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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I - FERMENTATION AND DOWNSTREAM PROCESSING
SBTA5203

The Ranges of Fermentation Processes

1. Processes that produce microbial enzymes
2. Processes that produce microbial metabolites
3. Processes that produce microbial cells (biomass) as the products
4. Processes that produce recombinant products
5. Processes that modify substrates (transformation process**)**

1. Processes that produce microbial enzymes

- Microbes, plants and animal are the major source of enzymes
- Commercial production of many enzymes exploiting these sources have been achieved
- As being produced in large quantities by the fermentation processes, microbial enzymes have the enormous economic potential
- Microbes are more prone to change in its genetics to enhance its productivity compared to plant or animal system
- It is possible to produce enzymes of eukaryotes into the prokaryote systems with the help of recombinant DNA technology
- It is possible to control and improve microbial enzyme production by introducing inducers and activators in the production medium
- It is also possible to increase the copy number of gene coding for the a specific enzyme using principles of recombinant DNA technology

2. Processes that produce microbial metabolites

The growth of a microbial culture can be divided into major four phases. These are.

1. Lag phase
2. Log phase
3. Stationary phase
4. Death phase

1. Lag phase

- Once the inoculation of the cells into fresh medium is done, the bacterial population remains temporarily unchanged
- There is no cell division during this phase
- The cells grow in volume and mass by synthesizing the population remains temporarily unchanged etc.

- Metabolic activity is at high rate
- This period is known as the period of adaptation
- **T**here are various factors that affects the this phase are size of inoculum, time required to recover shock in the transfer, time required for synthesizing essential coenzymes and other factors
- Time required for synthesis of necessary new enzymes to metabolize the substrates present in the medium

2. Phase of exponential growth

- This period is also known as the phase of exponential growth
- During this period, the growth rate of the cells gradually increases
- The cells grow at a constant, maximum rate
- Cells are growing in geometric progression dividing by binary fission
- The incubation conditions and composition of the growth medium control the rate of cell division

3. Stationary phase

- During this phase growth cease
- In a batch culture (in test tube or EM flask) , exponential growth cannot be continued forever
- Various factors like exhaustion of available nutrients, accumulation of inhibitory, metabolites or end products and lack of biological space limit the growth during this phase
- During this phase the number of dividing cells equals the number of dyeing cells
- This is not a quiescence period like lag
    - **Death phase**
- This phase is the reverse of the log phase
- The viable cell population declines exponentially during this phase

Base on the various products produced, the phases of bacterial growth can be categorized into two phases. These are

(i) The Trophophase

(ii) The Idiophase

1. Tropho phase

- Metabolites which are essential to the growth of the cells like amino acids,

nucleotides, proteins, nucleic acids, lipids, carbohydrates are produced during the log phase of the growth

- The products (metabolites) produced during this phase (log phase) are known as **primary metabolites** and the phase in which they are produced(equivalent to the log, or exponential phase) is referred to as the ***trophophase***
- The primary metabolites are also known as central metabolites
- Several primary metabolites are of economic importance and can be produced in large quantity by fermentation process
- The synthesis of primary metabolites by wild-type micro- organisms aims to meet the requirements of the organism
- The industrial production these metabolites can be achieve by providing appropriate cultural conditions to the wild-type organism to increase and improve the productivity of these compounds
- Productivity can also be improve by modifying interested genes by the help of recombinant DNA technology
- Following are few economically important primary metabolites which can be produced at large scale

Idiophase

- During the stationary phases several microbial cultures produce certain compounds (these compunds are not produced during the "***trophophase"*** and which do not appear to have any obvious function in cell metabolism). These compounds are called the secondary compounds of metabolism. The phase during which these compounds are produced (equivalent to the stationary phase) as the "***idiophase*"** (Bu'Lock et al., 1965)
- The secondary metabolism is also known as "**special metabolism**"
- The products of secondary metabolism are not absolutely required for the survival of the organisms
- All microorganisms do not undergo secondary metabolism. It is common amongst the filamentous bacteria and fungi and the spore forming bacteria
- The taxonomic distribution of secondary metabolism is different from that of primary metabolism
- The physiological role of secondary metabolism and hence secondary metabolites in the producer cells has been the subject of considerable debate

- The large scale production of secondary metabolites focus on the importance of these metabolites on organisms other than those that produce them
- Secondary metabolites play an important physiological role several ways. Many secondary metabolites possess antimicrobial activity, some acts as specific enzyme inhibitors and growth promoters and many have pharmacological properties
- Thus, due to a huge economic potential, the industrial production of these metabolites have formed the basis of a number of fermentation processes
- As the wild-type microorganisms produce very low concentrations of secondary metabolites, the large scale synthesis can be controlled by induction, catabolite repression and feed-back systems
- Following is the outline of inter-relationships between primary and secondary metabolism and their respective products

3. Processes that produce microbial cells (or biomass) as the product

The commercial microbial biomass production can be divided into two major processes:

1. The production of yeast to be used in the baking industry and
2. The production of microbial cells which can be used as human and/or animal food (single-cell protein)

- Bakers' yeast has been produced on a large scale since the early 1900s and yeast was produced as human food in Germany during the First World War
- However, it was not until the 1960s that the production of microbial biomass as a source of food protein was explored to any great depth
- A few large-scale continuous processes for animal feed production were established in the 1970s. These processes were based on hydrocarbon feedstocks which could not compete against other high protein animal feeds, resulting in their closure in the late 1980s

4. Recombinant products

- Recombinant DNA molecules are also known as chimeric DNA, as they consist genes (DNA) of two different species
- The nucleotide sequences used in the construction of recombinant DNA (rDNA) molecules can be from any species. For instance, plant or human DNA may be combined with bacterial DNA, or human DNA may be joined with fungal DNA
- Genes from higher organisms can be inserted into microbial cells in such a way that the

recipients are capable of synthesizing ***'foreign'*** proteins

- The advancement in the application of rDNA technology has made possible to produce a range of recombinant products by the fermentation process
- A wide range of microbial cells have been used as hosts for such systems including Escherichia coli, Saccharomyces cerevisiae and filamentous fungi
- Recombinant DNA is widely used in research, agriculture, medicine and biotechnology
- Several products that result from the use of rDNA technology are found in almost every pharmacy, medical testing laboratory, doctor‟s as well as and veterinarian‟s office, and biological research laboratory
- Following are the recombinant products that produced by genetically engineered organisms organisms

  Human Growth Hormone (rHGH) Biosynthetic Himan Insulin (BHI) Envelope protein of the Hepatitis B virus Follicle Stimulating Hirmeone (FSH) Blood clotting Factor III Erythropoietin (EPO)

  Granulocyte Colony-Stimulating Factor(G-CSF) Alpha-galactosidae

  Alpha-L-iduronidase

  N-acetylgalactosamine-4-sulfatase Dornasealfa

  Tissue Plasminogen Activator(TPA) Glucocerebrosidase

  Interferon (IF)

  Insulin-like growth factor 1 (IGF-1) Bovine somatotropin (bST)

  Porcine somatotropin Bovine chymosin

5. Processes modifying substrates (Transformation Process)

- Many microbial cells may be exploited to convert a compound into a structurally related, financially more valuable compounds
- As microbes can behave as catalysts with high positional specificity and stereospecificity, microbial processes are more specific than purely chemical ones
- These microbial processes enable the removal, addition and/or modification of various functional groups at predefiend specific sites on a complex molecule without the use of chemical protection
- The reactions which may be catalyzed include *Dehydrogenation, Oxidation, Hydroxylation, Dehydration and Condensation, Decarboxylation, Amination, Deamination and Isomerization*
- As microbial processes can be operated at a relatively low temperatures and

pressures have the additional advantage over chemical processes which require high temperatures, more pressures and presence of heavy-metal catalysts-a potential environmental pollutant

- Production of vinegar is the most well-established microbial transformation process (conversion of ethanol to acetic acid)
- Many transformation processes have been rationalized by immobilizing either the whole cells, or the isolated enzymes on an inert support which catalyze the reactions
- The immobilized cells or enzymes may be reused many times

The Preservation of Industrially Important Microbes

Microbes are required for the production of fermentation products. They are very valuable for specific product. One product produced efficiently by specific microbe will not be given by all the microbes.

The isolation of a desired organism for a fermentation process may be a time consuming and very expensive procedure and it is therefore essential that it retains the desirable characteristics that led to its selection. Also, the culture used for the fermentation process should remain viable and free from contamination. Thus, industrial cultures must be preserved and maintained in such way as to eliminate genetic change, protect against contamination and retain viability.

Different techniques are used for maintenance and preservation of different organisms based on their properties. Selected method should also conserve the properties of the organisms.

Techniques for the Preservation of microbes are broadly divided into two

1. **Methods where organisms are in Continuous metabolic active state**
2. **Methods where organisms are in Suspended metabolic state**

Continuous metabolic active state preservation technique

In this technique organisms are preserved on nutrient medium by repeated sub-culturing. In this technique any organisms are stored by using general nutrient medium. Here repeated sub-culturing is required due to depletion or drying of nutrient medium. This technique includes preservation by following methods.

- **Periodic transfer to fresh media**

  Organisms are grown in general media on slant, incubated for particular period of time at particular temperature depending on the characteristics of the selected organisms, then it is stored in refrigerator. These cultures can be stored for certain interval of time depending on the organism and its growth conditions. After that time interval again these organisms are transferred to new fresh medium and stored in refrigerator.

- **Overlaying culture with mineral oil**

  Organisms are grown on agar slant then they are covered with sterile mineral oil to a depth of 1 cm. above the tip of the surface. This method is simple; one can remove some organisms in aseptic condition with the help of sterile wire loop and still preserving the initial culture. Some species have been preserved satisfactorily for 15 – 20 years by this method.

Storage in sterile soil

This method is widely used for preserving spore forming bacteria and fungi. In this method organisms will remain in dormant stage in sterile soil. Soil is sterilized then spore suspension is added to it aseptically, this mixture is dried at room temperature and stored in refrigerator. Viability of organisms has been found around 70 – 80 years.

Saline suspension

Normal Saline is used to provide proper osmotic pressure to organism‟s otherwise high salt concentration is inhibitory for organisms. Organisms are kept in screw cap bottles in normal saline and stored at room temperature, wherever required transfer is made on agar slats and incubated.

Methods where organisms are in Suspended metabolic state Organisms are preserved in suspended metabolic state either by drying or storing at low temperature. Microbes when dried or kept at low temperature care should be taken so that their revival is possible.

- **Drying in vacuum**

  In this technique organisms are dried over chemical instead of air dry. Cells are passed over $CaCl_2$ in a vacuum and then stored in refrigerator. Organisms survive for longer period of time.

Lyophilization

Lyophilization is vacuum sublimation technique. Cells are grown in nutritive media

and then placed in small vial, which are then immersed in a mixture of dry ice and alcohol at -78^{o}C. These vials are immediately connected to a high-vacuum line, and when they are completely dried each vial is sealed under vacuum. This is most effective and widely used technique due to long time survival less opportunity for changes in characteristics of organisms and small storage area. Organisms can survive for period of 20 years or more.

Use of Liquid nitrogen

Culture of Microorganisms are grown in nutritive media and then frozen with Cryoprotective agents like Glycerol and Dimethyl Sulfoxide. Frozen culture is kept in liquid Nitrogen refrigerator. Organisms can remain alive for longer period of time.

- **Storage in silica gel**

Both bacteria and yeast can be stored by this method. By this technique organisms can survive for 1 – 2 years. Finely Powdered Heat sterilized Silica powder is mixed with thick suspension of cell at low temperature.

Note:

- Cells should be harvested when actively growing (mid logarithmic phase)
- One method may be used for few organisms or specific organism; all the organisms cannot be preserved by any one technique mentioned above.

Quality control of the preserved stock culture

Whichever technique is used for the preservation and maintenance of industrially important organisms it is essential to check the quality of the preserved organisms stocks. Each batch of newly preserved cultures should be routinely checked to ensure their quality. A single colony is transferred into a shake-flask to ensure growth of particular kind of microorganism; further shake-flask subculture is used for the preparation of huge quantity of vials. For the assessment of purity, viability and productivity of cultures few vials are tested. If samples fail any one of these tests the entire batch should be destroyed. Thus, by the use of such a quality- control system stock cultures many be retained, and used, with confidence.

Table 4.4: Examples of International Depository Authorities for the deposition of cultures for patent purposes under the Budapest Treaty

American Type Culture Collection (ATCC) Rockville, Maryland, USA	Algae, pathogenic and nonpathogenic bacteria, protozoa, fungi, phages, plasmids, cell lines, hybridomas, animal and plant viruses
Northern Regional Research Lab. NRRL) Peoria, Illinois,USA	Fungi and bacteria that can be freeze-dried (no human or plant pathogens)
Centraalbureau voor Schimmelcultures (CBS) Baarn, Netherlands	Fungi and actinomycetes
Deutsche Sammlung von Mikroorganismen (DSM) Braunschweig, Federal Republic of Germany	Bacteria, fungi, phages (no human pathogens)
Fermentation Research Institute Ibaragi, Japan	Fungi, bacteria (no human pathogens)
National Collection of Yeast Cultures (NCYC) Norwich, England, UK	Yeasts that can be freeze-dried (no pathogens)
National Collections of Industrial and Marine Bacteria (NCIMB) Aberdeen, Scotland, UK	Bacteria and phages (no pathogens)
National Collection of Type Cultures (NCTC) London, England, UK	Bacteria pathogenic to man or animals
The Culture Centre of Algae and Protozoa (CCAP) Cambridge, England, UK	Algae (but not large seaweeds), free living and parasitic protozoa (no pathogens)
Culture Collection of Commonwealth Mycological Institute (CMI), Kew, England, UK	Fungi (no human pathogens)

Inoculum preparation

Industrial fermentations utilizing yeasts are the brewing of beer, the production of Baker‟s Yeast (biomass) and recent processes have also been established for the production of recombinant products.

Brewing

- Yeast can be used to inoculate a fresh batch of wort from previous fermentation or from propagator.
- It is common practice in the British brewing industry to use the yeast from the previous

fermentation.

- The brewing terms used to describe this process and 'crop', referring to the harvested yeast from the previous fermentation, and 'pitch', meaning to inoculate.
- One of the major factors contributing to the continuation of this practice is the wort-based excise laws in the United Kingdom where duty is charged on the sugar consumed rather than the alcohol produced.
- Thus, dedicated yeast propagation systems are expensive to operate because duty is charged on the sugar consumed by the yeast during growth.
- The problems with this technique are chances of contamination and degeneration of strains, the most common problem with the degenerated cell is the change in the degree of flocculence and weakening of abilities of the yeast.
- In breweries employing top fermentations in open fermenters these dangers are minimized by collecting yeast to be used for future pitching from 'middle skimmings"'.
- As the head of yeast develops, the surface layer (the most flocculent and highly contaminated yeasts) is removed and discarded and the underlying cells (the 'middle skimmings') are harvested and used for subsequent pitching.
- Therefore, the 'middle skimmings' contain cells which have the desired flocculence and which have been protected from contamination by the surface layer of the yeast head.
- The pitching yeast may be treated to reduce the level of contaminating bacteria and remove protein and dead yeast cells by such treatments as reducing the pH of the slurry to 2.5 to 3, washing with water, washing with ammonium persulphate and treatment with antibiotics such as polymixin, penicillin and neomycin.
- However, traditional open vessels are becoming rare and the bulk of beer is brewed using cylindro-conical fermenters.
- In these systems the yeast flocculates and collects in the cone at the bottom of the fermenter where it is subject to the stresses of nutrient starvation, high ethanol concentration, low water activity, high carbon dioxide concentration and high pressure, which decreases the viability and physiological state of the yeast crop, would not be ideal for an inoculum.
- The situation is further complicated by the fact that the harvested yeast is stored rapidly to about 1°, before it is used as inoculum suspending in beer and storing in the absence of oxygen.
- One of the key physiological features of yeast inoculum is the level of sterol in the

cells. Sterols are required for membrane synthesis but they are only produced in the presence of oxygen.

- Thus, we have the irregularity of oxygen being required for sterol synthesis; yet anaerobic conditions are required for ethanol production.
- This irregularity is resolved traditionally by aerating the wort before inoculation.
- The difficulties outlined above and the likelihood of strain degeneration and contamination mean that are rarely used for more than five to ten consecutive fermentations which necessitates the periodical production of a pure inoculum.
- Pure inocula can be prepared by a yeast propagation scheme utilizing a 10% inoculum volume at each stage in the programme and employing conditions similar to those used during brewing.
- Continuous aeration may be used during the propagation stage which seems to have little effect on the beer produced in the subsequent fermentation.
- Yeast inoculum produced in this way would also be sterol rich, obviating the need for aerated wort.
- The simplest type of propagator is a single stage system resembling an unstirred, aerated fermenter which is inoculated with a shake-flask culture developed from a single colony.
- Two-stage systems propagator could be operated semi- continuously. It consisted of two linked vessels, 1.5 and 150 dm^3 respectively.
- The smaller vessel is filled with wort, sterilized, cooled, aerated and inoculated with a flask-grown culture. After growth for 3 to 4 days the culture was forced by air pressure into the second vessel which had been filled with sterilized, cooled wort and aerated.
- An aliquot of 1.5 dm^3 was forced back into the first vessel after mixing. In a further 3 to 4 days the larger vessel contained sufficient biomass to pitch a 1000 dm^3 fermenter and the first vessel contained sufficient inoculum for another second stage.
- However, although this procedure should produce a pure inoculum there is a danger of strain degeneration occurring in such a semi- continuous system.
- **Baker's Yeast**
- The commercial production of bakers' yeast involves the development of an inoculum through a large number of aerobic stages.
- Although the production stages of the process may not be operated under strictly aseptic conditions a pure culture is used for the initial inoculum, thereby keeping

contamination to a minimum in the early stages of growth.

- The development of inoculum for the production of bakers' yeast involve eight stages, the first three being aseptic while the remaining stages were carried out in open vessels.
- The yeast may be pumped from one stage to the next or the seed cultures may be centrifuged and washed before transfer, which reduces the level of contamination.
- The yields obtained in the first five stages are relatively low because they are not fed-batch systems, whereas the last three stages are fed-batch.

The Development of Inocula for Bacterial Processes Introduction

- The main objective of inoculum development for traditional bacterial fermentations is to decrease lag phase.
- A long lag phase is not only is wastage of time but also medium is consumed in maintaining a viable culture prior to growth.
- The length of the lag phase is affected by the size of the inoculum and its physiological condition.
- Bacterial inocula should be transferred, when the cells are still metabolically active.
- The age of the inoculum is particularly important in the growth of sporulating bacteria, for sporulation is induced at the end of the logarithmic phase and the use of an inoculum containing a high percentage of spores would result in a long lag phase in a successive fermentation.
- 5% inoculum of thermophilic Bacillus in logarithm phase is used for the commercial production of proteases.
- A two-stage inoculum development programme is used for the production of proteases by Bacillus subtilis. Inoculum for a seed fermenter was grown for 1 to 2 days on a solid or liquid medium and then transferred to a seed vessel where the organism was allowed to grow for a further ten generations before transfer to the production stage.
- The lag phase in plant fermenters could be almost completely eliminated by using inoculum medium of the same composition as used in the production fermenter and employing large inocula of actively growing seed cultures in the production of bacterial enzymes.
- The inoculum development programme for a pilot-plant scale process for the production of vitamin B12 from ***Pseudomonas denitrificans*** is shown below (Spalla et

al., 1989).

STOCK CULTURE

Lyophilised with skim milk

↓ MAINTENANCE CULTURE

Agar slope incubated 4 days at 28°

↓

SEED CULTURE - FIRST STAGE

2 dm^3 flask containing 0.6 dm^3 medium inoculated with culture from one slope; incubated with shaking for 48h at 28°

↓

SEED CULTURE - SECOND STAGE

40 - 80 dm^3 fermenter containing 25 - 50 dm^3 medium inoculated with 1 - 1.2% first stage seed culture. Incubated 25 - 30h at 32°

↓

PRODUCTION CULTURE

- 500 dm^3 fermenter with 300 dm^3 medium inoculated with 5% second stage seed culture. Incubated at 32° for 140 - 160 h
- The acetic-acid bacteria used in the vinegar process are extremely sensitive to oxygen starvation therefore it is essential to use an inoculum in an active physiological state.
- The cells at the end of fermentation are used as inoculum for the next batch by removing approximately 60% of the culture and restoring the original level with fresh medium.
- In this process there are enough chances of strain degeneration and contaminant accumulation.
- However, strain stability is a major concern in inoculum development for fermentations employing recombinant bacteria.
- Plasmid stability and productivity in ***E.coli*** biotin fermentation was improved if stationary, rather than exponential phase, cells were used as inoculum due to loss of plasmid in fermentation.

- In the lactic-acid fermentation the producing organism may be inhibited by lactic acid. Thus, production of lactic acid in the seed fermentation may result in generation of poor quality inoculum.
- High quality inoculum of ***Lactococcus lactis*** 10^{-1} on a laboratory scale is obtained using electrodialysis which reduced the lactate in the inoculum and reduced the length of the lag phase in the production fermentation.

Development of Inocula for Anaerobic Bacterial Processes

- Clostridial Acetone-Butanol fermentation is anaerobic process.
- Though the process was outcompeted by the petrochemical industry but there is still considerable interest in reestablishing the fermentation.
- The inoculum development programme described by McNeil and Kristiansen (1986) is given as below

Heat-shocked spore suspension inoculated into 150 cm^3 of
potato glucose medium
↓
Stage 1 culture used as inoculum for 500 cm^3molasses medium
↓
Stage 2 culture used as inoculum for 9 dm^3 molasses medium
↓
Stage 3 culture used as inoculum for 90,000 dm^3 molasses medium

- The stock culture is heat shocked to stimulate spore germination and to eliminate the weaker spores.
- The production stage is inoculated with a very low volume.
- The use of such small inocula necessitates the achievement of as near perfect conditions as possible to prevent contamination and to avoid an abnormally long lag phase.

Batch and Continuous sterilization of medium

Media may be sterilized by

1) Filtration,
2) Radiation,
3) Ultrasonic treatment,
4) Chemical treatment
5) Heat

6) Out of these methods, heat or steam is the most useful method for the sterilization of fermentation media.

A number of factors influence the success of heat sterilization

1. The number and types of microorganisms present
2. The composition of the culture medium
3. The pH value and the size of the suspended particle

Filtration is used for the sterilization of medium which is exception for the medium containing heat labile components

Sterilization process i.e. killing of microbes by steam under pressure is a first-order chemical reaction and, thus, may be written as

$$-dN/dt = kN$$

Where

N is the number of viable organisms present,

t is sterilization treatment time,

K is the reaction rate constant of the reaction, or the specific death rate

regardless of the volume of the batch, the minimum number of organisms to contaminate a batch is one.

On integration of equation (5.1) the following expression is obtained….

$$N_t / N_o = e^{-kt}$$

N_o is the number of viable organisms present initially

N_t is the number of viable organisms present after a period treatment, t

On taking natural logarithms, equation (5.2) is reduced to

$$\ln(N_t / N_o) = -kt \quad kt = \ln (N_0 / Nt)$$

A plot of the natural logarithm of N_t / N_o against time yields a straight line, the slope of which equals $-k$

This kinetic description makes two predictions which appear abnormal

1. Sterile condition is achieved in An infinite time (i.e. $N_t = 0$)
2. After a definite time number of cells present will be less than one

The relationship displayed and observed only for pure culture in certain metabolic state, under ideal sterilization conditions

The value of k also indicates physiological state of organisms with kind of species, but dependent on the physiological form of the cell; for example, the endospores of the genus Bacillus are far more heat resistant than the vegetative cells

Richards (1968) produced a various graphs illustrating the deviation from theory which may be experienced in practice

The deviation in the above graph is due to the induction of spore germination by the heat and moisture of the initial period of the sterilization process.

Time for the sterilization is dependent on the type of the population. If the sensitive organisms are more in number than whole culture sterilization will be equal to that of the sensitive culture. But if the number of the resistant organisms is more than the sterilization of whole culture is equal to that of the sterilization of the resistant organisms.

Now by considering that contaminant may be by not a single type of organism but by different types of organisms. Sterilization of media required destruction of all types of organisms. The destruction of organisms in sterilization process is given by the factor called Del factor.

As first order reaction, as temperature increases, reaction rate increases due to an increase in the reaction rate constant, which, in case of the destruction of microorganisms is the specific death rate (k)? Thus k is true constant only under constant temperature conditions.

The relationship between temperature and reaction rate constant was demonstrated by Arhenius and may be represented by the equation

$$\mathbf{D \ln k = E \, dT \, RT^2}$$

E – Activation energy R – gas constant

T – Absolute temperature On Integration

$K = A. e^{-E/RT}$

Therefore $kt = A. t. e^{-E/RT}$ On taking natural

logarithm $\ln k = \ln A - E/RT$

Plot of ln k versus 1/T gives straight line

Such a plot is Arhenius plot and enable calculation of activation energy and prediction of the reaction rate for any temperature.

