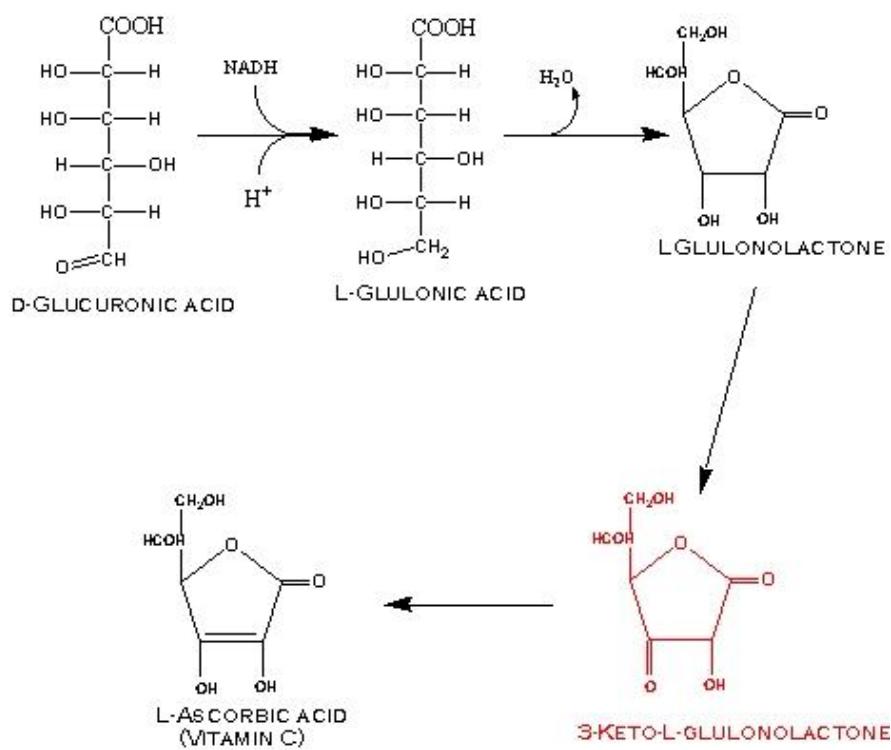
1. by chemical treatment of chlortetracycline
2. by carrying out fermentation in a chloride free culture medium
3. by employing mutants in which chlorination reaction does not occur
4. by blocking chlorination reaction by the addition of inhibitors eg-thiourea, 2-thiouracil.

### 4.3. VITAMINS

Vitamins are organic compounds that perform specific biological functions for normal maintenance and optimal growth of an organism. These cannot be synthesized by higher organisms and have to be supplied through diet. Microorganisms are capable of synthesizing vitamins.

#### 4.3.1. VITAMIN C

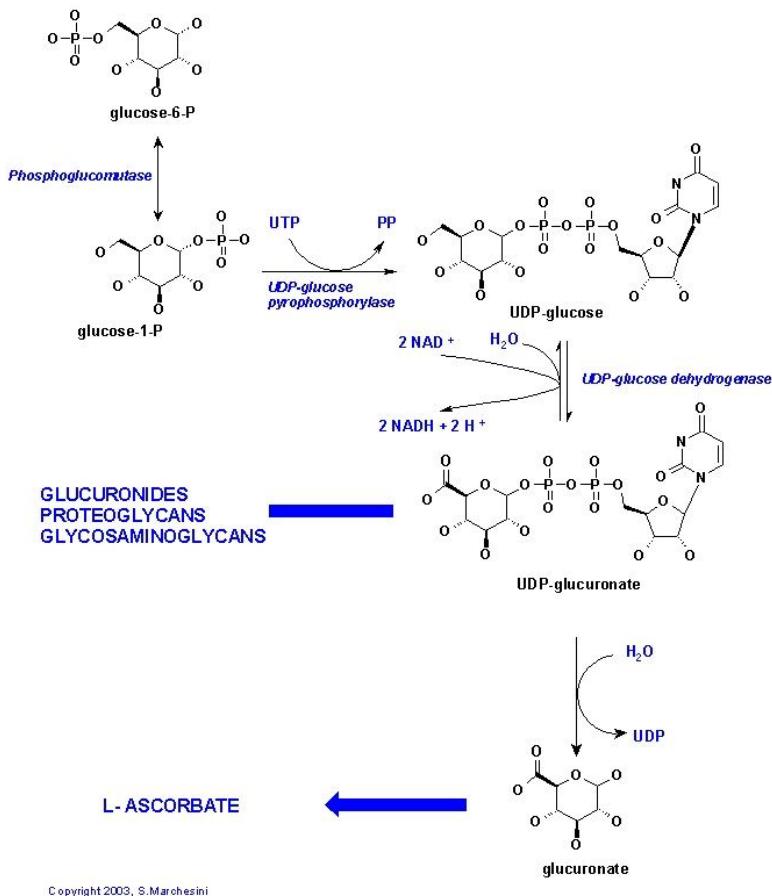
This vitamin forms a redox system and participates in several biological processes. It is intimately involved in the biosynthesis of collagen, the most abundant protein in the human body. It protects body against carcinogenic nitrosamines and free radicals. It is widely used in food and pharmaceutical industries.



**Fig 6 ASCORBIC ACID PRODUCTION FROM GLUCORONIC ACID**

#### 4.3.1.1. INDUSTRIAL PRODUCTION OF ASCORBIC ACID

Ascorbic acid is commercially produced by a combination of several chemical steps, and one reaction is biotransformation brought out by microorganisms. This process is referred to as Reichstein-Grüssner synthesis. D-glucose is first converted to D-sorbitol. Oxidation of D-sorbitol to L-sorbose is carried out by *Acetobacter xylinum* or *A. suboxydans*. A submerged bioreactor fermentation process is ideal for this reaction. It takes about 24 hours at temperature 30-35°C. Sorbose by a couple of chemical reaction can be finally converted to L-ascorbic acid.



**Fig 7 ASCORBIC ACID PRODUCTION FROM GLUCOSE**

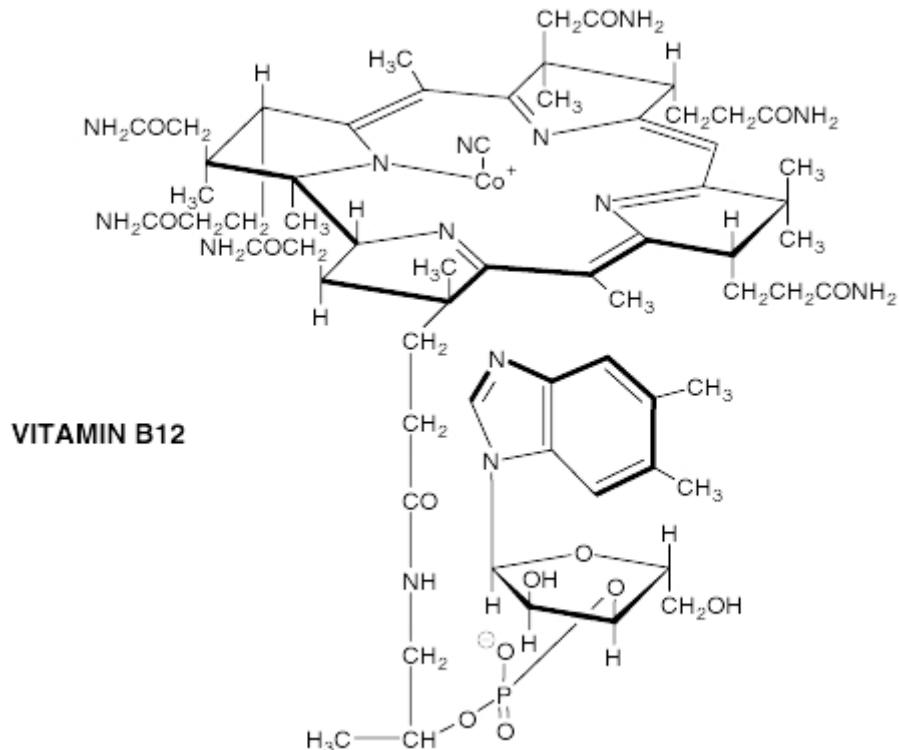
Two step fermentation process: in this, D-glucose is converted to 2,5-diketogluconic acid by *Erwinia*, *Acetobacter* or *Gluconobacter* sp. In the second step *Corynebacterium* sp converts 2,5-diketogluconic acid to 2-keto-L-gluconic acid. It is also possible to involve *Bacillus megaterium* for converting L-sorbose to 2-keto-L-gluconic acid. The latter, by chemical reactions, can be converted to ascorbic acid.

Production via L-gulonolactone: Ascorbic acid can also be synthesized via gulonolactone which can be directly converted to L-ascorbic acid by the enzyme L-gulonolactone dehydrogenase.

#### 4.3.2. VITAMIN B<sub>12</sub>

Vitamin B<sub>12</sub> is present in animal tissue at a very low concentration. It occurs mostly in the coenzyme forms methylcobalamin and deoxyadenosylcobalamin. Isolation of vitamin B<sub>12</sub> from animal tissue is highly expensive. Deficiency causes pernicious anemia. It is water soluble vitamin with complex structure. It consists of a corrin ring with a central cobalt atom. The corrin ring has four pyrrole units and bonded with four pyrrole nitrogens with cobalt. Microorganisms used for

commercial synthesis of vitamin are *Propionibacterium freudenreichii*, *Pseudomonas denitrificans*, *Bacillus megaterium*, *Streptomyces olivaceus*.



**Fig 8 STRUCTURE OF VITAMIN B<sub>12</sub>**

#### 4.3.2.1. COMMERCIAL PRODUCTION OF VITAMIN B<sub>12</sub>

It is commercially produced by fermentation. It was first obtained as a byproduct of *Streptomyces* fermentation in the production of certain antibiotics (streptomycin, chloramphenicol or neomycin).

Production using *Propionibacterium* sp: *P.freudenreichii* and *P.shermanii* and their mutant strains are used for vitamin synthesis. Process is carried out by adding cobalt in two phases.

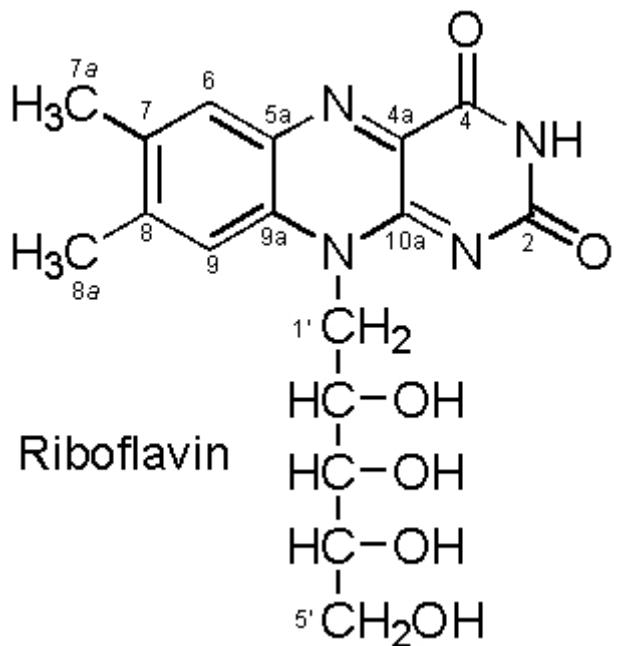
Anaerobic phase: this is preliminary phase that may take 2-4days. In the anaerobic phase 5'-deoxyadenosylcobinamide is predominantly produced. Aerobic phase: in this phase, 5,6-dimethyl benzimidazole is produced from riboflavin which gets incorporated to finally form coenzyme of vitamin B<sub>12</sub> namely 5'-deoxyadenosylcobalamin. In recent years, some fermentation technologists have successfully clubbed both an anaerobic and aerobic phases to carry out the operation continuously in two reaction tanks. The bulk production of vitamin is done mostly by submerged bacterial fermentation with beet molasses medium supplemented with cobalt chloride.

#### 4.3.2.2. RECOVERY OF VITAMIN B<sub>12</sub>

The cobalamins produced by fermentation are mostly bound to the cells. They can be solubilized by heat treatment at 80-120°C for about 30minutes at pH 6.5-8.5. The solids and mycelium are filtered or centrifuged and the fermentation broth collected. The cobalamins can be converted to more stable cyanocobalamins.

#### 4.3.3. RIBOFLAVIN

It is a water soluble enzyme, essential for the growth and reproduction in animals and man. Deficiency causes cheilosis, glossitis and dermatitis. It occurs in milk and its products, meat, eggs, liver and kidney. It occurs in free form in milk and as flavoproteins in other type foods. It contains 6, 7-dimethyl isoalloxazine attached to D-ribitol by a nitrogen atom. The ring participates in the oxidation –reduction reactions brought by coenzymes FAD and FMN. *Ashbya gossypii* and *Eremothecium ashbyii* are the microorganisms that produce the vitamin.



**Fig 9 STRUCTURE OF RIBOFLAVIN**

#### 4.3.3.1. PRODUCTION PROCESS OF RIBOFLAVIN

It is mostly carried out with simple sugars glucose and corn steep liquor. Glucose can be replaced by sucrose or maltose for the supply of carbon. Lipids such as corn oil when added to the medium for energy purpose have profound influence on riboflavin production. Further supplementation of the medium with yeast extract, peptone, glycine, inositol, and purine also increase production. The initial pH of the medium is adjusted to 6-7.5. Optimal fermentation conditions are – temperature 26-28°C, aeration rate 0.3vvm. The process is carried out for 5-7days by submerged aerated fermentation.

## 4.4. PROBIOTICS

Probiotics are dietary supplements containing potentially beneficial bacteria or yeasts. According to the currently adopted definition by FAO/WHO, probiotics are: ‘*Live microorganisms which when administered in adequate amounts confer a health benefit on the host*’. Lactic acid bacteria (LAB) are the most common type of microbes used. LAB have been used in the food industry for many years, because they are able to convert sugars (including lactose) and other carbohydrates into lactic acid. This not only provides the characteristic sour taste of fermented dairy foods such as yogurt, but acts as a preservative, by lowering the pH and creating fewer opportunities for spoilage organisms to grow. Strains of the genera *Lactobacillus* and *Bifidobacterium*, are the most widely used probiotic bacteria. Probiotic bacterial cultures are intended to assist the body's naturally occurring gut flora to reestablish them. They are sometimes recommended by doctors and, more frequently, by nutritionists, after a course of antibiotics, or as part of the treatment for gut related candidiasis. Claims are made that probiotics strengthen the immune system.

The rationale for probiotics is that the body contains a miniature ecology of microbes, collectively known as the gut flora. A number of bacterial types are thought to be thrown out of balance by a wide range of circumstances including the use of antibiotics or other drugs, excess alcohol, stress, disease, or exposure to toxic substances. In cases like these, the bacteria that work well with our bodies may decrease in number, an event which allows harmful competitors to thrive, to the detriment of our health.

Maintenance of a healthy gut flora is, however, dependent on many factors, especially the quality of food intake. Including a significant proportion of prebiotic foods in the diet has been demonstrated to support a healthy gut flora and may be another means of achieving the desirable health benefits promised by probiotics.

**4.4.1. Trade names:** Bifido Factor (Natren), DDS-Acidophilus (UAS Laboratories), DDS-Junior (UAS Laboratories), DDS-Plus (UAS Laboratories), Digesta Lac (Natren), Healthy Trinity (Natren), Life Start (Natren), Mega Dophilus (Natren), Multiflora-ABF (UAS Laboratories), Probiata (Wakunaga Consumer), Probioplus-DDS (UAS Laboratories).

## 4.4.2. MECHANISM OF ACTION

*Lactobacillus plantarum* 299v, which is derived from sour dough and which is used to ferment sauerkraut and salami, has been demonstrated to improve the recovery of patients with enteric bacterial infections. This bacterium adheres to reinforce the barrier function of the intestinal mucosa, thus preventing the attachment of the pathogenic bacteria to the intestinal wall. *Bifidobacterium breve* was found to eradicate *Campylobacter jejuni* from the stools of children with enteritis, although less rapidly than in those treated with erythromycin. *Lactobacillus GG* was found to eradicate *Clostridium difficile* in patients with relapsing colitis, and supplementation of infant formula milk with *Bifidobacterium bifidum* and *Streptococcus thermophilus* reduced rotavirus shedding and episodes of diarrhea in hospitalized children.

The antimicrobial activity of probiotics is thought to be accounted for, in large part, by their ability to colonize the colon and reinforce the barrier function of the intestinal mucosa. Probiotics, such as *Lactobacillus bulgaricus*, which do not adhere as well to the intestinal mucosa, are much less effective against enteric pathogens. In addition, some probiotics have been found to secrete antimicrobial substances. These substances are known as bacteriocins. Such a bacteriocin has been isolated from *Lactobacillus plantarum* ST31, a probiotic derived from sour dough. The substance was found to be a 20 amino acid peptide. A different bacteriocin was isolated from another strain of *Lactobacillus plantarum*. The bacteriocin has 27 amino acids and contains lanthionine residues. This type of bacteriocin is classified as a lantibiotic.

*Lactobacillus casei* has been demonstrated to increase levels of circulating immunoglobulin A (IgA) in infants infected with rotavirus. This has been found to be correlated with shortened duration of rotavirus-induced diarrhea. *Lactobacillus GG* has also been shown to potentiate intestinal immune response to rotavirus infection in children. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* appear to enhance the nonspecific immune phagocytic activity of circulating blood granulocytes. This effect may account, in part, for the stimulation of IgA responses in infants infected with rotavirus. In healthy individuals, *Lactobacillus salivarius* UCC118 and *Lactobacillus johnsonii* LA1 were demonstrated to produce an increase in the phagocytic activity of peripheral blood monocytes and granulocytes. Also, *Lactobacillus johnsonii* LA1, but not *Lactobacillus salivarius* UCC118, was found to increase the frequency of interferon-gamma-producing peripheral blood monocytes.

*Lactobacillus GG* has been shown to inhibit chemically induced intestinal tumors in rats. The probiotic appears to alter the initiation and/or promotional events of the chemically-induced tumors. *Lactobacillus GG* also binds to some chemical carcinogens.

*Saccharomyces boulardii* has been shown to prevent antibiotic-associated diarrhea and also to prevent diarrhea in critically ill tube-fed patients. The mechanism of this anti diarrheal effect is not well understood. *S. boulardii* has been found to secrete a protease which digests two protein exotoxins, toxin A and toxin B, which appear to mediate diarrhea and colitis caused by *Clostridium difficile*. The protective effects of *S. boulardii* on *C. difficile*-induced inflammatory diarrhea may, in part, be due to proteolytic digestion of toxin A and toxin B by a secreted protease.

Dietary antigens may induce an immuno inflammatory response that impairs the barrier function of the intestine, resulting in aberrant absorption of intraluminal antigens. This may account, in part, for food allergies. Probiotics that colonize the colon may be helpful in the management of some with food allergies by reinforcing the barrier function of the intestinal mucosa. *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12 were found to produce significant improvement of atopic eczema in children with food allergies. The decrease in the signs and symptoms of atopic eczema occurred in parallel with a reduction in the concentration of circulating CD4+ T lymphocytes and an increase in transforming growth factor beta1 (TGF-beta1), indicating suppressive effects on T cell functions in this disorder. These probiotics may help restore the Th1/Th2 balance in atopic eczema.

*Lactobacillus GG* was found to scavenge superoxide anion radicals, inhibit lipid peroxidation and chelate iron *in vitro*. The iron chelating active of *Lactobacillus GG* may account, in part, for its antioxidant activity. Other lactic acid bacteria, including strains of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium longum* and *Streptococcus thermophilus*, have also demonstrated antioxidative ability. Mechanisms include chelation of metal ions (iron, copper), scavenging of reactive oxygen species and reducing activity.

#### **4.4.3. PHARMACOKINETICS**

The effectiveness of probiotics is related to their ability to survive in the acidic stomach environment and the alkaline conditions in the duodenum, as well as their ability to adhere to the intestinal mucosa of the colon and to colonize the colon. Some probiotics, such as *Lactobacillus GG* and *Lactobacillus plantarum* 299v, are better able to colonize the colon than others. After passage through the stomach and the small intestine, those probiotics that do survive become established transiently in the colon.

#### **4.4.4. THERAPEUTIC APPLICATIONS OF PROBIOTICS**

##### **4.4.4.1. PREVENTION OF COLON CANCER**

In laboratory investigations, some strains of LAB have demonstrated anti-mutagenic effects thought to be due to their ability to bind with heterocyclic amines; carcinogenic substances formed in cooked meat. Animal studies have demonstrated that some LAB can protect against colon cancer in rodents, though human data is limited and conflicting. Most human trials have found that the strains tested may exert anti-carcinogenic effects by decreasing the activity of an enzyme called  $\beta$ -glucuronidase (which can generate carcinogens in the digestive system). Lower rates of colon cancer among higher consumers of fermented dairy products have been observed in some population studies.

##### **4.4.4.2. CHOLESTEROL LOWERING**

Animal studies have demonstrated the efficacy of a range of LAB to be able to lower serum cholesterol levels, presumably by breaking down bile in the gut, thus inhibiting its reabsorption (which enters the blood as cholesterol). Some, but not all human trials have shown that dairy foods fermented with specific LAB can produce modest reductions in total and LDL cholesterol levels in those with normal levels to begin with, however trials in hyperlipidemic subjects are needed.

##### **4.4.4.3. LOWERING BLOOD PRESSURE**

Several small clinical trials have shown that consumption of milk fermented with various strains of LAB can result in modest reductions in blood pressure. It is thought that this is due to the ACE inhibitor-like peptides produced during fermentation.

#### **4.4.4.4. IMPROVING IMMUNE FUNCTION AND PREVENTING INFECTIONS**

LAB are thought to have several presumably beneficial effects on immune function. They may protect against pathogens by means of competitive inhibition (i.e., by competing for growth) and there is evidence to suggest that they may improve immune function by increasing the number of IgA-producing plasma cells, increasing or improving phagocytosis as well as increasing the proportion of T lymphocytes and Natural Killer cells. Clinical trials have demonstrated that probiotics may decrease the incidence of respiratory tract infections and dental caries in children LAB foods and supplements have been shown to be effective in the treatment and prevention of acute diarrhea; decreasing the severity and duration of rotavirus infections in children as well as travelers diarrhea in adults.

#### **4.4.4.5. REDUCING INFLAMMATION**

LAB foods and supplements have been found to modulate inflammatory and hypersensitivity responses, an observation thought to be at least in part due to the regulation of cytokine function. Clinical studies suggest that they can prevent reoccurrences of inflammatory bowel disease in adults, as well as improve milk allergies and decrease the risk of atopic eczema in children.

#### **4.4.4.6. SYNBIOTICS**

It is also possible to increase and maintain a healthy bacterial gut flora by increasing the amounts of prebiotics in the diet such as inulin, raw oats, and unrefined wheat. As probiotics are mainly active in the small intestine and prebiotics are only effective in the large intestine, the combination of the two may give a synergistic effect. Appropriate combinations of pre- and probiotics are synbiotics. Synbiotics have also been defined as metabolites produced by ecoorgan or by synergistic action of prebiotics and probiotics e.g. short chain fatty acids, other fatty acids, amino acids, peptides, polyamines, carbohydrates, vitamins, numerous antioxidants and phytosterols, growth factors, coagulation factors, various signal molecules such as cytokine-like bacteriokines.

### **4.5. THERAPEUTIC PROTEINS**

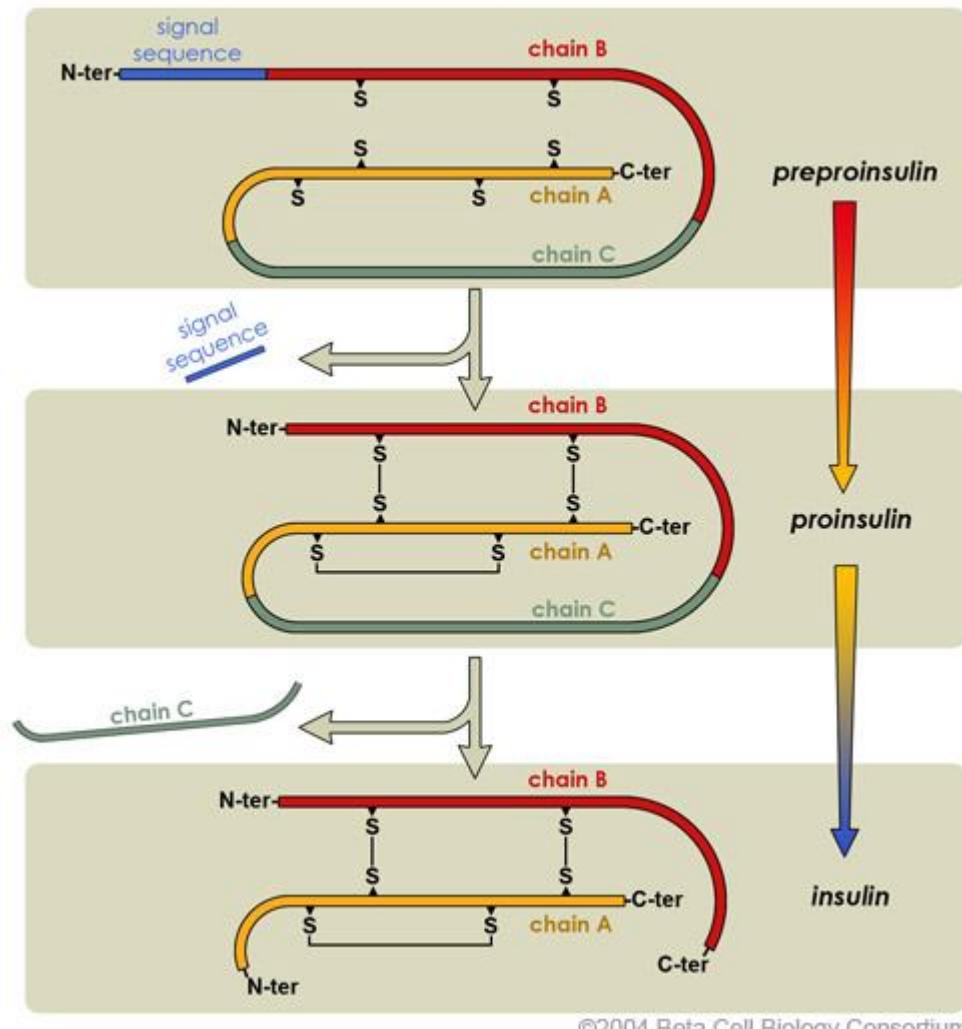
#### **4.5.1. INSULIN**

The hormone insulin is produced by the  $\beta$ - cells of islets of Langerhans of pancreas. Human insulin contains 51 amino acids, arranged in two polypeptide chains. The chain A has 21 and B has 30 amino acids. Both are held together by disulphide bonds. Insufficient of insulin leads to genetically linked disease Diabetes mellitus. Insulin facilitates the cellular uptake and utilization of glucose for the release of energy. In the absence of insulin glucose accumulates in the blood stream at higher concentration and excreted through urine. The more serious complications of uncontrolled diabetes mellitus include nephropathy, retinopathy, neuropathy and atherosclerosis, stroke.

In the early years, insulin isolated and purified from pancreases of pigs and cows was used for the treatment of diabetics. There is a slight structural difference between animal and human insulin resulting in allergy when administered for diabetics.

#### 4.5.1.1. PRODUCTION OF RECOMBINANT INSULIN

Attempts to produce insulin by recombinant DNA technology started in late 1970s. The basic technique consisted of inserting human insulin gene and the promoter gene of lac operon on to the plasmids of *E.coli*. In 1986, Eli Lily company received approval to market human insulin under trade name Humulin.



**Fig 10 SCHEMATICS OF RECOMBINANT INSULIN PRODUCTION**

The original technique of insulin synthesis in *E.coli* has undergone several changes, for improving the yield. Example, addition of single peptide, synthesis of A and B chains separately. The procedure employed for the synthesis of two insulin chains A and B. The genes for insulin A chain and B chain are separately inserted to the plasmids of two different *E.coli* cultures. The lac operon system consisting of inducer gene, promoter gene, operator gene and structural gene z for  $\beta$ -galactosidase is used for expression of both the genes. The presence of lactose in the culture medium induces the synthesis of insulin A and B chains in separate cultures. The so formed insulin chains can be isolated, purified and joined together to give full pledged human insulin.

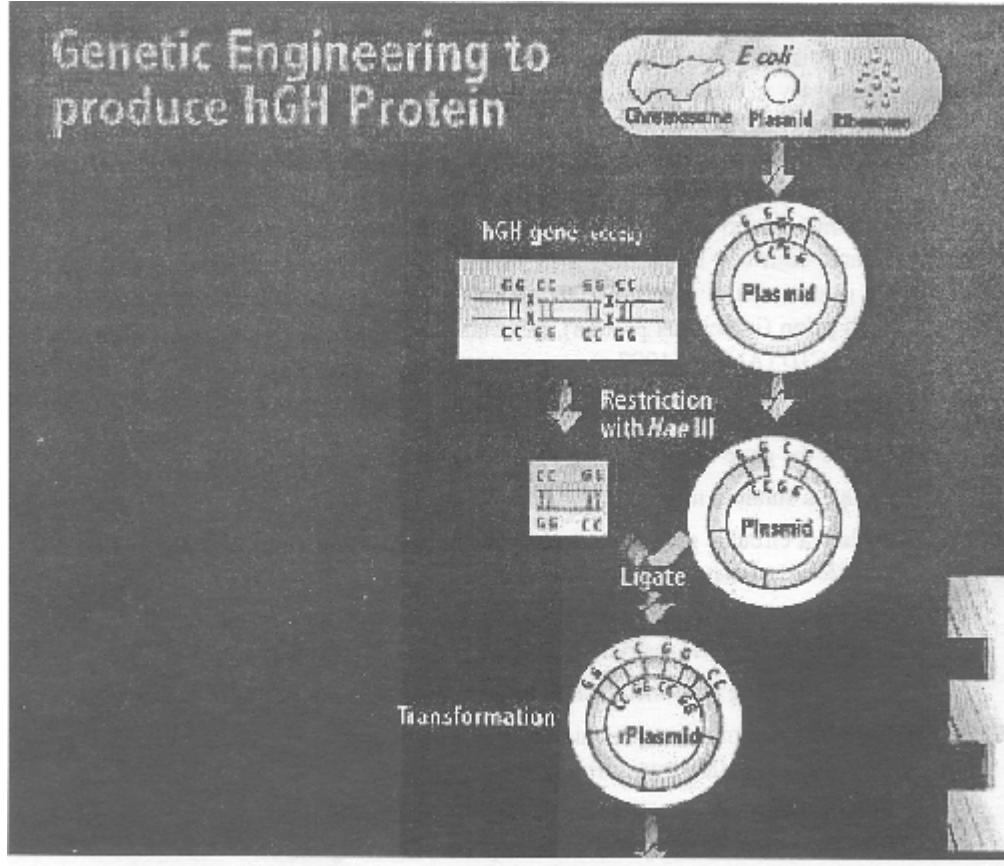
After injecting the insulin, the plasma concentration of insulin rises slowly. Therefore, insulin injection has to be done 15 minutes before meal. Further, decrease in the insulin level is slow, exposing the patients to a danger of hyperinsulinemia. All this is due to the existence of therapeutic insulin as a hexamer, which dissociates slowly to the biologically active dimer or monomer. A large number of second generation insulin called insulin muteins have been constructed with an objective of faster dissociation of hexamers to biologically active forms. Among these is insulin lispro, with modified amino acid residues at position 29 and 30 of the B-chain of insulin.

#### **4.5.2. HUMAN GROWTH HORMONE**

Growth hormone is produced by the pituitary gland. It regulates the growth and development. Growth hormone stimulates overall body growth by increasing the cellular uptake of amino acids, and protein synthesis and promoting the use of fat as body fuel. Insufficient human growth hormone (hGH) in young children results in retarded growth, clinically referred to as pituitary dwarfism. As traditional treatment of this disease, regular injections of growth hormone extracted from the brains of deceased human.

##### **4.5.2.1. PRODUCTION OF RECOMBINANT hGH**

The production procedure essentially consists of inserting hGH gene into *E.coli* plasmid, culturing the cells and isolation of the hGH from the extracellular medium. Limitation in hGH production: the hGH is a protein comprised of 191 amino acids. During the course of its natural synthesis in the body, hGH is tagged with a single peptide (26 amino acids). The signal peptide is removed during secretion to release the active hGH for biological functions. The entire process of hGH synthesis goes on in an orderly fashion in the body. Signal peptide interrupts hGH production by rDNA technology. The cDNA synthesized from the mRNA encoding hGH is inserted into the plasmid. The plasmid containing *E.coli* when cultured, produces full length hGH along with signal peptide where *E.coli* cannot remove the signal peptide.



*Fig (16) Genetic engineering technique to produce Human Growth Hormone hGH*

### **Fig 11 GENETIC ENGINEERING TECHNIQUE TO PRODUCE hGH**

A novel approach for hGH production: Biotechnologists have resolved the problem of signal peptide interruption by a novel approach. The base sequence in cDNA encoding signal peptide (26 amino acids) plus the neighboring 24 amino acids is cut by restriction endonuclease ECoRI. Now a gene (cDNA) for 24 amino acid sequence of hGH (that has been deleted) is freshly synthesized and ligated to the remaining hGH cDNA. The so constituted cDNA, attached to a vector, is inserted into a bacterium such as *E.coli* for culture and production of hGH.

Recombinant hGH was approved for human use in 1985. It was first marketed as Protropin by Genetech company and Humatrop by Eli Lily company.

Use of recombinant hGH for farm animals: Recombinant hGH is now administered to farm animals to promote early growth and development. Such farm animals yield linear meat, and increased milk production.

#### **4.5.3. CLOTTING FACTOR VIII**

The clotting factor VIII is required for proper blood clotting process. A genetic defect in the synthesis of factor VIII results in the disorder hemophilia A. The victims have prolonged clotting time and suffer from internal bleeding.

#### **4.5.3.1. PRODUCTION OF RECOMBINANT FACTOR VIII**

The gene for the formation of factor VIII is located on X-chromosome. It is a complex gene of 186kb in size, organized into 26 exons, many introns are present. The introns vary in their size, starting from 200 base pairs to as high as 32,000 base pairs. Biotechnologists were able to isolate mature mRNA (containing only exons) that is responsible for the synthesis of factor VIII. This mRNA contains 9000 bases and synthesizes the protein, factor VIII. It contains 2332 amino acids, with carbohydrate molecules attached at least at 25 sites. DNA technologists synthesized the cDNA for mature mRNA of factor VIII. This cDNA can be inserted into mammalian cells or hamster kidney cells for the production of recombinant factor VIII. Since 1992, factor VIII is available in the market under the trade name Recombinate and Kogenate.

#### **4.5.4. INTERFERONS**

Interferon is an antiviral substance and is the first line of defense against viral attacks. The term interferon has originated from the interference of this molecule on virus replication. It was originally discovered in 1957 by Alick Isaacs and Jean Lindemann and was considered to be a single substance. It is now known that interferon actually consists of a group of more than twenty substances with molecular weight ranging from 20-30kD. All the interferons are proteins in nature and many of them are glycoproteins. They are broadly classified into three group viz., IFN  $\alpha$ , IFN  $\beta$ , IFN  $\gamma$  based on their structure and function.

##### **4.5.4.1. MECHANISM OF ACTION**

Interferons are produced by mammalian cells when infected by viruses. As the virus releases its nucleic acid into cellular cytoplasm, it stimulates the host DNA to produce interferons. These interferons, secreted by the cells bind to the adjacent cells thus stimulate the cellular DNA to produce series of antiviral enzymes.

##### **4.5.4.2. PRODUCTION OF RECOMBINANT INTERFERONS**

The cDNA was synthesized from the mRNA of specific interferon. This is inserted to a vector which is introduced into *E.coli*. Interferon can be isolated from the culture medium. Production of interferons by yeast: the yeast *Saccharomyces cerevisiae* is more suitable for the production of recombinant interferons. Yeasts have the mechanism to carry out glycosylation of proteins similar to that occurs in mammalian cells which are lacked in bacteria. The DNA sequence coding for specific human interferon can be attached to the yeast alcohol dehydrogenase gene in a plasmid and introduced into 4 yeast cells. Yield is several times higher than *E.coli*. Production of hybrid interferon: several attempts have been made to produce hybrid interferons. This is advantages since different interferons with different antiviral activities can be combined to produce a more efficient interferon. Further, glycosylation step can be bypassed, and bacteria can be used to produce hybrid interferon. These are more reactive in their functions. These genes are digested by restriction endonucleases resulting fragments ligated to generate hybrid interferon.

#### 4.5.4.3. THERAPEUTIC APPLICATIONS OF INTERFERONS

IFN  $\alpha$ , IFN  $\beta$ , IFN  $\gamma$  were respectively approved for therapeutic use in human in the years 1986, 1993, 1990. A Swiss biotechnology firm was first to market IFN  $\alpha$  with a trade name Intron. Interferons are used for the treatment of large number of viral diseases and cancer. The cancer includes leukemia, kaposi's sarcoma, bladder cancer, head and neck cancer, renal cell carcinoma, skin cancer and multiple myeloma. The other diseases employing interferons as therapeutic agent are AIDS, multiple sclerosis, genital warts, hepatitis C, herpes zoster. In case of cancer, the mechanism of action of interferons is brought by stimulating the action of natural killer cells, a specialized form of lymphocytes that can destroy cancerous cells.

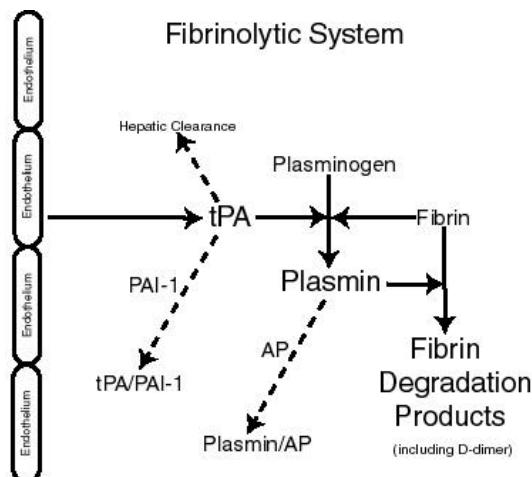
#### 4.5.5. INTERLEUKIN

The term interleukin (IL) is frequently used to represent cytokines. There are more than a dozen interleukins (IL1-IL12), produced by different cells with wide range of functions. The main function (directly or indirectly) of cytokines is to amplify immune responses and inflammatory responses. These are group of proteins that bring about communication between different cell types involved in immunity. They are low molecular weight glycoproteins and are produced by lymphoid and non lymphoid cells during the course of immune response. Cytokines may be regarded as soluble messenger molecules of immune system. They can act as short messengers between the cells or long messengers by circulating in the blood affecting cells at far off sites. The latter function is compared to that of hormones.

#### 4.5.5.1. THERAPEUTICS OF INTERLEUKINS

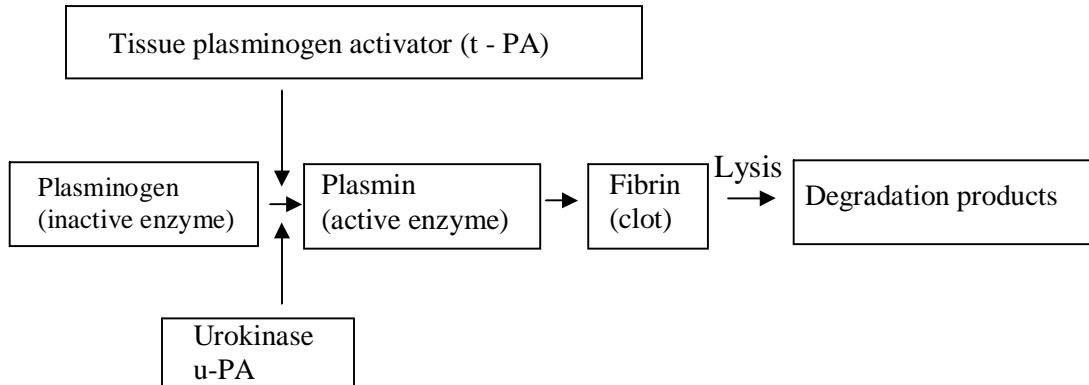
IL 2 is used in cancer immunotherapy, and in the treatment of immunodeficiency diseases. They induce the proliferation and differentiation of T- and B- cells, besides increasing the cytotoxic capacity of natural killer cells.

#### 4.5.6. TISSUE PLASMINOGEN ACTIVATORS



**Fig 12 ACTIVITY OF TISSUE PLASMINOGEN ACTIVATOR**

The blockage of blood vessels by clots is the commonest cause of death in the developed world. The processes involved in the formation and subsequent destruction of blood clot (thrombus) are complex and finely balanced. An array of enzymes and other substances ensure that clots normally form only when and where they are required to prevent blood loss, and broken down once their task is completed. The basis of blood clot is a network of fibres of the protein, fibrin. This network can be destroyed by the action of plasmin, a fibrinolytic enzyme, which is formed in the blood from an inactive precursor, plasminogen. The role of plasminogen activators (PAs) is to remove part of the plasminogen molecule to yield plasmin.



**Fig 13 ACTIVATION OF PLASMINOGEN AND BREAK DOWN OF BLOOD CLOTS**

#### 4.5.6.1. SOURCES OF PLASMINOGEN ACTIVATORS

PAs are found in blood, tears, saliva, urine, semen, and cerebrospinal and other body fluids. They are also found in many tissues and cells, including cancer cells. The tissue specific PAs (t-PAs) are made by many different types of cell, including several types of cancer.

#### 4.5.6.2. TISSUE PLASMINOGEN ACTIVATOR

Tissue plasminogen activator (tPA) is a naturally occurring protease enzyme that helps to dissolve blood clots. tPA is a boon for patients suffering from thrombosis. tPAs are not well characterized and have reported molecular weights in the range of 25 – 100kD. Recently a tPA obtained from a melanoma cell line has been cloned and expressed in *Escherichia coli*, and its complete amino acid sequence (562 amino acids) deduced from cDNA fragments. It is the first therapeutic product synthesized using mammalian cell culture. Recombinant tPA has been used since 1987 for treatment of acute myocardial infarction or stroke. Genetech was the first to market tPA by trade name Activase.

#### 4.5.6.3. PRODUCTION OF RECOMBINANT tPA

DNA technologists synthesized the cDNA molecule for tPA. This cDNA was then attached to a synthetic plasmid and introduced into mammalian cells. They are cultured and tPA producing cells were selected by using methotrexate to the medium. tPA producing cells were transferred to fermenter. Genetic manipulation may also be used at the post translational level to increase the yield of a product. Low yields of tPA is attributed to its association with GRP78 in rough endoplasmic reticulum of CHO (Chinese Hamster Ovary) cells. GRP78 is a 78kD glucose

regulated stress response protein. It has been shown that co expression of an antisense GRP78 message (due to cloned antisense RNA sequence) resulted in smaller quantities of GRP78 and faster tPA secretion.

Alteplase and Reteplase are the second generation recombinant tPAs. They have increased in vivo half lives and are functionally more efficient.

#### **4.5.6.4. ANTIBODY – PLASMINOGEN ACTIVATOR CONJUGATES**

An antibody against fibrin (antifibrin antibody) can be conjugated with tissue plasminogen activator. This conjugate is approximately regarded as immunotherapeutic thrombolytic agent. It quickly and specifically binds to fibrin clots and locally increases the conversion of plasminogen to plasmin to dissolve fibrin. In fact, antifibrin monoclonal antibodies have been synthesized, conjugated with tPA and tried for solubilising blood clots.

#### **4.5.6.5. ADVANTAGES OF tPA AS THROMBOLYTIC AGENT**

tPA acts on blood clots without reducing the blood clotting capability elsewhere. This is in contrast to the action of other plasminogen activators like Urokinase and streptokinase which are more generalized in their action. Further, tPA can be administered intravenously unlike Urokinase and streptokinase which have to be administered directly to the blocked blood vessel. tPA action is faster than other thrombolytic agents with reduced side effects.

#### **4.5.7. ERYTHROPOIETIN**

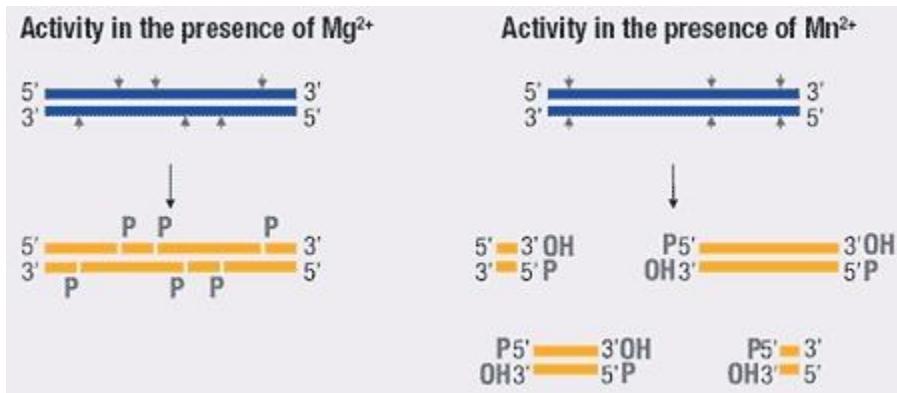
Erythropoietin is a hormone synthesized by the kidney. It stimulates the stem cells of bone marrow to produce mature erythrocytes. Biotechnologists were successful in producing recombinant erythropoietin. An approval for its therapeutic use in human was obtained in the year 1989. Amgen Inc. first marketed erythropoietin with a trade name Epogen. It is useful in treating the patients with severe anemia that accompanies kidney disease. Another firm Ortho Biotech produced Procrit, a genetically engineered erythropoietin in 1997. Procrit acts like the natural hormone and stimulates the production of erythrocytes. It is used in anemic patients undergoing non cardiac, non vascular surgery. Procrit administration before surgery serves as an alternative to blood transfusion.

Erythropoietin produced by Chinese hamster ovary cells can be subjected to post translational modification by the transfer of a gene for  $\beta$ -galactosidase  $\alpha$  2,6 sialyl transferase. This enzyme causes sialyl  $\alpha$  2,6 galactosyl linkage on surface glycoprotein and can convert erythropoietin into a kind more closely resembling human erythropoietin which is rich in these linkages.

#### **4.5.8. DEOXYRIBONUCLEASE I (DNaseI)**

The enzyme DNaseI hydrolyses long DNA chains into shorter oligonucleotides. The biotechnology firm Genetech isolated and expressed the gene to produce recombinant DNaseI. This enzyme is very useful in the treatment of common hereditary disease cystic fibrosis. DNaseI

cleave DNA preferentially at phosphodiester linkages adjacent to a pyrimidine nucleotide, yielding 5'-phosphate terminated poly nucleotides with a free hydroxyl group on position 3', on average producing tetra nucleotides. It acts on single stranded DNA, double stranded DNA, and chromatin. DNaseI is also a polymerization initiator for the cytoskeletal protein actin.



**Fig 14 DNase I ACTIVITY**

Cystic fibrosis is one of the most common genetic diseases. Patients of cystic fibrosis are highly susceptible to lung infections by bacteria. The presence of live or dead bacteria leads to the accumulation of thick mucus in the lungs making breathing very difficult. The major constituent of this mucus is the bacterial DNA (released on bacterial lysis). Administration of the enzyme DNaseI to the lungs of cystic fibrosis patients decreases the viscosity of the mucus, and the breathing is made easier.

#### 4.5.9. ALGINATE LYASE

Alginate lyases, also known as alginases or alginate depolymerases, catalyze the degradation of alginate by a  $\beta$ -elimination mechanism that has yet to be fully elucidated. Alginate is a copolymer of  $\beta$ -L-guluronate (G) and its C5 epimer  $\beta$ -D-mannuronate (M), arranged as homopolymeric G blocks, M blocks, alternating GM or random heteropolymeric G/M stretches. Although all lyases perform essentially the same depolymerization action on alginate, each enzyme is defined by its individual characteristics and its preference for the glycolytic bond connecting M and G monomers.

##### 4.5.9.1. SOURCES OF ALGINATE LYASE

Alginate-degrading enzymes with various substrate specificities have been isolated from many sources, including marine algae, marine mollusks, and a wide range of microorganisms. Alginate lyases have been isolated from a variety of marine bacteria, soil bacteria, and fungi. Most of these organisms are able to use M and G units from depolymerized alginates as carbon and energy sources.

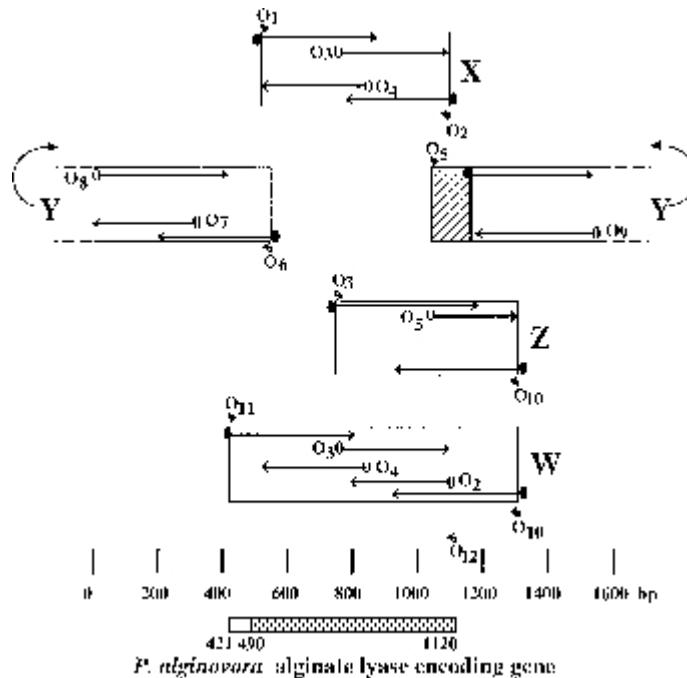
#### 4.5.9.2. CLASSIFICATION

Alginate lyases are classified, based on their dominant cleaving action on M-rich or G-rich alginates, as EC 4.2.2.3, poly(M) lyase [(1-4)- $\beta$ -D-mannuronan lyase] or EC 4.2.2.11, poly(G) lyase [(1-4)- $\alpha$ -L-guluronan lyase].