Now $\ln Nt/No = -kt$ So, $\ln No/Nt = kt$

Therefore $\ln No/Nt = A. t. e^{-E/RT}$

Deindoerfer and Humphrey (1959) used the term In N_o / N_t as a design criterion for sterilization, which has been variously called the Del factor, Nabla factor and sterilization criterion represented by the term ▼

Thus, the Del factor gives idea about fractional reduction in viable organism count produced by a definite heat in particular time

Now ▼= ln No/Nt

But from above equation ln No/Nt = A. t. $e^{-E/RT}$

Thus ▼= A. t. $e^{-E/RT}$ On rearranging

equation ln t = E/RT + ln (▼/A)

This graph is used to obtain definite ▼ value with certain absolute temperature.

If we plot the graph from above line equation then we will get idea about time and absolute temperature required to achieve sterilization.

According to Deindoerfer and Humphrey, Richards Banks and Corbett a risk factor of one batch in a thousand being contaminated is frequently used in fermentation industry – that is, the final microbial count in the medium after sterilization should be 10^{-3} viable cells.

To apply kinetics it is necessary to know the thermal death characteristics of all the taxa contaminating the fermenter and unsterile medium, this is an impossibile and, therefore, the assumption may be made that the only microbial contaminants present are spores of *Bacillus stearothelmophilus* - that is, one of the most heat-resistant microbial types known

Thus, by adopting *B. stearothennophilus* as the design organism a considerable safety factor should be built into the calculations

It should be remembered that *B. stearothennophilus* is not always adopted as the design organism

If the most heat-resistant organism contaminating the medium ingredients is known, then it may be advantageous to base the sterilization process on this organism

The initial rise in yield is due to some components of the medium being made more available to the process micro-organism by the 'cooking effect' of a brief sterilization period (Richards, 1966).

Reaction contributing the decrease in nutrient value during sterilization

Interactions between nutrient components of the medium

- Maillard-type browning reaction discoloration of the medium as well as deterioration of nutrient value caused by the reaction of carbonyl groups and amino groups from reducing sugars, and amino acids and proteins respectively

Degradation of heat labile components

- Certain vitamins, amino acids and proteins may be degraded during a steam

sterilization regime

- Thus heat labile compounds can be sterilized by filtration
- However, for the vast majority of fermentations these problems may be resolved by the judicious choice of steam sterilization regime

The activation energy for thermal destruction of *Bacillus steareothermophilus* spores is more than for thermal destruction of nutrient.

Thus it would be advantageous to employ high temperature for shorter period of time to achieve desired probability of sterility, yet causing minimum degradation of nutrients.

Batch sterilization is not possible as high temperature cannot be kept for short period of time by this method thus only solution to this problem is continuous stream sterilization

Advantages of continuous sterilization over batch sterilization

Better protection of medium value

1. Ease of scale-up - discussed later
2. Easier automatic control
3. The decrease of flow ability for steam
4. The reduction of sterilization cycle time
5. Under certain conditions, the decrease in corrosion of fermentor

Advantages of batch sterilization over continuous sterilization

1. Lesser assets apparatus expenditure
2. Less chance of contamination - processes require the aseptic inoculums transfer of the sterile broth to the sterile vessel
3. Easier manual control
4. Easier to use with media having a high amount of solid material

The Design of Batch Sterilization Processes

- Main aim of batch sterilization process is still to attain the necessary chance of getting sterility with the least change in nutrient value of the medium
- Continuous sterilization process is better than batch sterilization process in avoiding the damage of nutrients than a continuous sterilization process
- The maximum temperature which is possible in batch sterilization is 121°C therefore a method should be adopted so that medium is exposed to this high temperature for a short period of time

- High temperature and short time sterilization is attained by taking into consideration the heating and cooling time of the batch sterilization

  Deindoerfer and Humphrey (1959) offered a method to evaluate the role of heating and cooling periods in sterilization process

  The following point should taken into consideration for a batch sterilization process

1. How much temperature of the fermentation medium is increased during heating or decreased during cooling periods of the batch sterilization
2. The initially number of micro-organisms in the medium
3. The thermal death rate of the selected organism

   Requisite Del factor can be designed by knowing the initial number of organisms in the medium and the danger of contamination.

   Commonly accepted threat of contamination is 1 in 1000, which means number of living organisms after time t is 0.001

   For example if any unsterile broth contain 10^{11} number of cells then Del factor for that situation is 32.2

- However, the killing of cells take place during both the heating period and cooling period of the sterilization process in addition to during holding period at 121°C
- So, Del factor can be
- **▼overall = ▼heating + ▼holding + ▼cooling**
- Knowing the temperature and time required to reach that temperature during heating period and cooling period of sterilization process it is possible to determine the overall Del factor by these periods
- Thus, from the Del factors contributed by heating and cooling periods, it is possible to estimate the holding time that may be required for overall Del factor
- **Batch sterilization Methods**
- The batch sterilization of the medium for a fermentation may be achieved either in the fermentation vessel or in a separate mash cooker
- Richards (1966) considered the relative merits of *in situ* medium sterilization and the use of a special vessel

Advantages of a separate medium sterilization vessel

- The medium may be sterilized in a cooker in a more concentrated form than would be used in the fermentation and then diluted in the fermenter with sterile water prior to inoculation. This would allow the construction of smaller cookers

- One cooker may be used to serve several fermenters and the medium may be sterilized as the fermenters are being cleaned and prepared for the next fermentation, thus saving time between fermentations
- In some fermentation, the medium is at its most viscous during sterilization and the power requirement for agitation is not alleviated (lessen) by aeration. Fermenter equipped with a powerful motor would provide sterile medium for several fermenters. The fermenter would be spared the corrosion which may occur with medium at high temperature.

Disadvantages of a separate medium sterilization vessel

- The cost of constructing a batch medium sterilizer is much the same as that for the fermenter
- If a cooker serves a large number of fermenters complex pipe work would be necessary to transport the sterile medium, with the inherent dangers of contamination
- Mechanical failure in a cooker supplying medium to several fermenters would render all the fermenters temporarily redundant (unneeded). The provision of contingency equipment may be prohibitively costly

The design of continuous sterilization process

- The plan of continuous sterilization system may be advance precisely the same as batch sterilization systems
- In continuous sterilization medium is heated to reach to the sterilization temperature (121^oC), holding this temperature to particular period of time and then cooling the medium to reach to the temperature of the fermentation process
- The temperature of the medium is increased in a continuous heat exchanger and is maintained for the holding time in an shielding winding holding coil
- The extent of the holding time is stated by the coil length and the medium stream speed
- The medium after holding time is cooled to the temperature required for fermentation using two chronological heat exchangers
    - the first using the coming medium as the cooling source and the second using water
- In continuous process high temperature is used which reduce the holding time and nutrient loss

- The necessary Del factor required may be attained by the proper temperature and holding time which decrease the amount of nutrient loss
- Additionally, a continuous process engage heating of small amount of medium and cooling of small amount of medium which is very less in contrast with batch system

1. There are two types of continuous sterilizer:
2. The indirect heat exchanger
3. The direct heat exchanger (steam injector)

Continuous Sterilizer

A. Indirect Heat Exchanger Double spiral type

The most appropriate indirect heat exchanger is double-spiral type

- consist of two sheets of high-grade stainless steel, they are mould around central axis in such a way that they form a double spiral, as shown in Fig. 5.8.

- Steam and Medium is passed through two different plates in opposite direction to attain sterilization temperature
- This sterilizer is also use for cooling of the medium after proper holding time
- Incoming unsterile medium is partially heated which itself is a cooling agent for medium which is there in the sterilizer
- The major advantages of the spiral heat exchanger are

1. There is less chances of contamination between medium and liquid used for cooling or steam, as they are separately moving in a compartment formed by stainless steel plates with gasket seals at the end of the plates.
2. Exchanger will be cleaned by the steam or the liquid used for cooling and continues movement of the media, so that there is less chances of sedimentation, fouling and „burning on“

- Alternate plates of heat exchanger allow two different liquid or steam to circulate through them in opposite direction
- Two plates are divided by gasket, problem with this gasket can lead to cross contamination of various streams
- This sterilizer is useful for completely soluble media as any suspended solids can block the plates
- Ability of this Sterilizer can be increased by adding extra plates to it
- In continuous stem injector, steam can be directly injected into the unsterile broth

Advantages Indirect heat exchanger

(i) Immediate) heating up times

(ii) Media containing solids can be sterilized by this exchanger

(iii) Less investment

(iv) Easy to maintain and clean

(v) Efficient in using steam

Disadvantages Indirect heat exchanger

(i) Heating may cause foams

(ii) Steam is in direct contact with medium, so medium should be enough concentrated and steam should be free from any agent responsible for anticorrosion

B. Direct Heat Exchanger

- In direct heat exchanger medium is heated with the help of steam and cooled by sudden expansion of the medium in vacuum compartment
- Cooling happens almost instantaneously
    - Hot water is passed through system for the sterilization of the plant before sterilization of the medium
- Steam is used to sterilize pipe work and fermentor
- Heat is conserved by using incoming media which will cool sterile medium, which in turn get preheated before reaching the sterilizer **Advantages of continuous steam injector**

1. very short heating up time
2. it may be used for media containing suspended solids
3. low capital cost
4. easy cleaning and maintenance
5. high seam utilization efficiency

Disadvantages of continuous seam injector

1. foaming may occur during heating
2. direct contact of medium with steam require that allowance be made for condensate dilution and require „clean" steam, free from anticorrosion additives

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UNIT – II - FERMENTATION AND DOWNSTREAM PROCESSING
SBTA5203

GROWTH KINETICS

The growth of a microbial culture can be divided into major four phases. These are.

- Lag phase
- Log phase
- Stationary phase
- Death phase

1. Lag phase

- Once the inoculation of the cells into fresh medium is done, the bacterial population remains temporarily unchanged
- There is no cell division during this phase
- The cells grow in volume and mass by synthesizing the population remains temporarily unchanged etc.
- Metabolic activity is at high rate
- This period is known as the period of adaptation
- **T**here are various factors that affects the this phase are size of inoculum, time required to recover shock in the transfer, time required for synthesizing essential coenzymes and other factors
- Time required for synthesis of necessary new enzymes to metabolize the substrates present in the medium

2. Phase of exponential growth

- This period is also known as the phase of exponential growth
- During this period, the growth rate of the cells gradually increases
- The cells grow at a constant, maximum rate
- Cells are growing in geometric progression dividing by binary fission
- The incubation conditions and composition of the growth medium control the rate of cell division

3. Stationary phase

- During this phase growth cease
- In a batch culture (in test tube or EM flask) , exponential growth cannot be continued forever
- Various factors like exhaustion of available nutrients, accumulation of inhibitory, metabolites or end products and lack of biological space limit the growth during this phase

- During this phase the number of dividing cells equals the number of dyeing cells
- This is not a quiescence period like lag

4. Death phase

- This phase is the reverse of the log phase
- The viable cell population declines exponentially during this phase

Base on the various products produced, the phases of bacterial growth can be categorized into two phases. These are

(i) The Trophophase

(ii) The Idiophase

1. Tropho phase

- Metabolites which are essential to the growth of the cells like amino acids, nucleotides, proteins, nucleic acids, lipids, carbohydrates are produced during the log phase of the growth
- The products (metabolites) produced during this phase (log phase) are known as **primary metabolites** and the phase in which they are produced(equivalent to the log, or exponential phase) is referred to as the ***trophophase*** (Bu'Lock *et al.,* 1965)
- The primary metabolites are also known as central metabolites
- Several primary metabolites are of economic importance and can be produced in large quantity by fermentation process
- The synthesis of primary metabolites by wild-type micro- organisms aims to meet the requirements of the organism
- The industrial production these metabolites can be achieve by providing appropriate cultural conditions to the wild-type organism to increase and improve the productivity of these compounds
- Productivity can also be improve by modifying interested genes by the help of recombinant DNA technology
- Following are few economically important primary metabolites which can be produced at large scale

Idiophase

- During the stationary phases several microbial cultures produce certain compounds (these compunds are not produced during the "***trophophase"*** and which do not appear to have any obvious function in cell metabolism). These compounds are called the

secondary compounds of metabolism. The phase during which these compounds are produced (equivalent to the stationary phase) as the "***idiophase*"** (Bu'Lock et al., 1965)

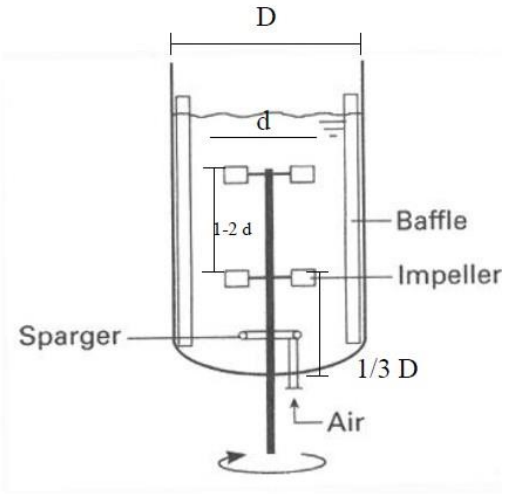
- The secondary metabolism is also known as "**special metabolism**"
- The products of secondary metabolism are not absolutely required for the survival of the organisms
- All microorganisms do not undergo secondary metabolism. It is common amongst the filamentous bacteria and fungi and the spore forming bacteria
- The taxonomic distribution of secondary metabolism is different from that of primary metabolism
- The physiological role of secondary metabolism and hence secondary metabolites in the producer cells has been the subject of considerable debate
- The large scale production of secondary metabolites focus on the importance of these metabolites on organisms other than those that produce them
- Secondary metabolites play an important physiological role several ways. Many secondary metabolites possess antimicrobial activity, some acts as specific enzyme inhibitors and growth promoters and many have pharmacological properties
- Thus, due to a huge economic potential, the industrial production of these metabolites have formed the basis of a number of fermentation processes
- As the wild-type microorganisms produce very low concentrations of secondary metabolites, the large scale synthesis can be controlled by induction, catabolite repression and feed-back systems
- Following is the outline of inter-relationships between primary and secondary metabolism and their respective products.

Type of operation	Advantages	Disadvantages
Batch	**Versatile**: can be used for different reaction everyday **Safe**: can be properly sterilised. Little risk of infection or strain mutation. Complete conversion of substrate is possible	**High labour cost**: skilled labour is required **Much idle time**: Sterilisation, growth of inoculum, cleaning after fermentation **Safety problem**: when filling, emptying, cleaning
Continuous, steady-state (chemostat)	**Works all the time**: low labour cost, good utilisation of reactor **Often efficient**: due to the autocatalytic nature of microbial reactions, the productivity can be high. Automation may be very appealing. Constant product quality.	**Often disappointing**: promised continuous production for months fails due to (a) infection, e.g. a short interruption of the continuous feed sterilisation. (b) spontaneous mutation of microorganism to non producing strain. **Very inflexible**: can rarely be used for other productions without substantial retrofitting. **Downstream**: all the downstream process equipment must be designed for low volumetric rate, continuous operation.
Semi-batch (fed-batch)	Combines the advantages of batch and continuous operation. Excellent for control and optimisation of a given production criterion.	Some of the advantages of both batch and continuous operation but the advantages far outweigh the disadvantages, and fed-batch is used to produce both biomass (baker's yeast) and important secondary metabolites (e.g. penicillin).

I. Bioreactor configurations

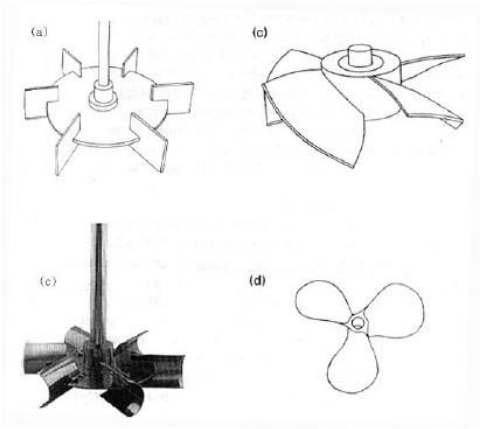
Stirred tank reactors

Features:



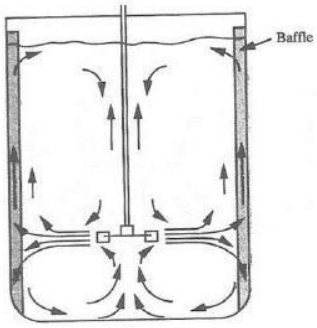
- Microbial reactors have impellers to provide agitation and generally have 4 baffles from the walls to prevent vortexing of the fluid, the baffle width is 1/10 or 1/12 of the tank diameter.
- The vortex and circular flow result in little mixing between fluids at different heights. At high speeds the vortex may reach down to the impeller so that gas from the surrounding is drawn into the liquid➔high mechanical stress in the stirrer shaft, bearings and seal.
- Bioreactors for animal cell cultures usually do not have baffles (especially for small scale reactors) to reduce turbulence.
- The aspect ratio (height-to-diameter ratio) of the vessel is 3-5 for microbial cultures but is normally less than 2 for animal cell culture.
- Sparger: gas is sparged at the bottom using a perforated pipe ring sparger.

- Number of impellers depends on the aspect ratio. The bottom impeller is located at a distance about 1/3 of the tank diameter above the bottom of the tank. Additional impellers are spaced approximately 1 to 2 impeller diameter (d) distances apart.
- The superficial aeration velocity (the volume flow rates of gas divided by the cross-sectional area of the vessel) in stirred vessel must be lower than that can flood the impeller (an impeller is flooded when it receives more gas than it can effectively disperse) otherwise the mixing is poor. Superficial aeration velocities generally do not exceed 0.05 m/s.
- Impellers: choice often depends on the viscosity of the liquid and sensitivity of the cells to mechanical shear.
  - Rushton (6-flat-blade) disc turbine (a) and concave bladed impeller (b): impeller diameter is about 1/3 of the vessel diameter and is often used for bacterial cultures. Rushton turbine is most commonly used in fermentation technology.
  - Hydrofoil impeller (c): diameter is about 0.5 to 0.6 times the tank diameter and is an effective mixer for highly viscous mycelial broths.

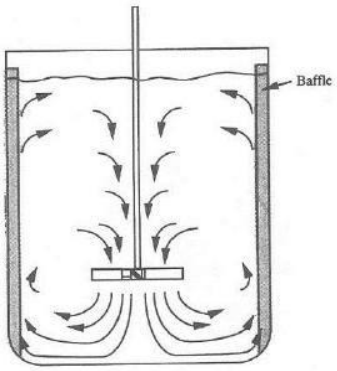


broths.

- Marine impeller (d): usually single, large diameter, low shear, used for animal cell culture.



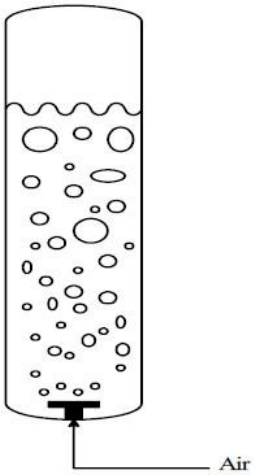
Flow pattern of Rushton turbine



Flow pattern of marine impeller (promotes axial flow)

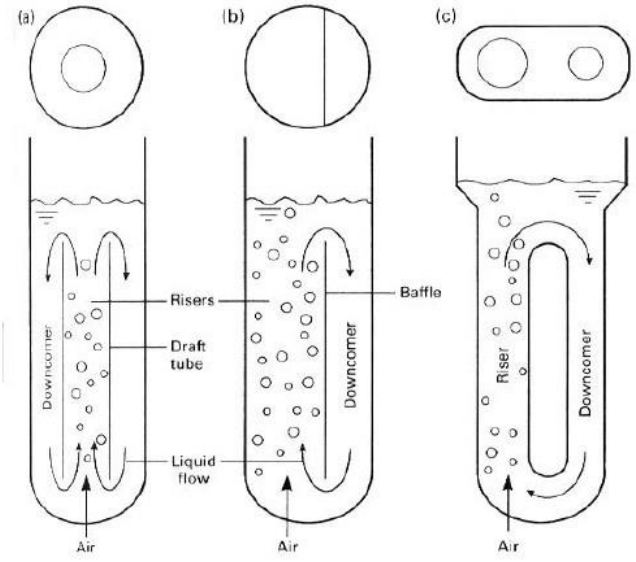
- Impeller speed:
  - Usually <120 rpm for animal cell cultures even for vessels >50 liters. Higher stirring speeds can be used for microbial cultures.
  - The impeller tip speed (3.14 × impeller diameter × speed of rotation) is usually less than 7.6 m/s for filamentous fungi.

Bubble column reactor



- Usually the height-to-diameter ratio is 4-6.
- Gas is sparged at the base through perforated pipes or plates or metal porous spargers.
- O_2 transfer, mixing and other performance factors are influenced mainly by gas flow rate and rheological properties of the fluid.
- Mixing and mass transfer can be improved by placing perforated plates or vertical baffles in the vessel.

Airlift bioreactor

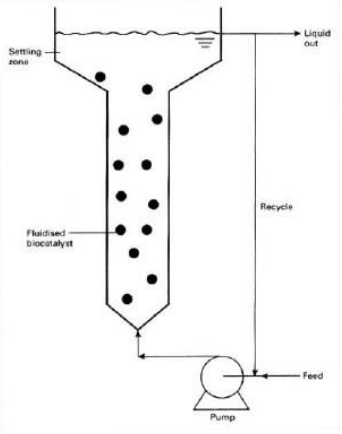


- Separated as two zones: the sparged zone is called the riser, and the zone that receives no gas is the downcomer. The bulk density in the riser region is lower than that in the downcomer region, causing the circulation (so circulation is enhanced if there is little or no gas in the downcomer).
- For optimal mass transfer, the riser to downcomer cross-sectional area ratio should be between 1.8 and 4.3.
- Highly energy efficient and productivities are comparable to those of stirred tank bioreactors.
- The rate of liquid circulation increases with the square root of the height of the airlift device. Consequently, the reactors are designed with high aspect ratios.
- A gas-liquid separator in the head-zone can reduce the gas carry-over to the downcomer and hence increase the liquid circulation.

Fluidized bed reactor

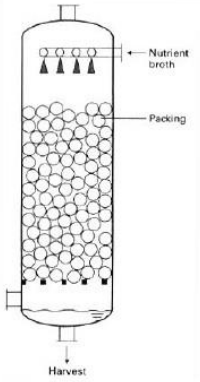
- Suitable for reactions involving a fluid-suspended particulate biocatalyst such as immobilized enzyme and cell particles

- Similar to the bubble column reactor except that the top section is expanded to reduce the superficial velocity of the fluidizing liquid to a level below that needed to keep the solids in suspension. Consequently, the solids sediment in the expanded zone and drop back, hence the solids are retained in the reactor whereas the liquid flows out.



Packed bed bioreactor

- A bed of particles are confined in the reactor. The biocatalyst (or cell) is immobilized on the solids which may be rigid or macroporous particles.
- A fluid containing nutrients flows through the bed to provide the needs of the immobilized biocatalyst. Metabolites and products are released into the fluid and removed in the outflow.
- The flow can be upward or downward. If upward fluid is used, the velocity can not exceed the minimum fluidization velocity.



II. Bioreactor design features

- Medium or feed nozzle (19).
- Vertical sight glass (15) and ports for pH, temperature and DO sensors (6).
- Connections for inoculum, acid and alkali (for pH control) and antifoam agents are located above the liquid level in the reactor vessel (16).
- O_2 and other gases (CO_2 or NH_3 for pH control; N_2 for O_2 control) can be

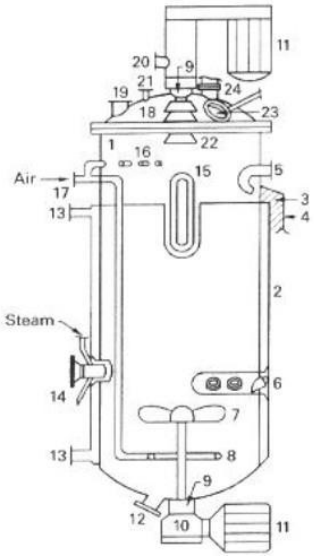


Fig. 7.7 A typical bioreactor: (1) reactor vessel; (2) jacket; (3) insulation; (4) shroud; (5) inoculum connection; (6) ports for pH, temperature and dissolved oxygen sensors; (7) agitator; (8) gas sparger; (9) mechanical seals; (10) reducing gearbox; (11) motor; (12) harvest nozzle; (13) jacket connections; (14) sample valve with steam connection; (15) sight glass; (16) connections for acid, alkali and antifoam chemicals; (17) air inlet; (18) removable top; (19) medium or feed nozzle; (20) air exhaust nozzle; (21) instrument ports (several); (22) foam breaker; (23) sight glass with light (not shown) and steam connection; (24) rupture disc nozzle.

introduced through a sparger at the bottom (8).