#### 4.5.9.3. MECHANISM OF ACTION

Alginate lyase catalyzes the degradation of alginate by a  $\beta$ -elimination mechanism, targeting the glycosidic 1 - 4 O-linkage between monomers. A double bond is formed between the C4 and C5 carbons of the six-membered ring from which the 4-O-glycosidic bond is eliminated, depolymerizing alginate and simultaneously yielding a product containing 4-deoxy-L-*erythro*-hex-4-enopyranosyluronic acid as the non reducing terminal moiety. Gacesa proposed a catalytic mechanism for alginate lyase that described a three-step reaction to depolymerize alginate. This mechanism may also be shared with epimerase, another enzyme that acts on the alginate polymer. The two reactions differ only in the last step of the three-stage transformation of alginate.

The three steps include (a) removal of the negative charge on the carboxyl anion—essentially neutralizing the charge by a salt bridge (lysine may be the candidate residue); (b) a general base-catalyzed abstraction of the proton on C5 (aspartic acid, glutamic acid, histidine, lysine, and cysteine have been suggested for this role), where one residue may be required as the proton abstractor and another as the proton donor, although the proton may be derived from the solvent environment; and (c) a transfer of electrons from the carboxyl group to form a double bond between C4 and C5, resulting in the  $\beta$  -elimination of the 4-Oglycosidic bond. In the proposed mechanism for epimerase, the replacement of the proton at C5 (epimerization) takes place in step c.



**Fig 15 ALGINATE LYASE ENCODING GENE**

#### **4.5.9.4. APPLICATIONS FOR ALGINATE LYASES**

Alginate has been widely used in the food, textile, printing, and chemical industries. Various genera of the *Phaeophyceae* (brown seaweed) have been the major sources of industrially useful alginate. Bacterial sources have not been extensively used. Because *P. aeruginosa* and other alginate-producing *Pseudomonad* do not synthesize polymers with G-blocks, which are essential for forming gels, they have not been of much interest for industrial use. However, the alginate from *A. vinelandii* may have some gelling properties and, consequently, has been investigated for various applications. Alginate has a lucrative worldwide market in its native form as does its organic derivative, propylene glycol alginate, which is used principally in the food industry.

##### **(i) PAST AND PRESENT APPLICATIONS**

Alginate lyases from both marine and bacterial sources have been used to analyze alginate fine structure to understand how chemical composition influences the physical properties of this important industrial polysaccharide.

Although the established commercial extraction of alginate from marine algae has used various mechanical and chemical methods, alginate lyases have now been used in the protoplasting of seaweed. Both the resulting alginate, extracted from the cell wall and the protoplast are used in the food industry and for manufacturing of other industrial materials. Alginate lyases from marine and bacterial sources have been applied successfully to the extraction of protoplasts for food research and regeneration of a variety of algal species. Although green algae can be protoplasted with cellulases and pectinases, red or brown algae cannot.

Thus, the presence of alginate as a major constituent of the red and brown algae cell wall requires the use of alginate lyase in combination with other carbohydrate enzymes for cell wall degradation and protoplasting.

Alginate lyases have also been used in studying *Fucus* cell wall development and as part of a depolymerase system in the study of Azoto-phage adsorption kinetics. Alginate lyases from *Beneckia pelagia* and *K. pneumoniae* have also been used in the investigation of mannuron C5-epimerase in *A. vinelandii* and *P. aeruginosa*.

More recent applications have included the use of an M lyase from a *Photobacterium* sp. to produce poly(M) and poly(MG) blocks from acetylated and deacetylated *P. aeruginosa* alginate that could then be used to investigate the substrate specificities of other lyases. Similarly, a G-specific lyase from *Flavobacterium multivolum*, which degrades poly (G) and poly (M/G) alginate, has been applied toward the preparation of poly (M) blocks from sodium alginate for substrate specificity studies.

## (ii) FUTURE APPLICATIONS

The use of alginate lyase for the treatment of alginate polysaccharide build-up in the lungs of Cystic fibrosis sufferers still remains one of the most important goals of studying alginate lyase. Mrsny et al described the complex distribution of DNA and alginate within the mucin matrix of Cystic fibrosis sputa. Inhalation therapy with a recombinant human deoxyribonuclease has proven to be clinically useful in reducing the viscosity of Cystic fibrosis sputa. Studies with an M-lyase from *P. aeruginosa* to reduce sputum viscosity in vitro were disappointing because  $\text{Ca}^{2+}$  and  $\text{Zn}^{2+}$  levels in most Cystic fibrosis sputa inhibited lyase enzyme activity. However, in some sputum samples, the combination of alginate lyase and deoxyribonuclease demonstrated an additive reduction of the sputum visco elasticity, which suggests that this approach deserves further study.

ALY1-III from *Sphingomonas* sp. has been reported to be highly effective against acetylated *P. aeruginosa* alginate and may show promise as a therapeutic agent. It is likely that lyase would be used in conjunction with other chemotherapeutics, such as deoxyribonuclease and antibiotics so that, after degrading the alginate-rich mucoid layer, *P. aeruginosa* would be more susceptible to macrophage phagocytosis and antibiotic therapy. Soothill has shown that highly lytic bacteriophages could protect against fatal doses of pathogenic *P. aeruginosa* in mice, and *P. aeruginosa*-specific phages have also been effective in preliminary studies to treat infection at burn wound sites. Phage therapy, using *P. aeruginosa*-specific bacteriophages that encode extracellular alginate lyases, might be an alternative for treating mucoid *P. aeruginosa* infections and controlling biofilm formation.

Alginate lyases may also be used as tools for generating defined products for innovative endeavors. Depolymerized alginates with low *Mr* act like oligosaccharines in their ability to regulate physiological processes in plants. Oligomeric alginate (average *Mr*, 2000) obtained from lyase degradation of high-*Mr* alginate, can promote growth of *Bifidobacteria* spp. and thus has been proposed for use as a physiological food source. Furthermore, alginate lyase-degraded products (average *Mr*, 1800) greatly enhanced germination and shoot elongation in plants, although repressing the growth of *Chlamydomonas* spp. and HeLa cells. Trisaccharides or alginate lyase-lysate has also been found to promote root growth in barley. In the presence of epidermal growth factor, dimers, trimers, and tetramers that possessed guluronic acid at the reduced ends highly induced the proliferation of keratinocytes. Other alginate polymers (average *Mr*, 230,000) have antitumor effects and enhance phagocytic activity of macrophages. Poly (M) block-rich alginate exhibited high antitumor activity and could stimulate production of cytokines by human monocytes. The conformational properties of the macromolecules and the anionic character and molecular weight of the polysaccharide were suggested to be important in the effectiveness of their antitumor activity. Therefore, alginate lyases are crucial in the generation of such useful oligomeric products. The promising use of calcium alginate beads as a biomaterial in wide-ranging applications extends further the potential for alginates and alginate lyases.

Calcium alginate, with or without a polylysine, polyarginine, or chitosan protective coating, has been used for the encapsulation of a variety of materials including drugs for controlled delivery; DNA and oligonucleotides for tumor development studies, gene delivery, gene therapy, and antisense oligonucleotide therapeutic agents; yeast cells coentrapped with

lipase for the production of flavor esters; plant tissue, with the aim of developing artificial seed technology; *P. flourescens* as a biocatalyst for phenolic-compound removal from wastewater; and entomopathogenic nematodes for agricultural biocontrol. Alginate in combination with other biomaterials like hyaluronate and chitosan can be highly effective in many medical applications, including use in wound dressing impregnated with antibiotics and encapsulation of chondrocytes to engineer cartilage tissues *in vitro* for cartilage transplant and repair. Alginate with G content of >70% and average G-block length of >15, and with low polyphenol contamination, provides the most suitable characteristics for immobilization beads.

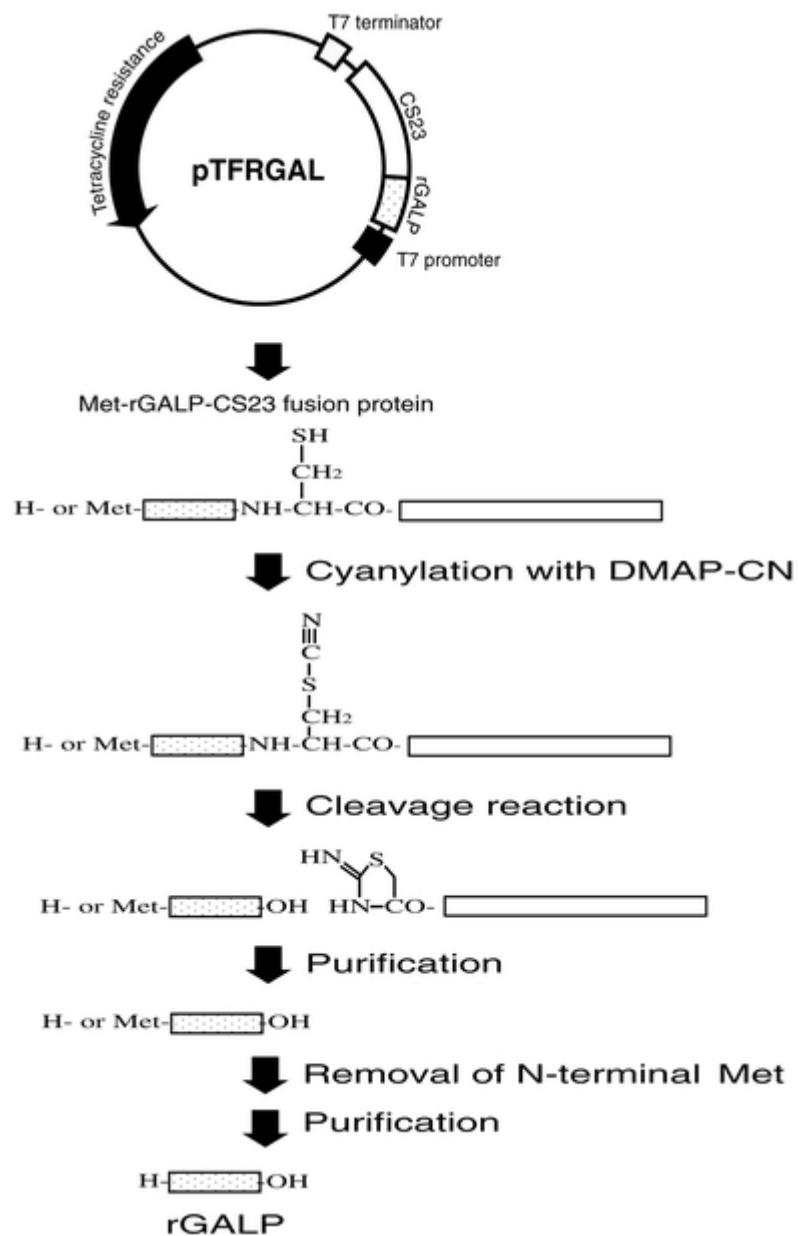
Apart from generating alginate with a predictable monomeric sequence from native sources, it is possible to use a combination of D-mannuronan C5-epimerase and alginate lyase on a particular alginate substrate to engineer novel alginate polymers of defined composition. The composition and properties of alginate gels are still being extensively studied by a wide variety of methods, including the use of quantitative magnetic resonance imaging to investigate the materials. The collective information will provide a guideline for the design of these novel polymers.

#### **4.5.10. MUTEINS**

By employing site directed mutagenesis, the amino acid sequence of a recombinant protein can be suitably modified by protein engineering. The mutated proteins are collectively called as muteins. These are second generation therapeutic proteins. Examples are insulin, lispro, Alteplase.

Example - IL-11 muteins, of which site I hydrophobicity has been increased. These muteins act as IL-11 agonist or hyperagonist, and are notably useful as anti-thrombocytopenia agents, and as agents improving the resistance of an organism to the deleterious *in vivo* effects induced by radiation or chemotherapy during the treatment of cancer or for the preparation of patient to transplantation.

The inventors have designed and produced IL-11 muteins wherein the hydrophobicity at site I has been substantially increased by replacement of at least two IL-11 site I hydrophilic amino acids by hydrophobic counterparts. The IL-11 muteins bind to IL-11R $\alpha$  with an enhanced affinity and retain the ability to recruit gp130 through site II. As an advantageous feature, they retain the ability to induce *in vitro* proliferation of various IL-11 dependent cells. A mutein of the invention; namely the H182V+D186A hIL-11 mutein has further been shown to be 60 to 400 fold more active than wild-type IL-11 on the *in vitro* proliferation of 7TD1 murine hybridoma cells. These muteins retain *in vivo* biological activity which are further much higher than wild-type IL-11. An injection of the H182V+D186A hIL-11 mutein at a 10-fold lower dose than the wild-type hIL-11 has been shown to delay the death of irradiated mice for the same duration. Compared to single-point mutation IL-11 muteins such as a D186A IL-11 mutein, the double point muteins prove to have *in vitro* and *in vivo* unexpected effect and advantages. They notably induce much higher survival rates upon exposure to radiation (i.e. upon inhibition of micro vascular endothelial apoptosis). Thus, muteins are therefore useful in every biological, medical or clinical application in which wild-type IL-11 is useful, and can even show an enhanced



**Fig 16 SYNTHESIS OF MUTEINS**

efficiency. The muteins are more particularly useful in radioprotection (e.g. radioprotection of the small intestine during abdominal irradiation), in decreasing chemotherapy deleterious effects (e.g. during 5-fluorouracil chemotherapy), in anti-inflammatory therapy, in resistance to septic shock, to diabetes, and in hematopoiesis stimulation.

#### **4.6. LET US SUM UP**

- The availability of therapeutic products was very limited due to cost and cumbersome procedure involved in their isolation or production.
- With the advent of recombinant DNA technology, it has been easy for us to synthesize the therapeutic agents of sufficient quantities for human use.
- Thus, in the present unit, the pharmaceutical products which are of economic importance with their application and production having been discussed - Various therapeutics, Methodologies involved in the production of therapeutics, Therapeutics applications.
- These pave way for the exploration of recombinant DNA technology in future for the production of more new therapeutic agents for the disorders of human.

#### **4.7. POINTS FOR DISCUSSION**

1. Substantiate the use of Interferon.
2. Evaluate the use of Probiotics.
3. Discuss the application of alginate zyaces.

#### **4.8. LESSON - END ACTIVITIES**

1. Discuss about the production of Human growth hormone
2. Explain the synthesis of penicillin and streptomycin
3. What are tissue plasminogen activators?
4. Explain mureins and probiotics.
5. What are the various types of vitamins and explain their production?

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## UNIT V WASTE MANAGEMENT

### **CONTENTS**

- 5.0. AIMS AND OBJECTIVES**
- 5.1. INTRODUCTION**
- 5.2. PRIMARY TREATMENT**
- 5.3. SECONDARY TREATMENT**
- 5.4. TERTIARY TREATMENT**
- 5.5. SOLID WASTE MANAGEMENT**
- 5.6. LET US SUM UP**
- 5.7. POINTS FOR DISCUSSION**
- 5.8. LESSON - END ACTIVITIES**
- 5.9. REFERENCES**

### **5.0. AIMS AND OBJECTIVES**

This unit explains the management of the wastes eliminated from the industries which are involved in the production of economic important products.

#### **5.1. INTRODUCTION**

Apart from drinking water, in urban areas, the household consumption of water is about 150L/day per person. The domestic water consumption may vary with the lifestyle of community and availability of water. Most of the water taken into the house and industries may be returned as waste water and effluent respectively through drainage system. All these waste water contain organic and inorganic wastes as suspended or dissolved matter. In addition they may also contain microorganisms, including those of faecal origin and pathogenic nature. The waste water discharged through the drainage system has to be properly disposed. They cannot be simply disposed off into water bodies or landscapes because of the oxygen demand they exert and also due to the presence of pathogenic microorganisms in them. So before disposal waste water, has to be properly treated either by physical, chemical or biological methods. These aspects will be discussed in detail.

##### **5.1.1. CHARACTERISTICS OF SEWAGE**

Sewage or waste water is the water borne human (faecal matter), domestic (food wastes, wash water) and farm wastes (pesticides). It may include industrial effluent (acids, oils, greases and animal and vegetable matter), subsoil or surface waters.

##### **5.1.2. CHEMICAL CHARACTERISTICS**

A typical raw sewage contains about 99.9% water. The remaining 0.1% is made up of about 70% organic and 30% inorganic solids. The solid content occurs in both suspended and dissolved forms. The inorganic components include ammonia, chloride, grit, salts and metals which are contributed from metal industries and mines. Organic compounds may be either nitrogenous compounds such as carbohydrates and lipids contributed mainly from plant and

animal wastes which are different in composition. Animal sewage is relatively high in proteins and lipids when compared to plants which are rich in cellulose and lignin. Cellulose in sewage mainly comes from the cell wall of plants and plant products. Other contributors are paper, cotton and certain other plastic products. Since cellulose cannot be digested in human digestive system, major proportion of cellulose in the human diet is excreted as undigested waste. Hemicellulose, pectin, starch and lignin are the other carbohydrate materials found in sewage.

The next important chemical group found in sewage is lipid. Fat and fatty acids which escape digestion, adsorption or deposition in human digestive system accounts for the lipids in faeces. Proteins are the next group which mainly originates from the excreta of humans and animals. In addition, dead/decomposing plants, insects and microorganisms also provide proteinaceous substances.

Since waste waters from different sources accumulate in sewage, its chemical composition varies depending upon the sources. Moreover, since the microorganisms are also part of sewage, they carry out certain changes in the chemical composition of sewage irrespective of the treatment processes. Industrial wastes from slaughter houses, sugar factories and paper mills add organic matters.

### **5.1.3. MICROBIAL CHARACTERISTICS**

The composition of sewage varies depending upon the source of waste water. This also causes variation in the microbial flora of sewage. Almost all groups of microorganisms, algae, fungi, protozoa, bacteria and viruses are present. Raw sewage may contain millions of bacteria per ml. the bacterial group comprises mainly the soil borne organisms, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus mycoides*, *Pseudomonas fluorescens*, *Achromobacter spp.* And *Micrococcus spp.* Bacteria of intestinal origin also occurs in sewage in large numbers. Mostly these are harmless organisms. Examples of this type are *Escherichia coli*, and other coliforms, *Proteus* and *Serratia* species. Potential pathogens include enterococci (*Streptococcus faecalis*) and *Clostridium perfringens*. Pathogenic bacteria which cause serious illness like *Vibrio cholerae*, *Salmonella typhi*, *Salmonella paratyphi* and *Shigella dysenteriae* may also occur in sewage.

Viruses which are released in the faeces from infected host are also occasionally found in sewage, for example, *Poliomyelitis* virus, infectious *Hepatitis* virus and *Coxsackie* virus. Bacteriophages also occur in comparatively large numbers. During treatment process the microbial flora may be dominated by the corresponding physiological groups.

### **5.1.4. SEWERAGE SYSTEM**

Sewerage is a system of sewers and ancillary works to convey sewage from its point of origin to a treatment plant or to other places of disposal. Sewer is a pipeline to carry sewage or other wastes and normally flowing full. There are three kinds of sewerage systems viz., sanitary sewers, storm sewers and combines sewers.

Sanitary sewers: these carry domestic and industrial waste water.

Storm sewers: these are designed to carry off surface and storm (atmospheric) water.

Combined sewers: these carry all waste waters through a single system of sewers.

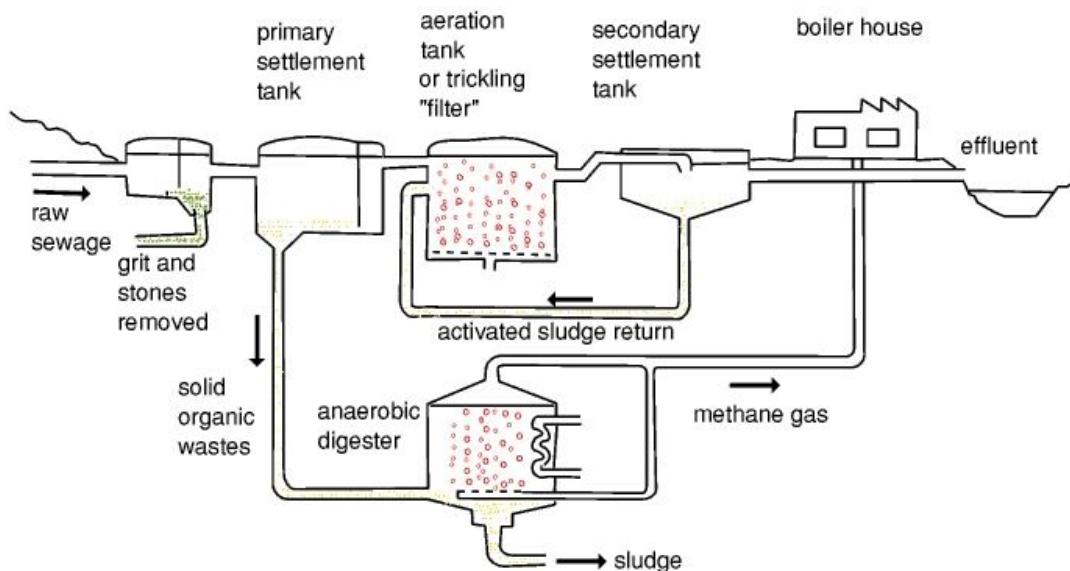
### 5.1.5. SEWAGE SYSTEM

Sewage treatment can be defined as an artificial process in which sewage is subjected to remove or alter its constituents to render it less offensive or dangerous.

Objectives of the sewage treatment are:

1. To convert waste and waste water into a readily reusable resource.
2. To prevent pollution of any water body to which treated or reused water enters.
3. To reduce the BOD of sewage from 30mg/L to 20mg/L in the final effluent.
4. To destroy the causative agents of water borne diseases.

If the waste water is inadequately treated and disposed into water bodies it may lead to a number of complicated problems. Since waste waters at times contain pathogenic microorganisms which can be removed or reduced to a non – infectious level by conventional disinfection process, inadequate treatment may provide a greater possibility for the dissemination of pathogenic microorganisms. The conditions may become more dangerous if the inadequately treated water is disposed into natural water bodies which are used for drinking into natural water bodies which are used for drinking water supplies.



**Fig 1 SEWAGE SYSTEM**

If the inadequately treated waste water is used for culturing oysters and other shellfish, they may harbor pathogenic microorganisms and thus become unsafe for human consumption. If the feeding grounds of water fowls are polluted by these waste waters, it may lead to great losses in the water fowl populations.

If the waste water is discharged without proper treatment into water bodies used for swimming and other recreational water sports, dangerous consequences may result.

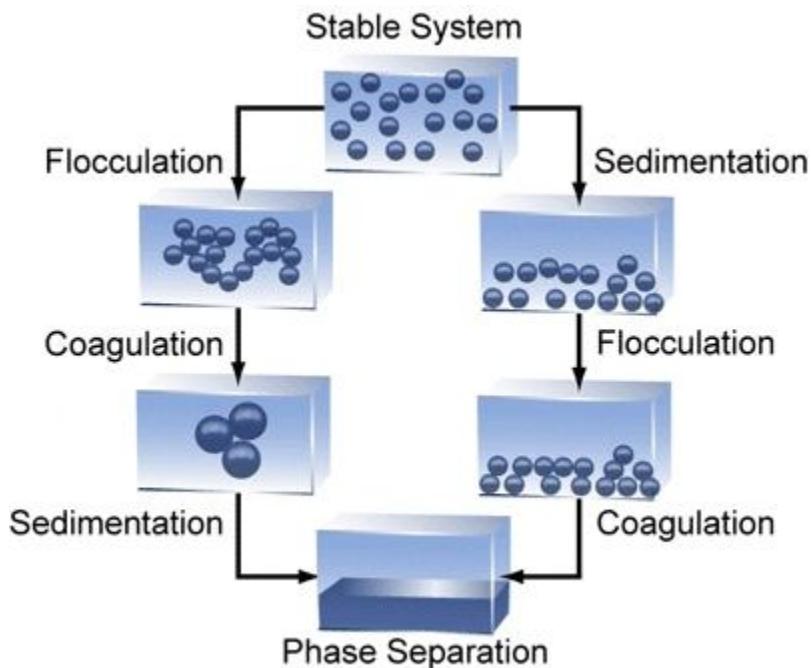
Depletion of dissolved oxygen may result in water bodies due to the accumulation of organic matter from sewage. This may ultimately kill the aquatic life. Moreover, toxic chemicals from industrial effluents may accumulate in water bodies used for drinking supplies; dissemination of toxic chemicals may threaten human health. Accumulation of debris and wastes in untreated water may create objectionable conditions such as offensive odor etc.

## 5.2. PRIMARY TREATMENT

To protect the main units of a treatment plant and to ensure their efficient operation it is necessary to remove large floating and suspended solids like leaves, twigs, paper, rags and other debris from waste water. This can be achieved in preliminary treatment process in the following ways.

### 5.2.1. SCREENING AND STRAINING

The first stage of preliminary treatment process involves screening or straining to remove large solids. Coarse screens with openings of about 75mm are used to prevent large objects. Main screens are provided with openings of 5-20mm through which the incoming water passes. To prevent overloading of the main screen with collected solids, the screen is slowly rotated so that the solids can be removed at regular intervals. In waste water treatment, the high content of papers and rags in waste water treatment makes it difficult to keep the mesh screen clean. Hence instead of mesh screens bar screen arrangements, with a spacing of 20-60mm between bars, are used. These screens can be cleaned automatically. Screenings from sewage are usually disposed of by burial or incineration. Alternatively they may be passed to a macerator which shreds them to a small size. Then they are returned to the flow so that they can be removed along with other settled solids during the main treatment process.