- Foam breaker (22) is used when antifoam is ineffective or the antifoam interferes with downstream processing (antifoam tends to foul the membrane during filtration).
- Can be sterilized in-place using saturated steam (14) at a minimum absolute pressure of 212 kPa. Over–pressure protection is provided by a rupture disc (24) on the top of the reactor, which cracks to relieve the pressure to avoid explosion.
- Maximum allowable working pressure is ≈377-412 kPa (absolute)[1], allowable temperature is usually 150-180°C (>121°C for sterilization). The vessel should withstand full vacuum or it could collapse while cooling after sterilization.

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UNIT – III - FERMENTATION AND DOWNSTREAM PROCESSING
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1.0 INTRODUCTION

The widespread use of advanced control and process automation for biochemical applications has been lagging as compared with industries such as refining and petrochemicals whose feedstocks are relatively easy to characterize and whose chemistry is well understood and whose measure- ments are relatively straightforward.

Biological processes are extraordinarily complex and subject to con- siderable variability. The reaction kinetics cannot be completely detemiined in advance in a femientation process because of variations in the biological properties of the inoculant. Therefore, information regarding the activity of the process must be gathered as the femientation progresses. Directly measuring all the necessary variables which characterize and govern the competing biochemical reactions, even under optimum laboratory condi- tions, is not yet achievable. Developing mathematical models which can be utilized to infer the biological processes underway from the measurements available, although useful, is still not sufficiently accurate. Add to this the constraints and compromises imposed by the manufacturing process and the task of accurately predicting and controlling the behavior of biological production processes is fomiidable indeed.

The knowledge base in fermentation and biotechnology has expanded at an explosive rate in the past twenty -five years aided in pan by the development of sophisticated measurement, analysis and control technology. Much of this research and technology development has progressed to the point where commercialization of many of these products is currently' undevout.

The intent of this chapter is to survey some of the more innovative measurement and control instrumentation and systems available as well as to review the more traditional measurement, control and information analysis technologies currently in use.

2.0 MEASUREMENT TECHNOLOGY

Measurements are the key to understanding and therefore controlling any process. As it relates to biochemical! engineering, measurement technology can be

separated into three broad categories. Those arc biological, such as ccll grout rate, florcscenco, and protein sjmthcsis ratc; chemical, such as glucose concentration. dissolved ox gen. pH and offgas concentrations of CO_2› OF. N_2 ethanol, ammonia and vaoous other organic substanccs; and physical, such as temperature, level, pressure, how rate and mass. The most prevalent are the physical sensors while the most promising for the field of biotechnology are the biological sensors.

One concern ashen considering measuring biological processes is thc maintenance of a sterile environment. This is necessary to prevent foreign organisms from contaminating the process. In-line measurement devices must conform to the AAA Sanitaq Standards specify'ing the extcrior surface and materials of construction for the "whetted parts." Instruments must also be able to withstand steam sterilization is'hich is needed periodically to prevent bacterial buildup. Devices located in process lines should bc Ctted with sanitary connections to facilitate their removal during extensive ciean- in-placc and stcrilizc-in-place operations. Sample ports, used for the removal of a small portion of the contents fTom the bioreactor for analjsis in a laboratory, must be equipped with sterilization systems to ensure organisms are not inadvertentl›' introduced during the removal of a sample.

3.0 BIOSENSORS

Biosensors arc literally the fusing of biological substrates onto electric circuits These have long been envisioned as the next generation of analytical sensors measuring specific bimolecular interactions The basic principle is first to immobilize one of the interacting molecules, the ligand, onto an tries substrate such as a dextran matrix which is bonded (eovalently bound) to a metal surfacc such as gold or platinum. This reaction must then be convened into a measurable signal typically by taking advantage of some transducing phenomenon. Four popular transducing techniques are'

Potcntiometnc or amperometrie, where a chemical or biological reaction produces a potential difference or current flow across a pair of electrodes.

Ermwe thermistors, where the thermal effect of the chemical or biological reaction is transduced into an clcctrical resistance change.

Optoelectronic, where a chemical or biological reaction evokes a change in light transmission.

Electrochemically sensitive transistors whose signal de-
pends upon the chemical reactions underlay

One example is the research ' to produce a biomedical device which can bc implanted into a diabetic to control the flow of insulin by monitoring the glucose level in the blood via an electrochemical reaction. One implantable glucose sensor, designed by Leland Clark of thc Children's Hospital Research Center in Cleveland, utilizes a microprobe where the outside call is constructed of glucose-permeable membrane such as cuprophan. Inside, an enzyme which breaks the glucose doom to hydrogen peroxide is affixed to an into substrate The hydrogen peroxide then passes through an inner membrane, constructed of a material such as cellulose acetate, where it reacts with platinum producing a current 'which is used to monitor the glucose concentration.

A commercial example of a biosensor, introduced by Pharmacia Biosensor AB^2, is utilizing a photoelectric principle called *surface Plasmon resonance* (SPR) for detection of changes in concentration of macromolecule- lar reactants. This principle relates the energy transferred from photons bombarding a thin gold film at the resonant angle of incidence to electrons in the surface of the gold. This loss of energy results in a loss of reflected light at the resonant angle.

The resonant angle is affected by changes in the mass concentration in the vicinity of the metal's surface which is directly correlated to the binding and dissociation of interacting molecules.

Phannacia claims its BIA core system can provide information on the affinity, specificity, kinetics, multiple binding patterns, and cooperativity of a biochemical interaction on line 'without the need of washing, sample dilution or labeling of a secondary interactant Their scientists have mapped the epitope specificity patterns of thirty monoclonal antibodies (Mabs) against recombinant cote HIV-1 core protein.

4.0 CELL SIASS MEASUREMENT

The on-line direct measurement of cell mass concentration b5' using optical densiñ principles promises to dramatically improve the Wowledge ofthe mctabolic processes underway within a bioreactor . This measurement is most cffcctive on spherical cells such as fi. Colt. The measurement technology is packaged in a sterilizable stainless steel probe which is inserted directly into the bioreactor itself via a flange or quick-disconnect mounting (Fig. 1).

By comparing the mass overtime, cell growth rate can be determined This measurement can be used in conjunction ix'ith metabolic models which employ such

physiological parameters as oxygen uptake rate (OFF), carbon dioxide evolution rate (CER) and respirator' quotient (RQ) along with direct measurements such as dissolved oxygen concentration, pH, temperature, arid off gas analysis to more precisely control nutrient addition, aeration rate and agitation. Harvest time can be directly determined as can shifts in metabolic pathways possibly indicating the production of an undesirable by-product.

Cell mass concentrations of up to 100 grams per liter are directly measured using the optical densin probe. In this probe, light of a specific wavelength, created by laser diode or passing normal light through a sapphire crystal, enters a smple chamber containing a representative sample of the bioreactor booth and then passes to optical detection electronics. The density is determined by measuring the amount of light absorbed, compensating for backscatter Commercial versions such as those manufactured by Cerex, WGdg ood, and Monitoc are packaged as stainless steel probes that can be mounted directly into bioreactors ten liters or greater, and oiTer features such as sample debubblcrs to eliminate interference from entrained air.

Another technique used to determine cell density is spectrophotometric titration which is a laboratoq' procedure which employs the same basic principles as the probes discussed above. This requires a sample to be withdraw from the broth during reaction and therefore exposes the batch to contamination.

5.0 CHEMICAL COMPOSITION

The most widely used method for determining chemical composition is chromatography. Several categories have been developed depending upon the species being separated. These include gas chromatography and several varieties of liquid chromatography including low pressure (gel permeation) and high pressure liquid chromatography and thin layer chromatography. The basic principle behind these is the separation of the constituents traveling through a porous, sorptive material such as a silica gel, The de8ree of retardation of each molecular species is based on its particular afhnity for the sorbent. Proper selection of the sorbent is the most critical factor in determining separation. Other environmental factors such as temperature and pressure also play a key role. The chemical basis for separation may include adsorption, covalent bonding or pore size of the material.

Gas chromatography is used for gases and fof liquids o'ith relative1› lori boiling points. Since many of the constituents in a biochemical reaction are of cOnsiderable

molecular weight, high pressure liquid chromatography is the most commonly used. Specialized apparatus is needed for performing this analysis since chromatograph pressures can rangc as high as 10.000 psi.

Thin later chromatography requires no pressure but Instead rclies on the capillay action of a solvent through a paper-like sheet of sorbent Each constimenttravcls a different distanccand the constituents are thus separated Analysis is doric manuall›. Bpicall various coloring or fluorcscing reagcnts.

Gel pemcation chromatography utilizes a sorbcnt bed and depends on gravin to pro› idc the driwng force but usually requires a considerable time to effect a separation.

All of these analyses are ñpically performed in a laboratop ': thercforc they require the removal of samples. As the reaction is conducted in a stcrilc environment, special precautions and sample rcmoval procedures must be utilized to prevent contaminating the contcnts of the rcactor.

6.0 DISSOLVED OXYGEN

Dissolved oxygen is one ofthe most impoaant indicators in a fermen- tation or bioreactor process. It determines the potential for grosith The measurement of dissoh ed oxygen is made by a steri!izable probe inseoed directly into the aqueous solution ofthe rcactor Two principles of operation

In the amperomctric (polarographic) approach, oxygen again perme- ates a diffusion barrier and encounters an electrochemical cell immersed in basic aqueous solution A potential difference of approximately 1,3 V is maintained between the anode and cathode. As the oxygen encounters the cathode, an clcctrochemical reaction occurs:

$$0_s + 2H;O \quad 4c' \text{—+} \quad 4OH' \text{ (at cathode)}$$

The hydroxyl ion then travels to the anode where it completes the electro-chemical reaction process:

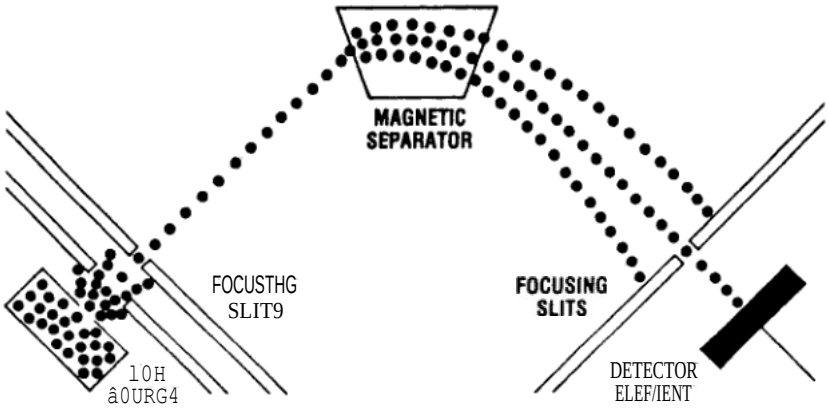
$$4OH' \text{—›} O_2 + 2H_2O + 4e \text{ (at anode)}$$

The concentration of oxygen is directly proportional to the amount of current passed through the cell.

7.0 EXHAUST GAS ANALYSIS

Much can be lcarncd front the exchange of gases in the metabolic process such as 0 2, CO_2 , N,, NH_3 , and ethanol In fact, most ofthe predicti› e analysis is based upon such calculations as oxygen uptake rate, carbon dio›ude exchange rate or respiratory' quotient This information is best
oblaincd by a component material balance across the reactor. A key factor in detcnnining this is thG analysis ofthe biorcactor offgas and the best method for measuring this is with a mass spectrometer because of its high resolution Two methods of operation are utilized. These are magnetic deflection and quadrapole. The quadrapole has become the primal commercial sj stem because of its enhanced sensitivity and its ability to filter out all gases but the one being analyzed.

Nfagnctic deflection mass spectrometers inject a gaseous sample into an inlct port, bombard the sample with an electron beam to iorrizc ihe particles and pass the sample through a magnetic separator. The charged particles are deflected by the magnet in accordance with its mass-to-energy (or charge) ratio-the greater this ratio, the less the deflection Detectors are located on the opposin8 wall of the chamber and are located to correspond to the trajectory of specific components as shorn in Fig. 3 As the ionized particles strike the detectors, they'generate a i oltage proportional to thcir charge This information is used to dctcrmine the percent concentration of each of the gasses.



Flgure 3. Magnetic deflection principle.

The quadrapole mass spectromcter also employs an electron beam to ionize the particles using the quadrapole instcad of a magnet to deflect the path of the particles and filter out all but the spccific componcnt to be analyzed. Thc quadrapole is a set of four sunilar and parallcl rods (scc Fig.

4) ss4th oppositc rods electrically connccted. A radio frequency and dc charge of equal potential, but opposite charge, is applied to each set ofthe rods. By varying the absolute potential applicd to the rods, it is possible to eliminate all ions except those of a specific mass-to-energy ratio Those ions which successfully travel the length ofthe rods strike a Faraday plate which releases electrons to the ions thercby gencrating a measurable change in EMF. For a given component the strength of the signal can be compared to references to determine the concentration.

The quadrapole. when used in conjunction is’ith a gas chromatograph to separate the components, can measure a n ide range ofgases, qpicallv from 50 to 1000 atomic mass units (amu)

As mass spectrometers are relati› eIy expensive, the exhaust gas of three or more bioreactors is qpicallj directed to a single analyzer. This is possible because the off gas analysis is done outside the bioreactors them- selves Hou’evcr, the multiplexing of the streams results in added complexity with regard to sample handling and routing,

particularly if concerns of cross contamination need be addressed. The contamination issue is usually handled by placing ultrafiltcrs in the exhaust lines, Care. ho»c›er, must be takcn to ensure that these filters don’t plug resulting in excessive backpressure. Pcriodic measurement calibration utilizing reference standards must be sent to thc spectrometer to chcck its calibration.

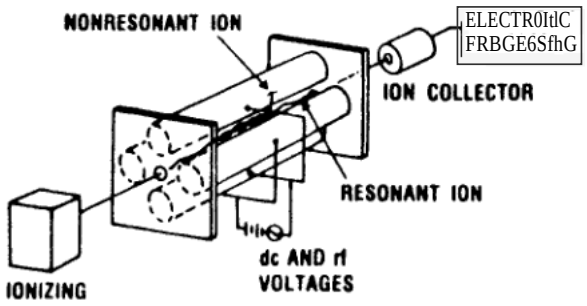


Figure 4. Quadrajnle principle.

MEASUREMENT OF pH

Metabolic processes are typically highly susceptible to even slight changes in pH, and therefore, proper control of this parameter is critical Precise manipulation of pH can determine the relative yield of the desired species over competing by-products. Deviations of as little as 0.2 to 0.3 may adverselyaffect a batch in some cases Like the cell mass probe and dissolved oxygen probes described earlier, the pH probe (see Fig. 5) is packaged in a sterilizible men casing uâth permeable electrode facings for direct insertion into the bioreactor. The measurement principle is the oxidation reduction potential ofthe hydrogen ion and the electrode materials are selected for that purpose.

Figure 5. Ingold sterilizable pH probe. *(Courtesy of Ingold Electrodes, Inc., Wilmington, Mass.)*

Precise temperature control and profiling are key factors in promoting biomass growth and controlling yield. Temperature is one of the more traditional measurements in bioreactors so there is quite a variety of techniques.

Filled thermal systems, Fig. 6, are among the more traditional temperature measuring devices. Their operating principle is to take advantage of the coefficient of thermal expansion of a sealed fluid to transduce temperature into pressure or movement. This has the advantage of requiring essentially no power and therefore is very popular in mechanical or pneumatic control loops. Although the trend in control is toward digital electronic, pneumatic and mechanical systems are still very popular in areas where solvent or other combustible gases may be present and therefore represent a potential safety hazard. The primary constraint in these types of systems is that the receiver (indicator, recorder, controller) must be in close proximity to the sensor.

Figure 6. Filled thermal system assembly for temperature measurement. *(Courtesy of the Foxboro Co., Foxboro, Mass.)*

Thermocouple assemblies, Fig. 7, are a popular measurement choice in electronic systems or in pncumatics where the sensor must be remote. The thcrmoclectric principle, referred to as the *Seebeck Effect,* is that two dissimilar metals, when fomcd into a closed circuit, generate an electromo- tive force when thejunctioo points ofthe metals are at different temperatures. This conversion of thermal energy to electric cncr generates an electric current, Therefore, if the temperature of one juncture pornt (the cold junction) is known, the tcmpcrature of the hot juncmre point is determined by the current flow through the circuit. Depending upon the alloys chosen, thennocouples can measure a wide temperature range (-200 to +350°C for copper, constantan) and are quite fast acting assuming the assembly doesn't contribute too much lag in its absorbance and dissipation ofheat. Its primal disadvantages are its lack of sensitivity (copper, constantan generates only 40.5 microvolts per °C) and requirement for a precise co!d junction temperaturc reading.

COVER

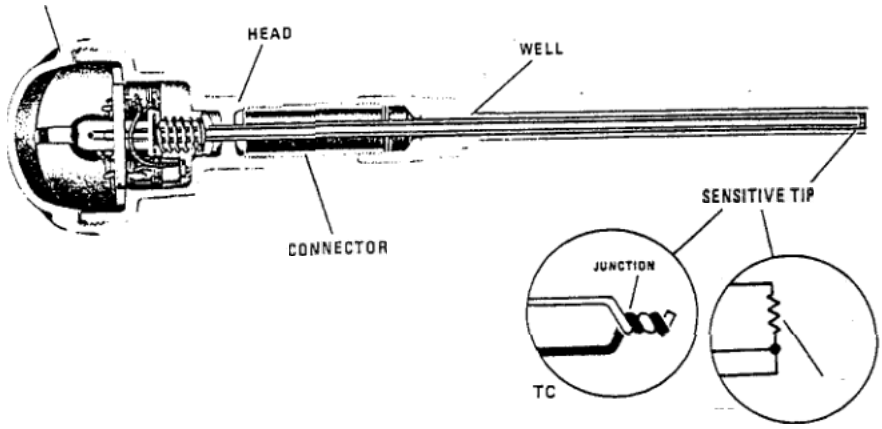


Figure 7. Cotaw'ay view' of thcnnocouple or resistance temperature detector probe for temperature measurement.

Rcsistance temperatuw detectors, RTD’s, are more sensitive than thcrmocouplcs especially when measuring small temperature ranges. As a result, thsj are preferred for accurate and precise measurements The principle behind these devices is based on the use of materials, such as platinum or nickel, whose resistance to current flo» changes with tempera- ture, Thcsc materials arc used as one leg in a›iheatstone bridge circuit with the other legs being known precision resistors. A voltage is applicd across the bridge and the › oltage drop midu’ay through each path of the circuit is compared. The potential difference at the midway point is directly related to the ratio ofeach set of resistances in series. Since three ofthese are known, the resistance ofthe RTD can be calculated and the temperature inferred If the RTD is remote from the bridge circuit, the resistance of lead wires can affect thc measurement. Therefore, for highly precisc mcasurements, compensating circuits are included u’hich require increasing the n iring for this measuring devicc from two to as mam as four leads.

Thermistors are a special class of RTD’s and arc constructed from semiconductor material Their primary advantage is their greater sensitivity to changes in temperature, therefore mAing them a more precise measuring method Their disadvantage is their nonlinear rcsponse to ternperaturc changes This form of RTD is gaining populantr for narrow rangc applications, particularly in laboratory end ironments.

11.0 PRESSURE

Pressure is an important controlled variable. The measurement is obtained by exposing a diaphragm surface or seal to the process via a flange or thrcaded tap through the › cssel wall. The signal is translated through a filled capillaq to a measurement capsule which is'ill transducc the signal to one measurable by an electroniG circuit b› one of set eral methods One method is to cmploy a piezoclectric phenomenon whereby the pressure excited on an asymmetric cv stal creates an elastic deformity is hich in turn causes the flow of an electric charge. A second technology is variable resistance whercby floxurc on a seniiconductii’e wafer affects its resistivity which is measured in a similar fashion to RTD’s. The third, shone in Fig. 8, is the use of a vibrating ›v’ire ›i here changes in the tcnsion of the » ire changes its resonant frequency which is measured as a change in pulse rate

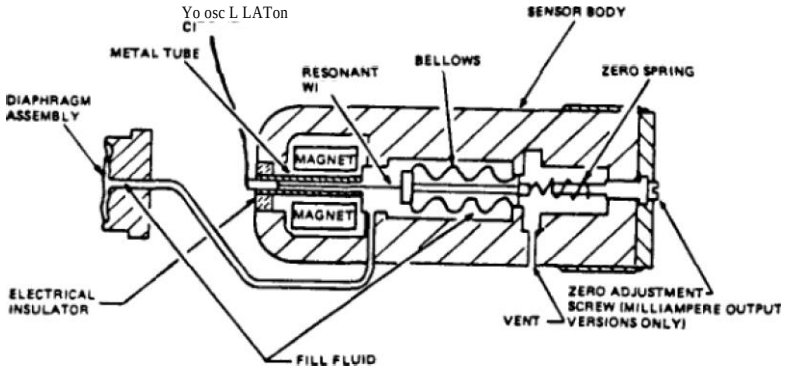


Figure 8. Diagram of resonant w4te tccJuiology pressure meosureincni. NourffS)' oJf/ffl *I'oxboro Co., Foxhoro, :t Pass.*

Sevcral wees of pressure measurcments can be taken. These include absolutc pressure, where one side ofthe capsule is exposed to 0 psia in a sealed chamber. Gauge pressure is measured with one side of the capsule i ented to atmosphere. Vapor pressure transmitter seals one side ofthe capsule, filling it with the chemical composition of the vapor to be measured The i apor pressurc in the scaled chamber is comparcd «ith the process pressure (at thC same temperature). If equal, the compositions are inferred to be equal. This techruque is used primarily for binary mixtures as multicomponent compositions have too degrees of freedom.

12.0 MASS

fi cigh ceils or load cells are ti picallx used to measure the mass of the contents ofa vessel. These are electromechanical dcvices which convert forcG or w'eight into an electrical signal The technique is to construct a i›heatstone bridge sunilar to that used inthc RTD Circult u ith one resistor being a rheostat svhich changes resistance based on load

Three configurations are popular These arc the column, i›hcrcthc cell is interposed between one leg of thc vessel and thc ground (sec Fig. 9) and is

qpically used for weights exceeding 5000 pounds. The second is the cantilever design, » here the weight is applied to a bending bar and is used for weights under 500 pounds. The third is the shear design, n'here the weight is applied to the center of a dual strain gage arrangement.

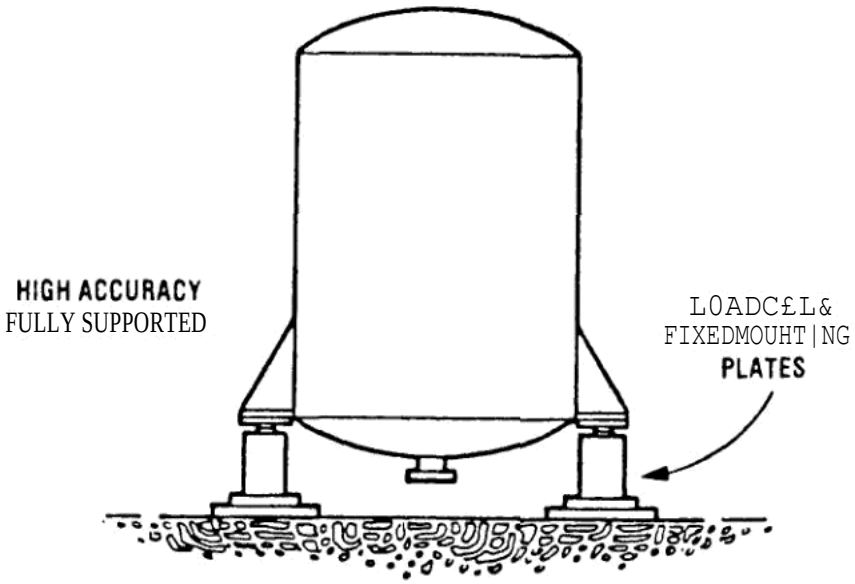


Figure 9. Schemetic of the installation of a load cell

13.0 MASS FLOW RATE

A Coriolis meter utilizes a mcasurement technologv ix’hich is capable of directly measuring mass flow (instead of inferring mass Cow from volumetric flow and dcnsiq’), The Coriolis effect is thc subtle correction to the path of moving objects to compensate for the rotation of the earth. This appears as a force exerted perpendicular to the direction ofoiotion and creates a counterclocRvise rotation in the Northern Hemisphere and a clockwise rotation in the Southern Hemisphere. This phenomenon is used by the mass flO›v meter to create a vibration whose frequency is proportional to the mass ofthe fluid flomng through the meter This is accomplished via the geometry of the meter (Fig. 10),

specificalh the bends to which the fluid is subjected as it travcls through the meter

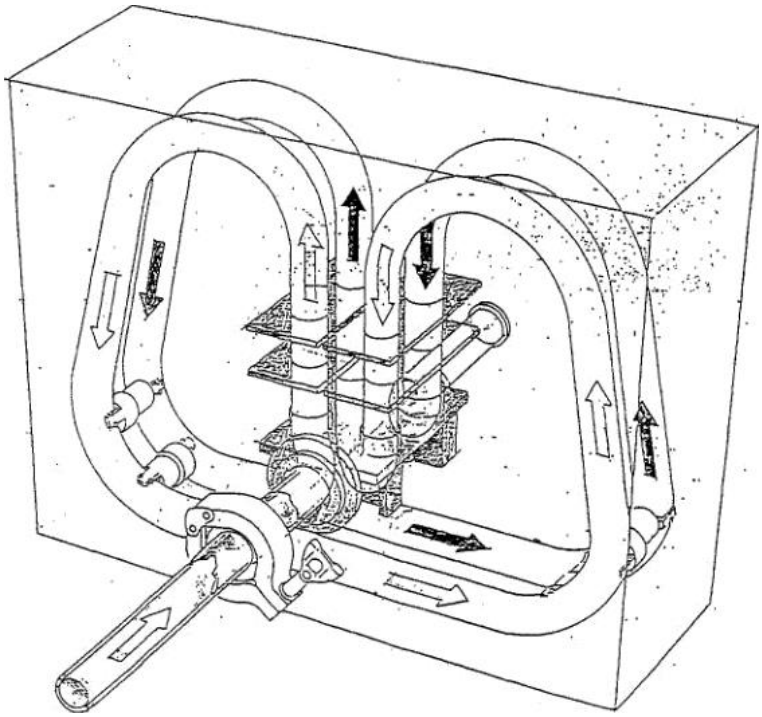


Figure 10. Schematic of Coriolis Metet fto«path. *(Courtesy of the Foxboro Co., Foxboro, .Pass.)*

14.0 VOLUMETRIC FLOW RATE

Quite a number of technologies are available for measuring volumetric flow rates. These include differential pressure transmitters. vortex meters and magnetic flow meters. Each has its advantages and disadvantages.

The differential pressure transmitter is the most popular md has been in use the longest Its measurement principle is quite simple. Create a restriction in the line iVith a» orifice plate and measure the pressure drop across the restriction. The measurement takes advantage of the physical relationship bemeen pressure drop and flow. That is, the fluid velocity is proportional to the square root ofthe pressure drop, and in turbulent flow, the Volumetric flow rate is essentially the velocity of the fluid multiplied by the cross-

sectional area of the pipe (Fig. 11).

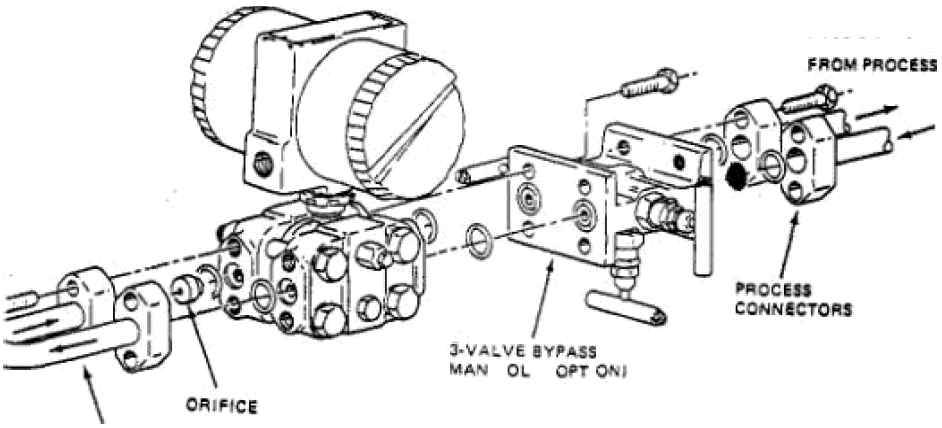


Figure 11. Integral flow orifice assembly, U-bend configuration.

Inaccuracies with regard to transmitting the pressures between the sensor and transducer occur at yep low flow' rates. therefore closely coupled units have been designed for this purpose. Using this approach and small bore orifice plates, extremcly ion flo»s can be measured. A 0.38 millimeter diameter bore can accurately measure fiows in the 0.021iters per minute range for liquids and 0.03 cubic meters per hour for gases. Jewcled orifice plates can have a bore as small as 0 05 millimeters in diameter. The primal disadvantages of the differential pressure producing tion' measurCnicnts arc the permansnt pressure drop caused by the restriction in the line, sediment buildup behind the orifice plate (which could bea source of bacterial buildup) and loss of accuracy over time as the edge ofthe plate is om bj passing fluid and sediment. This age of transmitter typically has a limited range *(iurfldo»n* usually a 4 to l ratio bebveen its maximum and minimum accurate fiow rates.

Vortex mctcrs utilize a precision constructed bar or bluff through thc diameter ofthe flou' path to create a disruption in trots’ which manifests itself as eddy currents or vortices being generaled, starting at the downstream side of the bar (Fig. 12) The frequency at which the vortices are created are directly proportional to velocity ofthe fluid. Although these devices contain a line obstruction, the turbulence created by the vortices make the bluff self- cleaning and they are available for sanitary applications Also, their linear nature makes them a node-range device with a ratio of as much as 20: l between the maximum and minimum flow’ rate Line sizes as small as 1 " are a› ailablc ivhlch are capable of

reading flow' rates as low' as 0.135 liters per minute.

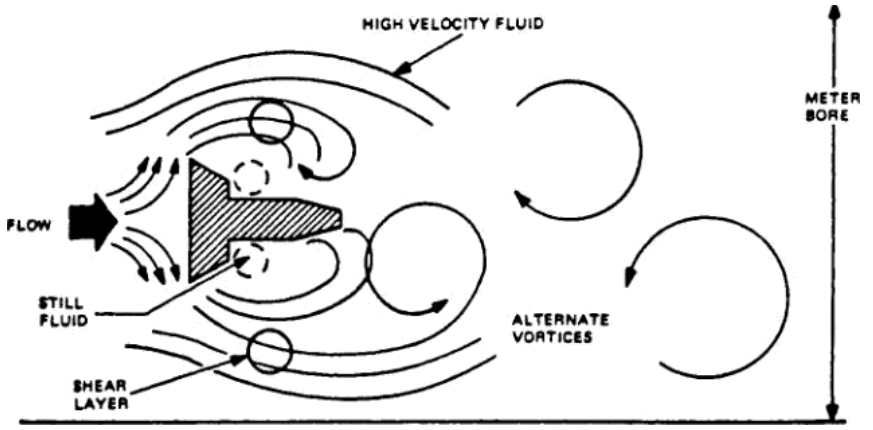


Figure 12. Vortex creation via shedding bluff

Magnetic flowmeters take advantage of the electrolytes in an aqueous to induce a magnetic field in the surrounding the meter's flow tube. see Fig. 13. The faster the flow rare, the greater the induced field. Interestingly, the ionic strength of the electrolytes has only negligible effect on the induced field so long as it is above the threshold value of 2 microsiemens per centimeter. Because these meters create no obstructions to the flow path the are the preferred mum for sanitary applications.

15.0 BROTH LEVEL

The introduction of tiny sterilised air bubbles at the bottom of the liquid and from mixing by the impeller, it has a tendency to foam. This can be a serious problem as the level may rise to the point where it enters the exhaust gas lines clogging the ultrafilters and possibly jeopardizing the sterile environment within the reactor. Various antifoam strategies can be employed to this situation, however, detection of the condition is first required.

600H•SCR TO 601H•SCR SANITARY, CERAMIC-LINEO FLOWTUBES

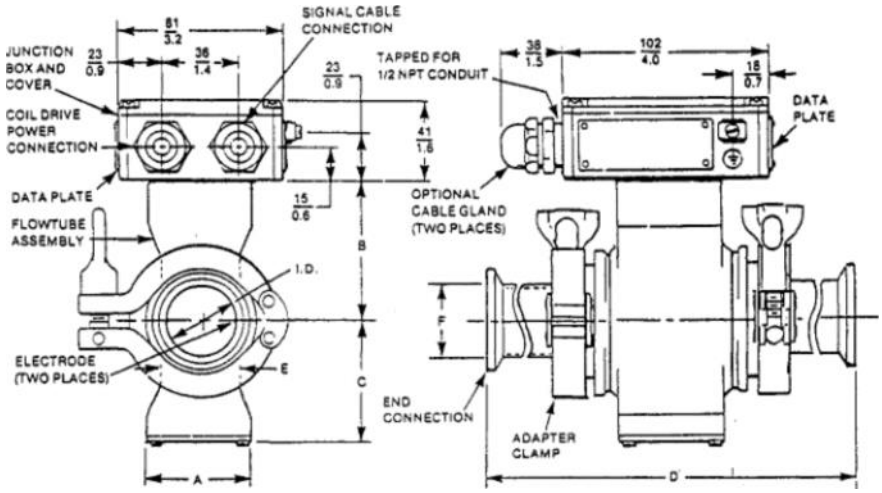


Figure *13.* Cutaway schematic of a sanitary magnetic flowmeter.

Capacitance probes (Fig. 14) are one means to accomplish this. The basic principle is to measure the charge befn'een two conductive surfaces maintained at different voltage potentials and separated by a dielectric material. The construction of the probe provides an electrode in the center surrounded by an insulator, air, and a conductive shell. The length of the probe is from the top ofthe reactor to the lowest level measuring point. As the level in the reactor rises the broth displacesthe air between the capacitance plates and thereby changes the dielectric current between the plates to the level ofthe broth. The result isa change in the charge on the plate. Ifthevessel wall can act as a plate (is sufficiently conductive), the preferred approach would be to use anunshielded probe (innere1ectrode with insulator)toprevent erroneous readings resulting from fouling of the probe. Because of the uncertain dielectric character ofthe broth, this measurement should only be used as a gross approximation of level for instituting antifoaming strategies.

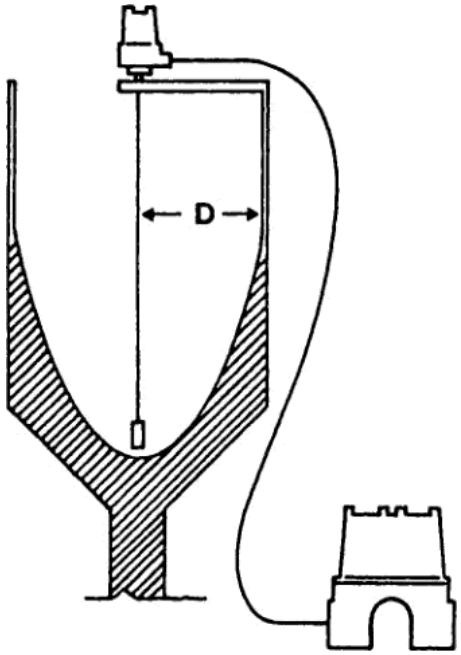


Figure 14. Installation schematic of a capacimnce probe in a vessel

Several other forms of level measurement technologies arc available. One is the float and cable system, where the buoyancy of the float determines the air-broth interface bounding rind the length of the cable determines the level The density of the broth may render this measurement questionable. A second is hydrostatic gauging, where level is inferred from pressure. Again, density, particularly if tu'o phases exisi (aqueous and foam), may render this approach questionable,

A third is some, which computes the distance from the device to the broth surface based on the time it takes for the sound is' avc initiating from the device to reflect off the surface of the air-liquid boundary and return.

Several other ingenious variations of these basic approaches are commercially available as well

16.0 REGULATORY CONTROL

Automatic regulatory control systems (Fig. 15) have been in use in the process industries for over fifty years.

Utilizing simple feedback principles, measurements were driven to- ward their setpoints by manipulating a controlled variable such as flow rate through actuators like throttling control valves. Through successive refinements m first mechanical, then pneumatic, then electronic and finally' digital electronic systems, control theory and practice has progressed to a highly sophisticated state.

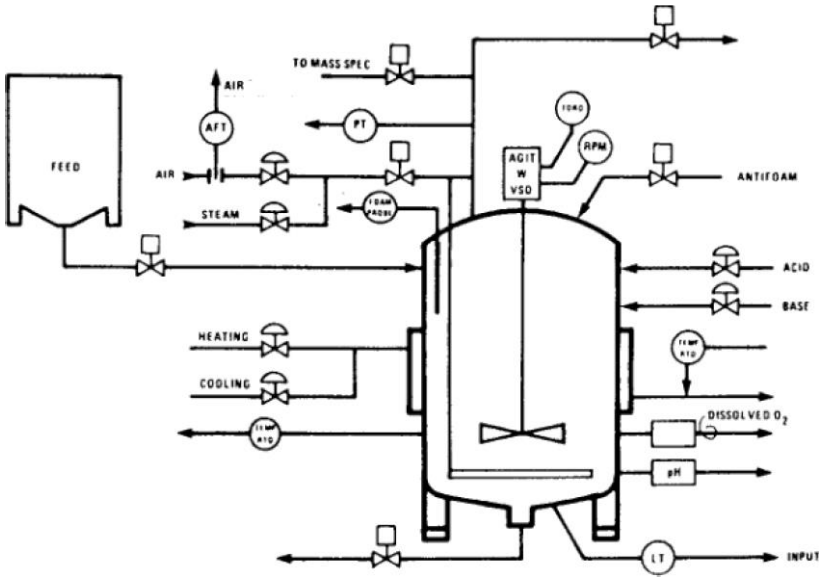


Figure 15. Typical instrument configuration around a fermented.

16.1 Single Stage Control

The fundamental building block has been the proportional plus integral plus derivative (PID) controller whereby the proportional term would adjust the manipulated variable to correct for a deviation bet 'ecu measurement and target or setpoint; the integral tern would continue the action of the proportional turn over tune until the measurement reached the setpoint md the derivative term would compensate for lags in the action in the measurement in responding to actions of the manipulated variable. The classic equation is:

$$m = 100/GB\ (e + l/fi\ edt - D\ de/dt)$$

judicious application of this control strategy on essentially linear single variable control systems which don't exhibit a prolonged delay (dead time) between action by the manipulated variable and measured response by the controlled variable has prox'en quite effective. Fortunately, most single loop control systems exhibit this behavior.

In highly› nonlinear applications such as pH Control, or in situations where the dynamics of the process change over time as occurs in mans chemical reactions, adjustments to the tuning coefficients are needed to adequately control the modified process dynamics. Self-tuning controllers employing expert rule sets for dynamic retuning the PID settings arc available for this class of problem. These arc also used by many users to dcterfniuc tlic optimum settings for the linear systems described above. One such rule system is the EXACT controller bj FoxbOfO (Fig 16), which automatically adjusts the controller tuning parameters based on the pattern ofthe measure- ment signal received.

When the process under control exhibits significant dead time, the problem is considerably more difficult. One approach is to use a simple model-based predictor corrector algorithm such as the Smith predictor which is interposed between the manipulated and controlled variable in parallel is'ith a conventional controller and conditions the measurement signal *iO* the controller based on time conditioned changcs to the manipulated variable made by the controller This works exceedingly self if property tuned. but is sensitive to changes in process dynamics. Another scheme, introduced by Shinsky ' recently, utilizes a standard PID controller unit a dcad time function added to the external reset feedback potion of thc loop This appears to be lcss sensitive to changes in process conditions

Figure 16. Model 761 Controller with EXACT tuning. (Courtesy of the Foxboro Co. Foxboro, Mass.)

A control system which anticipates adjustments to the manipulated variables based on changes to one or more controlled variables can be constructed by combining single station controllers with signal characterizers, dynamic compensators and computational elements such as summers and multipliers. Simpler implementations, such as cascade control, will minimize the effect of a deviation of a controlled variable from its target value while dynamic models will anticipate changes to process conditions and adjust the control strategy to compensate based on a leading indicator. A simple example would be the effect on the draw rate and energy input to a distillation column based on a change to its feed rate. The dynamic model in this case would be a material and energy balance around the column compensating for the time delays encountered on each tray as the increased flow rate works its way through the column.

17.0 DYNAMIC MODELING

L TIVARIABLE CONTROL

Characterizing a process as a set of nonlinear time dependent equations and then developing a strategy which manipulates sets of outputs based on changes to the inputs is another approach gaining momentum in other industries such as petroleum refining. One approach is called Dynamic Matrix Control [12] (DMC) which first automates the process of determining the coefficients for the set of nonlinear equations based on sets of controlled and manipulated variables declared Tic method perturbs each of the manipulated variables and determines the corresponding response of the controlled variables Once the model is constructed, the information is represented in a relative gain matrix to predict the control actions necessary to correct for changing process conditions. Once the DMC is correctly tuned, including dynamic compensations, a predictor corrector algorithm is applied to compensate to changes in the process dynamics over time

This technique has bun applied quite successfully to reaction processes in the petroleum industry including fluid catalytic cracking units and catalytic reformers.

18.1 Batch Control

Batch is a general term given to a diverse set of time dependent control strategies including:

State variable control. such as the opening and closing of a solenoid or the starting md stopping of a motor, including the use of an timing circuits which may be used for alarming in the event the action doesn't achieve its specified results in the allotted time.

The interlocking, sequencing or coordinating of systems of devices to ensure their proper and coordinated operation. Examples include interlocking a discharge pump to the opening of the discharge valve and the alignment of pumps and valves to transfer materials from one vessel to another. This may include actions such as the resetting and starting of totalizers to ensure the proper amount of material was successfully transferred

The modification of selected process variables in accordance with a prespecified time-variable profile Two examples are the changing of the reactor temperature over time to conform 'ith a specified profile or the timed periodic addition of nutrient into the bioreactor.

Conducting event driven actions such as adding antifoam upon the detection of excess foam

or invoking an emergencv shut down routine if an exothennic reaction goes beyond controllable limits.

Performing a sequence of operations in a coordinated manner to produce the desired changes to the contents of a process unit. This would typically include combinations of ths above mentioned activities on various sets of equipment associated with the unit.

The instrument Society of America Committee Group SP88, Batch Control Structure, is drafting a specification which decomposes batch control into a hierarchal set of activities each with their own purview and problem definition. The objective is to define the properties of the control problem at each level and identify conceptually the appropriate control and information management tools needed for each level Once defined, a building block approach is taken whereby successively higher levels rely on the foundation established by the controls implemented at the low er levels A strategy directed at the operation of a reflux condenser would rely on the definitions already in place for throttling flow to achieve proper temperature control and would merely direct the devices (such as PID controllers) as to the actions required.

This hierarchy is currently' depicted '" as:

Lonp Device, F.lement I evel, which deals with the real-time dev'ices which interface directly with the process

Equipment Module Level, which utilizes combinations *OF* loops and devices to manage an equipment function such as a reflux condenser within a reactor.

Unit Level, which coordinates thc equipment modules to manage the process unit.

TraJn'Line Level, Which coordinates a set of units to manufacture a batch of specified product.

Area Level, which coordinatcs the manufacture of sets of products being made at thc train/line level so as to ensure adequate availability of resources and the optimum utili- zation of capital equipment.

Plant Levvl is the integration of the manufacturing pro- cess with other plant functions such as accounting, quality' control, management, purchasing, etc.

Corporafe Level is the coordination of various plants to ensure a proper manufacturing balance with market needs and financial goals.

19.0 ARTIFICIAL INTELLIGENCE

A considerable amount of attention is being given to the use of various forms of artificial intelligence for the control of bioreactor systems. Two forms of systems are currently being explored. These are expert systems and neural networks. Expert systems combine stored knowledge and rules about a process with inference engines (forward and backward chaining algorithms) to choose a best or most reasonable approach among a large number of choices when no correct answer can be deduced and in some situations the information may appear to be contradictory

Neural networks arc also being seriously explored for certain classes of optimization applications. These employ parallel solution techniques which are patterned after the way the human brain functions. Statistical routines and bank propagation algorithms are used to force closure on a set of cross linked circuits (equations). Weighting functions are applied at each of the intersections.

The primal' advantage for using neural networks is that no model of the problem is required (some tuning of the weighting functions may facilitate “letting”, however). The user merely furnishes the system with cause and effect data which the program uses to learn the relationships and thereby model the process from the data. Given an objective function, it can assist in the selection of changes to the causes (manipulated variables) to achieve the optimum results or effect (controlled variables).

At BPEC, the Engineering Research Center of Excellence at MIT, advanced computer control of bioprocesscs is being researched visit an eye

toward industrial commercialization. Professor Charles Cooney has directed the effort to develop expert systems and artificial neural networks to achieve this goal. One of the products resulting from this effort is the Bioprocess Expert developed by Dr. Gregor O'Connor. President of Bioproccss Automation, Inc in Cambridge. This uses an expert sj stem called G2 from Gensym Corporation, also located in Cambridge.

20.0 DISTRIBUTED CONTROL SYSTEMS

As the knowledge ofthe physiology and reaction kinetics of biochemical processes has progressed and the measurement systems for monitoring their activiq'has inproved, the need for sophisticated systems able to execute coordinated control strategies including batch has increased, Fortunately the state of the an of control systems has rapidly evolved to the point sphere al) of the control strategies described above can be embodied in a Distributed Control System (DCS), see Fig, 17. This transformation has been facilitated to a great extent by the technology breakthroughs in computer, communica- tions, and software technology.

Distributed control systems arc organized into five subsystems

Process interface, which is responsible for the collection of process data from measurement instruments and the issuing of signals to actuating devices such as pumps. motors and valves.

Process control, which is responsible for translating the information collected ffOm the process interface subsist- tem and determining the signals to be sent to the process interface subsystem based on preprogrammed algorithms and rules set in its memory.

Process operations. which is responsible for communicating with operations personnel at all levels including operator displays, alarms, trends of process variables md activities, summat reports, and operational instructions and guidelines. It also tracks process operations and product batch lots.

OPEN INDUSTRIAL SYSTEM

NETWORK ARCHITECTURE

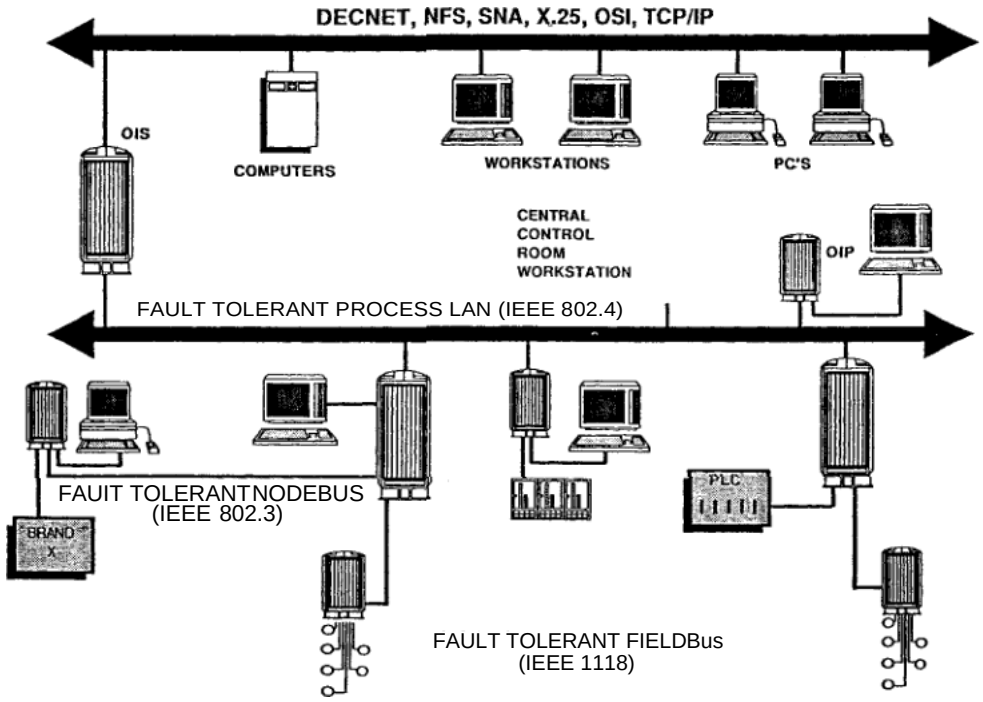


Figure 17. Schematic drawing of Foxboro Distributed Control System called intelligent Automation.

Applications engines, which arc the repository for all of the programs and packages for the system from control, display and report configuration tools to program language compilers and program libraries to specialized packages such as database managers, spreadsheets and optimization or expert system packages to repositories for archived process information.

Communications subsystems, which enable information flow between the various DCS subsystems as nell as to other computerized systems such as laboratory information management systems (LIMS); plant inventory management and scheduling systems such as MRP II; plant maintenance systems and business systems

The integration of these systems into a cohesive whole has dramatically increased the level of automation possible to improve the qualm', productivity and economics of manufacturing.

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SBTA5203

REMOVAL OF INSOLIBALES FOAM SEPARATION

Foam separation is a chemical process which falls into a category of separation techniques called "Adsorptive bubble separation methods". It is further divided into froth flotation and foam fractionation. A variety of materials can be concentrated as well as separated from one another using foam separation techniques that make use of the tendency of surface-active components in a solution to preferentially concentrate at the solution/gas interface. Nonsurface active agents that are capable of associating with surface-active agents can also be separated using these techniques. The various anions such as alkyl benzyl sulfonate; chromate; cyanide and phenolate; cations of, for example, dodecylamine. mercury, lead, and strontium; proteins; microorganisms; and minerals. The attractive feature of this group of techniques is its effectiveness in the concentration range that is too dilute for the successful use of most other techniques. Furthermore. these techniques are ideally suitable for also treating materials that are too sensitive to changes in temperature.