## **Fig 2 TECHNIQUES INVOLVED IN PRIMARY TREATMENT**

### **5.2.2. MICRO STRAINING**

Micro strainer is a drum screen with a fine woven stainless steel mesh. This can also be used in the final tertiary stage to produce a high quality sewage effluent.

### **5.2.3. GRIT REMOVAL**

Considerable amount of grit is carried along with the flow of sewage and these pose problems in most sewage systems, in particularly with combined sewers. If not removed these grit particles may damage the mechanical parts of the treatment system. Grit particles are of large size and have a high density when compared with the organic particles in sewage. Thus they can be removed using the principle of differential settling. Grit particles, with a diameter of 0.2mm and a specific gravity of 2.56, settle at a velocity of about 1.2m/min whereas most of the suspended solids in sewage have considerably lower settling velocities. With a retention time of 30-60 seconds, the flow of sewage through a channel of sufficient length can allow the settling of grit particles at the bottom while the remaining suspended solids continue to flow through the channel. The settled grit is removed at intervals, washed and then disposed off. Other types of grit removal employ a short retention settling tank.

### **5.2.4. SEDIMENTATION**

Sedimentation or clarification is the settling and removal of suspended particles that take place when water stands still in or flows slowly through a basin or tank. Particles that have high density higher than that of water will settle down under the influence of gravity and form sludge layer whereas the clarified water will be collected through the outlet. Thus sedimentation units play dual role – the removal of settle able solids and the concentration of the removed solids into a small volume of sludge. Sedimentation can be used to remove suspended particles down to a size of about 50 $\mu\text{m}$  depending upon density.

#### **5.2.4.1. RECTANGULAR HORIZONTAL FLOW TANK**

Rectangular horizontal flow sedimentation tanks are normally built of bricks or concrete and are rectangular in plan and cross section. The inlet for the raw water and the outlet for clarified water are made at opposite ends of the tanks near the top surface. Turbulence or cross circulation of the influent water within the tank will reduce the efficiency of the sedimentation process. This can be overcome by settling a special inlet structure which ensures an even distribution of the influent water over the full width and depth of the tank. Similarly the outlet structure ensures the even collection of clarified water. In between inlet and outlet is the settling or sedimentation zone. The sediment materials form a sludge layer at the bottom of the tank. To facilitate the sludge removal, the tank bottom is built in such a way that it slopes slightly towards the inlet end where the opening for sludge collection is situated. The sludge has to be cleaned out regularly. This can be done by manual cleaning in which the tank must be drained first. Alternatively the bottom of the tank can be provided with a continuous belt system with scrapper.



#### **5.2.4.2. EFFICIENCY OF SEDIMENTATION TANKS**

The sedimentation velocity ( $V_s$ ) of a particle can be determined by calculating the depth of the tank ( $H$ ) it traverses during the detention time ( $T$ )

$$V_s = H/T \text{ (m/hr)}$$

Detention time can be calculated as

$$T = AH/Q \text{ where, } A - \text{surface area of tank (m}^2\text{), } H - \text{depth of tank (m), } Q - \text{influent flow rate (m}^3/\text{hr)}$$

by substituting the  $AH/Q$  for  $T$  in the equation for  $V_s$ ,

$$V_s = Q/A \text{ (m}^3/\text{m}^2\text{hr} = \text{m/hr)}$$

Thus the sedimentation efficiency of a tank depends only upon the ratio between the influent flow rate and the surface area of tank. This ratio is called as surface loading and is independent of the depth of tank. Generally there is no difference in the settling efficiency between a shallow and a deep tank.

When the sedimentation is used without pre treatment, as in case of clarification of river water, the process is called plain sedimentation. The surface loading for the process will generally be in the range from 0.1 – 1m/hr. For water treated by chemical coagulation and flocculation, a higher surface loading is possible between 1 and 3m/hr. In both cases, lower the surface loading better the clarification of water and settled water will have less turbidity.

Since the purpose of sedimentation tanks is to remove suspended solids their efficiency can also be expressed by the percentage of removal of solids. Although suspended solid particles are of size down to a few microns, floc particles smaller than  $100\mu\text{m}$  are usually not removed by sedimentation. Hence it is clear that a sedimentation tank will never remove all the suspended solids from sewage. Thus the normal range of suspended solids removal from sewage by sedimentation is 50-60%.

#### **5.2.4.3. MODIFICATION OF SEDIMENTATION TANKS**

Since the sedimentation efficiency depends on surface area and not on depth, installation of an extra bottom in the settling zone will increase sedimentation efficiency. This set up can further be improved by using more trays.

The modification has demerits that the removal of sludge deposits will be difficult as the space between the trays is very small. This can be overcome by using jet washing or by setting self cleaning plates. In latter case the plates are set steeply at an angle of  $40\text{-}60^\circ$  to the horizontal. Such installations are called tilted plate settling tanks also closed PVC pipes can be used instead.

#### **5.2.5. FLOTATION**

Small size, low density particles in waste water have low settling velocities and cannot be settled by conventional processes. An alternative clarification process for them is to allow the particles to float to the surface where they can be removed as a scum. If the density of the suspension is close to that of the fluid in which they are suspended they may float without any encouragement. Even if the density of the particles is greater than that of the suspending fluid they can be made to float by the addition of a floatation agent which produces positive buoyancy.

Air bubbles are the effective flotation agents. A number of techniques are available to generate air bubbles and commonly used one is the dissolved air flotation.

In dissolved air flotation process a portion of the effluent from the flotation tank is recycled through a saturator where air is injected at high pressure. The pressurized recycle flow is introduced to the inlet at the bottom of the tank where it is mixed with the incoming flow. This sudden drop in the pressure produces super saturation of the water with air and a cloud of air bubbles, which attach themselves to the particles in the suspension, are released. These air bubbles along with the particles float at the top of tank as scum and can be removed. The cycle is repeated to produce an effective removal of suspended particles.

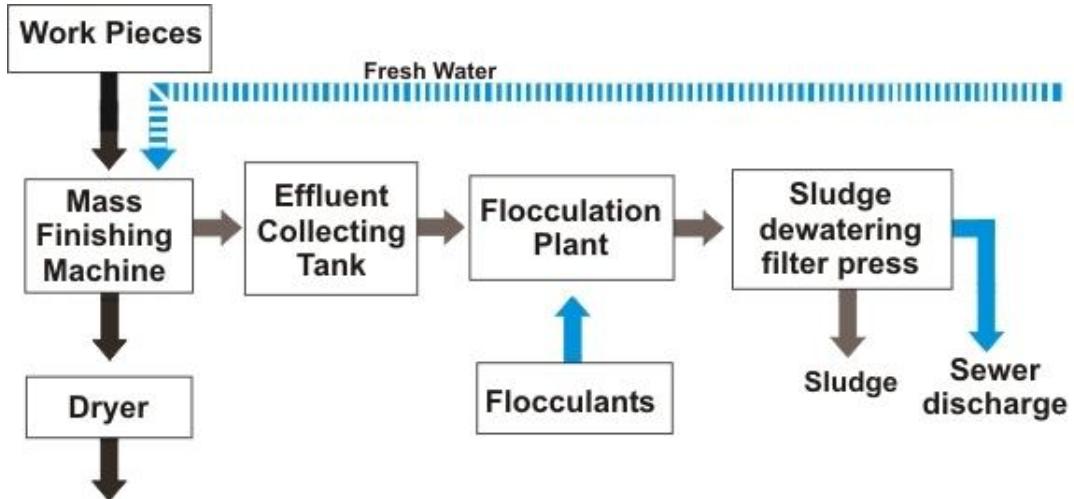
Dissolved air flotation has certain advantages over the sedimentation tank processes. They are effective in water treatment when chemical coagulation is used to remove dissolved substances such as colour, iron and manganese. They produce lower turbidity output. Dissolved air flotation units can be brought into full operation within a short period of time whereas the conventional sedimentation tanks take several days. The scum removed from flotation units is normally of a higher solid content than that produced by settlement of the same suspension. Thus the volume of the sludge is significantly reduced. The capital cost of flotation units is less than that of the equivalent settling tank but operating costs are higher because of the power required for pumping and compressed air injection. However total cost of dissolved air flotation will be less than that of the conventional sedimentation tank.

### **5.2.6. COAGULATION AND FLOCCULATION**

Many impurities in waste water are present as colloidal solids. Smaller particles have very low settling velocities and cannot be removed by sedimentation. These smaller particles can be removed if they are allowed to agglomerate. Agglomeration by flocculation with or without the use of a coagulant may lead to an increase in the size of particles and the particles are ultimately removed by sedimentation or flotation. With colloidal particles in high concentration, flocculation will be effective and for the ones in low concentration a coagulant may be required.

#### **5.2.6.1. FLOCCULATION**

In quiescent liquids fine particles will collide because of Brownian movement. Collision may also occur when rapidly settling solids overtake the slowly settling particles. As a result of these collisions larger particles are produced but in fewer number. Also the process is slow. In flocculation, collision between particles is improved by gentle agitation so that larger settleable particles (flocs) are produced which can be readily removed by settling or filtration. Agitation of water produces velocity gradients and the number of collisions between particles is directly related to the velocity gradients. Thus, the intensity of velocity gradients controls the degree of flocculation. Flocculation can be carried out in specially designed tanks where the influent liquid is subjected to mechanical stirring. The normal retention time in flocculation tank is 30-45 min. Flocculation and sedimentation may be combined in a single unit.



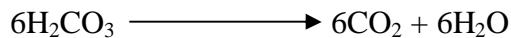
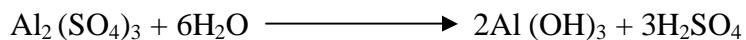
**Fig 3 FLOCCULATION TANK**

#### 5.2.6.2. COAGULATION

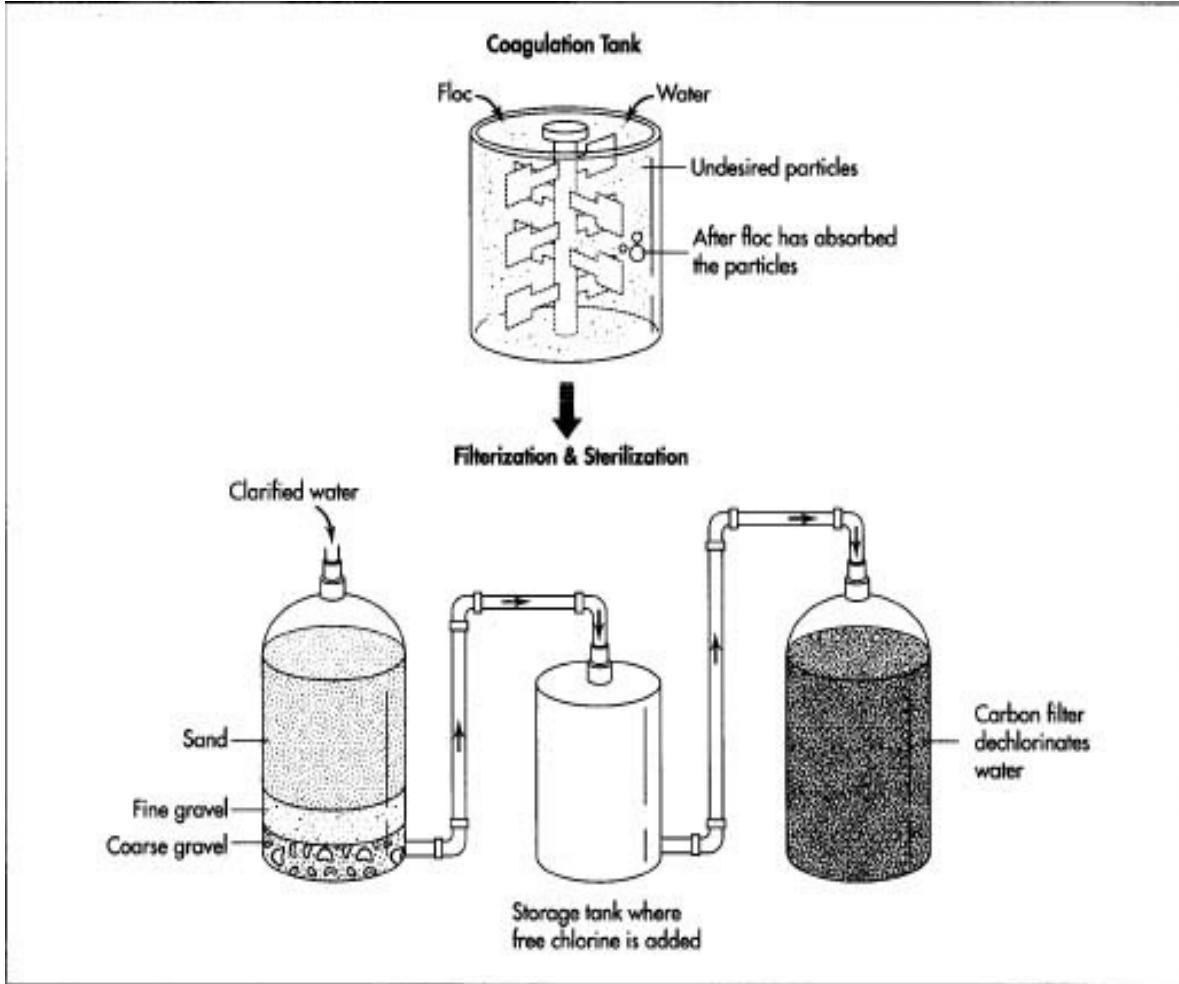
Flocculation of dilute colloidal suspensions provides only infrequent collisions and agglomeration does not occur significantly. Clarification can be achieved in such cases using a chemical coagulant followed by flocculation and sedimentation.

The coagulant is a metal salt which reacts with alkalinity in the water to produce an insoluble metal hydroxide floc which incorporates the colloidal particles. This fine precipitate is then flocculated to produce settle able solids. The coagulant is usually required in doses of 30-100mg/l.

The commonly used coagulant for water treatment is aluminium sulphate which is commercially called as alum. Complex reactions take place when alum is added to water.



For satisfactory coagulation and to leave a suitable residual in the treated water sufficient alkalinity must be available to react with alum. The solubility of  $\text{Al}(\text{OH})_3$  is pH dependent and is low between 5-7.5. Outside this range coagulation with aluminium salts is not successful. Other coagulants include ferrous sulphate commonly known as copperas  $[\text{FeSO}_4 \cdot 7\text{H}_2\text{O}]$ , ferric sulphate  $[\text{Fe}(\text{SO}_4)_3]$  and ferric chloride  $[\text{FeCl}_3]$ . When copperas is treated with chlorine it gives a mixture of ferric sulphate known as chlorinated copperas. Ferric salts give satisfactory coagulation at pH above 4.5 and ferrous salts are suitable above 9.5. Iron salts are popular because of the toxic effect of aluminium in water and are cheaper than alum. Precipitation must be complete when using iron salts otherwise residual iron in water will be troublesome.



**Fig 4 COAGULATION TANK**

Coagulant has to be dispersed throughout the body of water before flocculation occurs. A rapid mixing chamber with a high speed turbine can be used. Floc formation will be difficult if the concentration of colloidal matter is very low. This can be overcome by the use of coagulant aids. These are simple additives like clay particles which form nuclei for precipitation of the hydroxide or polyelectrolyte. Heavy long chain synthetic polymers can also be used as coagulant aid which when added in small amounts ( $<1\text{mg/l}$ ) promote agglomeration.

Alternative way to conventional coagulation process is suspension of finely divided magnetite used to adsorb colour, turbidity, iron and aluminium from water. This reaction takes place in presence of pH control reagents and a coagulant aid. Then the flow is allowed to pass through a strong magnetic field which causes agglomeration of magnetic particles. Finally these agglomerates are removed in an upward flow settling tank. The separated magnetic sludge can be reused after desorption of the contaminants.

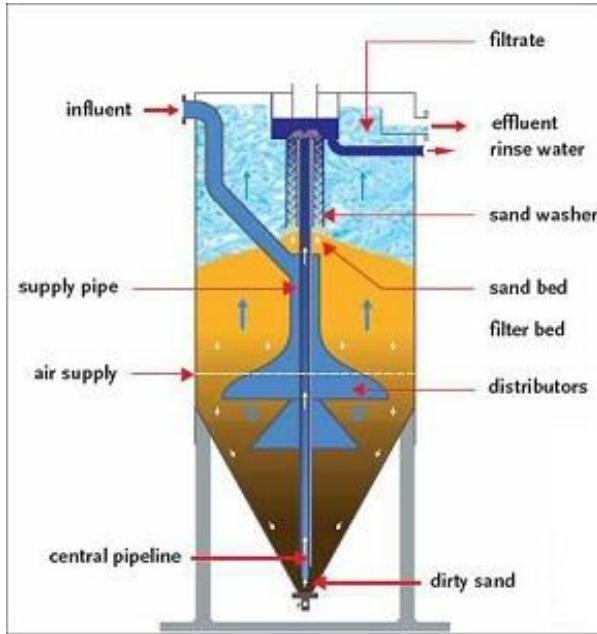
## 5.2.7. FILTRATION

Filtration is a process where water is purified by passing through porous material. Removal of solids depends upon mechanisms such as gravity, diffusion, sedimentation and hydrodynamic processes. A bed of fine sand can act as medium for filtration and called as sand filters. These are highly efficient and remove 99% of microorganisms from raw water. Two types of sand filter viz., rapid sand filters and slow sand filters.

### 5.2.7.1. RAPID SAND FILTERS

Rapid filters are usually built open with the water passing down the filter but by gravity. Filtration rate is  $5-15\text{m}^3/\text{m}^2/\text{hr}$ . tank is made of either steel or concrete with depth of 3-3.5m. Coarse sand of size 0.4-1.5mm used as filter medium, thickness of bed is 0.45-0.75m thick supported by a coarse gravel of 0.60m thickness. There is a inlet through which water to be filtered enters. Inlet valve controls the inward flow of raw water. In the lowest layer of gravel, there is a collector system of perforated pipes connected to main collecting channel. Outlet valve controls the outward flow of filtered water.

The removal of impurities from raw water in rapid filtration is affected by a combination of different processes like straining, sedimentation, adsorption and bacterial and biochemical processes. Adsorption of impurities having an electric charge, onto the filter bed grains with an opposite charge is the only effective process taking place in rapid sand filtration. The filter bed material itself has a neutral static charge; in addition, high flow rate of water produces electro kinetic charges.



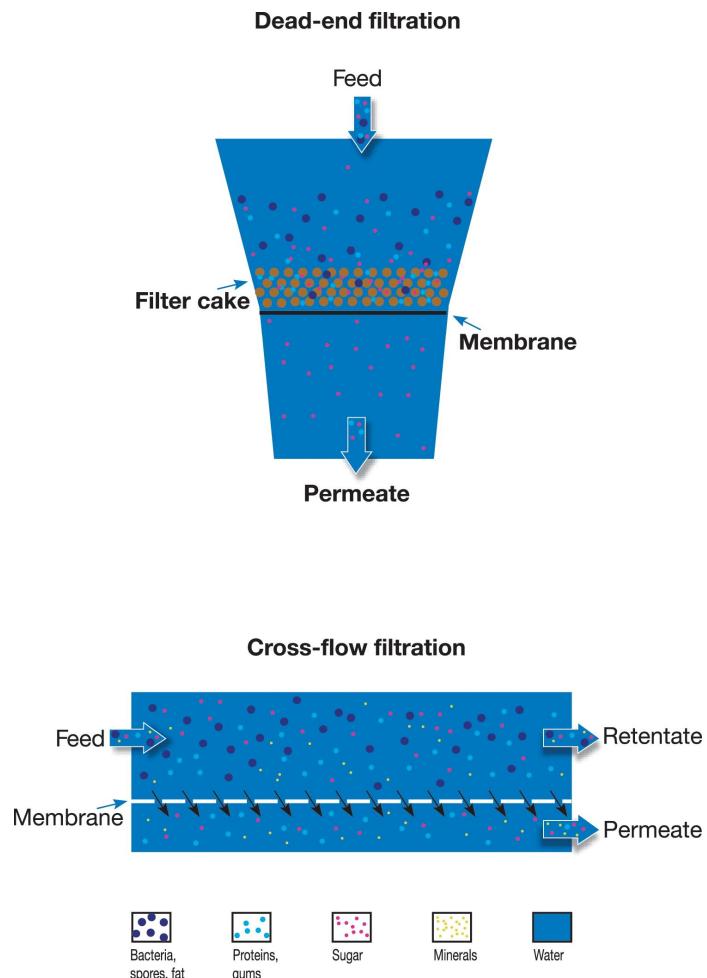
**Fig 5 SAND FILTER**

Types of rapid filter based on the operating conditions viz., pressure filters, upflow filters, multiple media filters.

**5.2.7.1.1. PRESSURE FILTERS:** filter bed and filter bottom are enclosed in a water tight steel pressure vessel. High water pressure applied on the filter bed brings out the filtration process. Draw backs are installation; operation and maintenance are not easy.

**5.2.7.1.2. UPFLOW FILTERS:** employs a coarse to fine filtration process. Sand alone is used as single filter medium. Bottom layer is made of coarse sand which filters out most impurities. Upper layer contains fine sand and filters out the remaining impurities. Used generally for the pretreatment of water which is further treated by gravity type rapid sand filters or slow sand filters.

**5.2.7.1.3. MULTIPLE-MEDIA FILTERS:** these are down flow filters. Filter bed composed of several different materials which are placed coarse-to-fine in the direction of flow. Dual media filter, a kind of multiple media filters comprises of sand layer of about 0.3-0.5m thickness with an effective size of 0.4-0.7mm as bottom layer and topped by anthracite or crushed coconut husks of 0.5-0.7m thickness with an effective size of 1-1.6mm. These are effective as final treatment filters.



**Fig 6 FILTRATION PRINCIPLE AND PROCESS**

### **5.2.7.2. SLOW SAND FILTRATION**

A typical filter consists of 3m deep tank which is open at the top. Area varies with amount of water to be treated and is from ten to several hundred square meters. An under drain system called filter bottom is placed at the bottom of the tank. In addition the ungraded fine sand of 0.3-1.6mm size which is free from clay or loam. The filter bed has a total thickness of about 1-1.2m above which the water to be treated stands to a height of about 1-1.5m. The slow sand filter has numerous inlet and outlets.

In this type, removal of impurities is brought by a combination of different processes such as straining, sedimentation, adsorption and biochemical and microbiological processes. As filtration proceeds day after day a slimy, gelatinous film accumulates around each grain of sand and in the interstices. This slimy layer is composed of millions of bacteria, protozoa, filamentous algae and other microorganisms and is called as Schmutzdecke. This layer is usually formed in the upper three or four inches of sand. This slowly closes up pores between sand grains thus making filtration more effective.

Charged sand particles adsorb particles of opposite charge by electrostatic attraction. Clean quartz sand has a negative charge and binds positively particles such as flocs of carbonates, aluminum hydroxide and cations of iron and manganese. They cannot bind negatively charged bacteria, organic colloidal particles and other anions. However excessive binding of positively charged particles leads to a reversal of the charge thus negatively charged particles adsorb to the positive charge bed.

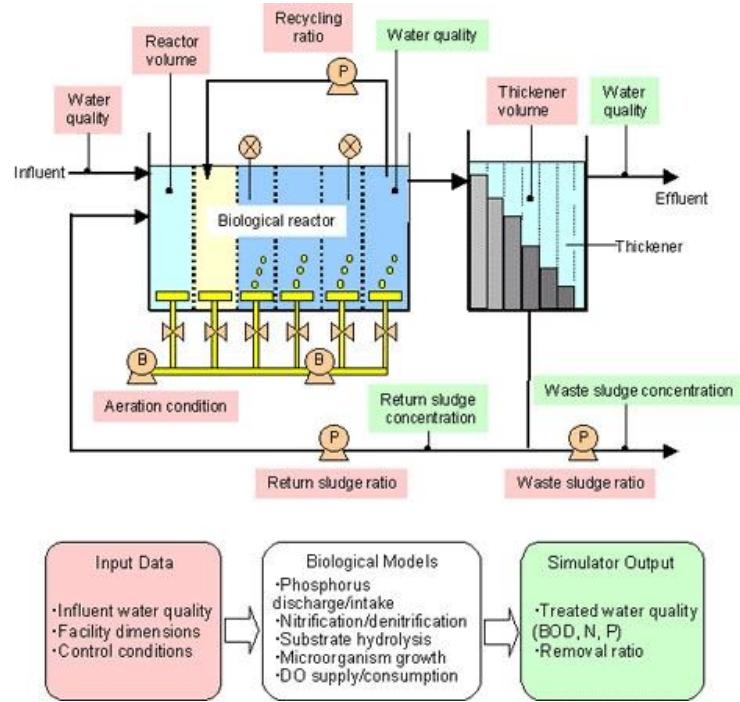
### **5.2.8. AERATION**

Aeration is a water treatment process in which water is brought into intimate contact with air. This is to increase the oxygen content, reduce the carbon dioxide content and to remove methane,  $H_2S$  and other volatile organic compounds responsible for taste and odour. Aeration is used to treat water containing high iron and manganese. The oxygen converts the dissolved ferrous and manganous compounds into insoluble ferric and manganic hydroxides. These are further removed by sedimentation and filtration. Formation of precipitates is difficult if organic content is high in the water. Two methods are available viz., waterfall and bubble aerators.