PRECIPITATION

Ammonium sulfate precipitation is a method of protein purification by altering the solubility of protein. Ammonium sulfate is commonly used due to its high solubility that allows salt solutions with high ionic strength. At sufficiently high ionic strength, the protein will be almost completely precipitated from the solution. Differential precipitation of proteins by ammonium sulfate is one of the most widely used preliminary purification procedures. It is based on proteins having differing solubility in ammonium sulfate solutions and can result in a two- to five-fold increase in specific activity. Provided that appropriately buffered ammonium sulfate solutions are used to protect the desired activity, recoveries approaching 100% can be expected. A typical protocol consists of adding ammonium sulfate to give specific percentage saturation, followed by a period of time for proteins to precipitate and a centrifugation step to collect the precipitate.

SEDIMENTATION:

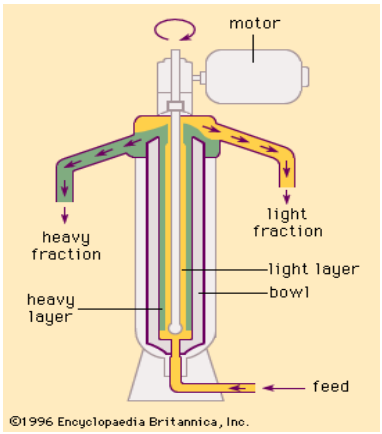
Sedimentation is the tendency for particles in suspension to settle out of the fluid in which they are entrained and come to rest against a barrier. This is due to their motion through the fluid in response to the forces acting on them: these forces can

be due to gravity, centrifugal acceleration, or electromagnetism. In geology, sedimentation is often used as the opposite of erosion, i.e., the terminal end of sediment transport. In that sense, it includes the termination of transport by saltationor true bedload transport. Settling is the falling of suspended particles through the liquid, whereas sedimentation is the termination of the settling process.Sedimentation may pertain to objects of various sizes, ranging from large rocks in flowing water to suspensions of dust and pollen particles to cellular suspensions to solutions of single molecules such as proteins and peptides. Even small molecules supply a sufficiently strong force to produce significant sedimentation.The term is typically used in geology to describe the deposition of sediment which results in the formation of sedimentary rock, but it is also used in various chemical and environmental fields to describe the motion of often-smaller particles and molecules. This process is also used in the biotech industry to separate cells from the culture media.

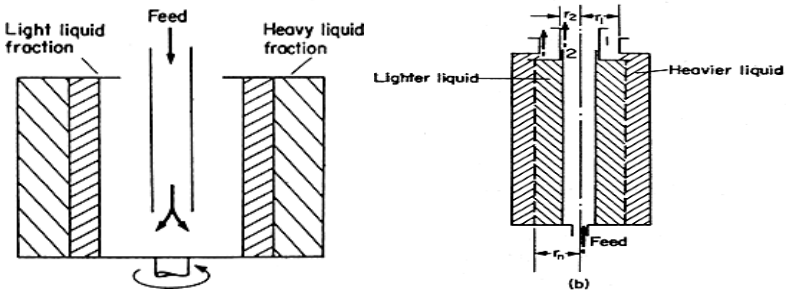
CENTRIFUGATION

Centrifugation is a process which involves the application of the centripetal force for the sedimentation of heterogeneous mixtures with a centrifuge, and is used in industrial and laboratory settings. This process is used to separate two immiscible substances. More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis. Chemists and biologists may increase the effective gravitational force on a test tube so as to more rapidly and completely cause the precipitate (pellet) to gather on the bottom of the tube.

- Feed added to spinning bowl
- Sedimentation of particles occurs in centrifugal field
- Flow is upwards at a particular rate which determines residence time in device
- Separation happens if sedimentation velocity is high enough for particle to reach side of bowl within residence time
- Large particles have higher settling velocities than small particles
- Both large and small are still particles, have small Reynolds no.s (<1) and obey Stokes‟ Law



- **Separation of milk into skimmed milk and cream is done with a centrifuge**



Centrifugal Motion

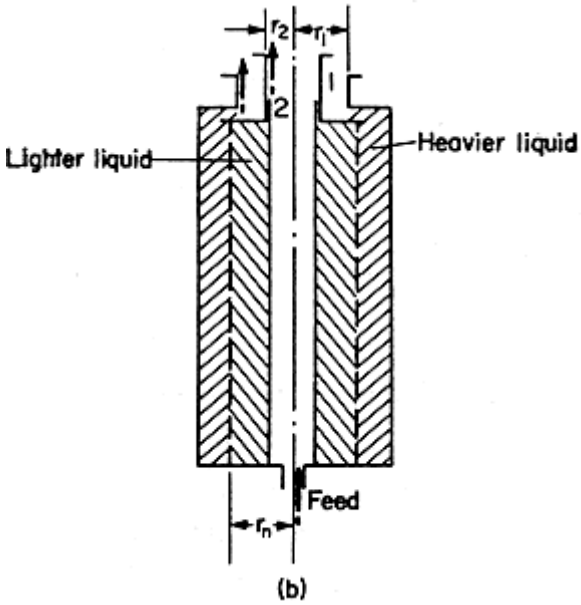
- Centrifugal acceleration = $r\omega^2$
- ω is the angular velocity in rad/s
- r is the radius of rotation
- Centrifugal force = $mr\omega^2$
- m is the mass of the particle

Sigma Factor

- The capacity of a centrifuge is defined by Σ
- Q is the throughput (m^3/s) at which all particles with a terminal velocity $\geq u_T$ (m/s) are retained
- Σ has units of m^2 and is equivalent to the cross sectional area of a thickener with the same capacity

- **The contents of a fermenter are discharged to a centrifuge**
- Volume of material is 100 m^3
- Centrifugation time is 5 hrs
- Particle size is 3 □m – all particles of this size are separated
- Density of solid phase 1090 kg/m^3

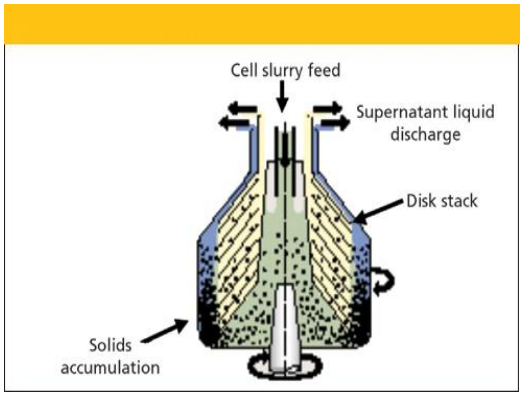
- Cell free liquid density 1025 kg/m^3
- Cell free liquid viscosity 0.005 Pa.s
- **Calculate the capacity factor, Σ**



Bowl Centrifuge

Sigma Factor – Bowl Centrifuge

The Disc Stack Centrifuge



Large particles have higher settling velocities than small particles Cellular debris ends up at the outer edge of the bowl

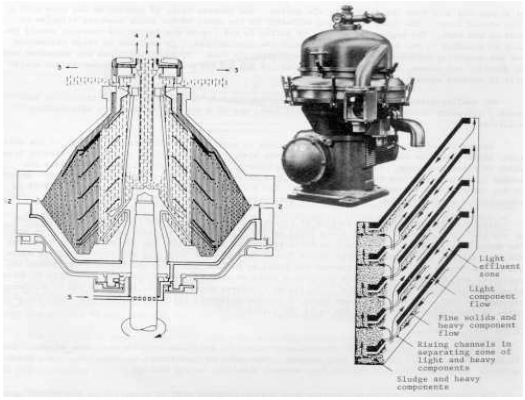
Soluble intracellular material passes through with the clarified liquid Discs give a higher sigma factor

Benefit of Discs

The discs split the stream into a large number of very thin layers thereby improving separation

Solids flow downwards on bottom face of disc Liquid flows upwards on top face of disc

Sigma factor no. of discs



Disc Stack Centrifuge Capacity

For the disc stack centrifuge:

ω is the angular velocity (rad/s)

n is the number of discs

R is the outer radius of the discs

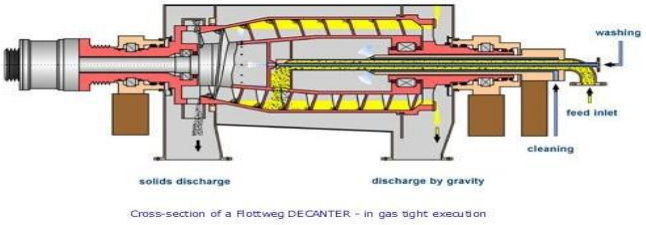
(m) r is the inner radius of the discs

(m)

θ is the angle between disc and vertical

(rad) g is the acceleration due to gravity

(m/s^2) **Decanter Centrifuge**



Cross-section of a Flottweg DECANTER - in gas tight execution

FILTRATION

Filtration plays an important role in the natural treatment of groundwater as it percolates through the soil. It is also a major part of most water treatment. Groundwater that has been softened, or treated through iron and manganese oxidation, requires filtration to remove floc created by coagulation or oxidation processes. Since surface water is subject to run-off and does not undergo natural filtration, it must be filtered to remove particles and impurities.

Filtration can be compared to a sieve or micro-strainer that traps suspended material between the grains of filter media. However, since most suspended particles can easily pass through the spaces between grains of the filter media, straining is the least important process in filtration. Filtration primarily depends on a combination of complex physical and chemical mechanisms, the most important being adsorption. Adsorption is the process of particles sticking onto the surface of the individual filter grains or onto the previously deposited materials. Forces that attract and hold particles to the grains are the same as those that work in coagulation and flocculation. In fact, coagulation and flocculation may occur in the filter bed, especially if coagulation and flocculation before filtration was not properly controlled. Incomplete coagulation can cause serious problems in filter operation.

Theory of filtration

Depending on dispersing medium filtration is divided in two parts: 1) gas filtration and 2) liquid filtration. Gas filtration theory It mainly includes filtration of aerosols and lyosols. Membrane filters and nucleopore filters are based on these below mechanisms. Mechanism of gas filtration (Wilson & Cavanagh, 1969) Diffusion deposition: The trajectories of individual small particles do not coincide with the streamlines of the fluid

because of Brownian motion. With decreasing particle size the intensity of Brownian motion increases and, as a consequence, so does the intensity of diffusion deposition. Direct interception: This mechanism involves the finite size of particles. A particle is intercepted as it approaches the collecting surface to a distance equal to its radius. A special case of this mechanism is the so-called sieve effect, or sieve mechanism. Inertial deposition: The presence of a body in the flowing fluid results in a curvature of the streamlines in the neighbourhood of the body. Because of their inertia, the individual particles do not follow the curved streamlines but are projected against the body and may deposit there. It is obvious that the intensity of this mechanism increases with increasing particle size and velocity of flow. Gravitational deposition: Individual particles have a certain sedimentation velocity due to gravity. As a consequence, the particles deviate from the streamlines of the fluid and, owing to this deviation; the particles may touch a fiber. Electrostatic deposition: Both the particles and the fibers in the filter may carry electric charges. Deposition of particles on the fibers may take place because of the forces acting between charges or induced forces. Liquid filtration theory (Melia & Weber, 1972). The term solid-liquid filtration covers all processes in which a liquid containing suspended solid is freed of some or the entire solid when the suspension is drawn through a porous medium.

Kozeny-Carman equation: $Idv/Adt= \Delta P/ r \mu (l+L)$

Where, A = filter area; V = total volume of filtrate delivered; t = filtration time; ΔP = pressure drop across cake and medium; r = specific cake resistance; μ = filtrate viscosity; l = cake thickness; L = thickness of cake equivalent to medium resistance.

TYPES OF FILTERS

Several types of filters are used for water treatment. Early slow sand filters typically have filter rates of 0.05 gpm/ft2 of surface area and require large filter areas. The top several inches of the sand has to be removed regularly-- usually by hand--due to the mass of growing material „schmutzdecke" that collects in the filter. Sand removed is usually washed and returned to the filter. These filters are still in use in some small plants, especially in the western United States as well as in many developing countries. They may also be used as a final step in wastewater treatment.Modern filters are classified as: Gravity Filters (Rapid Sand or High Rate-Dualmedia-Multi- media) or Pressure Filters (Sand or Multi-

media).

RAPID SAND FILTERS

Rapid sand filters have filter rates 40 times those of slow sand filters. The major parts of a rapid sand filter are:

•Filter tank or filter box

•Filter sand or mixed-media

•Gravel support bed

•Underdrain system

•Wash water troughs

•Filter bed agitators

The filter tank is generally constructed of concrete and is most often rectangular. Filters in large plants are usually constructed next to each other in a row, allowing piping from the sedimentation basins to feed the filters from the central pipe gallery. Some smaller plants are designed with filters forming a square of four filters with a central pipe gallery feeding the filters from a center wall.

Filter Sand

The filter sand used in rapid sand filters is manufactured specifically for the purpose of water filtration. Most rapid sand filters contain 24-30 inches of sand, but some newer filters are deeper. The sand used is generally 0.4 to 0.6 mm in diameter. This is larger than the sand used in slow rate filtration. The coarser sand in the rapid filters has larger voids that do not fill as easily.

PRESSURE FILTERS

Pressure filters fall into two categories: pressure sand and diatomaceous earth filters. Pressure filters are used extensively in iron and manganese removal plants. A pressure sand filter is contained under pressure in a steel tank, which may be vertical or horizontal, depending on the space available. As with gravity filters, the media is usually sand or a combination of media, and filtration rates are similar to gravity filters.

Groundwater is first aerated to oxidize the iron or manganese, and then pumped through the filter to remove the suspended material.

FILTER AIDS

When water passes through a filter, the floc sometimes is torn apart into smaller particles that penetrate deeply into the filter media, causing premature turbidity breakthrough. This requires more frequent filter backwashing of the filter and large volumes of backwash water to be able to remove the floc that has penetrated deeply into the filter bed.

A filter aid is a material that adds strength to the floc and prevents its breakup. Generally, a polymer is used as a filter aid because it creates strong bonds with the floc. Polymers are water soluble, organic compounds that can be purchased in either wet or dry form.

Polymers have very high molecular weight and cause the floc to coagulate and flocculate quickly. Polymers can have positive or negative charges, depending on the type needed to cause attraction to the specific floc filtered.

When used as a filter aid, the polymer strengthens the bonds and prevents the shearing forces in the filter from breaking the floc apart. For best results, the polymer should be added just ahead of the filter. A normal dose of polymer for filter aiding will be less than 0.1 ppm, but the exact dose will be decided by the result of a jar test and by experimentation in the treatment plant. Too much polymer will cause the bonds to become too strong, which may then cause the filter to plug, especially the top few inches of the filter media.

COAGULATION AND FLOCCULATION PROCESS FUNDAMENTALS

Coagulation and Flocculation Groundwater and surface water contain both dissolved and suspended particles. Coagulation and flocculation are used to separate the suspended solids portion from the water. Suspended particles vary in source, charge, particle size, shape, and density. Correct application of coagulation and flocculation depends upon these factors. Suspended solids in water have a negative charge and since they have the same type of surface charge, they repel each other when they come close together. Therefore, suspended solids will remain in suspension and will not clump together and settle out of the water, unless proper coagulation and flocculation is used. Coagulation and flocculation occurs in successive steps, allowing particle collision and growth of floc. This is

then followed by sedimentation (see Sedimentation Chapter). If coagulation is incomplete, flocculation step will be unsuccessful, and if flocculation is incomplete, sedimentation will be unsuccessful.

COAGULATION Coagulant chemicals with charges opposite those of the suspended solids are added to the water to neutralize the negative charges on non-settlable solids (such as clay and color-producing organic substances). Once the charge is neutralized, the small suspended particles are capable of sticking together. These slightly larger particles are called microflocs, and are not visible to the naked eye. Water surrounding the newly formed microflocs should be clear. If not, coagulation and some of the particles charge have not been neutralized. More coagulant chemicals may need to be added. A high-energy, rapid-mix to properly disperse coagulant and promote particle collisions is needed to achieve good coagulation. Over-mixing does not affect coagulation, but insufficient mixing will leave this step incomplete. Contact time in the rapid-mix chamber is typically 1 to 3 minutes.

Alum A12(SO4)3 + 3 Ca(HCO3)2 ----------> 2 Al(OH)3 + 3CaSO4 + 6 CO2 Aluminum + Calcium gives Aluminum + Calcium + Carbon Sulfate Bicarbonate Hydroxide Sulfate Dioxide (present in the water to treat) Ferric Sulfate Fe2(SO4)3 + 3 Ca(HCO3)2----------> 2 Fe(OH)3 + 3CaSO4 + 6 CO2 Ferric + Calcium gives Ferric + Calcium + Carbon Sulfate Bicarbonate Hydroxide Sulfate Dioxide (present in the water to treat) Ferric Chloride 2 Fe Cl3 + 3 Ca(HCO3)2 ----------> 2 Fe(OH)3 + 3CaCl2 + 6CO2 Ferric + Calcium gives Ferric + Calcium + Carbon Chloride Bicarbonate Hydroxide Chloride Dioxide (present in the water to treat) Polymers Polymers (long-chained, high-molecular-weight, organic chemicals) are becoming more widely used. These can be used as coagulant aids along with the regular inorganic coagulants. Anionic (negatively charged) polymers are often used with metal coagulants. Low-to-medium weight cationic (positively charged) polymers may be used alone, or in combination with alumor ferric coagulants to attract suspended solids and neutralize their surface charge. Manufacturers can produce a wide range of polymers that meet a variety of source-water conditions by controlling the amount and type of charge and the polymers molecular weight. Polymers are effective over a wider pH range than inorganic coagulants. They can be applied at lower doses, and do not consume alkalinity. They produce smaller volumes of more concentrated, rapidly settling

floc. Floc formed from use of a properly selected polymer will be more resistant to shear, resulting in less carryover and a cleaner effluent. Polymers are generally several times more expensive in price per pound than inorganic coagulants. Selection of the proper polymer requires considerable jar testing under simulated plant conditions, followed by pilot or plant-scale trials. All polymers must also be approved for potable water use by regulatory agencies.

Precipitation:

Precipitation is the most commonly used technique in industry for the concentration of macromolecules such as proteins and polysaccharides. Further, precipitation technique can also be employed for the removal of certain unwanted byproducts e.g. nucleic acids, pigments.

Neutral salts, organic solvents, high molecular weight polymers (ionic or non-ionic), besides alteration in temperature and pH are used in precipitation. In addition to these non-specific protein precipitation reactions (i.e. the nature of the protein is unimportant), there are some protein specific precipitations e.g., affinity precipitation, ligand precipitation.

Neutral salts:

The most commonly used salt is ammonium sulfate, since it is highly soluble, nontoxic to proteins and low-priced. Ammonium sulfate increases hydrophobic interactions between protein molecules that result in their precipitation. The precipitation of proteins is dependent on several factors such as protein concentration, pH and temperature.

Organic solvents:

Ethanol, acetone and propanol are the commonly used organic solvents for protein precipitation. They reduce the dielectric constant of the medium and enhance electrostatic interaction between protein molecules that lead to precipitation. Since proteins are denatured by organic solvents, the precipitation process has to be carried out below 0°C.

Non-ionic polymers:

Polyethylene glycol (PEG) is a high molecular weight non-ionic polymer that can precipitate proteins. It reduces the quantity of water available for protein solvation and precipitates protein. PEG does not denature proteins, besides being non-toxic.

Ionic polymers:

The charged polymers such as polyacrylic acid and polyethyleneimine are used. They form complexes with oppositely charged protein molecules that causes charge neutralisation and precepitation.

Increase in temperature:

The heat sensitive proteins can be precipitated by increasing the temperature.

Change in pH:

Alterations in pH can also lead to protein precipitation.

Affinity precipitation:

The affinity interaction (e.g., between antigen and antibody) is exploited for precipitation of proteins.

Precipitation by ligands:

Ligands with specific binding sites for proteins have been successfully used for selective precipitation.

Cell Disruption:

Physical methods of cell disruption:

The microorganisms or cells can be disrupted by certain physical methods to release the intracellular products.

Ultra sonication:

Ultrasonic disintegration is widely employed in the laboratory. However, due to high cost, it is not suitable for large-scale use in industries.

Osmotic shock:

This method involves the suspension of cells (free from growth medium) in 20% buffered sucrose. The cells are then transferred to water at about 4°C. Osmotic shock is used for the release of hydrolytic enzymes and binding proteins from Gram-negative bacteria.

Heat shock (thermolysis):

Breakage of cells by subjecting them to heat is relatively easy and cheap. But this technique can be used only for a very few heat-stable intracellular products.

High pressure homogenization:

This technique involves forcing of cell suspension at high pressure through a very narrow orifice to come out to atmospheric pressure. This sudden release of high pressure creates a liquid shear that can break the cells.

Impingement:

In this procedure, a stream of suspended cells at high velocity and pressure are forced to hit either a stationary surface or a second stream of suspended cells (impinge literally means to strike or hit). The cells are disrupted by the forces created at the point of contact. Micro fluidizer is a device developed based on the principle of impingement. It has been successfully used for breaking E. coli cells. The advantage with impingement technique is that it can be effectively used for disrupting cells even at a low concentration.

Grinding with glass beads:

The cells mixed with glass beads are subjected to a very high speed in a reaction vessel. The cells break as they are forced against the wall of the vessel by the beads. Several factors influence the cell breakage-size and quantity of the glass beads, concentration and age of cells, temperature and agitator speed. Under optimal conditions, one can expect a maximal breakage of about 80% of the cells. A diagrammatic representation of a cell disrupter employing glass beeds is shown in Fig. 20.6. It contains a cylindrical body with an inlet, outlet and a central motor-driven shaft. To this shaft are fitted radial agitators. The cylinder is fitted with glass beads. The cell suspension is added through the inlet and the disrupted cells come out through the outlet. The body of the cell disrupter is kept cool while the operation is on.

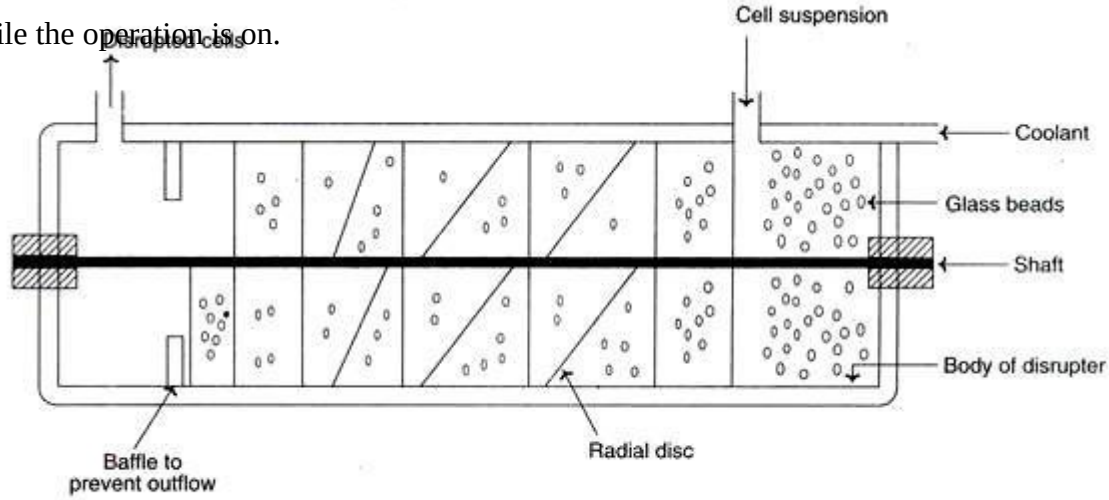


Fig. 20.6 : Diagrammatic representation of a cell disrupter.

Mechanical and non-mechanical methods:

Among the physical methods of cell disruption described above, ultra sonication, high-pressure homogenization, impingement and grinding with glass beads are mechanical while osmotic shock and heat shock are non-mechanical. The chemical and enzymatic methods (described below) are non- mechanical in nature.

Chemical methods of cell disruption:

Treatment with alkalies, organic solvents and detergents can lyse the cells to release the contents.

Alkalies:

Alkali treatment has been used for the extraction of some bacterial proteins. However, the alkali stability of the desired product is very crucial for the success of this method e.g., recombinant growth hormone can be efficiently released from E. coli by treatment with sodium hydroxide at pH 11.

Organic solvents:

Several water miscible organic solvents can be used to disrupt the cells e.g., methanol, ethanol, isopropanol, butanol. These compounds are inflammable; hence require specialised equipment for fire safety. The organic solvent toluene is frequently used. It is believed that toluene dissolves membrane phospholipids and creates membrane pores for release of intracellular contents.