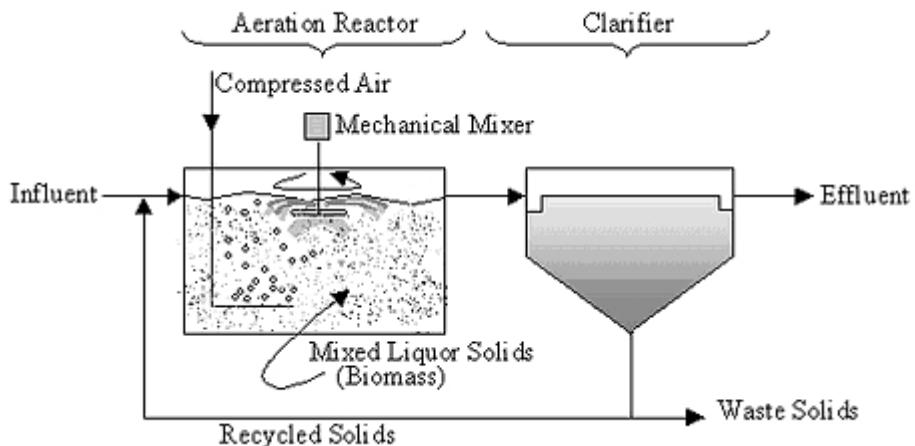
In waterfall aerators, water is dispersed through the air in thin sheets or fine droplets. Simplest and inexpensive type is multiple tray aerators. Consists of about 4-8 trays with perforated bottom which are arranged at intervals of 30-50cm. Water is evenly sprayed over the upper tray through perforated pipes. When the water trickles down, the droplets are dispersed and re-collected at each tray. Trays are made of asbestos. A layer of coke added to promote precipitation of iron from the water by acting as catalyst. In spray aerators a distribution grid is connected with stationary nozzles which spray water into the surrounding air.

In bubble aerators, water is mixed with dispersed air. Simplest type is venture aerator. Aerator is kept higher than the pipe carrying the raw water. In the throat region the velocity of flow is so high that the water pressure is less than the atmospheric pressure. As a result air is sucked into the water. After the throat region water flows through a wider pipe. In this region the

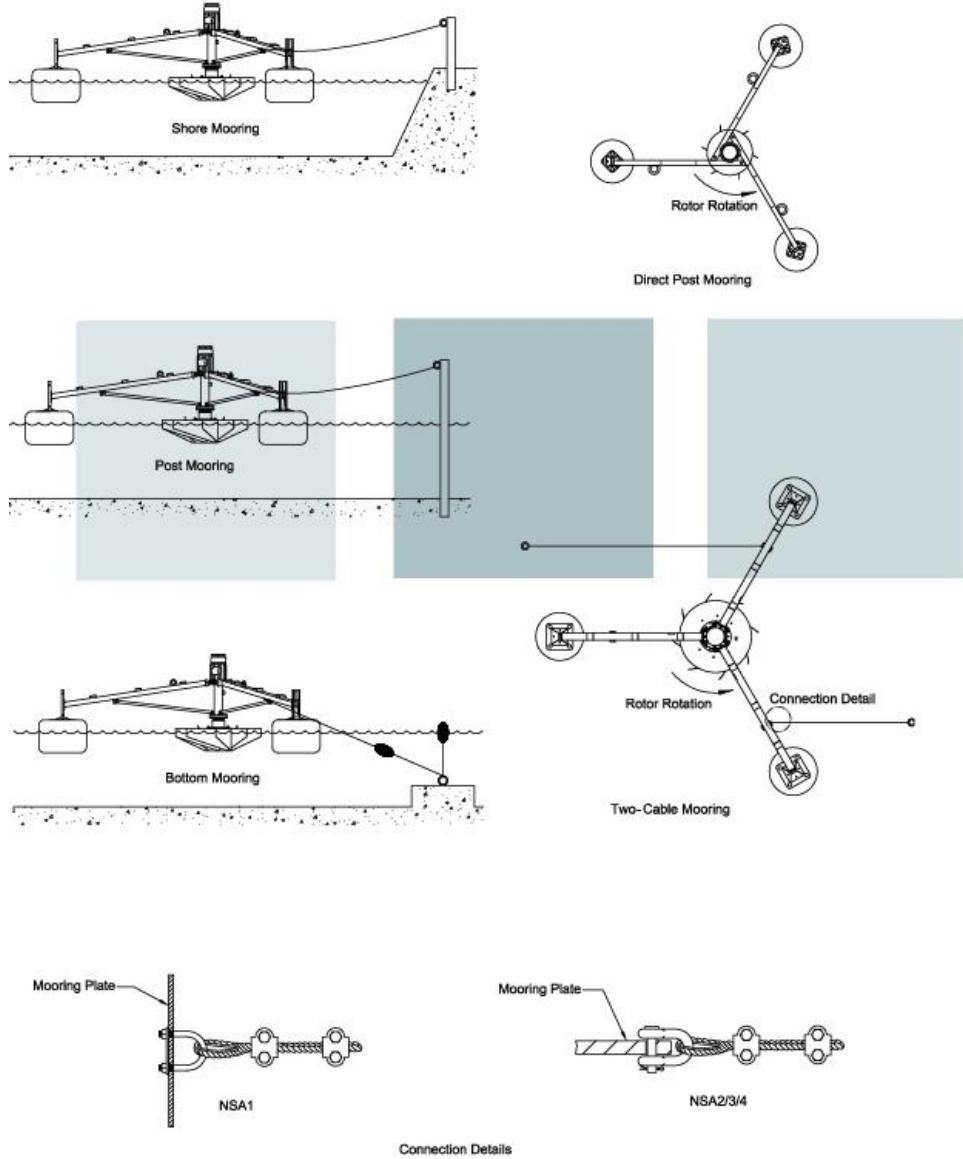
velocity of flow decreases and the water pressure increases. The air bubbles are mixed intimately with water and oxygen is absorbed into the water.



**Fig 7 DIGRAMMATIC REPRESENTATION OF AERATION PROCESS IN BIOREACTOR**



**Fig 8 AERATION REACTOR**



**Fig 9 TYPES OF AERATORS**

### 5.2.9. DISINFECTION

It is the final process to which water is subjected prior to distribution. Two types available are chemical and physical disinfection.

#### 5.2.9.1. CHEMICAL DISINFECTION

Various chemical agents are used which includes chlorine, chlorinated lime, oxidants like ozone and potassium permanganate and halogens.

**5.2.9.1.1. CHLORINE:** chlorine and its compounds are common chemical disinfectants. They are less harmful and more effective comparative to other agents. Chlorination process is of two types namely pre-chlorination and post-chlorination. In the former method chlorine is applied prior to any other treatment, usually for controlling algae, taste and odour. In the later method chlorine is applied after other treatment processes, especially after filtration. The chlorine dosage must be sufficient to leave a residual of 0.2 – 2.0mg/l free chlorine in water is diethyl para phenylene diamine method and ortho toluidine method.

**5.2.9.1.2. CHLORINATED LIME:** it is commonly known as bleaching powder. Before the discovery of liquid chlorine, chlorinated lime was widely used for chlorination. It is loose combination of slaked lime and chlorine gas. When added to water it decomposes to give hypochlorous acid. Chlorinated lime is unstable and on exposure to sun, light and moisture reduces the chlorine content rapidly.

**5.2.9.1.3. OZONE:** it is powerful oxidizing agent and highly unstable. It must be manufactured on site by passing dry air through a high voltage high frequency electrical discharge. It has more rapid effect than chlorine in destroying viruses and bacteria including spores. It is also effective in eliminating compounds that give objectionable taste and colour to water. The treatment with ozone should leave 1-2mg/l residual ozone. But it usually leaves very low level of residuals and thus there is no protection against new contamination of the water after disinfection. The high installation and operation costs further reduce its use as disinfectant.

**5.2.9.1.4. POTASSIUM PERMANGANATE:** it is also powerful oxidizing agent. It has been found to be effective against cholera pathogen but not other pathogens. It is not satisfactory disinfectant as it leaves stain in vessels.

**5.2.9.1.5. HALOGENS:** bromine is used in swimming pools. Fluorine and iodine also used as disinfectants sometimes.

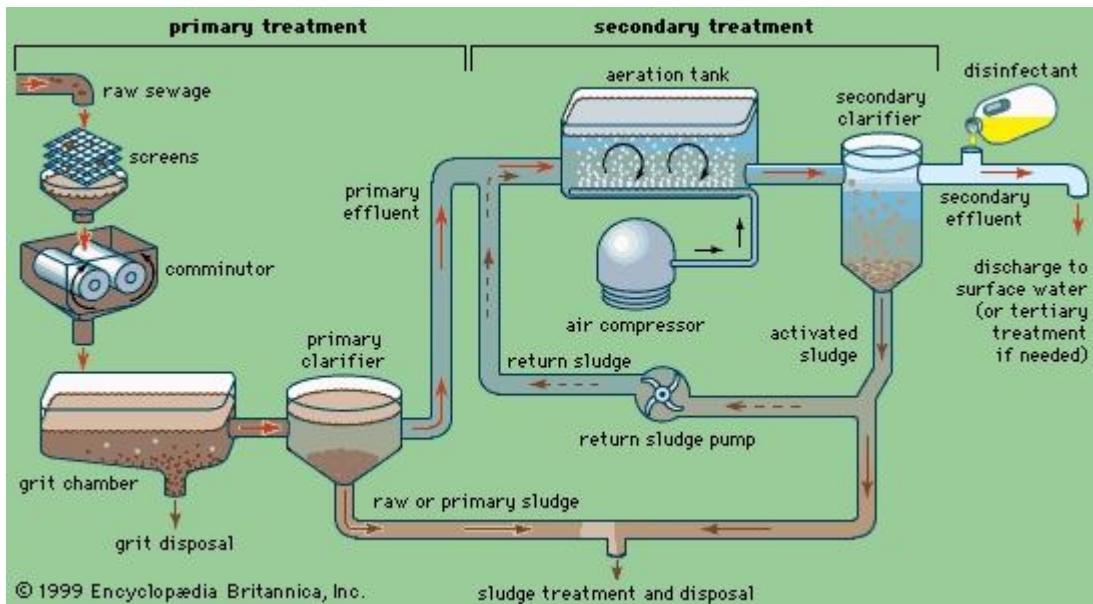
## 5.2.9.2. PHYSICAL DISINFECTION

Two important methods are ultraviolet radiation and boiling of water.

**5.2.9.2.1. UV RADIATION:** Electromagnetic radiation of UV range can be used to destroy microorganisms. This process is effective in certain small water supplies where the water is highly polished i.e., filtered and dematerialized. The process is also used in industries. Eg., breweries, pharmaceuticals, fish hatcheries and aquariums. Actually irradiation must strike the organism to kill it. In this process some of the radiation energy is absorbed by the organism and other constituents in the medium surrounding the organisms. So if sufficient dosages of UV reach the organism water can be disinfected. The germicidal effect of UV energy is thought to be associated with its absorption by certain organic components essential for the functioning of cells. Dissipation of energy by excitation causes disruption of unsaturated bonds, particularly of the purines and pyrimidines, thus leads to lethal biochemical changes.

UV treatment does not alter the water chemically. Only energy is added, which produces heat, resulting in a temperature rise in the treated water. UV rays can penetrate the cell walls of

microorganism. The germicidal efficiency of UV rays is maximum at the wavelength of 250-260nm. There is an abrupt decrease in the efficiency at 290-300nm and continues upto visible range.



**Fig 10 PRIMARY AND SECONDARY TREATMENT PLANT**

### 5.3. SECONDARY TREATMENT

Secondary treatment involves the aerobic oxidation of the effluent waters from the preliminary treatment processes and anaerobic digestion of sludge from both primary and secondary treatment processes.

#### 5.3.1. AEROBIC PROCESSES

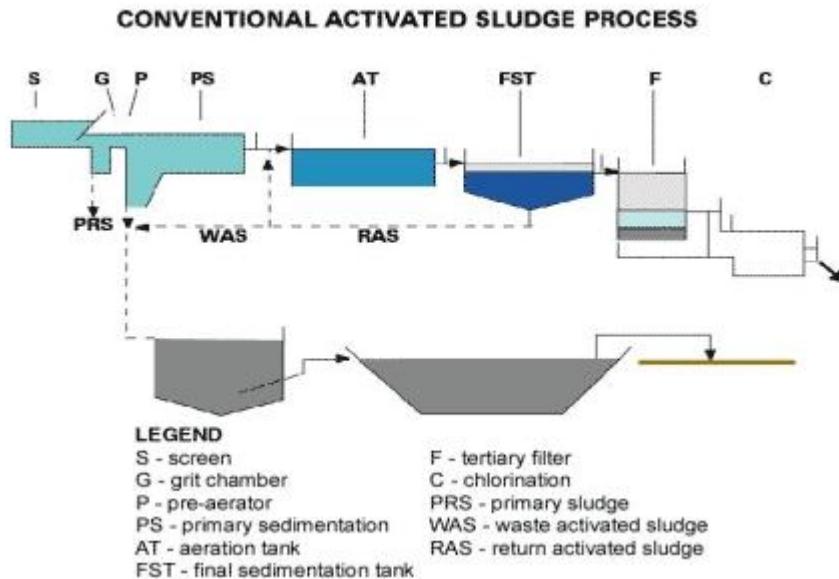
Effluent waters from the preliminary treatment processes still have a high content of heterogenous organic matters. The availability of dissolved oxygen in various water bodies can not support the self-purification taking place in them when the effluent is disposed off into them. Hence, the effluent waters are aerobically treated in oxidation tanks which provide a large population of microorganisms in the form of a slime or sludge organic matters from the effluent, when adsorbed onto the microbial surfaces oxidized to aerobic end products. Since these oxidation processes are carried out by microorganisms they are usually called as biological oxidations. There are four different types of aerobic oxidation processes.

##### 5.3.1.1. ACTIVATED SLUDGE PROCESS

Activated sludge process is a highly efficient system for the aerobic biological treatment of industrial or municipal wastes. The process depends on the use of a high concentration of microorganisms in the form of floc which is kept in suspension by agitation. Agitation is provided either by mechanical means or by aeration. In this process, a portion (about 20%) of the

separated sludge along with the native population of living microorganisms is added to the incoming effluent as inoculum. This added sludge is often referred to as activated sludge and carries out the actual oxidation. Thus a constant microbial population is maintained in the activated sludge tank.

The activated sludge tank is simple in design. It is an oblong deep tank, provided with an inlet at the top of one end and an outlet at the bottom of the other end. Aeration is provided either by an air diffuser located at the bottom of the tank or by agitators at the surface of waters along both sides of the tank.



**Fig 11 CONVENTIONAL ACTIVATED SLUDGE PROCESS**

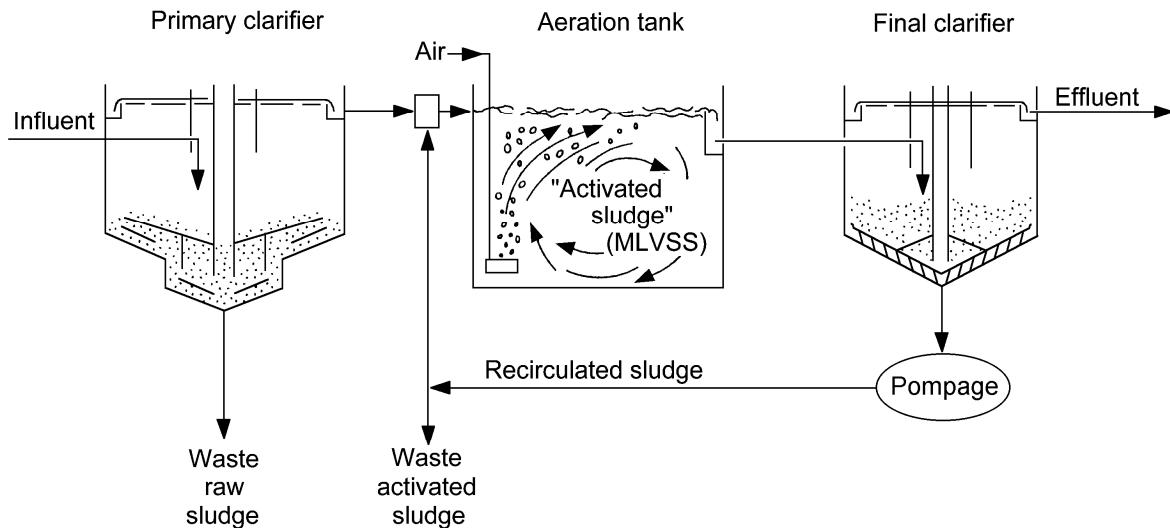
### 5.3.1.1.1. MICROORGANISMS INVOLVED

Various microorganisms are involved in the activated sludge process. The most important one is the rod shaped bacterium, *Zooglea ramigera*, which produces a copious extracellular slime matrix. This organism is the main agent for flocculation. Among other bacterial forms the major genera are *Pseudomonas*, *Nitrosomonas*, *Flavobacterium*, *Alcaligenes*, *Brevibacterium*, *Bacillus*, *Beggiatoa*, *Achromobacter*, *Corynebacterium* and *Sphaerotilus*. Other minor genera include *Aeromonas*, *Aerobacter*, *Micrococcus*, *Spirillum*, *Acinetobacter*, *Glucanobacter*, *Cytophaga* and *Hyphomicrobium*.

Among fungi, *Zoophagus*, *Arthrobotrys*, *Geotrichium*, *Pullularia*, *Alternaria*, *Penicillium* and *Cephalosporium* have been noted. In protozoa the dominant ciliates are *Opercularia*, *Vorticella*, *Aspidisca*, *Carchesium* and *Chilodonella*. In addition amoebae and flagellates are also seen. Yeasts and algae are of rare occurrence.

### 5.3.1.1.2. PROCESS DESCRIPTION

During the process, the effluent water from the primary sedimentation tank is mixed with a portion of the activated sludge from the final settling tank. The mixture of biomass and waste water is termed the mixed liquor and the concentration of biomass in the tank is usually expressed as the mixed liquor suspended solids (MLSS). The contents are thoroughly mixed and aerated by the agitators. Thus mixed liquor is maintained in suspension by the turbulence created by agitators. In the absence of agitation, the solids quickly settle to the bottom and lose contact with the organic matter in the liquid stage.



**Fig 12 PROCESS DESCRIPTION OF ACTIVATED SLUDGE**

Moreover, the settled solids, if not returned to the aeration zone, will become anaerobic. Sufficient air must be transferred to the mixed liquor to maintain dissolved oxygen of 1-2mg per litre. After a period of 4-8 hours the contents are transferred to a final settling tank. In this final settling tank the microbial biomass along with other suspended solids sediment as sludge. This sludge is usually referred to as secondary sludge, to differentiate it from the one in the primary treatment. The supernatant is discharged as effluent.

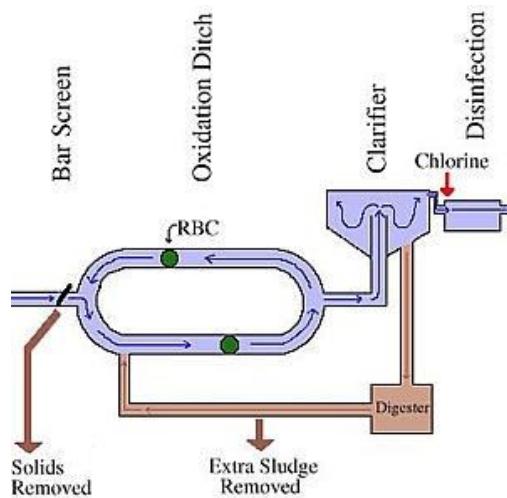
In activated sludge process the biomass exists in the form of freely suspended flocs in which the cells are embedded in a gelatinous matrix. Thus the basic unit of activity in activated sludge process is the floc, which in turn is dependent for its existence on the metabolism, growth and physical properties of the microbial cells present. The successful operation of the process is dependent on four major characteristics of the biomass. They are the ability of the floc to absorb substrates, assimilation and oxidation of organic matter, oxidation of nitrogen and the maintenance of good flocculation to permit efficient sedimentation of the secondary sludge.

### 5.3.1.1.3. ADVANTAGES AND DISADVANTAGES

The main advantage of activated sludge process is that it requires less space than the biological filter. The second advantage is that a final effluent of high quality is produced such that it does not require high dilution for disposal.

There are two disadvantages. Since the incoming effluent is introduced at one end of the tank, the BOD value will be higher at this end than the other. Moreover, the microorganisms at this end will be physiologically more active than those at the other end. These defects are rectified in the complete mixing activated sludge process.

### 5.3.1.2. OXIDATION DITCHES



**Fig 13 OXIDATION DITCH**

Oxidation ditches can be used to treat wastewater. After pretreatment, water flows through the oxidation ditches, where RBC's and microorganisms in returned activated sludge break down the B.O.D. Then the sludge is removed in a clarifier and the remaining water is disinfected. This process differs from that of a packaged plant by a longer retention time and by more types of microorganisms digesting the B.O.D.

Greater ammonia removal can be achieved by having two oxidation ditches, each at a different pH. Diversion basins can be used to prevent washouts during periods of heavy rainfall.

Oxidation ditches have the advantage of much more efficient sludge removal. But they are costly, not only in monetary terms, but also in terms of pollution to the environment.

Treatment of wastewater using an oxidation ditch is relatively similar to wastewater treatment in a packaged plant. But the oxidation ditch replaces the aeration basin and provides better sludge treatment.

The only pretreatment typically used in an oxidation ditch system is the bar screen. After passing through the bar screen, wastewater flows directly into the oxidation ditch.

The oxidation ditch is a circular basin through which the wastewater flows. Activated sludge is added to the oxidation ditch so that the microorganisms will digest the B.O.D. in the water. This mixture of raw wastewater and returned sludge is known as mixed liquor.

Oxygen is added to the mixed liquor in the oxidation ditch using rotating biological contactors (RBC's.) RBC's are more efficient than the aerators used in packaged plants. In addition to increasing the water's dissolved oxygen, RBC's also increase surface area and create waves and movement within the ditches.

Once the B.O.D. has been removed from the wastewater, the mixed liquor flows out of the oxidation ditch. Sludge is removed in the clarifier. This sludge is pumped to an aerobic digester where the sludge is thickened with the help of aerator pumps. This method greatly reduces the amount of sludge produced. Some of the sludge is returned to the oxidation ditch while the rest of the sludge is sent to waste.

#### **5.3.1.2.1. COMPARISON TO A PACKAGED PLANT**

The treatment of wastewater in an oxidation ditch is similar to treatment in a packaged plant. The two main differences between the processes are the retention time and the type of organisms which digest the wastewater. Retention time is much longer in an oxidation ditch. A packaged plant usually has a retention time of two to four hours while an oxidation ditch retains the wastewater for two days. Since the D.O. is higher in the oxidation ditch than in a packaged plant, a greater variety of microorganisms live in the oxidation ditch. In contrast, packaged plants usually depend upon only a few types of microorganisms to eat the sewage.

#### **5.3.1.2.2. VARIATIONS FROM THE TYPICAL PROCESS**

##### **5.3.1.2.2.1. AMMONIA REMOVAL**

Oxidation ditches can be set up to remove ammonia very effectively. Wastewater can be sent through two sets of ditches, each of which has a different pH. The different pH in the two ditches creates a niche for certain microorganisms. These microorganisms are very efficient at removing B.O.D and converting ammonia to nitrates. Oxidation ditches are much more efficient at ammonia removal than packaged plants are. As a result, most new treatment facilities are designed as oxidation ditches.

##### **5.3.1.2.2.2. DIVERSION BASINS AND WASHOUTS**

Some oxidation ditches, like the one located in Big Stone Gap, Virginia, have a diversion basin to hold the influent when flows increase because of excessive rainfall. The diversion basin holds the excess influent and allows more time for treatment.

Without a diversion basin, heavy rains could cause a washout to occur. A washout occurs when a large influx of influent rushes into the oxidation ditches. The ditches are unable to contain the extra water, so microorganisms, sludge, and wastewater are pushed through the plant and out into a river or stream before being properly treated.

Without diversion basins, total washouts can be prevented by shutting off the inner ditches and allowing the outside ditch to circulate the influent, providing primary treatment to the water before it is released. In periods of excessive rainfall, oxidation plants can be operated on high flow settings for a month at a time.

### **5.3.1.2.3. ADVANTAGES AND DISADVANTAGES**

The greatest advantage of an oxidation ditch is the efficiency of sludge removal. In an oxidation ditch, only about 15% of the original B.O.D. ends up as sludge, compared to a packaged plant where about 60% of the B.O.D. becomes sludge.

However, oxidation ditches are expensive to maintain. The monetary cost is very high per ton of B.O.D. removed. In some cases, the cost may reach nearly 350 dollars per ton.

Oxidation ditches have an additional environmental drawback. The water is moved through the ditches using rotors, and these rotors in turn use electricity. The electricity used to operate the plant causes sulphur dioxide and other contaminants to be released into the atmosphere from coal-burning electrical plants.

Oxidation ditches provide the most thorough process for treating sewage, but oxidation ditches are also one of the most costly forms of treatment.