Detergents:

Detergents that are ionic in nature, cationic-cetyl trimethyl ammonium bromide or anionic-sodium lauryl sulfate can denature membrane proteins and lyse the cells. Non-ionic detergents (although less reactive than ionic ones) are also used to some extent e.g., Triton X-100 or Tween. The problem with the use of detergents is that they affect purification steps, particularly the salt precipitation. This limitation can be overcome by using ultrafiltration or ion-exchange chromato- graphy for purification.

Enzymatic methods of cell disruption:

Cell disruption by enzymatic methods has certain advantages i.e., lysis of cells occurs under mild conditions in a selective manner. This is quite advantageous for product recovery. Lysozyme is the most frequently used enzyme and is commercially available (produced from hen egg white). It hydrolyses β-1, 4-glycosidic bonds of the mucopeptide in bacterial cell walls. The Gram- positive

bacteria (with high content of cell wall mucopeptides) are more susceptible for the action of lysozyme.

For Gram-negative bacteria, lysozyme in association with EDTA can break the cells. As the cell wall gets digested by lysozyme, the osmotic effects break the periplasmic membrane to release the intracellular contents. Certain other enzymes are also used, although less frequently, for cell disruption. For the lysis of yeast cell walls, glucanase and mannanase in combination with proteases are used.

AQUEOUS TWO-PHASE EXTRACTION

Aqueous two-phase extraction which is a special case of liquid-liquid extraction involves transfer of solute from one aqueous phase to another. The two immiscible aqueous phases are generated in-situ by addition of substances such as polymers and salts to an aqueous solution. Historically, gelatin-agar and gelatin- soluble starch were used. Two types of aqueous two-phase systems are commonly used:

1.Polymer-polymer two-phase system 2.Polymer-salt two-phase system

Tab. 1. Two-Phase Aqueous Polymer Systems

Polymer/Salt Combination
Polyethylene glycol (PEG)-dextran
PEG-phosphate
PEG-citrate
PEG-hydroxypropyl starch
Ethylhydroxyethyl cellulose-hydroxypropyl starch
PEG-polyvinyl alcohol
PEG-pullulan
PEG-maltodextrins

A polymer-polymer two phase system can for instance be obtained by mixing dextran and PEG at a certain composition. By adding specific amounts of these polymers to an aqueous feed phase (which contains the solute), two aqueous phases, one rich in PEG and the other rich in dextran can be obtained. Aqueous two-phase systems can also be generated using a polymer (e.g. PEG or

dextran) and a salt such as sodium or potassium phosphate. Aqueous two-phase separations take place at certain compositions only. The figure below shows a PEG-dextran phase diagram where a solubility curve separates the two- phase region (above the curve) from the single phase region (below the curve). Such "binary" phase diagrams which are based on the compositions of the two polymers (or polymer and salt) are used for determining the concentrations needed for an extraction process. These phase diagrams also predict the polymer/salt content of the raffinate and the extract phases. The composition of the individual phases generated can be obtained using tie-lines as shown in the figure.

Biomolecule	Recovery (%)
Xylanase	41
β-glucosidase	85 - 95
Polyphenol oxidase	50
Lipase	68
β-galactosidase	90
Glucoamylase	96
Horseradish peroxidase	75
Thaumatin	90 - 95
β-galactosidase	85 - 100
Fumarate hydratase (*Brevibacterium ammoniagenes*)	83
Fumarate hydratase (*E. coli*)	93
Aspartate-ammonia-lyase (*E. coli*)	96

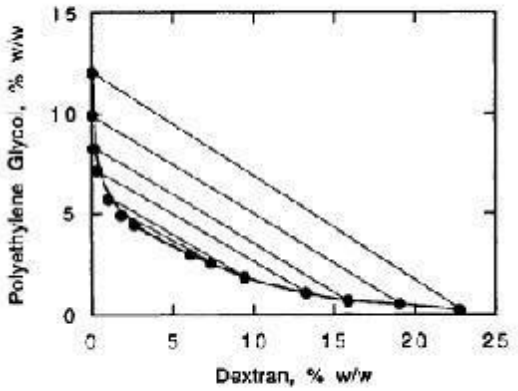


Fig. 2. Phase diagram for a PEG 6000-dextran D48 two-phase system at 20 °C (redrawn from Albertsson, 1986).

The partition of a solute between the two aqueous phases depends on its physicochemical properties as well as those of the two polymers (or polymer and salt).Factors Affecting Protein Partitioning in Two-Phase Aqueous Polymer Systems

1.Protein molecular weight

2.Protein charge, surface properties 3.Polymer molecular weight 4.Phase composition

5. Salt effects

6. Affinity ligands attached to polymers 7.pH

8.Temperature

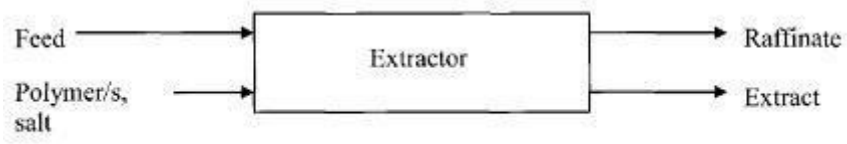


Fig. 7.4 Aqueous two-phase extraction

In PEG/dextran aqueous two-phase extraction of proteins, the partition behavior depends to a great extent on the relative polymer composition. It also depends on the solution pH and the molecular weight of the protein. Generally speaking, protein partitioning into the PEG rich phase is favored. When a polymer-salt combination is used to generate the aqueous two-phase system, a protein partitions favorably into the polymer rich phase. The general scheme for aqueous two-phase extraction is shown in Fig. 7.4.Extraction by an ATPS offers advantages for processing on a large scale, such as the possibility of obtaining a high yield, the possibility of continuous processing and a reduction in operational cost in relation to the costs of conventional processes.

PEG-DEXTRAN SYSTEMS

Effect of Polymer Molecular Mass (MM)

An increase in the molecular mass of dextran or of PEG will lower the concentration required for phase separation. The polymer molecular mass influences protein partitioning as a direct result of interactions between the two polymers. It has been found that the partitioned protein behaves as if it were more attracted by smaller polymer sizes and more repelled by larger polymers,

provided all other factors such as polymer concentrations, salt composition, temperature and pH are kept constant. It was observed that smaller protein molecules and amino acids were not affected as much as larger ones. For some proteins the partition coefficients increased as the MM of dextran increased if all other conditions were kept constant, but little effect was found for low MM proteins (Cytochrome C, 16,000). When the same proteins were partitioned in systems with different PEG MM, their partition coefficients decreased as the PEG MM increased, and for cytochrome C the effect was the smallest. This was attributed to the fact that when the PEG MM is increased, a weaker repulsion energy is required to cause phase separation. Repulsive interactions between the polymer and the protein become stronger as the polymer MM is increased, resulting in a distribution of the protein towards the phase containing the polymer with an unchanged MM. A weak net repulsion between the proteins and the polymer is sufficient to change the distribution when the polymer MM is changed.

Effect of Polymer Concentration

It was found that proteins with MM less than 20,000 showed a linear relationship between the ln K in PEG-dextran systems and a difference in PEG concentration between the phases, for any particular system. They found that it was possible to predict the partitioning of a protein at any concentration in that particular system if one partition coefficient in the system were known.

However, others found that for some proteins the partition coefficient was inversely correlated to phase concentration in a PEG-dextran system, showing that better separation could be achieved at high polymer concentrations. This, however, may also affect the concentration of proteins that can be manipulated in the system as polymer concentration has a directly inverse effect on protein solubility.

Effect of Salts

Salts can affect protein partitioning in different ways in PEG-dextran systems: one is by altering the physical properties of the systems the hydrophobic difference between the phases and the other is by the partitioning of ions between the phases, which affects the partitioning of proteins according to their molecular charge.

Salts have been added to PEG-dextran systems to increase the selectivity of

protein partitioning in the aqueous two-phase methodology application for biological separations. It was observed that salt ions partition differently between the phases, causing an uneven distribution in the system that generates a difference in electrical potential between the phases. This difference in electrical potential would be independent of salt concentration, but linearly dependent on the partition behaviour of the ions. It was also observed that polyvalent anions such as phosphate, sulphate and citrate partitioned preferentially into dextran-rich phases, while halides partitioned nearly equally. As an example, negatively charged materials have higher partition coefficients in phases containing sodium sulphate rather than sodium chloride, while the reverse holds for positively charged materials. Partition coefficients of negatively charged materials decrease when the cationic series is changed from lithium to sodium to potassium. The ratio between the phosphate ions, rather than the concentration, was decisive for the difference in electrical potential. This applies to multivalent ions, which show a series of pH-dependent dissociations and was clearly the reason for the potential difference found between the two phases (Kula et al., 1982).

PEG-SALT SYSTEMS

The formation of PEG-salt systems was first observed in the 1950s, but the theoretical fundamentals have not been well explained. It was found that for PEG solutions the addition of some inorganic salts (sulphates and carbonates) is more effective than the addition of others in reducing the critical concentration of cloud point curves. These inorganic salts dramatically reduced the PEG cloud point at high temperatures.

PEG-salts systems have been introduced for the practical application of large-scaleprotein separation because of the larger droplet size, greater difference in density between the phases, lower viscosity and lower costs, leading to a much faster separation than in PEG-dextran systems. Industrial application of PEG-

Biomolecule	Ligands attached to PEG	Recovery (%)
Lactate dehydrogenase	Tryazine dye-Cibacron Blue F3G-A	81.3
β-galactosidase	p-aminophenyl-β-D-thiogalactopiranoside – (APGP)	83
Protein A	IgG human	87
Lactate dehydrogenase	Eudrogit-Cibacron Blue	54
Penicillin acylase	Trimethylamina	97
Trypsin	Trypsin inhibitor	82

salt systems was improved by the availability of commercial separators, which allowed faster continuous protein.

Initially PEG-phosphate systems were widely used where scientists have studied ways of recycling the phosphate phase of the systems to minimize environmental pollution. The recycling of the phosphate phase was achieved by its separation from the solids by the use of alcohols. PEG from the top PEG-rich phase can also be successfully recycled.

More recently PEG-sulphate systems have begun to be used where separation of some biomaterial was achieved with PEG 4000 and (NH4)2SO4 at pH 7-7.5. The presence of 2% NaCl (0.17 M) made the separation much worse. With 4% NaCl (0.34 M), a poor separation was obtained (a tenfold decrease in K for aspartase). Since a pH or phase ratio change was not observed, the dramatic change in K was considered to be due to a change in hydrophobicity between the phases.

AFFINITY PARTITIONING

In the last 30 years, several groups have studied methods to increase partitioning by the use of biospecific interactions in ATPSs.

The initial works on affinity partitioning in ATPSs were to purify trypsin by usingPEG-bound ligand p-aminobenzamidine and S-23 myeloma protein by using dinitrophenol as ligand.The degree of affinity partitioning, Kaff, can be described by the ratio between the partition coefficients of proteins with and without a ligand:

This equation describes the increase in the partition coefficient of a protein by the binding of a specific ligand to the PEG-rich phase.

Affinity partitioning results in specific extractions of proteins, nucleic acids, membranes, organelles and even cells, mainly when biospecific ligands are used.

Large Scale Extraction Schemes

Extraction processes can be divided into two general schemes:

•Batch extractions

•Continuous extractions,

Continuous extractions can also be further divided into the following schemes:

•Single stage continuous extraction

•Multi stage continuous extraction

In turn, multi stage continuous extraction can be divided into two general modes as:

•Crosscurrent continuous extraction

•Counter current continuous extraction These will be studied in the following sections.

Batch extraction

In a batch extraction process a batch of feed solution is mixed with a batch of extracting solvent in an appropriate vessel. The solute distributes between the two phases depending on its partition coefficient. The rate at which the transfer of solute takes place from the feed to the extracting solvent depends on the mixing rate. Once equilibrium is attained, the mixing is stopped and the extract and raffinate phases are allowed to separate. The separation funnel commonly seen in chemistry laboratories is the simplest small-scale batch extraction device. Mixer-settler units are usually used for large-scale batch extraction. The basic principle of batch extraction using a mixer settler unit is shown in Fig. 7.5. The mixer unit must be able to generate high interfacial area, must provide high solute mass transfer coefficient and cause low entrainment of air bubbles. The settler unit must have a low aspect ratio (L/D), i.e. be of flat geometry, must allow easy coalescence and phase separation, and must allow for easy collection of raffinate and extract as separate streams. The antibiotic penicillin partitions favorably in an

organic solvent from an aqueous fermentation media at acidic conditions. However, at a neutral pH, the partitioning from organic phase to aqueous phase is favored. Thus the antibiotic could be purified by sequential reversed batch extraction, where the antibiotic is moved from aqueous to organic phase and back again (as shown in Fig. 7.6). This sequence is usually repeated a few times in order to obtain highly pure antibiotic.

If a batch of feed containing R volume of initial solvent and an initial solute concentration of C0 is mixed with S volume of pure extracting solvent, the concentration distribution in the extract and the raffinate at equilibrium is given by:

CE = KCR

Where

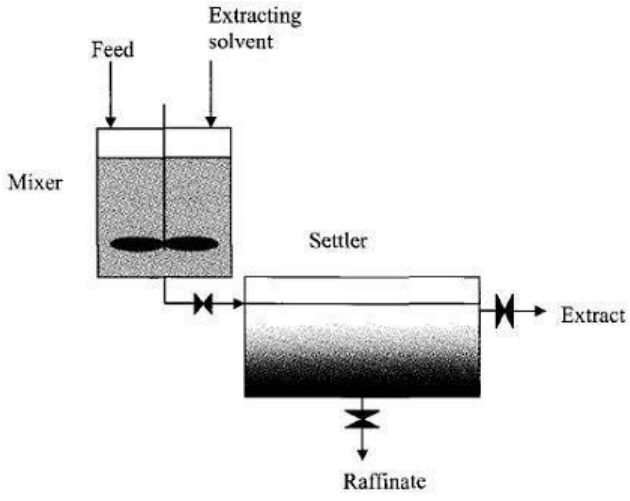


Fig. 7.5 Batch extraction using mixer settler unit

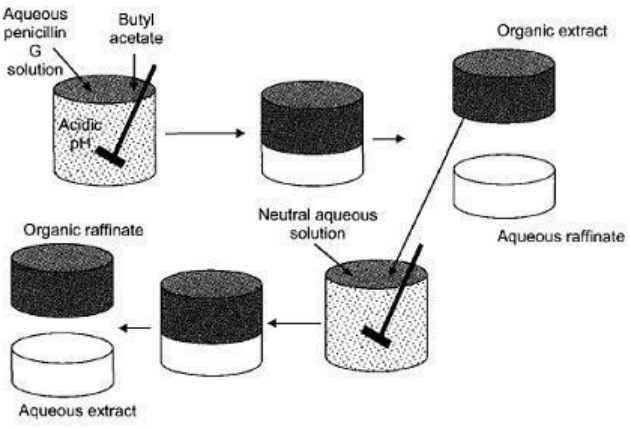


Fig. 7.6 Sequential reverse batch extraction

CE solute concentration in extract (kg/m3) CR solute concentration in raffinate (kg/m3)

Supercritical Fluid Extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. SFE can be used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). These essential oils can include limonene and other straight solvents. Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol ormethanol. Extraction conditions for Supercritical carbon dioxide are above the critical temperature of 31°C and critical pressure of 74 bar. Addition of modifiers may slightly alter this. The discussion below will mainly refer to extraction with CO_2, except where specified. The properties of a supercritical fluid can be altered by varying the pressure and temperature, allowing selective extraction. For example, volatile oils can be extracted from a plant with low pressures (100 bar), whereas liquid extraction would also remove lipids. Lipids can be removed using pure CO_2 at higher pressures, and then phospholipids can be removed by adding ethanol to the solvent. The same principle can be used to extract polyphenold and unsaturated fatty acids separately from wine wastes.

The system must contain a pump for the CO_2, a pressure cell to contain the sample, a means of maintaining pressure in the system and a collecting vessel. The liquid is pumped to a heating zone, where it is heated to supercritical conditions. It then passes into the extraction vessel, where it rapidly diffuses into the solid matrix and dissolves the material to be extracted. The dissolved material is swept from the extraction cell into a separator at lower pressure, and the extracted material settles out. The CO_2 can then be cooled, re-compressed and recycled, or discharged to atmosphere.

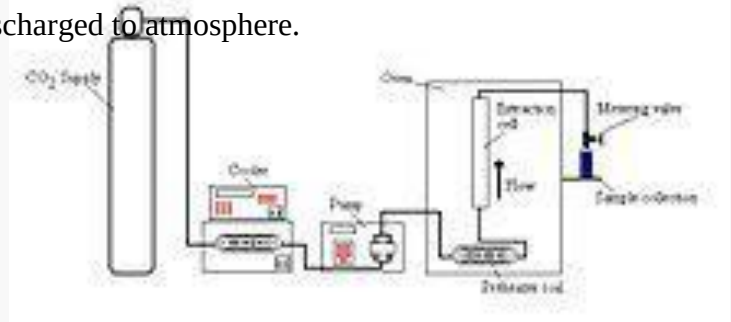


Figure 1. Schematic diagram of SFE apparatus

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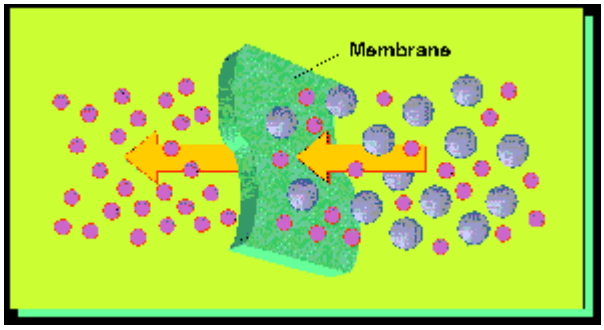
DEPARTMENT OF BIOTECHNOLOGY

UNIT – V - FERMENTATION AND DOWNSTREAM PROCESSING
SBTA5203

MEMBRANE SEPERATIONS

A **membrane** is a **selective barrier** that permits the separation of certain species in a fluid by combination of sieving and sorption diffusion mechanism. Separation is achieved by **selectively passing (permeating)** one or more components of a stream through the membrane while **retarding** the passage of one or more other components. See the Figure. Membranes can selectively separate components over a wide range of particle sizes and molecular weights, from macromolecular materials such as starch and protein to monovalent ions. Membranes have gained an important place in chemical technology and are used in a broad range of applications.

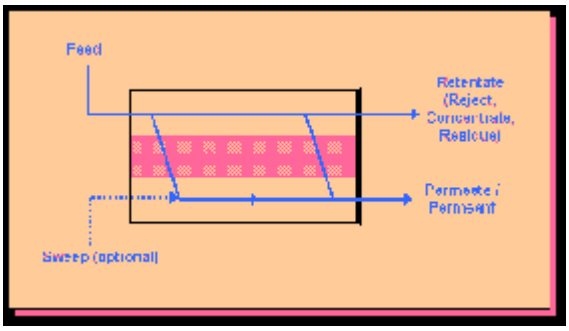


The key properties determining membrane performance are **high selectivity and fluxes, good mechanical, chemical and thermal stability under operating conditions, low fouling tendencies and good compatibility with the operating environment; and cost effective and defect-free production**.

Although the major uses of membranes are in the production of potable water and separation of industrial gases, they can be used for many other important applications such as filtration of particulate matter from liquid suspensions, air or industrial flue gas and the dehydration of ethanol azeotropes.

More specialised applications include ion separation in electrochemical processes, dialysis of blood and urine, artificial lungs, controlled release of therapeutic drugs, membrane-based sensors, etc.

Membrane processes are characterized by the fact that a feed stream is divided into 2 streams: retentate and permeate. The most general process can be depicted by the following Figure:



The **retentate** is that part of the feed that **does not pass through** the membrane, while the **permeate** is that part of the feed that **does pass through** the membrane. The optional "sweep" is a gas or liquid that is used to help remove the permeate. The component(s) of interest in membrane separation is known as the **solute**. The solute can be **retained** on the membrane membrane and removed in the retentate or passed through the membrane in the permeate.

It is important to note that there are 3 different mechanisms by which membrane can perform separations:

- By having holes or pores which are of such a size that certain species can pass through and others cannot. This mechanism is called size exclusion.
- By selective retardation by the pores when the pore diameters are close to molecular sizes. This mechanism is called pore flow.
- By dissolution into the membrane, migration by molecular diffusion across the membrane, and re-emergence from the other side. This is called solution diffusion.

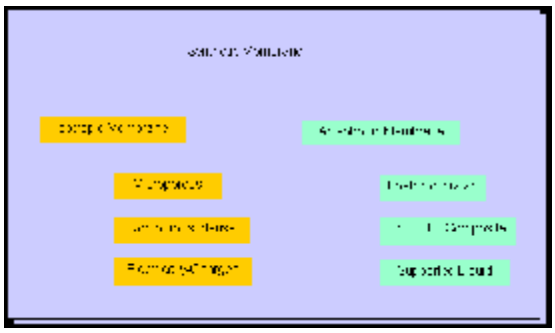
CLASSIFICATION OF SYNTHETIC MEMBRANES

A membrane can be natural or synthetic, thick or thin, its structure can be homogeneous or heterogeneous, transport across membrane can be active or passive, passive transport can be driven by various means (e.g. pressure, concentration, electrical difference), neutral or charged. As such, membranes can

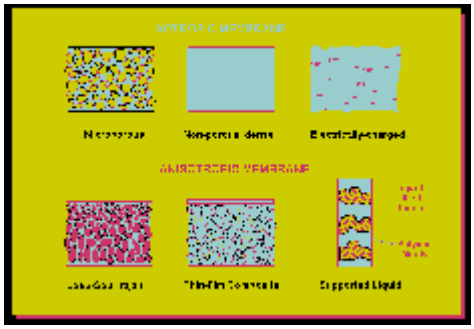
be **classified according to different viewpoints**. The first classification is by **nature**, i.e. **biological or synthetic** membranes. This is the clearest distinction possible. Synthetic membranes can be subdivided into organic (polymeric or liquid) and inorganic (e.g. ceramic, metal) membranes.

Another means of classifying membranes is by **morphology** or **structure** - for the case of solid synthetic membranes, the 2 types of membrane structures are the symmetric and asymmetric (anisotropic) membranes.

The principal types of **polymeric membranes** are classified as shown in the Figure below:



The Figure shows schematically the different types of membranes. This classification is, however, rather arbitrary, and many structures fit more than one of the above-mentioned classes. For example, a membrane may be microporous, asymmetric in structure, and have electrical charges.



The **IUPAC classification** for pores sizes are as follows:

Macropores	> 50 nm
Mesopores	2 – 50 nm
Micropores	< 2 nm

The following membranes are briefly discussed:

Isotropic Membranes

Microporous Membranes

Non-Porous, Dense Membranes

Electrically-Charged

Membranes

Anisotropic (Asymmetric) Membranes

Loeb-Sourirajan Membranes

Thin-Film (Composite)

Membranes Liquid Membranes

Membrane Materials

Almost all industrial membrane processes are made from natural or synthetic polymers. These membranes are sometimes known as organic membranes. Natural polymers include wool, rubber (polyisoprene) and cellulose. Examples of synthetic polymers include polyamide, polystyrene and polytetrafluoroethylene (Teflon).

Membranes can also be made from other non-polymeric materials. Such membranes include inorganic membranes (for example metal, ceramic, carbon and zeolites) and liquid membranes.

In addition, recent developments had led to the introduction of the so-called Hybrid Membranes (or Mixed Matrix Membranes, where both organic and inorganic components are used).

Another variation in membranes application is the Bipolar Membranes (BPM).

Polymeric Membranes

Synthetic polymers are produced by polymerisation of a monomer (condensation or addition) or by the co-polymerisation of 2 different monomers. The resulting polymer is categorised as having:

(1) a long, linear chain, such as linear polyethylene

(2) a branched chain, such as polybutadiene

(3) a three-dimensional highly cross-linked structure, such as phenol- formaldehyde

(4) moderately cross-linked structure, such as butyl rubber

The linear-chain polymers soften with an increase in temperature and are often soluble in organic solvents, and are referred to as thermoplastic polymers.

At the other extreme, high cross-linked polymers do not soften appreciably, are almost insoluble in most organic solvents, and are referred to as thermosetting polymers.

Material	Application					
	MF	UF	NF/RO	GS	PV	MD
Cellulose acetate	×	×	×	×	×	
Cellulose esters	×					
Cellulose nitrate	×					
Poly (vinyl alcohol)	×					
Polyacrylonitrile		×			×	
Poly (vinyl chloride)	×					
PVC copolymer	×	×				
Aromatic polyamide	×	×	×			
Aliphatic polyamide	×	×				
Polyimide	×	×	×	×		
Polysulfone	×	×				
Polyetheretherketone (PEEK)	×	×		×		
Polycarbonate	×					
Polyester	×					
Polypropylene	×				×	×
Polyethylene	×				×	×
Polytetrafluoroethylene (PTFE)	×	×			×	
Poly (vinylidene difluoride) (PVDF)	×	×			×	×
Polydimethylsiloxane (PDMS)				×	×	

Examples of polymers used in membrane fabrication are shown in the Figures below.