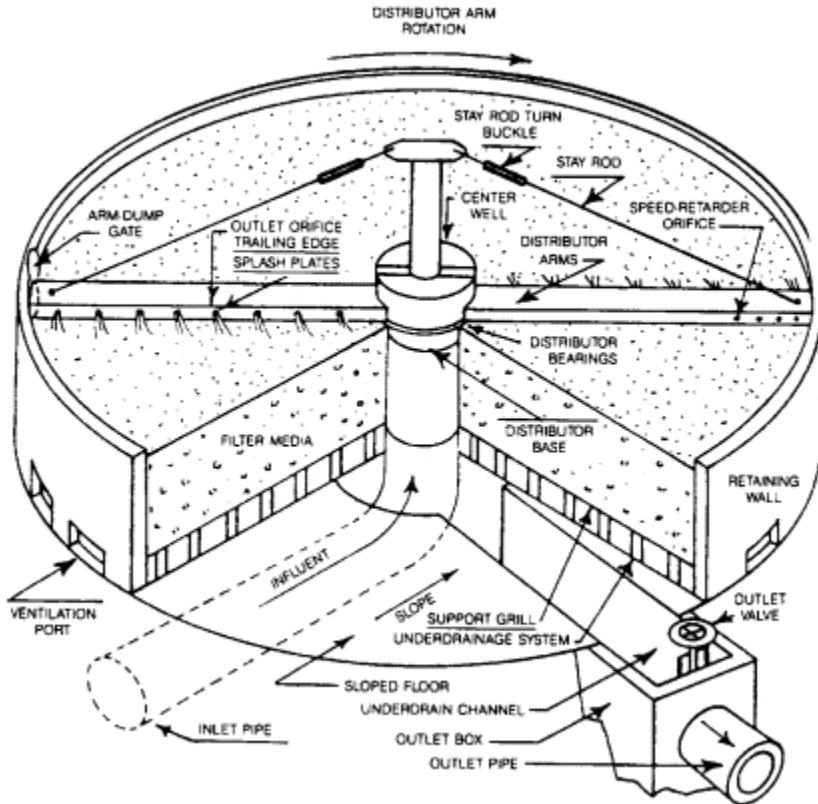
### **5.3.1.3. TRICKLING FILTERS**

Trickling filter is an oldest form of aerobic oxidation treatment. It is called as bacteria bed or percolating filters. It consists of a circular or rectangular bed of stone, gravel or synthetic material with a particle size of 50-10cm. the filter bed is usually 1.5-2m depths and is supported by a layer of large stones over the under drains. There is a rotating arm above the surface of filter bed with nozzles. The rotating arm sprays the settled sewage over the filter bed either continuously or intermittently. The spraying saturates the liquid with oxygen.

With the interstices of the medium the microorganisms grow in the form of a shine or filth. The film is often known as zoogloal film and consists of bacteria, fungi, protozoa and algae. A newly constructed bed must acquire the zoogloal film before it can function efficiently.

Effluent sprayed over the bed slowly trickles through the interstices in the medium which is coated with the zoogloal film. Actually the liquid flows over the film rather than through it. When the liquid flows over the microbial film the organic materials bind to it and are oxidized. Unlike the microorganisms in the activated sludge, which move along with the sewage, microorganisms in the trickling filter are stationary. Portions of the film may be sloughed off by the shear forces created by the flow of waste water. The treated effluent containing the sloughed

off biomass leaves the bed via the under drain into a sedimentation tank. This tank is known as humus tank and the sludge sedimented at the bottom of the tank is called humus sludge.



**Fig 14 INNER VIEW OF TRICKLING FILTER**

The highest rate of oxidation takes place in the upper regions of the bed where oxygen is supplied by natural ventilation. Below this level the rate of oxidation decreases due to the decreasing concentration of organic matter in the liquid phase. Trickle filters are mostly used for the treatment of industrial wastes, especially those from cannery and food industry.

#### 5.3.1.3.1. MICROORGANISMS INVOLVED

The upper regions of the trickling filter bed, where light is available to support photosynthesis, is dominated by algae of the genera *Phormidium*, *Chlorella* and *Ulothrix*. At times their growth may be so extensive that it impairs the operation of the filter.

As in the case of activated sludge, zoogloal film is developed because of the presence of filamentous bacteria like *Sphaerotilus natans* and *Beggiatoa*. Other bacterial genera which oxidize the organic matter are *Pseudomonas*, *Flavobacterium*, *Achromobacter* and *Alcaligenes*. In the lower level nitrifying organisms like *Nitrobacter* and *Nitrosomonas* predominate.

Many species of fungi and protozoa occur throughout the filter and their number is influenced by the availability of oxygen and nutrients. Fungal genera include *Fusarium*,

*Penicillium, Mucour, Geotrichum, Sporotrichum.* Among protozoa the predominant group is ciliates which includes *Opercularia, Epistylis* and *Verticella*. Other protozoan forms are *Amoeba, Euglena, Persanema, Trepomonas, Paramecium* and *Stenor*.

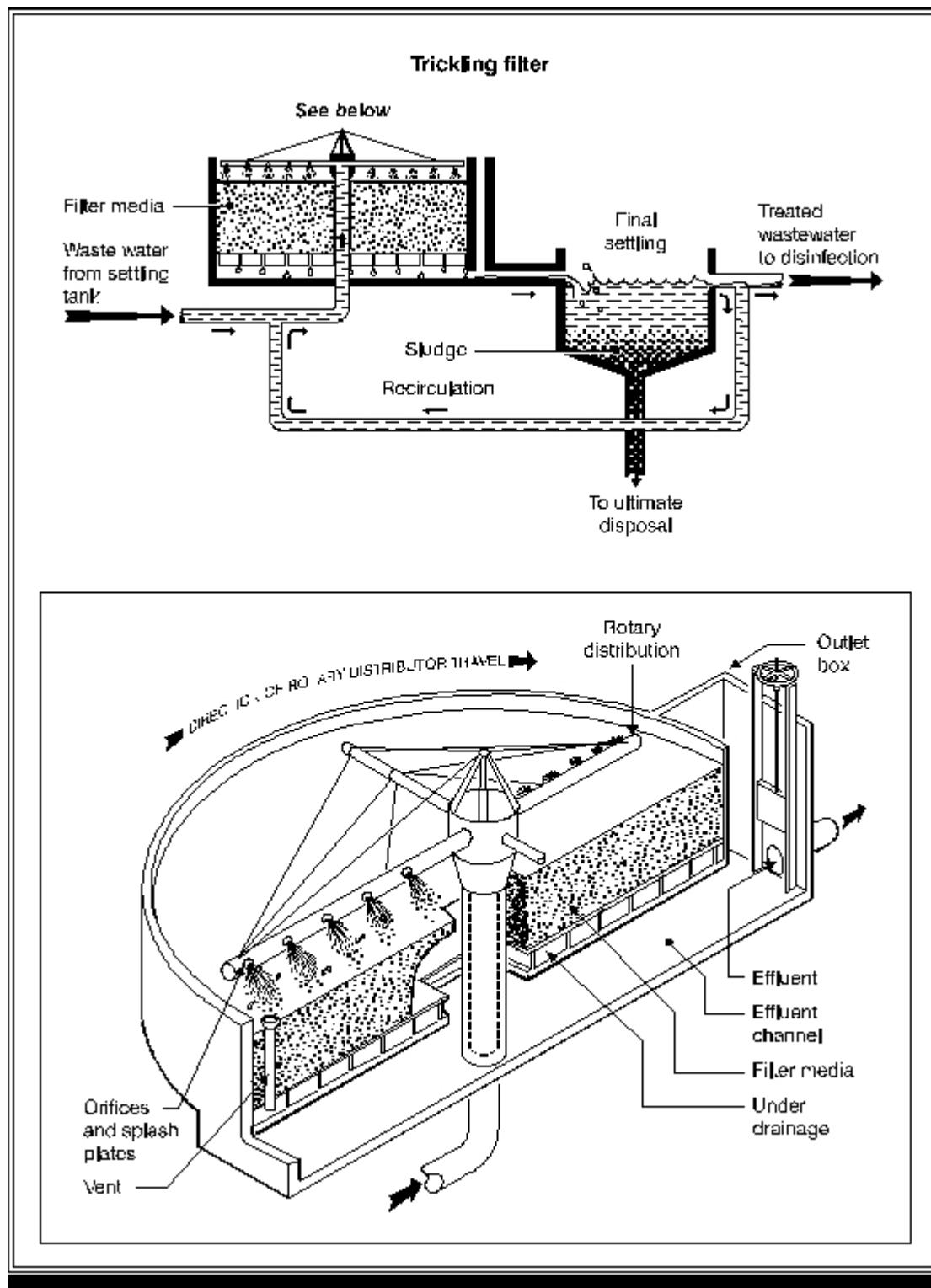
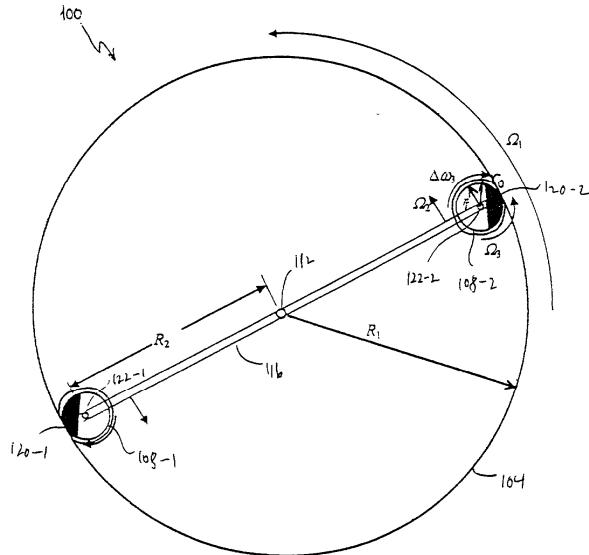
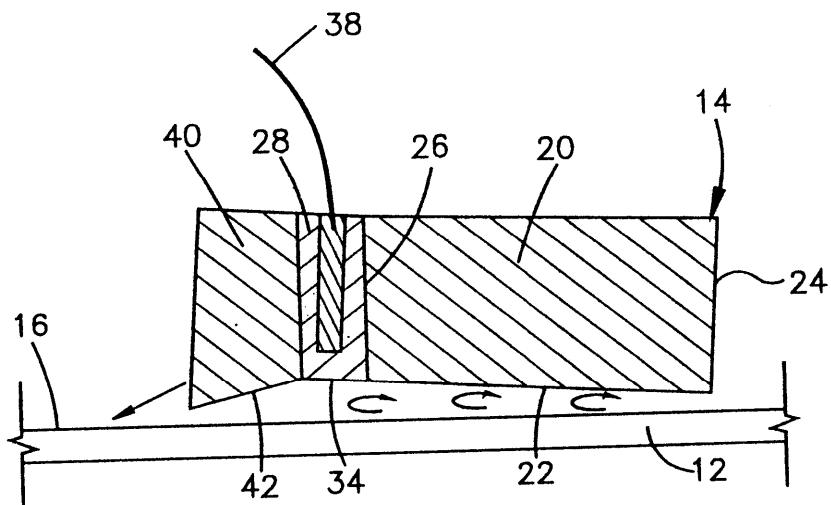


Fig 15 TRICKLING FILTER

### 5.3.1.4. ROTATING DISCS AND DRUMS



**Fig 16 ROTATING DRUM**



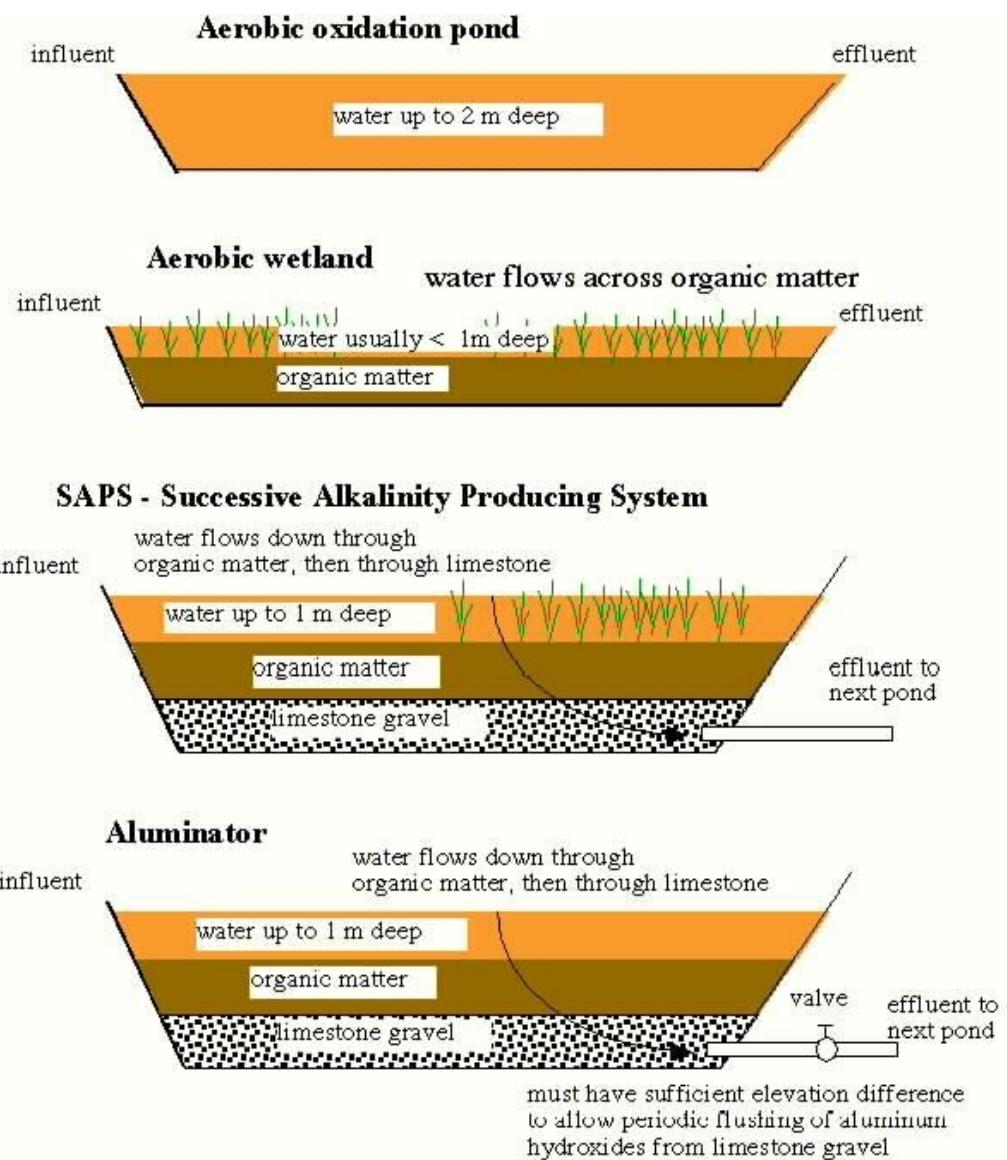
**Fig 17 ROTATING DISC**

Rotating biological contractor (RBC) consists of a number of rotating discs which are partially submerged in a semi circular receiving raw waste water. The discs serve as an excellent support for the biological film which grows at the expense of the organic matter thus bringing about the required stabilization. The discs while rotating alternatively dip into the waste water and are aerated when exposed to the atmosphere.

### 5.3.1.5. OXIDATION PONDS

Oxidation ponds are shallow water bodies in which natural stabilization of raw sewage takes place. This method can be followed where sufficient land area is available. In addition, the process depends upon the climatic condition and warm sunny climate which makes the process efficient. These ponds are also called as lagoons or stabilization ponds. Aerobic, biological oxidation takes place in these ponds.

Based on their activities oxidation ponds can be grouped into four. They are facultative ponds, maturation ponds, anaerobic ponds, aerated ponds.



**Fig 18 OXIDATION POND**

### **5.3.1.5.1. FACULTATIVE PONDS**

Oxidation pond, in which both aerobic and anaerobic activities are carried out in a single unit is called facultative pond. They are the most widely used oxidation ponds. Facultative ponds are mostly rectangular in shape with a length – breadth ratio of 3:1. They are usually 1-2m deep. Shallow areas near the edge of the tank should be avoided to prevent breeding of mosquitoes. Cutting the weeds or grasses and spraying of insecticides can also help. Banks of the ponds may be simply made of earth or in case of large ponds to prevent wave action slabs can be paved. The normal retention time for these ponds is 5-30 days depending upon the temperature.

In facultative ponds, as mentioned, both aerobic and anaerobic activities take place. Near the surface of the ponds chlorophyll bearing microorganisms, phytoflagellates and algae dominate. Representative genera of algae include *Chlorella*, *Chlamydomonas*, *Scenedesmus*, *Euglena* and *Oscillatoria*. These organisms perform photosynthesis utilizing the inorganic materials and carbon dioxide. Carbon dioxide is obtained either from bacterial decomposition of organic matters or from the atmosphere which gives rise to dissolved CO<sub>2</sub>. the oxygen produced by photosynthesis causes an increase in the dissolved oxygen (DO) level which favours aerobic bacteriological activity. Dominant member of the aerobic heterotrophic bacterial population are *Pseudomonas*, *Achromobacter* and *Flavobacterium* DO level may decrease after aerobic activities and it may also reach zero if the pond is overloaded.

In the bottom region, anaerobic condition is maintained and decomposition of detritus materials occurs. This anaerobic activity stabilizes the sludge to some extent and releases some of the organic matter in soluble form which is degraded in the aerobic zone.

Removal of bacteria occurs by natural death and also by settlement, because of the long retention time in the tank. Bacteria and phytoplankton can also be removed by the preying action of ciliates, rotifers and crustaceans. However some bacteria may escape these and are found in the effluent. Considerable removal of viruses also occurs and is enhanced by the light intensity. Sometimes large algal growth may occur in the tanks and may lead to a high suspended solid level in the effluent if not removed.

### **5.3.1.5.2. MATURATION PONDS**

These types of ponds are mostly used during the secondary stage of treatment following a facultative pond or other biological treatment units. They are shallow ponds and can be loaded with only a limited amount of organic material. Heavy algal growth is a problem with this type of pond; nevertheless, they are efficient in removing the pathogenic bacteria.

### **5.3.1.5.3. ANAEROBIC PONDS**

These are comparatively deep ponds with a depth of 3-5 meters to ensure anaerobic conditions. They can be operated with a high organic loading. Retention time for these ponds is about 30 days. They are generally used for the pretreatment of strong organic wastes before adding them to facultative ponds. The main problem with such anaerobic ponds is the production of odor.

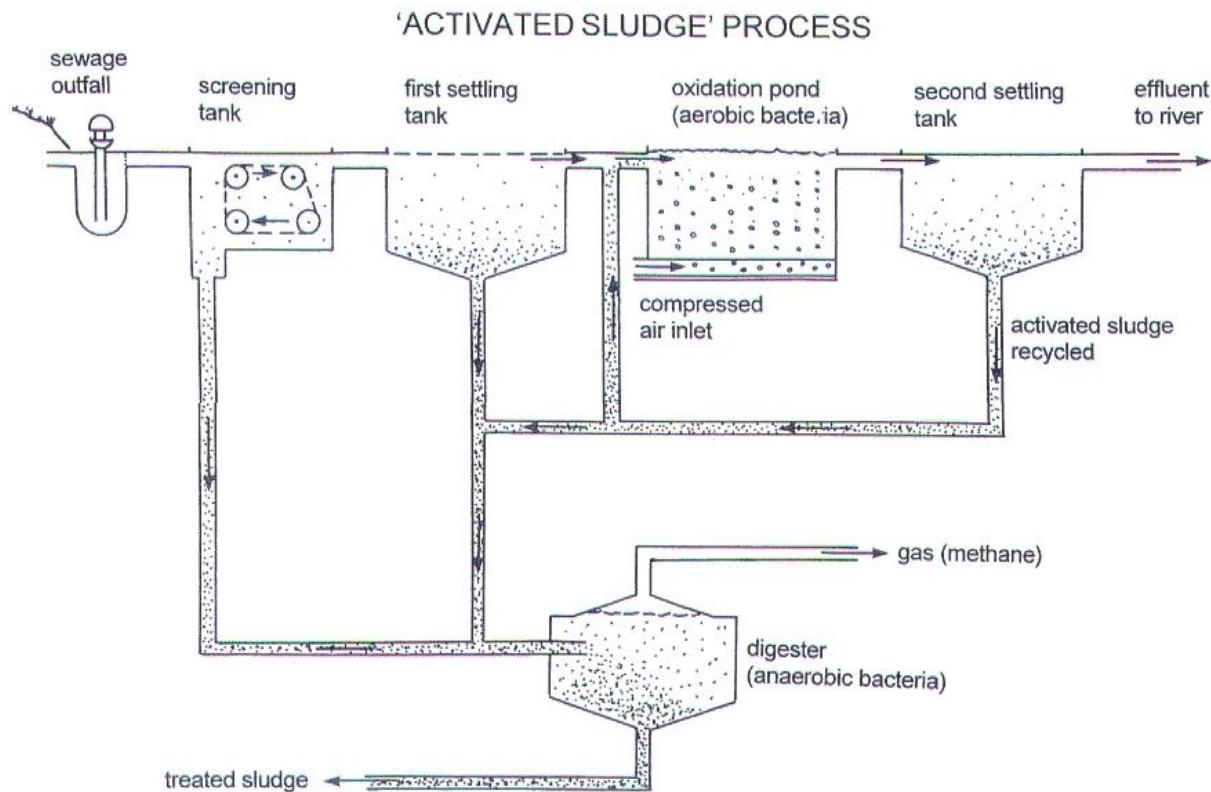
### 5.3.1.5.4. AERATED PONDS

Aerated ponds are less common use when compared to other oxidation ponds. They utilize floating aerators to maintain dissolved oxygen levels and to provide mixing. Retention time for those ponds is a few days. The process involves bacterial floc and produces good quality effluents.

### 5.3.2. ANAEROBIC PROCESSES

#### 5.3.2.1. ANAEROBIC SLUDGE DIGESTION

The solids which accumulate from the sedimentation tanks and the primary and secondary sludge are treated further in anaerobic digestion tank, since they cannot be degraded efficiently through aerobic processes. In cases, where the suspended solid content is very high, the sewage can directly be treated in an anaerobic digestion tank. The anaerobic biological reactions are carried out by microorganisms. The microbial action on the constituents of sludge is termed sludge digestion.



**Fig 19 ANAEROBIC ACTIVATED SLUDGE**

### **5.3.2.1.1. MICROORGANISMS INVOLVED**

Various physiological groups of bacteria occur in anaerobic sludge digestion tank. Although fungi and protozoa have been found, they occur in very low number. The digestion process itself is believed to be carried out by bacteria. There are three different groups of bacteria occurring in this process: hydrolytic, fermentative and methanogenic bacteria. However, there is some overlap between the members of the first two groups. Among the non methanogens *Bacillus*, *Pseudomonas*, *Lactobacillus*, *Spirillum*, *Vibrio*, *Klebsiella*, *Alcaligenes*, *Micrococcus* are the facultative anaerobes and *Clostridium* and *bacteroides* are the obligate anaerobes. Methanogens include *Methanospirillum* and *Methanomicrobium*.

### **5.3.2.1.2. DESCRIPTION OF THE TANK**

Anaerobic sludge digestion tank is a specially designed tank which is maintained free of oxygen. It may be a completely closed tank or may be provided with a lid at the top, floating over the contents of the tank. The contents are introduced into the tank through an inlet near the top. There is an outlet at the bottom to remove the digested sludge. There is also a vent in the lid through which any gas producing during the digestion escapes.

### **5.3.2.1.3. OPERATIONAL PARAMETERS**

The degree of mixing required is much less than in aerobic processes. Sludge digestion is a slow process and frequently requires heating to accelerate the biochemical reactions involved. The majority of the conventional digesters are operated in the mesophilic range, between 25-40°C. a neutral pH is required for the sludge digestion. The retention time for mesophilic and thermophilic digesters is in range of 25-35 days and in some cases 12-15 days.

### **5.3.2.1.4. PROCESS DESCRIPTION**

Anaerobic biochemical reactions take place in the anaerobic sludge digestion tank. Both anaerobic and facultative bacteria degrade the organic solids by converting them into soluble substances and gaseous products. The gases consist mainly of methane (60 -70%) and carbon dioxide (20-30%). In addition hydrogen and nitrogen are also produced during sludge digestion in small amounts. This gas mixture can be used for heating and also for operating power. The supernatant liquid portion, the effluent, can be sent to the aerobic treatment. The digested sludge is disposed off in a proper way.

## **5.3.2.2. UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS**

Upflow Anaerobic Sludge Blanket (UASB) technology, normally referred to as UASB reactor, is a form of anaerobic digester that is used in the treatment of wastewater.

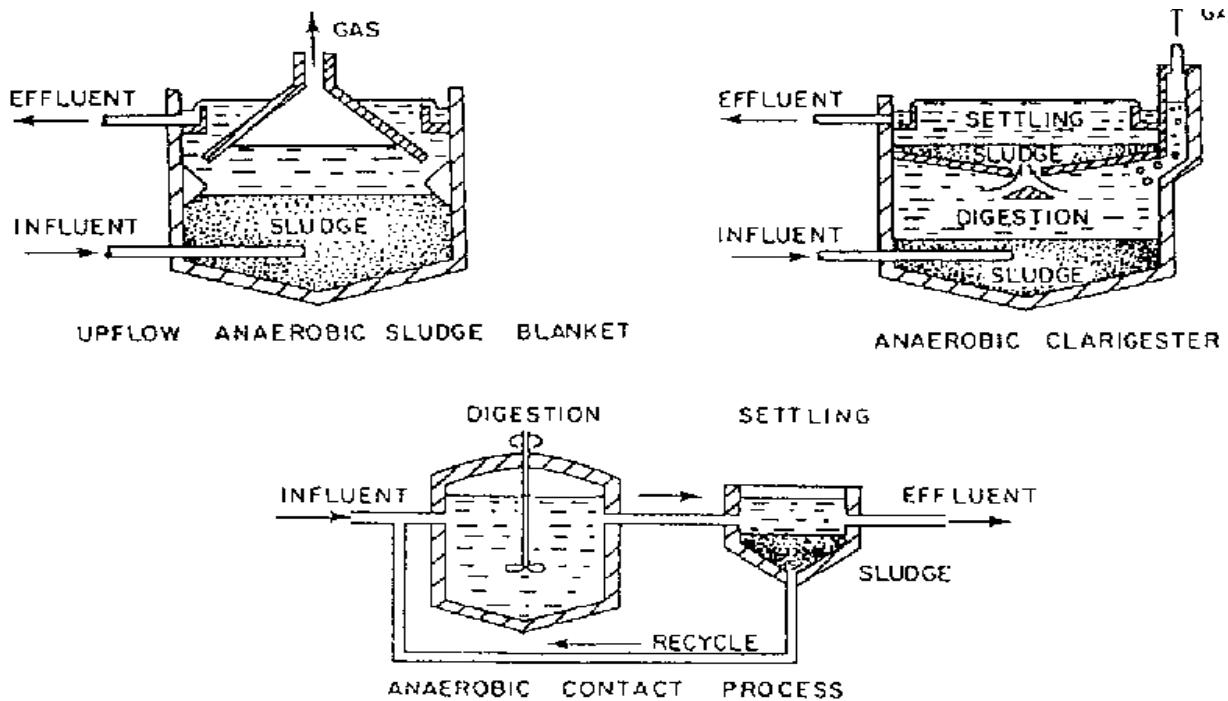
The UASB reactor is a methanogenic (methane-producing) digester that evolved from the anaerobic clarigester. A similar but variant technology to UASB is the expanded granular sludge bed (EGSB) digester. A diagrammatic comparison of different anaerobic digesters can be found here.

UASB uses an anaerobic process whilst forming a blanket of granular sludge and suspended in the tank. Wastewater flows upwards through the blanket and is processed by the anaerobic microorganisms. The upward flow combined with the settling action of gravity suspends the blanket with the aid of flocculants. The blanket begins to reach maturity at around 3 months. Small sludge granules begin to form whose surface area is covered in aggregations of bacteria. In the absence of any support matrix, the flow conditions creates a selective environment in which only those microorganisms, capable of attaching to each other, survive and proliferate. Eventually the aggregates form into dense compact biofilms referred to as "granules". A picture of anaerobic sludge granules can be found here.

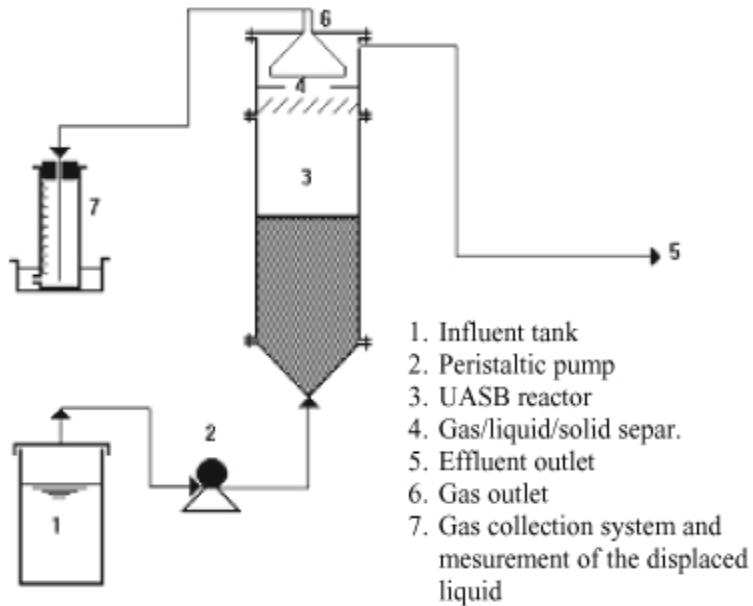
Biogas with a high concentration of methane is produced as a by-product, and this may be captured and used as an energy source, to generate electricity for export and to cover its own running power. The technology needs constant monitoring when put into use to ensure that the sludge blanket is maintained, and not washed out (thereby losing the effect). The heat produced as a by-product of electricity generation can be reused to heat the digestion tanks.

The blanketing of the sludge enables a dual solid and hydraulic (liquid) retention time in the digesters. Solids requiring a high degree of digestion can remain in the reactors for periods up to 90 days. Sugars dissolved in the liquid waste stream can be converted into gas quickly in the liquid phase which can exit the system in less than a day.

UASB reactors are typically suited to dilute waste water streams (3% TSS with particle size >0.75mm).



**Fig 20 UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS**



**Figure 1:** Schematic diagram of the laboratory UASB reaction system.

### Fig 21 SCHEMATIC REPRESENTATION OF UASB

## 5.4. TERTIARY TREATMENT

### 5.4.1. ADSORPTION

Adsorption is the accumulation of molecules from a substance dissolved in a solvent onto the surface of an adsorbant particle. Adsorption techniques are used to remove soluble organic from drinking waters and wastewaters.

Tiny concentrations of both natural and synthetic organic compounds produce serious taste and odour problems in water. Long term exposure to these organics may lead to potential health hazards. Thus it has to be removed. Biological treatment processes are efficient in removing organics but they are inefficient in removing small concentration of organics, as they form the nutrient source for the organisms.

Adsorption is a surface phenomenon and effective adsorbents have a highly porous structure so that their surface area to volume ratio is very high. The solute molecule is held in contact with the adsorbent by a combination of physical, ionic and chemical forces. When the adsorbent is left in contact with a solution the amount of adsorbed solute increases on the surface of the adsorbent and decreases in the solvent. When the number of molecules of solute is equal on the adsorbent and in the solvent it represents the adsorption equilibrium. The rate of adsorption is governed by the rate of diffusion of solute into the capillary pores of the adsorbent particle. The rate decreases with increasing particle size and increase with increasing solute concentration and temperature. Low molecular weight solutes are more easily adsorbed than the high molecular weight solutes.

#### 5.4.1.1. ACTIVATED CARBON

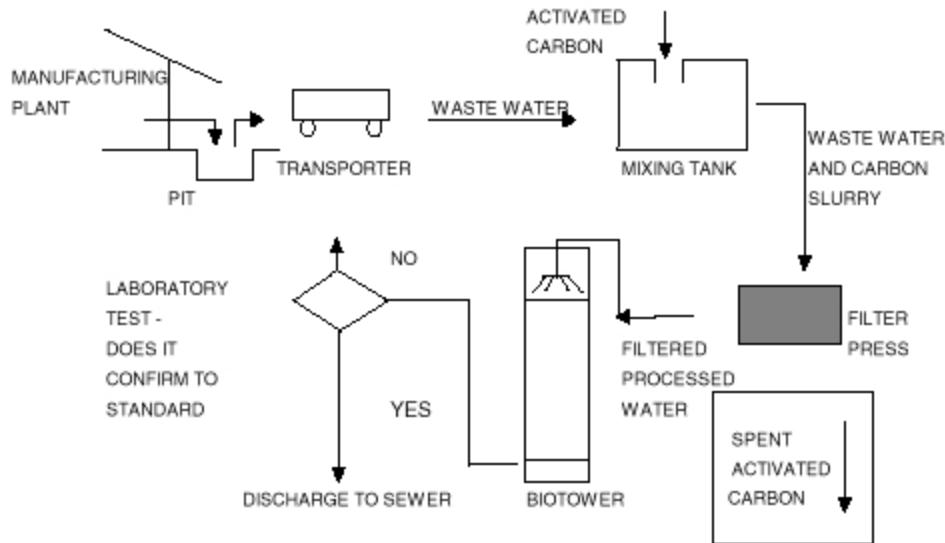
It is the most popular adsorbant and is produced from coal, wood, or vegetable fiber sources. Dehydration and carbonization are achieved by slow heating in the absence of air. Activation is done by the application of steam, air or carbon dioxide at a temperature of about 950°C. It is used in two forms namely powder form of activated carbon, granular activated carbon.

#### 5.4.1.2. POWDER FORM OF ACTIVATED CARBON (PAC)

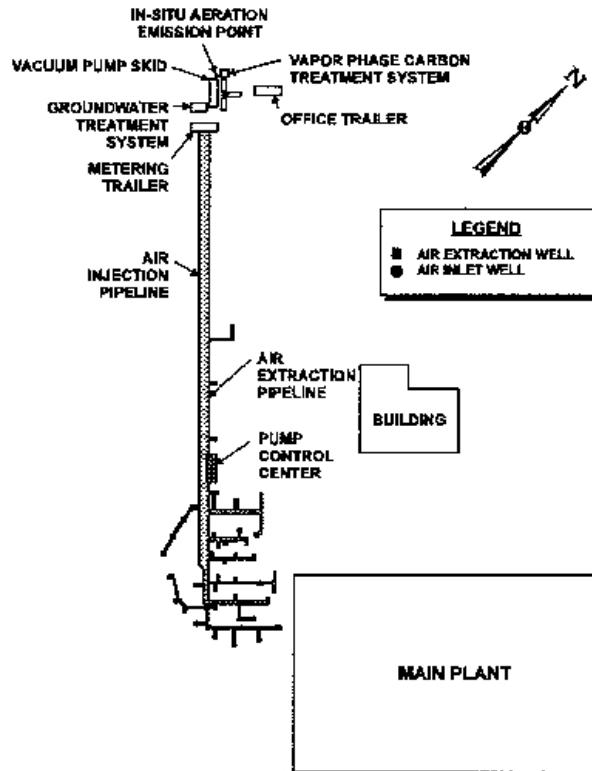
The particles are less than 100mm. This technique is used for intermittent removal of occasional organics by adsorption. PAC is added to sedimentation tanks or to the surfaces of sand filters. When the capacity of PAC for adsorption is lost it cannot be regenerated and must be discarded.

#### 5.4.1.3. GRANULAR ACTIVATED CARBON (GAC)

It is effectively used for the regular removal of organics. The size of the particle is about 0.5-2mm. GAC is used either as a down flow bed, similar to sand filters, instead of sand filters or if the water is turbid, as a separate stage after sand filtration. In the latter case, adsorption of carbon surface by turbidity particles reduces its adsorptive capacity. GAC form is expensive and can be regenerated by a process similar to its manufacture. Regeneration reduces the adsorptive capacity.



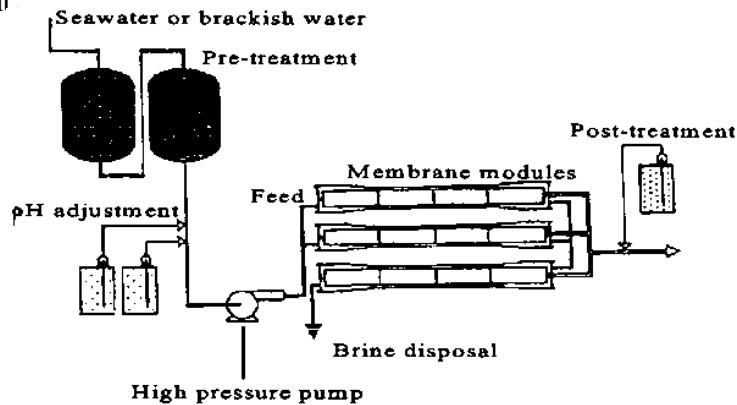
**Fig 22 WASTE WATER TREATMENT USING ACTIVATED CARBON PLANT**



**Fig 23 GRANULAR ACTIVATED CARBON PLANT**

#### 5.4.2. REVERSE OSMOSIS

When a semi permeable membrane separates fresh and saline water, the fresh water passes through the membrane and joins the saline water. In the case of reverse osmosis, when a pressure above the osmotic pressure is applied on the saline water, only the fresh water passes through the membrane. A wide range of membranes are available for the reverse osmosis. They include cellulose acetate, tricellulose acetate, polyamide, polyimide. Up to 60% of treated waste water can be recycled. The remaining 40% along with other streams which cannot be recycled can be collected into pond. The effluent so collected may be toxic and can be reduced by chemical treatment.



**Fig 24 SIMPLE REVERSE OSMOSIS PLANT SET UP**

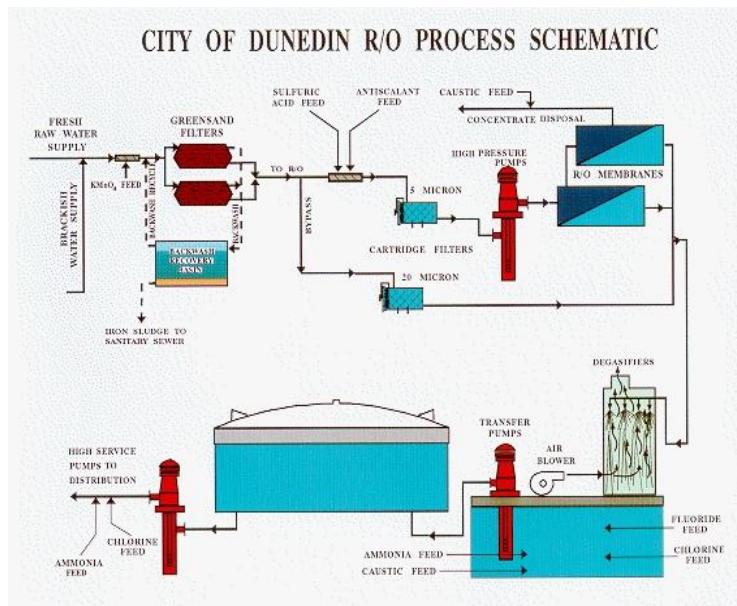
Application of RO in effluent treatment is industry specific. Examples are its application in paper and pulp industries. The chemical pulping processes usually produce effluents which are acidic, alkaline nature along with sulphate liquors. RO is mainly applied best for the treatment of sulphate liquors. Cellulose acetate membrane is used and life span is around one year. The RO treatment process is quite attractive in some sulphate mills where effluent has a solid content of 6-10 weight percent. These effluents can be concentrated to 19-25 weight percent solids by RO. In such cases the permeate streams contain about 0.01 weight percent dissolved solids and COD, about  $1000\text{g/m}^3$ . The permeate can be recycled to the process, while the retentate is further concentrated by evaporation before disposal.

Another application is in metal plating industry. RO has proved to be extremely useful in recovering metal ions from the effluent (mostly comprising the rinse stream). The idea behind employing the RO method is to concentrate so that it can be recycled for plating.

#### 5.4.2.1. REMOVAL OF INORGANIC CONTAMINANTS

The removal of inorganic contaminants by reverse osmosis membranes has been studied in great detail by many researchers using a variety of membrane types. Complex interactions occur in feed waters containing mixtures of ionic species. Nevertheless, general guidelines for the rejection of inorganic contaminants by reverse osmosis membranes can be given.

Ionic contaminants are more readily rejected than neutral species. For most membrane types, polyvalent ions are rejected to a greater extent than mono valent ions. If the polyvalent ion is strongly hydrated, rejection is even higher. Because electrical neutrality must be preserved, ions diffuse across the membrane as a cation-anion pair. As a consequence, rejection of a particular ion depends on the rejection of its counter ion.



**Fig 25 REMOVAL OF INORGANIC CONTAMINANTS BY REVERSE OSMOSIS PLANT**

### 5.4.3. ELECTRO DIALYSIS

Electro dialysis is diffusion of electrolyte through a porous partition under the action of electric current using strong acid cation and strong base anion resins. The equipment operates at low pressures and requires only 1kWh/Kg of TDS removed, is cheap and simple to operate.

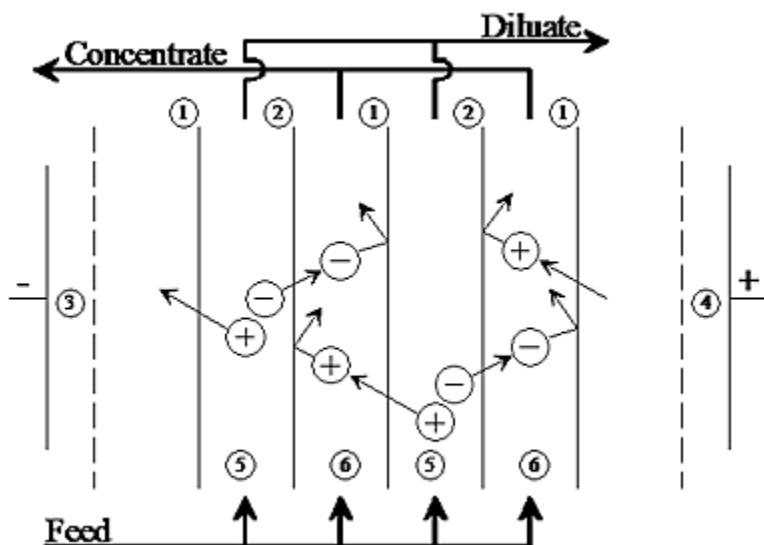
Electro dialysis (ED) is used to desalinate or concentrate a liquid process stream containing salts. ED is a highly efficient method for separating and concentrating salts. It is also very useful to reduce salt contents of process streams with high amounts of salts.

Liquid process streams must be free of particles and high organic content, since ED is subject to membrane fouling. For this purpose, Electro dialysis Reversal (EDR) is a possible solution. EDR is operated like ED, but when fouling has build to a certain level, the setup is altered by reversing the direction of the constant current driving the separation and switching the dilution and concentration chambers. This way, it is possible to prolong the ED operation without having to stop and clean the equipment.

ED is very useful for water treatment: removal of mineral salts, sulfate, nitrate, etc. from brackish water and sea water. ED is also useful for waste water reduction or recovery.

#### 5.4.3.1. ELECTRODIALYSIS PRINCIPLE

Electro dialysis differs from pressure-driven membrane processes by utilizing electrical current as the main driving force in matter separation. This limits the possible solutes targeted for recovery separation to charged particles. The charged particles must be mobile, and the separation media must be able to transfer the electrical current with relatively low resistance. Electro dialysis is almost exclusively carried out on liquids.



**Fig 26 PRINCIPLE OF SIMPLE ELECTRO DIALYSIS PROCESS**

Diagram shows the membrane configuration with alternating cation-selective (1) and anion-selective (2) membranes between two electrodes ((3) and (4)), one at each end of the stack.

The drawing shows a membrane configuration with alternating cation-selective (1) and anion-selective (2) membranes. A cation-selective membrane (cation-exchange membrane) permits only positive ions to migrate through it. An anion-selective membrane (anion-exchange membrane) permits only passage to negatively charged ions. At each end of the membrane stack, electrodes (a cathode (3) and an anode (4)) are placed, supplying a well distributed electrical field of direct current across the membrane stack. Between every membrane, spacers are placed. Spacers make sure that there is room between membranes for the liquid process streams to flow along the membrane surfaces. A special, conductive solution is added to each electrode chamber, where electrode processes are taking place. Usually, an electrode solution that does not result in unwanted reaction products is utilized for this purpose. A sulfuric solution as “electrode rinse” splits water molecules into hydrogen gas at the cathode (3) and oxygen gas at the anode (4).

For a simple desalting process, feed is entered into the flow channels and directed into the spacers. The electrical current influences the charged ions in the feed solution, and similar to ordinary electrolysis, cations are carried towards the cathode, while anions are carried towards the anode. The cations in every second spacer (5) are able to migrate through the cation-selective membrane (1) into the next spacers flow chamber (6). In these flow chambers (6), the cations are trapped, unable to migrate through the anion-selective membrane (2). The anions in the flow chambers (5) are able to migrate towards the anode through the anion-selective membrane (2) and into the alternating flow chambers (6). In these flow chambers (6), the anions are trapped, unable to migrate further, since they are faced with a cation-selective membrane (1). The two electrodes are kept separated from the processed solutions.

Thus, cations and anions are migrating out of every second flow chamber (5) into the remaining chambers (6). The result is that by collecting the outlet of the flow chambers (5) and (6) separately, a depleted and an enriched solution is created. This setup is common for concentrating seawater for table salt production or as pre-treatment for desalting brackish water to create drinking water.

A repeating section of the electro dialysis stack is named a cell pair. In the example of Figure 1.4, the cell pair consists of one cation-exchange membrane (1), anion-exchange membrane (2), one dilution chamber (5), and one concentration chamber (6). In commercial electro dialysis stacks, many hundred cell pairs can be stacked between one set of electrodes, thus improving energy efficiency and negating the electrode effects to great extent.

## **5.5. SOLID WASTE MANAGEMENT**

### **5.5.1. SEWAGE SLUDGE TREATMENT AND UTILIZATION**

Sewage cannot be simply disposed off due to their microbiological and chemical characteristics. Only after proper treatments they can be discharged, that too in a proper way. Disposal of treated sewage into water bodies and lands and recycling of waste water have raised objections from the public. So the methods of sewage disposal should ensure cent percent safety

to get public acceptance. The methods for the disposal of sludge and effluent are different from each other.

### **5.5.1.1. SLUDGE DISPOSAL**

During waste water treatment process large volumes of sludge is produced. The treatment and disposal of the sludge is one of the major problems in waste water treatment. The types of sludge produced in treatment process of waste water are as follows.

- Primary sludge from waste water sedimentation.
- Secondary sludge from biological waste water treatment.
- Digested forms of primary and secondary sludges.
- Hydroxide sludges from coagulation and sedimentation of water, industrial wastes.
- Precipitation sludges from softening plants and from industrial waste treatment.

#### **5.5.1.1.1. SLUDGE CONDITIONING**

Sludge particles contain enormous amount of bound water. To release these bound water and to allow agglomeration of solids a preliminary conditioning stage is required before the dewatering process. This preliminary process is termed as sludge conditioning and is essential to improve the efficiency of sludge dewatering. Various methods are available for sludge conditioning.

Thickening method is employed for various flocculent sludges. In this method additional flocculation of the sludge is effected by low speed stirring in a tank. These results in an increase in solid content and the supernatant can be drawn off.

The second method, chemical conditioning, employs chemical coagulants to promote agglomeration of floc particles. Chemical coagulants used in this method are aluminum sulphate, iron salts and aluminum chlorohydrate.

Elutriation is the third method and is applicable for digested sludge with high alkalinity. Alkalinity in the sludge exerts a high requirement for chemical conditioning. The excess alkalinity can be removed by mixing the sludge with water or effluent and allowing the sludge to settle.

In heat treatment method sludge is heated to about 190°C for 30min at a pressure of 1.5MPa. Then the sludge is transferred to thickening tank. The supernatant with high organic content is once again sent to the oxidation plant for stabilization.

#### **5.5.1.1.2. SLUDGE DEWATERING**

A number of methods are available for sludge dewatering and the choice of method depends on the total cost of the treatment process and on the method of sludge disposal.

### **5.5.1.1.2.1. DRYING BEDS**

The oldest and the most common method of sludge dewatering are drying beds. This method involves shallow rectangular beds which are divided into small compartments. The beds have porous bottoms made of materials such as ash. Sludge is poured over the porous bed upto a height of 125 to 250mm. Drainage of water occurs through the bed and finally collected and removed at the bottom. In addition to dewatering by drainage evaporation occurs at the surface under the action of wind and sun. Cracking of dried sludge provides further for evaporation. Dried sludge is removed either manually or mechanically. Deep drying beds can also be used but they have the disadvantage of prolonged drying period.

### **5.5.1.1.2.2. PRESSURE FILTRATION**

This is a type of batch process which has metal chambers lined with filter material like filter cloth. The metal back of chambers has grooves. Conditioning sludge is pumped into the chambers at a pressure of about 600-850KPa. At this pressure solids are retained adjacent to the cloth while the liquid drains via the grooves and drawn out at the bottom. Compressed sludge further acts as filter for incoming sludge.

### **5.5.1.1.2.3. VACUUM FILTRATION**

This method is a continuous process. There is a revolving segmented drum, partially submerged in a shallow tank which contains conditioned sludge. When a vacuum of about 90KPa is applied to the submerged segments, sludge is sucked onto the surface of the filter cloth. As the drum rotates the layer of sludge on the surface of filter comes out of the tank. Dewatering occurs and finally the sludge is removed by a scraper blade. The cycle is repeated again and again.

### **5.5.1.1.2.4. CENTRIFUGATION**

This method has not been practiced much as it is less efficient in dewatering and is a continuous process. It employs a solid-bowl type centrifuge. Conditioned sludge is fed to the centre of bowl. Rapid rotation of the bowl throws the solid to the outer edge of the bowl. Scraper blades remove the solids deposited on the edge.

In all the above four methods the liquid portion separated from the sludge must be properly disposed. Depending upon the organic content, it may be either disposed directly or sent back to the treatment plant for further stabilization.

## **5.5.2. REFUSE DISPOSAL**

### **5.5.2.1. DISPOSAL OF INCINERATION**

Where transport cost for sludge disposal is high, incineration is the best alternative. Incineration is also particularly useful in cases in which the sludge contains toxic chemicals making their disposal environmentally unacceptable through other routes.