Inorganic Membranes

Inorganic membranes refer to membranes made of materials such as **ceramic, carbon, silica, zeolite, various oxides** (alumina, titania, zirconia) and **metals** such as palladium, silver and their alloys. Inorganic membranes can be classified into 2 major categories based on its structure: porous inorganic membranes and dense (non-porous) inorganic membranes. Microporous inorganic membranes have 2 different structures: symmetric and asymmetric; and include both amorphous and crystalline membranes.

Microporous inorganic membranes can be obtained by coating of a porous support with a colloidal solution, called **sol**. The sol can consist of either dense spherical particles (colloids of oxides such as Al_2O_3, SiO_2 or ZrO_2) or polymeric macromolecules.

Application of dense inorganic membranes is primarily for highly selective separation of gases such as hydrogen and oxygen. However, dense membranes have limited industrial application due to their low permeability compared to porous inorganic membranes. Therefore, today's commercial inorganic membrane market is dominated by porous membranes.

Metal Membrane

Dense, metal membranes are being considered for the separation of hydrogen from gas mixtures. Palladium (Pd) and its alloys are the dominant material used, due to its high solubility and permeability for hydrogen. Palladium, however, is expensive. One alternative is to coat a thin layer of palladium on a tantalum or vanadium support film. Alternative to palladium and less expensive are tantalum and vanadium, which are also quite permeable to hydrogen.

Recent focus is on supported thin metallic membranes with thickness ranging from submicron to a few ten microns. The advantages include reduced material costs, improved mechanical strength and possibly higher flux. The main

developments have been the production of composite palladium membranes for use in catalytic membrane reactors (CMRs). This development is based on the concept of process intensification, one important aspect if which is the potential for combining the reaction and separation stages of a process in one unit. One such application is the CMR. Apart from the benefit inherent in cost reduction of plant and maintenance, there is also the potential attainment of higher conversions and product yields.

The composite palladium membrane used in the CMR is composed of a thin layer of Pd, or Pd alloy, deposited onto a porous substrate, such as a ceramic or stainless steel.

The composite palladium membrane is placed adjacent to a catalyst bed and effects the selective removal of hydrogen from the catalytic reaction source. Another application is the use of these membranes to control the feed rate during partial oxidation reactions (e.g. addition of hydrogen).

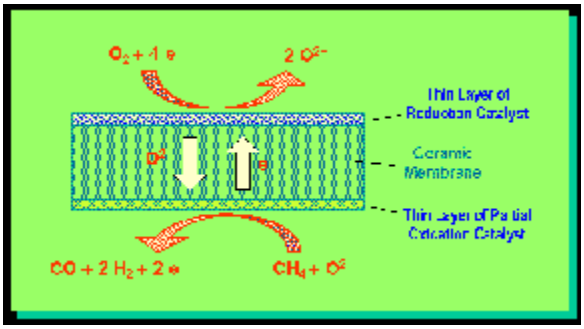
A major problem associated with metal membranes is the surface poisoning effects (e.g. by a carbon-containing source) which can be more significant for thin metal membranes.

Ceramic Membrane

These membranes are made from aluminum, titanium or silica oxides. They have the advantages of being chemically inert and stable at high temperatures. This stability makes ceramic microfiltration and ultrafiltration membranes particularly suitable for food, biotechnology and pharmaceutical applications in which membranes require repeated steam sterilization and chemical cleaning. Ceramic membranes have also been proposed for gas separations.

An example application of recent development is in the production and processing of syngas (synthetic gas - a mixture of hydrogen and carbon monoxide). The key part of the process involves the separation of oxygen from air in the form of ions to oxidize the methane.

A schematic representation of the process is given in the Figure.



Oxygen feeding from air is split at the perovskite-type membrane surface and is transported as O^{2-}. The advantage of the membrane-based process is that the production of syngas takes place in a single-step operation occurring on one of the membrane sides, This process eliminates the need for a separate oxygen production plant, and might lead to significantly lower energy and capital costs.

Some remaining problems include:

- Difficulties in proper sealing of the membranes in modules operating at high temperature
- Extremely high sensitivity of membranes to temperature gradient, leading to membrane cracking
- Chemical instability of some perovskite-type materials in the high temperature reduction conditions

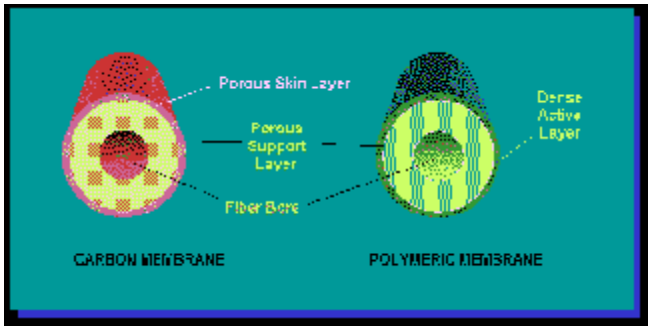
Carbon Membranes

Carbon molecular sieve (CMS) membranes have been identified as very promising candidates for gas separation, both in terms of separation properties and stability. Carbon molecular sieves are porous solids that contain constricted apertures that approach the molecular dimensions of diffusing gas molecules. As such, molecules with only slight differences in size can be effectively separated through molecular sieving.

Carbon membranes can be divided into 2 categories: supported and unsupported. Unsupported membranes have 3 different configurations: flat (film), hollow fiber

and capillary while supported membranes consisted of 2 configurations: flat and tube.

The Figure showed a comparison between carbon asymmetric membrane and polymeric asymmetric membrane where the main difference is in the skin layer.



CMS membranes can be obtained by pyrolysis of many thermosetting polymers such as poly(vinylidene chloride) or PVDC, poly(furfural alcohol) or PFA, cellulose triacetate, polyacrylonitrile or PAN, and phenol formaldehyde.

Zeolite Membranes

Zeolites are microporous crystalline alumina-silicate with a uniform pore size. Zeolites are used as catalysts or adsorbents in a form of micron or submicron-sized crystallites embedded in millimeter-sized granules. The zeolite type prepared most often as a membrane is MFI.

Main problem - relatively low gas fluxes compared to other inorganic membranes …. Due to the fact that relatively thick zeolite layers are necessary to get a pinhole-free and crack-free zeolite layer. Overcome: use thin layer supported on others.

Other problem: thermal effect of zeolites. The zeolite layer can exhibit negative thermal expansion, i.e. in the high temperature region the zeolite layer shrinks …. But the support continuously expands, resulting in thermal stress problems for the attachment of the zeolite layer to the support, as well as for the connection of the individual micro-crystals within the zeolite layer.

MEMBRANE PROPERTIES

Various membrane properties can be classified as follows:

Physical - porosity, pore size and pore distribution, thickness, tortousity, thermal stability, etc.

Separation - permeate flow rate or permeation flux, permeability, selectivity.

Surface and electrochemical - streaming potential, zeta potential (or electrokinetic potential), membrane potential (voltage potential difference), surface charge density, wettability (hydrophilic or hydrophobic), etc

Electrical - impedance measurements to determine the membrane conductance and capacitance

Separation properties of membranes depend not only on their physical properties, but also on their surface charge and electrical properties. Many of these properties are inter-related, and vary from membrane to membrane, as well as the environment in which the membrane is exposed, e.g. feed conditions (pH, temperature, concentration of solutes, various properties of solutes, etc). Suffice to say, each membrane-solute system is unique, and must be evaluated experimentally.

Membrane Characterization

Membrane processes can cover a wide range of separation problems with a specific membrane being required for every problem. Membranes may differ significantly in their structure and consequently in their functionality. To know what membrane to use in a particular separation process, different membranes must be characterized in terms of structure and mass transport properties. Because very different membranes are used, different techniques are required for characterization.

Membrane characterization is a very important part of membrane research and development because the design of membrane processes and systems depends on reliable data relating to membrane properties.

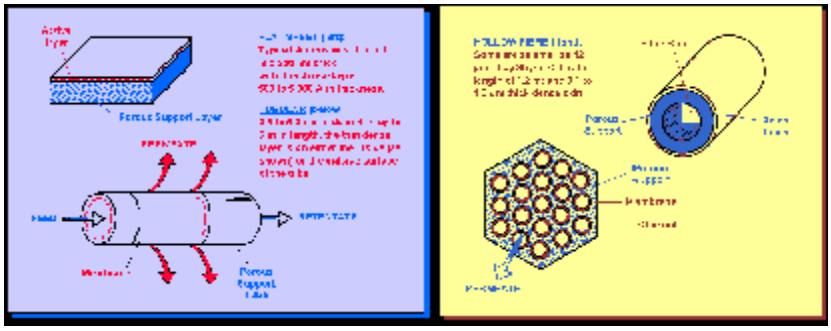
The following sections briefly discuss the following:

Characterization of Porous Membranes Characterization of Dense, Homogeneous Membranes Characterization of Charged Membranes

Membrane Shapes

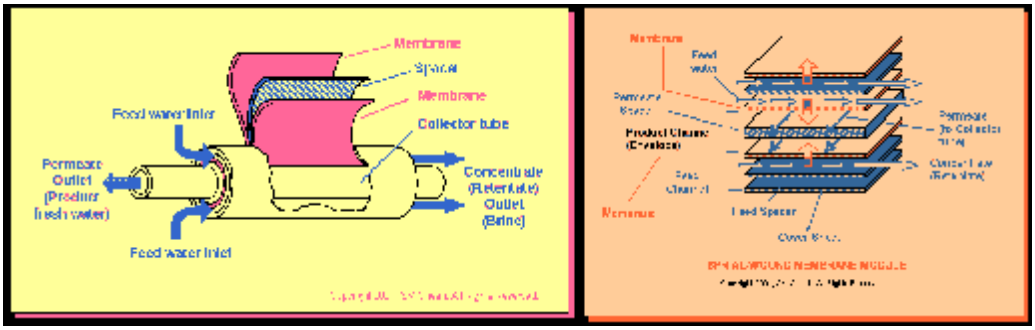
Membranes are configured for the most part in 3 ways: (1) as **long cylinders**

such as hollow fibres, capillaries or tubes, (2) as **sheets** which are either rolled up or maintained in a flat condition, and (3) as various **monolithic** designs. Common membrane shapes include flat sheet, tubular (Figure - left), hollow fiber and monolith (Figure - right).



Flat sheets used in **plate-and-frame modules** can be circular, square, or rectangle in cross-section. The sheets are separated by support plates that channel the permeate.

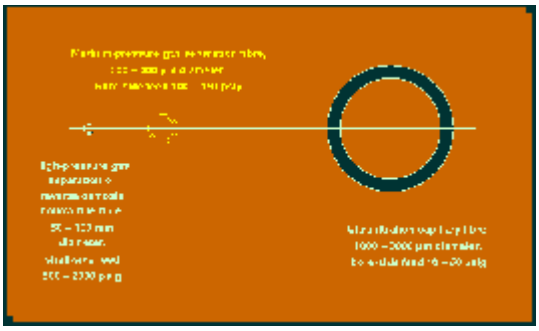
Flat sheets can also be fabricated into **spiral-wound modules**. The simplest design is the **single-leaf** spiral wound module. See the Figure (left). A "leaf" or laminate, consisting of 2 membrane sheets separated by a spacer sheet forms the channel for flow of feed or permeate. The leaf is attached to the axial collector tube and is wound around the tube. The configuration is sometimes described as a membrane "envelope". Some design can have 2 or more leaves. Permeate flows through the membrane into the product channel where it is conducted spirally to the collector tube. See the Figure (right) for the flow details.



An alternative to the flat sheet membranes is the **hollow-fibre membranes**. The diameter of hollow fibres varies over a wide range from 50 to 3,000 micrometer. Fibres can be made with a uniformly dense structure, but preferably are formed as a microporous structure having a dense selective layer on either the outside

(sometimes referred to as the shell side) or the inside surface.

The Figure showed the various hollow-fibre membranes:



The dense surface layer can be either integral with the fibre or a separate layer coated onto the porous support fibre. Many fibres can be packed into bundles and potted into tubes to form a membrane module.

Fibres of 50 - 200 micrometer diameter are usually called **hollow fine fibres**. Such fibres can withstand very high hydrostatic pressures applied from the outside (shell side), so they are used in reverse osmosis or high-pressure gas separation applications (up to 1,000 psi). The feed fluid is applied to the outside of the fibre and the permeate is removed down the fibre bore.

When the fibre diameter is 200 - 500 micrometer, the fluid is commonly applied to the inside bore of the fibre, and the permeate is removed from the outside. Thus method is used for low-pressure gas separations and for ultrafiltration or hemodialysis. Fibres with diameter greater than 500 micrometer are called **capillary fibres**.

Industrial Membrane Modules

Industrial membrane plants often require hundreds to thousands of square metres of membrane to perform the separation required on a useful scale. There are several ways to economically and efficiently package membranes to provide a large surface area for effective separation.

From an overall cost standpoint, not only is the cost of membranes per unit area important, but also the cost of the containment vessel into which they are mounted. Basically the problem is how one can pack the most area of membranes into the least volume, to minimise the cost of the containment vessel consistent with providing acceptable flow hydrodynamics in the vessel.

These packages are called membrane modules. The most important were: Plate-and-frame
Tubular
Spiral-
wound
Hollow fiber

Plate-and-Frame Modules

The plate-and-frame modules were one of the earliest types of membrane system, but because of their relatively high cost they have been largely replaced in most applications by spiral-wound modules and hollow-fiber modules. Plate-and-frame modules are now used only in electrodialysis and pervaporation systems and in a limited number of reverse osmosis and ultrafiltration applications with highly fouling conditions.

Tubular Modules

These modules are now generally limited to ultrafiltration applications, for which the benefit of resistance to membrane fouling outweighs the high cost. Tubular membranes contains as many as 5 to 7 smaller tubes, each 0.5 to 1.0 cm in diameter, nested inside a single larger tube. In a typical tubular membrane system, a large number of tubes are manifolded in series. The permeate is removed from each tube and sent to a permeate collection header. An example is shown in the Figure.

Spiral-wound modules

Industrial-scale modules contain several membrane envelopes, each with an area of 1 to 2 m^2, wrapped around the central collection pipe. Multi-envelope designs minimise the pressure drop encountered by the permeate travelling toward the central pipe. The standard industrial spiral-wound module is 8-inch in diameter and 40-inch long. An example of the 4-envelope (4-leaf) module is shown in the Figure.

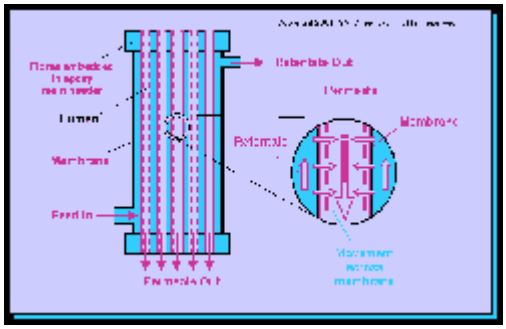
The module is placed inside a tubular pressure vessel. The feed solution passes across the membrane surface, and a portion of the feed permeates into the membrane envelope, where it spirals towards the centre and exits through the collection tube as shown in the Figure.

4 to 6 spiral-wound membrane modules are normally connected in series inside a

single pressure vessel. A typical 8-inch diameter tube containing 6 modules has 100 to 200 m^2 of membrane area. An example of a membrane tube with 2 modules is shown in the Figure.

Hollow-fibre Modules

Hollow-fibre modules are characteristically 4-8 inch (10-20 cm) in diameter and 3-5 (1.0-1.6 m) feet long. Hollow-fibre units are almost always run with the feed stream on the outside of the fibre. Water passes through the membrane into the inside or "lumen" of the fibre. A number of hollow-fibres are collected together and "potted" in an epoxy resin at both ends and installed into an outer shell. An example is shown in the Figure.



Hollow-fibre membrane modules are formed in 2 basic geometries: (a) shell-side feed design, and (b) bore-side feed design, as shown in the Figure.

In the shell-side feed design, a loop or closed bundle of fibres is contained in a pressure vessel. The system is pressurised from the shell side, and the permeate passes through the fibre wall and exits through the open fibre ends. This design is easy to fabricate and allows very large membrane areas to be contained in an economical system. Because the fibre wall must support considerable hydrostatic pressure, the fibres usually have small diameters and thick walls, typically 50-mm inner diameter and 100 - 200 mm outer diameter.

In the bore-side feed design, the fibres are open at both ends and the feed is circulated through the bore (annulus area) of the fibres. To minimise pressure drop inside the fibres, the diameter are usually larger than those of the fine fibres used in the shell-side feed design.

These so-called capillary fibres are used in ultrafiltration, pervaporation, and some low to medium pressure gas applications.

The single greatest advantage of hollow-fibre modules is the ability to pack a

very large membrane area into a single module. For example, in an 8-inch diameter, 40-inch long spiral-wound module would contain about 20 - 40 m^2 of membrane area. The equivalent hollow-fibre module filled with fibres of 100-mm diameter, will contain approximately 600 m^2 of membrane area.

Medical Applications of Membranes

Polymeric membranes have become widely used as components of medical devices and implants, drug delivery systems, diagnostic assays, biosensors, etc. Membrane processes are used effectively for treatment of patients with various pathologies for the removal of toxins from blood (e.g. hemodialysis) or for gas exchange with blood (e.g. blood oxygenation). Membranes of suitable molecular mass cut-off are used in bio-artificial liver, bio-artificial pancreas, and as selective barriers to prevent the immune system components from coming into contact with implants while allowing nutrients and metabolites to permeate freely to and from cells.

In medical devices, membranes exhibit in general the same properties they present in microfiltration and ultrafiltration processes.

Examples of medical applications of membranes

This section briefly discusses several applications, namely the following: Hemodialysis (Artificial Kidney)

Blood Oxygenation (Artificial Lung)

Artificial Pancreas

Controlled Drug Delivery (Controlled-Release Pharmaceuticals)

WHOLE BROTH PROCESSING

The concept of recovering a metabolite directly from an unfiltered fermentation broth is of considerable interest because of its simplicity, the reduction in process stages and the potential cost savings. It may also be possible to remove the desired fermentation product continuously from a broth during fermentation so that inhibitory effects due to product formation and product degradation can be minimized throughout the production phase (Roffler et al., 1984; Diaz, 1988).

Bartels et al. (1958) developed a process for adsorption of streptomycin on to a series of cationic ion-exchange resin columns directly from the fermentation broth, which had only been screened to remove large particles so that the columns would not become blocked. This procedure could only be used as a batch process.

Belter et al. (1973) developed a similar process for the recovery of novobiocin. The harvested broth was first filtered through a vibrating screen to remove large particles. The broth was then fed into a continuous series of well-mixed resin columns fitted with screens to retain the resin particles, plus the absorbed novobiocin, but allow the streptomycete filaments plus other small particulate matter to pass through. The first resin column was removed from the extraction line after a predetermined time and eluted with methanolic ammonium chloride to recover the novobiocin.

Karr et al. (1980) developed a reciprocating plate extraction column (Fig. 10.33) to use for whole broth processing of a broth containing 1.4 g dm"3 of a slightly soluble organic compound and 4% undissolved solids provided that chloroform or methylene chloride were used for extraction. Methyl-iso-butyl ketone, diethyl ketone and iso-propyl acetate were shown to be more efficient solvents than chloroform for extracting the active compound, but they presented problems since they also extracted impurities from the mycelia, making it necessary to filter the broth before beginning the solvent extraction. Considerable economies were claimed in a comparison with a process using a Pod-bielniak extractor, in investment, maintenance costs, solvent usage and power costs but there was no significant difference in operating labour costs.

An alternative approach is to remove the metabolite continuously from the broth during the fermentation. Cycloheximide production by Streptomyces griseus has been shown to be affected by its own feed-back regulation (Kominek, 1975). Wang et al. (1981) have tested two techniques at laboratory scale for improving production of cycloheximide. In a dialysis method (Fig. 10.34), methylene chloride was circulated in a dialysis tubing loop which passed through a 10 dm"3 fermenter. Cycloheximide in the fermentation broth was extracted into the methylene chloride. It was shown that the product yield could be almost doubled by this dialysis-solvent extraction method to over 1200 fig cm"3 as compared with a control yield of approximately 700 fig cm"3. In a resin method, sterile

beads of XAD-7, an acrylic resin, as dispersed beads or beads wrapped in an ultrafiltration membrane, were put in fermenters 48 hours after inoculation. Some of the cycloheximide formed in the broth is absorbed by the resin. Recovery of the antibiotic from the resin is achieved by solvents or by changing the temperature or pH. When assayed after harvesting, the control (without resin) had a bioactivity of 750 fig cm"3. Readings of total bioactivity (from beads and broth) for the bead treatment and the membrane-wrapped bead treatments

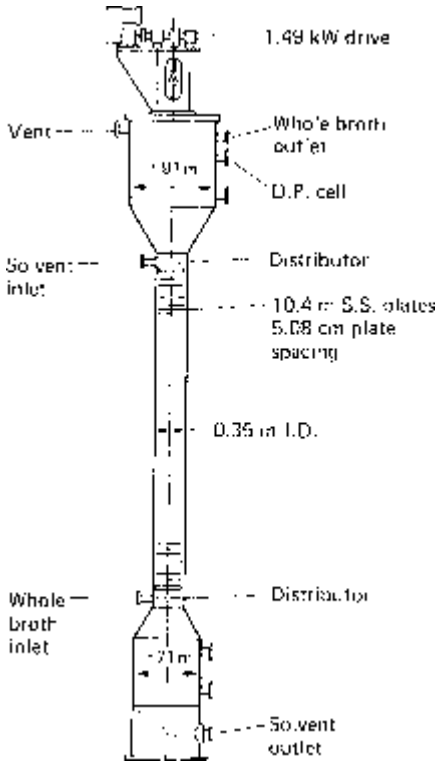
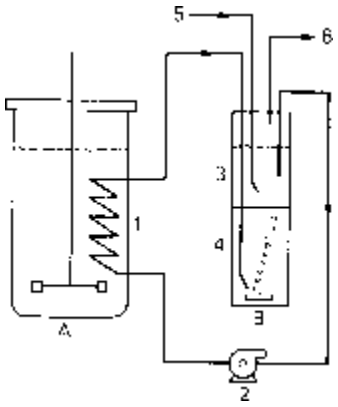


Fig. 10.33a. Diagram of a 0.35-m internal diameter reciprocating plate column (Karr et al, 1980).



A Fermenter B
Extractor 1 Dialysis
tubing
2 Pump Aqueous layer Solvent laysr Air inlet Air outlet

Fig. 10.34. Dialysis-extraction fermentation system (Wang et al 1981). A Fermenter B Extractor
1 Dialysis tubing
2 Pump Aqueous layer Solvent laysr Air inlet Air outlet

Fig. 10.33a. Diagram of a 0.35-m internal diameter reciprocating plate column (Karr et al, 1980).

Fig. 10.34. Dialysis-extraction fermentation system (Wang et al 1981).

(a) Vacuum and flash fermentations for the direct recovery of ethanol from fermentation broths.

(b) Extractive fermentation (liquid-liquid and two-phase aqueous) for the recovery of ethanol, organic acids and toxin produced by Clostridium tetani.

(c) Adsorption for the recovery of ethanol and cy-cloheximide.

(d) Ion-exchange in the extraction of salicylic acid and antibiotics.

(e) Dialysis fermentation in the selective recovery of lactic acid, salicylic acid and cycloheximide.

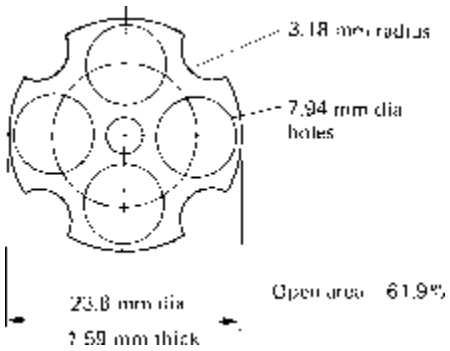


Fig. 10.33b. Plan of a 23.8-m stainless-steel plate for a 25-mm diameter reciprocating plate test column (Karr et al., 1980).

were 1420 ¿u,g cm"3 and 1790 /xg cm"3 respectively.

Roffler et al. (1984) reviewed the use of a number of techniques for the in-situ recovery of fermentation products:

Hansson et al. (1994) have used an expanded adsorption bed for the recovery of a recombinant protein produced by E. coli directly from the fermentation broth. The protein was produced in high yields (550 mg dm"3) and > 90% recovery together with concentration (volume reduction) and removal of cells was achieved on the expanded bed. Affinity chromatography was used for further purification, and again an overall yield of > 90% obtained.

What Is adsorption chromatography?

Adsorption chromatography is the oldest types of chromatography technique. It makes use of a mobile phase which is either in liquid or gaseous form. The mobile phase is adsorbed onto the surface of a stationary solid phase.