### **5.5.2.1.1. ROTARY MULTIPLE HEARTH TYPE INCINERATORS**

These are widely used for sludge incineration. Fluidized bed furnaces are also becoming popular. Dewatered sludge with about 30% solid content has sufficient calorific value to maintain self sustaining combustion. However, most incinerators require fuel oil for efficient combustion. After incineration the resulting inert ash is easily disposed. Still the sludge disposal by incineration process is inefficient it may lead to air pollution. Discharge of residual ash and gases produced during incineration has to meet emission standards proposed by environmental organizations. This requires complex equipments and skilled workers, thus making the process costly.

### **5.5.2.2. DISPOSAL BY LANDFILLING**

The increased costs of sludge disposal paved the way to develop new technologies which will reduce the amount of sludge produced in sewage treatment.

Water treatment produces less sludge than the sewage treatment. Moreover sludge from water treatment has low organic content and hence can be disposed by landfill without any objection. Sludge from softening process contains high content of calcium and in this way desirable for agricultural use. Sewage sludge can also be used as a landfill material thus becoming a useful way for land reclamation.

### **5.5.2.3. DISPOSAL INTO SEA**

In treatment plants which are located near coastal areas disposal of sludge into sea can be practiced. If the site of disposal is deep water areas there will not be any environmental problems. However, agitations have been raised worldwide against the disposal into the sea.

### **5.5.2.4. SLUDGE AS A FERTILIZER**

Considerable quantities of sludged is charged to land is used as a fertilizer for agriculture lands and the rest is used for landfill. In certain cases the sludge is mixed with domestic refuse and treated by various methods of composting. This provides compost containing nutrients and humans which can be used to condition the soil. Heat dried activated sludge is by far the best for use as a fertilizer. It has the advantages of reduced bulk, little odour, high nitrogen content, good source of organic matter, low pathogenic bacterial content and low grease content. They can be stored dry until use. It is recommended as a source of nitrogen. However sludge as a fertilizer has certain disadvantages. It has a moderate manurial value and has to be prepared in such a way that it is something more than low grade manure. Only small quantities of potassium and magnesium are present in sludge and these may have to be supplemented. Much of the nitrogen in digested sludge is in the form ammonia. Because of its solubility, ammonia can be lost during sludge dewatering.

Sludge contains mineral grease and potentially toxic chemicals which injure soil. The toxic chemicals which affect plant growth are the heavy metals like zinc, copper and iron. Other heavy metals like cadmium, lead and chromium can cause problems to the consumer rather than to the plants. Normally domestic sewage sludge contains low concentration of heavy metals.

Presence of pathogenic bacteria, particularly *Salmonella* and eggs of parasites like *Taenia*, make the sludge unsuitable as manure for crops which are eaten uncooked. Method of treatment of the sludge for use as fertilizer is too expensive to adapt.

#### **5.5.2.5. EFFLUENT DISPOSAL**

The most important method of effluent disposal is disposal by dilution into water bodies. Even though the sewage water is treated by various processes still it may have some organic wastes. When this effluent water is mixed with water bodies it will be diluted out so that it will not cause a high demand for dissolved oxygen in the natural water bodies. Thus dilution of treated water actually brings out further reduction in BOD. Microorganisms in the water will feed on the remaining organic compounds of the sewage and convert them into inoffensive compounds. Treated effluent can be discharged into sea, river or artificial lakes. This mode of disposal is called as disposal by dilution and is usually employed in cities and towns which are suited by sea or river. This method is the most effective one. It is the least offensive process. By far it is the cheapest method that can be employed.

Rivers: treated effluent can be released into river water. The organic matter and other polluting liquids contained in sewage will be rapidly oxidized during the flow of the river. The effluent and the diluting river water have to be mixed thoroughly and quickly.

Artificial lakes: treated effluent may also be disposed by mixing with a large body of still water. In a state of rest the heavier suspended matter settles to the bottom and the suspended colloidal matter undergoes a natural flocculation. In the presence of light and dissolved oxygen biological self purification takes place.

Sea out falls: this can be employed when places are near to the sea. Since the total area or volume of the sea is very large there will be effective dilution. But this method has some limitations. If the sewage is not treated properly microorganisms present may cause disease in the marine shellfish. Secondly treated effluent should not be discharged near a bathing beach unless there is a constant current toward the sea.

#### **5.5.2.6. RE-USE OF EFFLUENTS**

Effluents from sewage treatment plants, apart from discharging into water bodies for dilution, can also be reused for various other purposes. The first use of reclaimed waste water was for crop irrigation. These crops were fiber, fodder and seed crops and there was little opportunity for public contact. Later reclaimed waste water was used for landscape irrigation ie irrigation of parks, play grounds etc. water scarcity and lack of proper disposal sites for effluents leads to the phenomenon of water recycling.

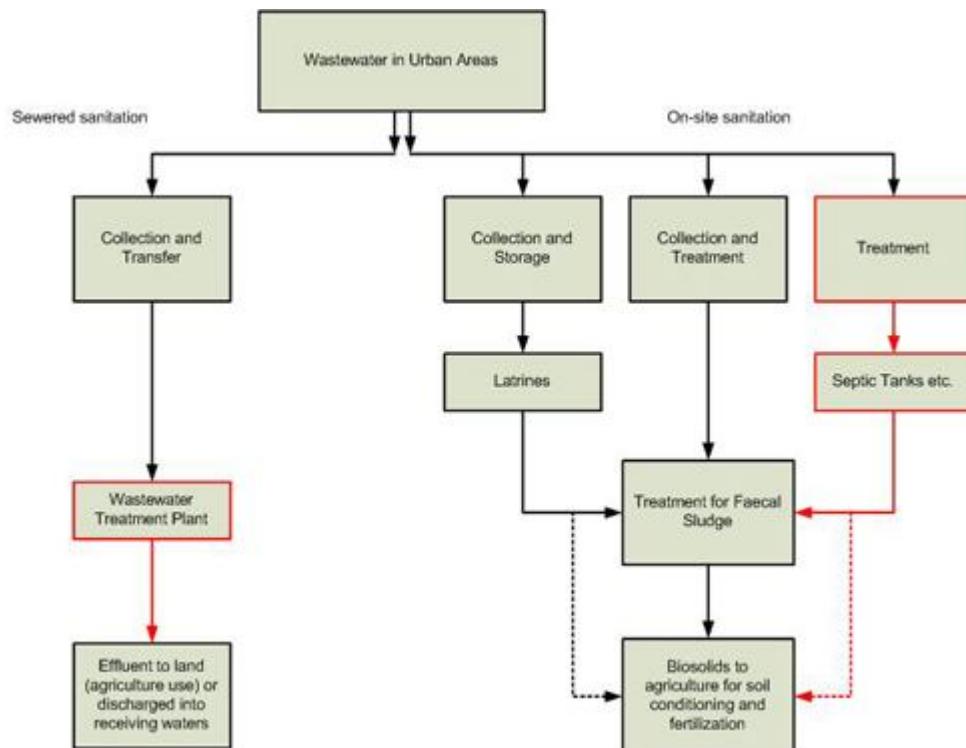
Manufacture of biological and biologically produced materials requires high quality water as a major raw material. Moreover in these cases recovery of the product involves expensive removal of large quantities of water as effluent. Hence, treatment of waste water within the process unit for reuse will not be economical as it adds up to the total cost. Thus,

recycling of waste water will be beneficial only if there is a large scale use for water with a quantity inferior to that of available water supply.

### 5.5.2.7. INDUSTRIAL RE-USE OF EFFLUENT

Generally, due to the cost of treatment processes, treatment and recycling of waste water will be practiced only when the effluent discharge is prevented or the local capacity for effluent discharge is insufficient.

There occurs several grades of waste water within the processing operation and depending upon the industrial processes different grades of water can be used. Thus recycling is normally practiced in heavy industries such as power generation and steel making where water of low quality can be used and minimum treatment of waste water is sufficient as water is used only for cooling. Higher grade waste water from sterilizing and food preparation processes can be recycled with a minimum treatment for a lower grade use, such as vegetable cleaning. After solids removal and sterilization biologically treated effluent can be used for washing vehicles.



**Fig 27 SCHEMATICS OF SEWAGE DISPOSAL**

### 5.5.3. EXCRETA DISPOSAL IN UNSEWERED AREA

#### 5.5.3.1. MUNICIPAL RE-USE OF EFFLUENTS

Recycling of domestic waste water is also becoming popular in arid areas. Schemes for any expansion in public water supply have to consider the use of reclaimed water as supplies of

upland water are heavily utilized. Used water from bathing, dishwashers and washing machines can be reused for flushing toilets after treatment with mild sterilizing agents like sodium hypochlorite.

### **5.5.3.2. AGRICULTURAL RE-USE OF EFFLUENTS**

Effluents from sewage treatment plants contain a wide range of inorganic plant nutrients. Thus effluent waters can be effectively used as a source of both water and nutrients for the plants of arid climates. However, there is a possibility for hazards like contamination by pathogenic microorganisms or accumulation of toxic chemicals.

Irrigation: treated waste water can be used for irrigation. It can be carried out in two different methods land filtration and broad irrigation.

Land filtration: this method can be followed in landscapes which are porous for filtration. This is an efficient method. In this method the effluent is irrigated into parallel trenches which are dug with an even depth to avoid the flow of water to the lowest point.

Broad irrigation: this method is followed when the ground is not sufficiently porous for land filtration. It is less efficient than land filtration and requires the use of more land. There is a main trench from which arise several side branches. The best crops for sewage forms are quick growing plants such as rye. Land which is overdosed with sewage becomes sewage sick. Such lands can be restored by a prolonged rest.

Although waste water recycling is widely followed it has certain potential problems. If the water is recycled many times accumulation of dissolved salts particularly anions such as nitrate, sulphate, phosphate and chloride from biological oxidation occurs. These may lead to corrosion and toxicity problems. Recycling of persistent pollutants, commonly termed recalcitrants, may also cause problems. Recent developments in waste water treatment employ microorganisms for the breakdown and scavenging of these recalcitrants.

### **5.5.4. COMPOSTING AND VERMICULTURE**

Composting is a thermophilic, aerobic decomposition process, in which solid substrates like municipal waste, raw sewage, or sludge can be degraded over a period of weeks by a succession of microbial population to yield a stable form of organic matter. After such a biodegradation, a dark brown, granular, humus like end product is formed, which is called as compost.

The primary objective of composting is to convert an unstable, offensive material into a stable end product. Thus it is an effective method of domestic refuse disposal. When compost is used as a soil conditioner it also provides a means of recycling solid wastes. The difference between anaerobic digestion of sewage sludge and composting is that the latter is an aerobic process.

Substrates: substrates for composting include a range of materials from municipal refuse to sewage sludge. Paper, garbage, agricultural wastes like leaves and even human faecal material in some cases can be composted. For producing stable compost the organic substrate must contain adequate nitrogen. A reasonable carbon: nitrogen ratio is about 50. Rubbish, the faecal material, has a very high content of carbonaceous materials and has too high C: N ratio. To make excellent compost the C: N ratio of rubbish can be lowered by mixing with garbage, sewage sludge, cow manure or wastes from fish farms or cannery.

#### **5.5.4.1. MICROBIOLOGY OF COMPOSTING**

Composting involves different groups of microorganisms which act on the substrates in succession. Although the decomposition is mainly carried out by the thermophilic microorganisms, the initial process is carried out by mesophilic microorganisms. In the initial stages of composting the heap of materials to be composted will be at ambient temperature and pH will be more or less neutral. At first the biodegradable materials, such as proteins and carbohydrates, are decomposed naturally by the mesophilic microorganisms. The degradation products are usually simple organic acids which cause a drop in pH. As the initial reactions proceed heat is produced which rises the temperature within the heap. The insulating effect of the heap further causes an increase in temperature. However, the maximum temperature achieved and the time taken to achieve it depend on various factors like composition of organic wastes, moisture content, size of heap and degree of aeration and agitation. As the temperature increases, the activity of mesophiles is gradually reduced and finally at about 60°C the mesophiles are killed by the high temperature. They may become the source of nutrients for higher forms like protozoa, rotifers and nematodes. They may even add to the total organic content. At this temperature, thermophilic microorganisms take over the degradation process. Hemicellulose, cellulose and lignin are degraded rather slowly. Thermophilic bacteria like *Thermonospora* and *Thermoactinomyces* and thermophilic fungi such as *Thermoascus* carry out these reactions.

Once the readily degradable materials are degraded, the reaction rate slows down. Eventually the temperature decreases once again to the mesophilic range through heat loss from the surface of the heap. Once again the mesophiles, either through re-invasion from outside or through germination of heat resistant spores, dominate.

The thermophilic process has certain advantages as follows: acceleration of the process, killing of pathogenic microorganisms, destruction of ungerminated seeds of weeds.

Although the pathogenic microorganisms are destroyed. Sometimes spores of the fungi *Aspergillus* may be found in the vicinity of composting areas. However, any adverse effects caused by these microorganisms are highly localized and hence there will not be any threat to residential areas. In certain cases, starter culture from old compost fermentations may be used as an inoculum for new ones.

#### **5.5.4.2. PROCESS**

The composting process involves five major stages. They are salvage, grinding and homogenization, degradation, drying and curing and finishing. The entire process may take one to three months to produce good compost.

**5.5.4.2.1. SALVAGE:** domestic as well as municipal refuse contain non biodegradable materials like glass, ceramic, plastic and metals in addition to other biodegradable organic materials. So they have to be removed. Ferrous materials can be removed magnetically. Non compliance materials like plastics are removed by hand – sorting (manual picking). Other very large objects are separated by mechanical screening.

**5.5.4.2.2. GRINDING, PULPING AND HOMOGENIZATION:** for effective composting the substrates should be a finely divided granular material, which permits aeration, mixing and invasion by the degradative microorganisms. Particles of size about 4 – 7cm will be suitable and this can be achieved by grinding or shredding. Alternatively, wet pulping method can also be used which produces a slurry containing about 5% solids. But this requires drying to about 50% solids to be suitable for composting. Sometimes agitation in the initial stages of composting causes the break down of fibres in the material to be composted. This reduces the pretreatment required.

A moisture level of about 40-50% is ideal for good composting. Low moisture content may inhibit microbial action. So, at this stage moisture content has to be adjusted. The required moisture content can be attained by adding water. Sewage sludge can also be used to attain required moisture and in this case the final product will be of a higher grade. The sludge may be raw, dewatered, digested or a combination of these types and the nature of resulting compost will depend on the sludge used. Generally raw primary or secondary sludge will be preferred to digested sludge because they can be easily dewatered and has a higher nutrient content. When municipal refuse and sewage sludge are combined for composting, the ratio of reuse to sludge should be approximately 2: 1 by weight.

#### **5.5.4.2.3. BIOLOGICAL DEGRADATION**

There are different methods of composting the commonly used local method involves piling up of the substrates into large heap. Bulking agents like large wood chips or barks can be mixed with the substrates to facilitate aeration. After the required period of composting, about 21 days, bulking agents are separated from the compost and the compost is ready for use.

The most primitive and simplest method is to pile the material into windrows, which are heaps resembling pyramids. Windrows are of about 3cm high with a base of 9m width. The mixture is turned regularly with mechanical shovels to promote aeration. After four to six weeks, depending on moisture and climate, stable compost is formed. Windrow composting has a number of disadvantages. Since it is done in the open air it is significantly affected by the local climate. Often the compost generates odors which may be difficult to control and this may raise complaints from local people. Windrow composting requires large land area and therefore unsuitable for urban.

An alternative method of composting involves mechanical mixing and aeration inside closed digesters. A number of processes are used in mechanical composting and all of them have equipments to control temperature, pH and C: N ratio. The most well known process is the Dano process. This employs drums which are about 30m long and 5m in diameter. The drums rotate slowly to facilitate mixing and aeration.

The advantages of these mechanical composting are they do not suffer the problems of windrow composting, with better control over mixing, aeration and temperature the composting process can be completed in a shorter period than that required for windrow composting.

**5.5.4.2.4. CURING AND DRYING:** the compost must be stabilized completely before it is added to the soil. Otherwise, it will remove the nitrogen, which is available to the plant, from the soil in the final stages of its degradation. In case of windrow composting, to ensure complete stabilization the compost is cured for a period of two weeks in shallow piles. In mechanical composting, curing periods can range upto three weeks or may be absent.

**5.5.4.2.5. FINISHING:** the type of finishing is largely dependent on the method of marketing or disposal of the compost. Often, the compost will be mechanically dried to a moisture content of less than 30% for agricultural or horticultural use. The consistency of the compost is also important. For this reason, regrinding and screening of the compost may be done to remove large pieces of debris. When sold in small quantities, the compost may be pelletized before bagging. Sometimes the compost is pasteurized to kill pathogens before sale to the farmer. In contrast, if the compost is to be disposed off by land filling no finishing will be required at all.

### 5.5.4.3. ADVANTAGES

Composting can be carried out with simple equipment and very little training. Well suited to many tropical environments. It is particularly appropriate for small cities and towns near to agricultural areas.

### 5.5.4.4. PROBLEMS OF COMPOSTING AND THEIR REMEDIES

Composting procedure any sometimes is associated with certain problems which make the process unsatisfactory.

1. Glass, metal, polythene bags and other non-biodegradable matter have to be separated from the refuse. This may be done mechanically or by hand before and after composting.
2. Farmers may refuse to use compost if it includes night soil.
3. There needs a market and low price for the compost.
4. Organic refuse and night soil may contain fly eggs and larvae before they come to the process. To prevent them from hatching the compost has to be turned often so that the surface materials are brought to the hot centre. More over the process should be carried out on a hard surface to prevent the larvae from burrowing into soil.

## **5.5.4.5. VERMICULTURE**

### **5.5.4.5.1. TYPES OF WORMS**

There are many different types of worms living in our soil. However only very few are well suited to composting. The most commonly used are *Eisenia foetida* or redworm (also known as ‘red wiggler’) and *Lumbricus rubellus*. These worms can be purchased at local bait shops or ordered through the mail or via the internet. One pound of redworms (about 1000) is needed for a typical worm bin. Night crawlers are not well suited for composting food waste.

### **5.5.4.5.2. OXYGEN**

As all living things, worms need oxygen to survive. Therefore the container in which you choose to house your worms should have adequate ventilation, but preferably a mesh of some description should be used over the vents in order to keep out unwelcome intruders such as flies. Worms breathe through their skin and because of this they require a moist environment in order for the exchange of air to take place.

### **5.5.4.5.3. pH**

The pH of the bedding is a very important factor in the smooth running of your worm bin. If it becomes either too acidic or too alkaline this will upset and possibly kill the worms. You should therefore check this regularly using litmus paper or a pH meter. A suitable level is around pH 7. If the level becomes unsuitable it can be lowered by adding a dilute mix of white vinegar or raised by adding baking soda or calcified seaweed.

### **5.5.4.5.4. TEMPERATURE**

Redworms can tolerate a temperature range of around 10 degrees C – 28 degrees C, although they will be most active at about 25 degrees C. If your worms freeze they will die. Consideration should therefore be given to the positioning of your worm bin. If you decide to keep it outside find a spot where it will not be in full sunlight during the summer months and remember to bring it indoors (garage or utility) during the winter. Obviously the other option is to keep it indoors all the time, in which case you should not have to worry about the temperature.

### **5.5.4.5.5. LIGHT**

Worms are sensitive to light and when they become exposed with it they will burrow into their bedding. This is useful when you want to harvest your worms.

### **5.5.4.5.6. BREEDING**

If the conditions are correct Redworms will reproduce quickly. They will reach adulthood in about six weeks and can reproduce up to three times a week for their life span, which is generally around a year. Each time they reproduce they will deposit a cocoon which can contain anything up to 40 baby worms, although this number is usually around four. The worms

population will be controlled by the size of their environment so you will never end up with too many worms.

#### **5.5.4.5.7. HOUSING**

Although there are a number of specially made worm bins available, many different containers can be used for the job. These can be a bucket with a lid, a plastic tub, homemade timber container or some other container. The important factors that should be considered are:

Worms will produce a lot of liquid so their home should either have drain holes in the bottom, or a layer of stone should be used to collect the liquid. If you choose the later you will need to fit a tap in order to drain it off at regular intervals. This liquid can then be diluted (1:10) to make an excellent plant food. Your container should preferably have a lid in order to keep out pests, such as flies. You must ensure that the container is suitably ventilated. Once you have chosen your container you must prepare the bedding ready to receive your worms. As mentioned earlier, worms need a moist environment in order to breathe. Therefore the bedding that you provide should be suitably dampened but not wet. Bedding can be made from a number of materials but probably the best and easiest is shredded newspaper. Once the newspaper (no ad inserts) has been torn into thin strips (a shredder works great for this) it should have water added to it until it is uniformly moist. A handful of sand, compost, or soil should also be added to aid the worms digestion process. When the mix is complete it can be added into your container, after which you should introduce your worms. A small amount of kitchen waste may then be added to the mix and covered with the bedding. Initially the worms will want to explore their new home, so to prevent them venturing up the sides of the bin it is a good idea to leave the lid off for a while until they get settled into the bedding.

#### **5.5.4.5.8. FEEDING**

Redworms are not that fussy when it comes to feeding time. Basically they can be fed most organic waste, although as they do not have teeth, their food must be soft in order for them to eat it. This does not mean that harder foods are unsuitable, only that it will take longer for them to be eaten.

They can be fed all types of fruit and vegetable waste. Coffee and tea bags may also be fed, although as these are acidic they should only be given in small quantities, as should citrus fruits. Grass clippings may also be given in small quantities, but if too much is fed it will generate heat and give off ammonia which will harm the worms.

Things to avoid feeding include meats & dairy products (the worms will eat this but it can cause bad odors and attract unwelcome insects), salty foods, manure from pets as these may contain antibiotics or harmful bacteria.

If your worm bin environment is satisfactory, you can expect the worms to eat up to their body weight in food each day. This means that a decent size worm bin should be able to cope with the kitchen waste produced by a family of four without too much trouble. If, however, you

find that you are over feeding your worms and odors are becoming apparent, you should stop feeding until the worms have had chance to catch up.

#### **5.5.4.5.9. HARVESTING WORMS**

With continued use of your worm bin, you will notice that the bin begins to fill up. When it eventually becomes full you will need to harvest your worms so the bin can be emptied. This is a relatively simple operation and should not cause any problems.

As the worms feed just under the layer of food, the majority can be removed simply by scooping off the top three or four inches of bedding. Place them in a bucket or other suitable container until you are ready to replace them.

With most of the worms removed the rest of the bedding can then be emptied from the bin. This will be top quality compost that can be used in your garden. If you find, as the bin is being emptied, that there is still a large number of worms in the compost, they can be removed by adopting the 'pyramid' method of harvesting.

For this you will need a plastic sheet and a light source ( the best idea is to do it outside on a sunny day ). Place the contents of the worm bin onto the plastic sheet and form into small piles. As the worms are sensitive to light they will burrow into the compost. Gradually scrape away the compost from the sides of the heap. As you do this the worms will continue to burrow away from the light towards the center. Keep scraping until all the compost has been removed, leaving you with a wriggling mass of worms, which can then be placed with those removed initially.

Once you have finished the harvest, your worms can be placed back in the bin, along with some fresh bedding, and the process can begin all over again. The worm castings or 'compost' can be added to your garden or houseplant soil for a wonderful soil amendment.

#### **5.5.4.5.10. TROUBLESHOOTING**

If your worm bin is maintained correctly you should not have any problems, however occasionally things can go wrong. Below are some possible problems and suggestions on how to remedy them.

**5.5.4.5.10.1. WORMS TRY TO LEAVE THE BIN:** Check the moisture and the pH of the bedding. Also check that the bin is not situated near a source of vibration. Be sure your worms are not being subjected to extremes of temperature.

**5.5.4.5.10.2. BAD SMELLS COMING FROM BIN:** Either the bedding is too wet or you may have over fed your worms. If they have been over fed stop feeding for a few days to let them catch up, and add some fresh bedding (shredded newspaper).

**5.5.4.5.10.3. BEDDING IS VERY WET:** Mix in some dry bedding until the excess moisture has been soaked up.

**5.5.4.5.10.4. FRUIT FLIES:** Cover fruit and vegetative matter with enough bedding material to thoroughly cover it or wet several sheets of newspaper and lay flat on top of the bedding. This helps keep moisture in also.

## 5.5. RADIO ACTIVE PRODUCT WASTE DISPOSAL

Radioactive compounds released from the companies are properly neutralized and are dumped in biological safety cabins and are disposed outside the populations restricted areas as land fills.

## 5.6. LET US SUM UP

- Recombinant products producing industries dispose or eliminate industrial wastes which are dangerous to the organisms present in the environment.
- It is thus necessary for us to manage the industrial wastes posing hazardous to human welfare.
- The various processes such as primary, secondary, tertiary treatments and their disposals are discussed in the chapter.
- The alternative usage of the treated wastes and the role of composting are explained.
- Radio active elements causing mutations also had to be treated and disposed in a safer manner. The methods involved are discussed in the chapter.

## 5.7 POINTS FOR DISCUSSION

1. Discuss the Biological degradation process.
2. Analyze the sewage disposal methods.
3. Substantiate the use of composting and vermiculate.

## 5.8. LESSON - END ACTIVITIES

1. Explain in detail about the various techniques involved in primary treatment of industrial waste.
2. What are the aerobic and anaerobic processes available for the treatment of wastes and explain them.
3. Explain activated carbon treatment and reverse osmosis techniques with applications.
4. Define following: compost, vermin compost. Explain the process of vermicomposting.
5. Explain the disposal of radioactive products.

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