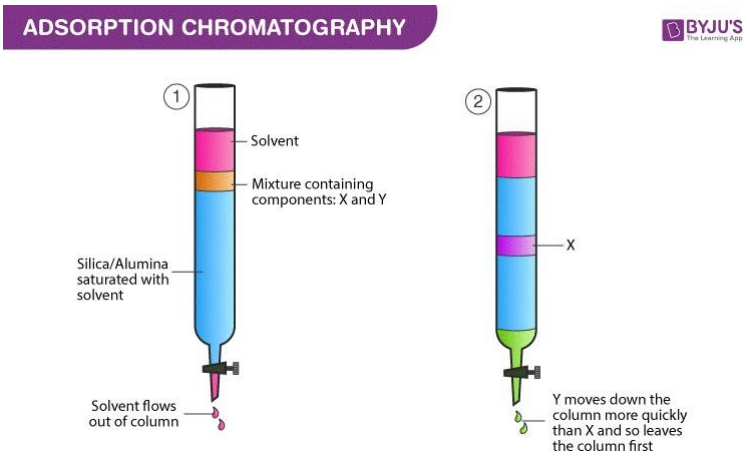
Adsorption Chromatography Principle

Adsorption Chromatography involves the analytical separation of a chemical mixture based on the interaction of the adsorbate with the adsorbent. The mixture of gas or liquid gets separated when it passes over the adsorbent bed that adsorbs different compounds at different rates.

Adsorbent – A substance which is generally porous in nature with a high surface area to adsorb substances on its surface by intermolecular forces is called

adsorbent. Some commonly used adsorbents are Silica gel H, silica gel G, silica gel N, silica gel S, hydrated gel silica, cellulose microcrystalline, alumina, modified silica gel, etc.

Adsorption Chromatography Diagram



Adsorption Chromatography Procedure

Before starting with the adsorption chromatography Experiment let us understand the two types of phases and the types of forces involved during the mixture separation process.

- Stationary phase – Adsorbent is the stationary phase in adsorption chromatography. The forces involved help to remove solutes from the adsorbent so that they can move with the mobile phase.
- Mobile phase – Either a liquid or a gas is used as a mobile phase in adsorption chromatography. Forces involved help to remove solutes from the adsorbent so that they can move with the mobile phase. When a liquid is used as a mobile phase it is called LSC (Liquid-Solid Chromatography). When a gas is used as a mobile phase it is called GSC (Gas-Solid Chromatography).

Apparatus –

Chromatography jar – The glass jar has a lid. It helps to maintain a proper environment during separation.

Thin layer chromatography plate – Borosilicate glass plate with size 20*20 cm, 20*5 cm, 20*10.

Capillary tube – Sample mixture is applied to TLC with the help of this tube.

Mobile phase – Liquid or gas

Stationary phase – Adsorbents

Adsorption Chromatography Experiment (TLC)

- Take a clean and dry chromatographic jar.
- To make sure that the environment in the jar is saturated with solvent vapors, a paper soaked in the mobile phase is applied to the walls.
- Add the mobile phase to the jar and close it.
- Maintain equilibrium
- Mark the baseline on the adsorbent.
- Apply sample to TLC plate with the help of a capillary tube and allow it to dry.
- Put the plates in the jar and close it.
- Wait until the solvent moves from the baseline.
- Take out the TLC plate and dry it.

Adsorption Chromatography Applications

- Adsorption chromatography is used for separation of amino acids.
- It is used in the isolation of antibiotics.
- It is used in the identification of carbohydrates.
- It is used to separate and identify fats and fatty acids.
- It is used to isolate and determine the peptides and proteins. Types of Adsorption Chromatography:

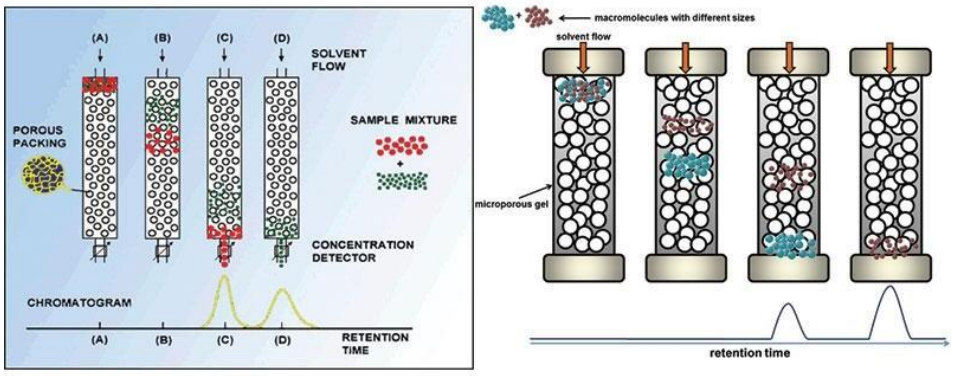
1. Thin Layer Chromatography – It is a chromatography technique where the mobile phase moves over an adsorbent. The adsorbent is a thin layer which is applied to a solid support for the separation of components. The separation takes place through differential migration which occurs when the solvent moves along the powder spread on the glass plates.

2. Mobile phase – This phase in TLC can either be a single liquid or mixture of liquids. Some commonly used liquids are Ethanol, acetone, methanol, chloroform. Stationary phase – Adsorbents
3. Column chromatography – the technique in which the solutes of a solution are entitled to travel down a column where the individual components are adsorbed by the stationary phase. Based on the affinity towards adsorbent the components take positions on the column. The most strongly adsorbed component is seen at the top of the column.
4. Gas-Solid chromatography – The principle of separation in GSC is adsorption. It is used for solutes which have less solubility in the stationary phase. This type of chromatography technique has a very limited number of stationary phases available and therefore GSC is not used widely.

Gel Permeation Chromatography

- Gel permeation chromatography is also called as gel filtration or size exclusion chromatography.
- In size exclusion chromatography, the stationary phase is a porous matrix made up of compounds like cross-linked polystyrene, cross-like dextrans, polyacrylamide gels, agarose gels, etc.
- The separation is based on the analyte molecular sizes since the gel behaves like a molecular sieve.
- This technique is used for the separation of proteins, polysaccharides, enzymes, and synthetic polymers.
- As a technique, size exclusion chromatography was first developed in 1955 by Lathe and Ruthven.

Gel Permeation Chromatography



Principle of Gel Permeation Chromatography

- It is a technique in which the separation of components is based on the difference in molecular weight or size.
- The stationary phase used is a porous polymer matrix whose pores are completely filled with the solvent to be used as the mobile phase.
- The molecules in the sample are pumped through specialized columns containing such microporous packing material (gel).
- The basis of the separation is that molecules above a certain size are totally excluded from the pores, while smaller molecules access the interior of the pores partly or wholly.
- The flow of the mobile phase hence will cause larger molecules to pass through the column unhindered, without penetrating the gel matrix, whereas smaller molecules will be retarded according to their penetration of the gel.

Components/ Instrumentation of Gel Permeation Chromatography

1. Stationary Phase
2. The Mobile Phase
3. The Columns
4. The Pump
5. Detectors

Stationary phase

It is composed of semi-permeable, porous polymer gel beads with a well-defined range of pore sizes.

It has the following properties:

- Chemically inert
- Mechanically stable
- With ideal and homogeneous porous structure (wide pore size give low resolution).
- A uniform particle and pore size. Examples of gel:

1. **Dextran** (Sephadex) gel: An α 1-6-polymer of glucose natural gel
2. **Agarose** gel: A 1,3 linked β-D-galactose and 1,4 linked 3,6-anhydro-α, L- galactose natural gel
3. **Acrylamide** gel: A polymerized acrylamide, a synthetic gel

B. The Mobile Phase

It is composed of a liquid used to dissolve the bio-molecules to make the mobile phase permitting high detection response and wet the packing surface.

C. Columns

Any of the following kinds may be used:

- Analytical column- 7.5–8mm diameters.
- Preparative columns-22–25mm
- Usual column lengths-25, 30, 50, and 60 cm.
- Narrow-bore columns- 2–3mm diameter have been introduced

D. Pumps

They are either syringe pumps or reciprocating pumps with a high constant flow rate.

E. Detectors

The detectors may be concentration sensitive detectors, bulk property detectors, refractive index (RI) detector, etc.

Steps in Gel Permeation Chromatography

It involves three major steps:

A. Preparation of column for gel filtration

It involves:

1. Swelling of the gel
2. Packing the column semi-permeable, porous polymer gel beads with a well-defined range of pore sizes.
3. Washing: After packing, several column volumes of buffer solution is passed through the column to remove any air bubbles and to test the column homogeneity.

B. Loading the sample onto the column using a syringe

C. Eluting the sample and detection of components

Applications of Gel Permeation Chromatography

1. Proteins fractionation
2. Purification
3. Molecular weight determination.
4. Separation of sugar, proteins, peptides, rubbers, and others on the basis of their size.
5. Can be used to determine the quaternary structure of purified proteins

Advantages of Gel Permeation Chromatography

- Short analysis time.
- Well defined separation.
- Narrow bands and good sensitivity.
- There is no sample loss.
- The small amount of mobile phase required.
- The flow rate can be set

Limitations of Gel Permeation Chromatography

- The limited number of peaks that can be resolved within the short time scale of
- the GPC run.
- Filtrations must be performed before using the instrument to prevent dust and other particulates from ruining the columns and interfering with the detectors.
- The molecular masses of most of the chains will be too close for the GPC separation to show anything more than broad peaks.

Affinity Chromatography

- Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis.
- It is a separation technique in which a mobile phase carrying a mixture is caused to move in contact with a selectively absorbent stationary phase.
- Affinity chromatography is a type of liquid **chromatography** for the separation, purification or specific analysis of sample components.
- It utilizes the reversible biological interaction or molecular recognition called affinity which refers to the attracting forced exerted in different degrees between atoms which cause them to remain in combination.
    - Example: Enzyme with and inhibitor, antigen with an antibody etc.
- It was discovered by Pedro Cuatrecasas and Meir Wilcheck.

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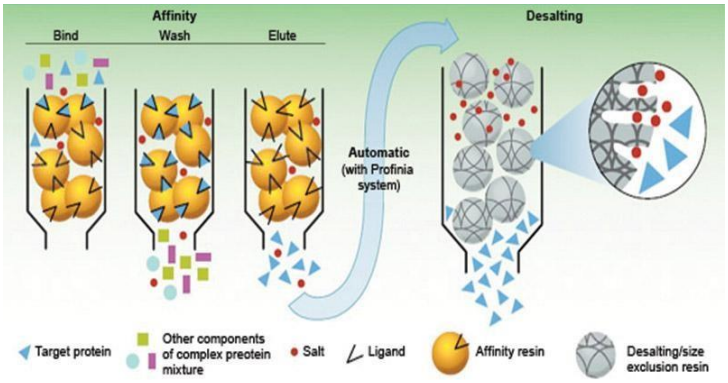


Principle of Affinity Chromatography

- The stationary phase consists of a support medium, on which the substrate (ligand) is bound covalently, in such a way that the reactive groups that are essential for binding of the target molecule are exposed.
- As the crude mixture of the substances is passed through the chromatography column, substances with binding site for the immobilized substrate bind to the stationary phase, while all other substances is eluted in the void volume of the column.
- Once the other substances are eluted, the bound target molecules can be eluted by methods such as including a competing ligand in the mobile phase or changing the pH, ionic strength or polarity conditions.

Components of Affinity Chromatography

1. **Matrix**

- The matrix is an inert support to which a ligand can be directly or indirectly coupled.
- In order to for the matrix to be effective it must have certain characters:
- Matrix should be chemically and physically inert.
- It must be insoluble in solvents and buffers employed in the process
- It must be chemically and mechanically stable.
- It must be easily coupled to a ligand or spacer arm onto which the ligand can be attached.

- It must exhibit good flow properties and have a relatively large surface area for attachment.
- The most useful matrix materials are agarose and polyacrylamide.

2. **Spacer arm**

- It is used to improve binding between ligand and target molecule by overcoming any effects of steric hindrance.

3. **Ligand**

- It refers to the molecule that binds reversibly to a specific target molecule.
- The ligand can be selected only after the nature of the macromolecule to be isolated is known.
- When a hormone receptor protein is to be purified by affinity chromatography, the hormone itself is an ideal candidate for the ligand.
- For antibody isolation, an antigen or hapten may be used as ligand.
- If an enzyme is to be purified,a substrate analog, inhibitor, cofactor, or effector may be used as a the immobilized ligand.

Steps in Affinity Chromatography

- Affinity medium is equilibrated in binding buffer.
- Sample is applied under conditions that favor specific binding of the target molecule(s) to a complementary binding substance (the ligand). Target substances bind specifically, but reversibly, to the ligand and unbound material washes through the column.
- Elution is performed specifically, using a competitive ligand, or non-specifically, by changing the pH, ionic strength or polarity. Target protein is collected in a purified, concentrated form.
- Affinity medium is re-equilibrated with binding buffer.

These events can be summarized into the following three major steps:

1. **Preparation of Column**

- The column is loaded with solid support such as sepharose, agarose, cellulose etc.

- Ligand is selected according to the desired isolate.
- Spacer arm is attached between the ligand and solid support.

2. **Loading of Sample**

- Solution containing a mixture of substances is poured into the elution column and allowed to run at a controlled rate.

3. **Elution of Ligand-Molecule Complex**

- Target substance is recovered by changing conditions to favor elution of the bound molecules.

Applications of Affinity Chromatography

- Affinity chromatography is one of the most useful methods for the separation and purification of specific products.
- It is essentially a sample purification technique, used primarily for biological molecules such as proteins.

Its major application includes:

- Separation of mixture of compounds.
- Removal of impurities or in purification process.
- In enzyme assays
- Detection of substrates
- Investigation of binding sites of enzymes
- In in vitro antigen-antibody reactions
- Detection of Single Nuceotide polymorphisms and mutations in nucleic acids

Advantages of Affinity Chromatography

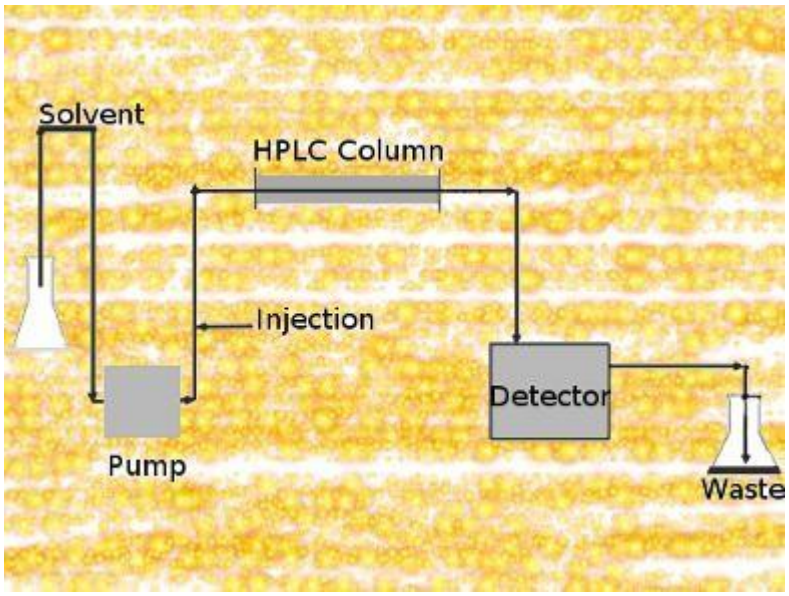
- High specificity
- Target molecules can be obtained in a highly pure state
- Single step purification
- The matrix can be reused rapidly.
- The matrix is a solid, can be easily washed and dried.
- Give purified product with high yield.

- Affinity chromatography can also be used to remove specific contaminants, such as proteases.

Limitations of Affinity Chromatography

- Time consuming method.
- More amounts of solvents are required which may be expensive.
- Intense labour
- Non-specific adsorption cannot be totally eliminated, it can only be minimized.
- Limited availability and high cost of immobilized ligands.
- Proteins get denatured if required pH is not adjusted.

HPLC



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The components of a mixture are separated from each other due to their different degrees of interaction with the absorbent particles. This causes different elution rates for the different components and leads to the separation of the components as they flow out the column. Compared to column chromatography, HPLC is highly automated and extremely sensitive. **Types of HPLC**

The two most common variants are normal-phase and reversed-phase HPLC.

Normal-PhaseHPLC

The column is filled with tiny silica particles, and a non-polar solvent, for example, hexane. A typical column has an internal diameter of 4.6 mm or smaller and a length

of 150 to 250 mm. Non-polar compounds in the mixture will pass more quickly through the column, as polar compounds will stick longer to the polar silica than non-polar compound will.

Reversed-PhaseHPLC

The column size is the same. The column is filled with silica particles which are modified to make them non-polar. This is done by attaching long hydrocarbon chains (8–18 C atoms) to its surface. A polar solvent is used, for example, a mixture of water and an alcohol such as methanol. Polar compounds in the mixture will pass more quickly through the column because a strong attraction occurs between the polar solvent and the polar molecules in the mixture.

Non-polar molecules are slowed down on their way through the column. They form varying degrees of attraction with the hydrocarbon groups principally through van der Waals dispersion forces and hydrophobic interactions. They are also less soluble in the aqueous mobile phase components facilitating their interactions with the hydrocarbon groups. Reversed phase HPLC is the most commonly used form of HPLC

LYOPHILIZATION

Historical Overview

Drying from the frozen state is not uncommon in nature. In the winter, snow vanishes along the roads in dry cold air without melting. In Central Siberia, scientists have found the large bodies of mammoths that have been progressively freeze-dried during the past 15,000 years. In the Peruvian high plateau, the Incas reportedly stored, in their tambos, meat that had been dried in the sun at the reduced pressure of the Andes Scientific interest in freeze-drying began at the turn of the twentieth century with a publication by Bordas and d'Arsonval at the French Academy of Sciences. Following that publication, Altman and later Gersh used this technique to prepare undistorted dry samples for microscopy. Ronald Greaves, in Cambridge, UK, began his work along those lines in the 1930s by preparing dry suspensions of living bacteria. However, this technique still was only familiar to a handful of scientists in isolated laboratories.

Then came World War II. With tens of thousands of casualties on the battlefields, human plasma was in great need, and freeze-drying again entered the limelight. Thanks to Greaves in England, François Henaff in France, and Earl Flosdorf in the United States, thousands of liters of blood were processed to isolate plasma, which was then preserved by freezing and drying. As the use of lyophilization expanded, the process began to be industrialized. Loire, Stokes, Edwards, and others designed and built the first equipment for the purpose. Called “lyophilization” by Flosdorf, the process faced its first major challenge under Sir Ernst Boris Chain, who used the technique to preserve antibiotics. Given Chain‟s results turned to lyophilization to prepare vaccines and, later on, to refine blood fractions. By the mid-1950s, many industries were already using freeze drying to preserve pharmaceutical and biological products, as were the physicians and surgeons who developed tissue-banking for plastic and reconstructive surgery. Drs. Hyatt, Bassett, and Meryman of the United States Navy were among the early pioneers in the field.

INTRODUCTION

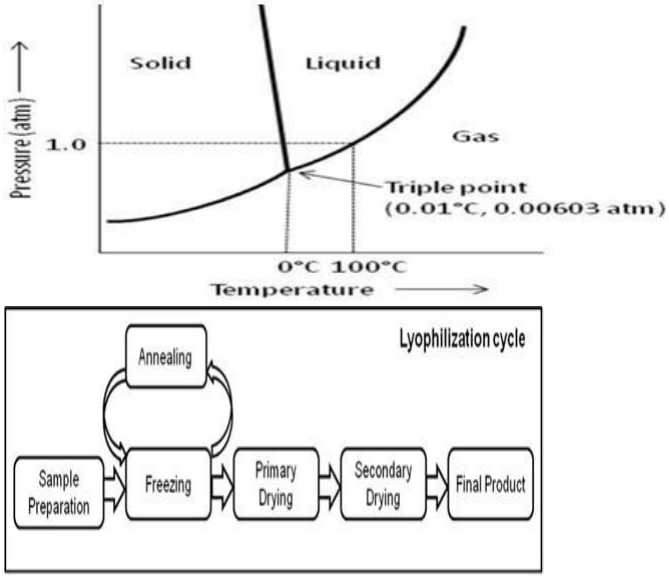
In Lyophilization, or freeze drying, there is a water is frozen, followed by its removal from the sample, initially by sublimation (primary drying) and then by desorption (secondary drying). In this process, the moisture content of the product is reduced to such a low level that does not support biological growth or chemical reactions which gives the stability to the formulation. This technique useful in formulation development of drugs which are thermolabile and/or unstable in aqueous medium.

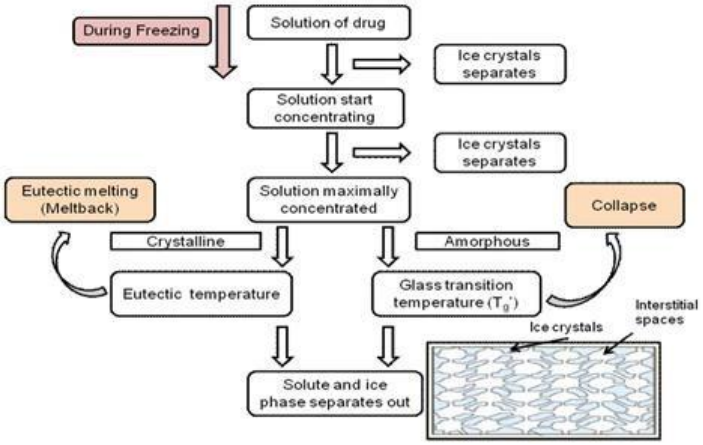
Lyophilization process is based on the principle of sublimation of ice, without entering the liquid phase. The phase diagram of water (Figure 1) represent that two phases coexist along a line under the given conditions of temperature and pressure, while at the triple point (0.0075 ?C at 0.61kPa or 610 Nm-2; 0.01 ?C at 0.00603 atm), all three phases coexist.

Figure 1- Phase diagram showing the triple point of water at 0.01°C, 0.00603 atm. Lyophilization is take place below the triple point.

This process is performed at temperature and pressure conditions below the triple point, to facilitate sublimation of ice. The entire process is performed at low temperature and pressure, so that useful for drying of thermolabile compounds.

various important Steps involved in lyophilization process which start from sample preparation followed by freezing, primary drying and secondary drying, to obtain the final dried product with desired moisture content (Figure 2).





The concentration gradient of water vapour between the drying front and condenser is the driving force for removal of water during lyophilization. The vapour pressure of water increases with an increase in temperature during the primary drying. Therefore, primary drying temperature should be kept as high as possible, but below the critical process temperature, to avoid a loss of cake structure. This critical process temperature is the collapse temperature for amorphous substance, or eutectic melt for the crystalline substance.During freezing, ice crystals start separating out until the solution becomes maximally concentrated. On further cooling, phase separation of the solute and ice takes place. If the solute separates

out in crystalline form, it is known as the eutectic temperature. In contrast, if an amorphous form is formed, the temperature is referred to as the glass transition temperature (Tg‟).

Determination of this critical temperature is important for development of an optimized lyophilization cycle. During primary drying, drying temperature should not exceed the critical temperature, which otherwise leads to „meltback‟ or

„collapse‟ phenomenon in case of crystalline or amorphous substance respectively (Figure 3)

ADVANTAGES-

Lyophilization process has various important advantages compared to other drying and preserving techniques.

1-It is a ideal drying technique for heat sensitive products,

2-It can be stored at ambient temperature over a 2 year shelf life, enhanced product stability in a dry state.

3- Easy reconstitution greatly reduces weight and makes the products easier to transport, maintains food/biochemical and chemical reagent quality.

4- Reconstitution of the dried product facilitates use in emergency medicine and safe application in hospitals.

5- It is not limited to products for parenteral use, but can also be used for fast dissolving sublingual tablets. Tablets can have very low disintegration time and have great mouth feel due to fast melting effect.

6- it is much easier to achieve sterility assurance and freedom of particles than using other drying methods or handling of dry powders.

7-lyophilized products sensitive to oxidation can be stoppered and sealed within an inert atmosphere (i.e. nitrogen) to minimize detrimental effects.

DISADVANTAGES-

Although lyophilization has many advantages compared to other drying and preserving techniques it has quite a few disadvantages. It is a long and cost intensive process, requires sterile diluents for reconstitution, it should only be used when product is unstable and heat-liable and the limited amount of vials processed in each run restricts the overall production capacity.

MATERIAL THAT CAN BE LYOPHILIZED-

The major type of material that can be lyophilized summarized bellow

1-Non-living bio products this comprises the major areas of application and include:

- Enzyme, hormones, antibiotics, vitamins, blood products, inactivated vaccines etc.

Foodstuffs where organoleptic properties are important

- Industrially useful bio-products.
- Bone and other body tissue for medical and surgical use.

2-Non-biological where the process is used to dehydrate and concentrate reactive and heat labile chemicals.

3- Living organism- where reconstituted cells after drying must be able to grow and multiply to produce new progency.