GURUNANAK INSTITUTE OF TECHNOLOGY

157/F, Nilgunj Road, Panihati

Kolkata -700114

Website: www.gnit.ac.in

Email: info.gnit@jisgroup.org

Approved by A.I.C.T.E., New Delhi

Affiliated to MAKAUT, West Bengal

ONLINE COURSE WARE

Paper Name: Principles of Biochemical Engineering

Paper Code: FT802A

Credit: 3

NAME OF COORDINATOR: Dr. Dolanchapa Sikdar (GNIT)

NAME OF TEAM MEMBER: Prof. Utpal Raychaudhuri (GNIT)

MODULE I

Lecture 1. Introduction to biochemical process industries

1.1 Introduction

Biotechnology usually implies the use or development of methods of direct genetic manipulation for a socially desirable goal. Such goals might be the production of a particular chemical, but they may also involve the production of better plants or seeds, or gene therapy, or the use of specially designed organisms to degrade wastes. The key element for many workers is the use of sophisticated techniques outside the cell for genetic manipulation. Others interpret biotechnology in a much broader sense and equate it with applied biology; they may include engineering as a subcomponent of biotechnology. Many words have been used to describe engineers working with biotechnology. Bioengineering is a broad title and would include work on medical and agricultural systems; its practitioners include agricultural, electrical, mechanical, industrial, environmental and chemical engineers, and others. Biological engineering is similar but emphasizes applications to plants and animals. Biochemical engineering has usually meant the extension of chemical engineering principles to systems using a biological catalyst to bring about desired chemical transformations. It is often subdivided into bioreaction engineering and bioseparations. Biomedical engineering has been considered to be totally separate from biochemical engineering, although the boundary between the two is increasingly vague, particularly in the areas of cell surface receptors and animal cell culture.

There is a difference between bioprocess engineering and biochemical engineering. In addition to chemical engineering, bioprocess engineering would include the work of mechanical, electrical, and industrial engineers to apply the principles of their disciplines to processes based on using living cells or subcomponents of such cells. The problems of detailed equipment design, sensor development, control algorithms, and manufacturing strategies can utilize principles from these disciplines. Biochemical engineering is more limited in the sense that it draws primarily from chemical engineering principles and broader in the sense that it is not restricted to well-defined artificially constructed processes, but can be applied to natural systems.

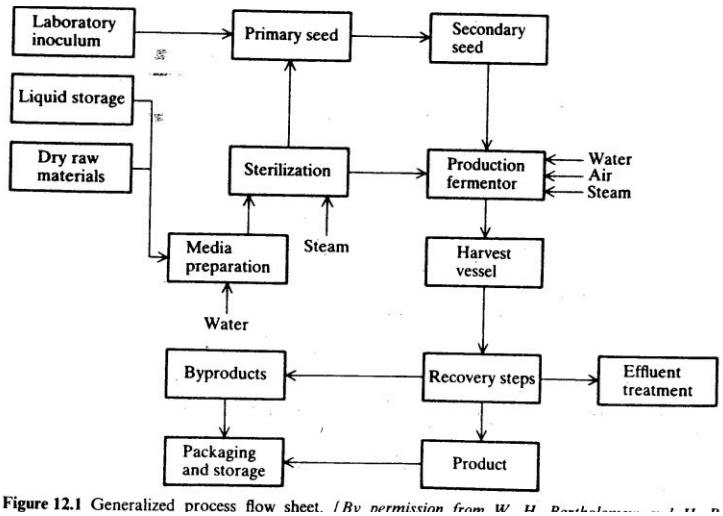


Figure 12.1 Generalized process flow sheet. *[By permission from W. H. Bartholomew et al. Ha...*

Lecture 2.Industrial alcohol production

2.1 Industrial Alcohol

The production of industrial alcohol, ethanol become commercially feasible on a large scale after 1906 when the Industrial Alcohol Act was passed. This act allowed the sale of tax-exempt alcohol, if it has first denatured to prevent its use in various Alcohol beverages. Industrial Alcohol is commonly employed as a solvent and to a lesser extent as a raw material for chemical synthesis. Smaller amounts are also used as a motor fuel like gasoline.

2.2 Microorganism:

Choice of fermentation microorganism for the alcohol production depends upon the type of carbohydrate employed in the medium. For example if starch and sugar are raw materials in the medium then specially selected strains of Saccharomyces crevisiae are utilized. Production from Lactose of whey is accomplished with Candida pseudotropicalis. If it is sulfur waste liquor fermentation the Candida utilis is the best organism, because of its ability to ferment pentoses. So particular strains of these various organisms actually employed for the fermentation are selected for several properties. They must grow rapidly, have higher tolerance to the high concentartions of sugar but at the same time they must be able to produce much larger amounts of alcohol and be resistant to the produced alcohol.

2.3 Media

The media for the commercial production includes:Blackstrap Molasses / Corn (Blackstrap molasses has greater use), Grains, Sulfite waste liquor, Whey, Patatoes, Wood Wastes

2.4 Fermentation process

For Molasses fermentation , molasses must be diluted with water to a sugar conc. between 10 - 18%. Concentrations greater than 20% are not employed as they could be detrimental to yeast. The pH of the medium is set between 4 -5 by adding sulfuric acids or lactic acids, or by employing Lactic acid bacteria to bring initial lactic acid fermentation. Microbial contaminants are usually inhibited by the low pH, high sugar conc. and anaerobic conditions of the fermentation and by the high alcohol production by the yeast.

Starchy media such as corn, rye and barley must undergo initial starch hydrolysis. This can be accomplished by mashing with barley malt, by addition of dilute acids or by utilizing fungal amylolytic enzymes (Aspergillus and Rhizopus).In most of the cases, malt is used to accomplish hydrolysis of starch By mixing 30% barley and 70% corn with water and carry on the mashing procedures similar to the wine or beer making procedures.

Fermentation is carried out in large reactors at a temperature between 21 - 27oC, but heat evolution might raise the temperature to 30oC, so cooling coils are used to bring the temperature down. Fermentation lasts for about 2-3 days, but actual time period depends upon the substrate utilized and temperature. The fermentation broth at completion of fermentation ranges from 6 -9 percent alcohol by volume and this alcohol reflects the yield 0f 90 -98% theoretical conversion of substrate sugar to alcohol.Soyileds should not be confused with "proof" as proofing means alcohol concentartion designation and it will be twice the percentage in vol of ethanol as dissolved in water e.g. 70% ethanol is 140 proof.

Lecture 3.Industrial Antibiotic Production

1.1 Structure of Penecillin

Penicillins are a group of β-lactam antibiotics consisting of natural penicillins and semisynthetic penicillins. The basic structure of all penicillins, natural and semisynthetic, is 6-aminopenicillanic acid composed of a four membered heterocyclic β-lactam ring fused with a five membered (benzylpenicillin), penicillin V (Phenoxymethyl penicillin), thiazolidine ring

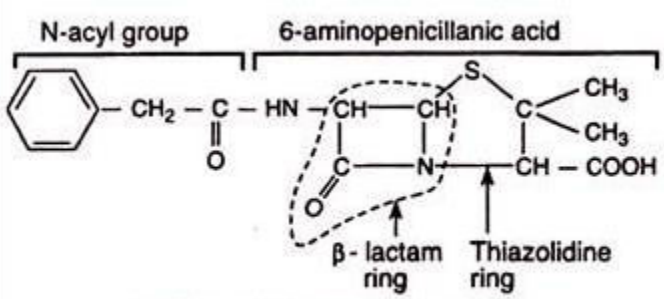


FIG. 45.6. Basic structure of penicillins.

3.2 Penecillin Production

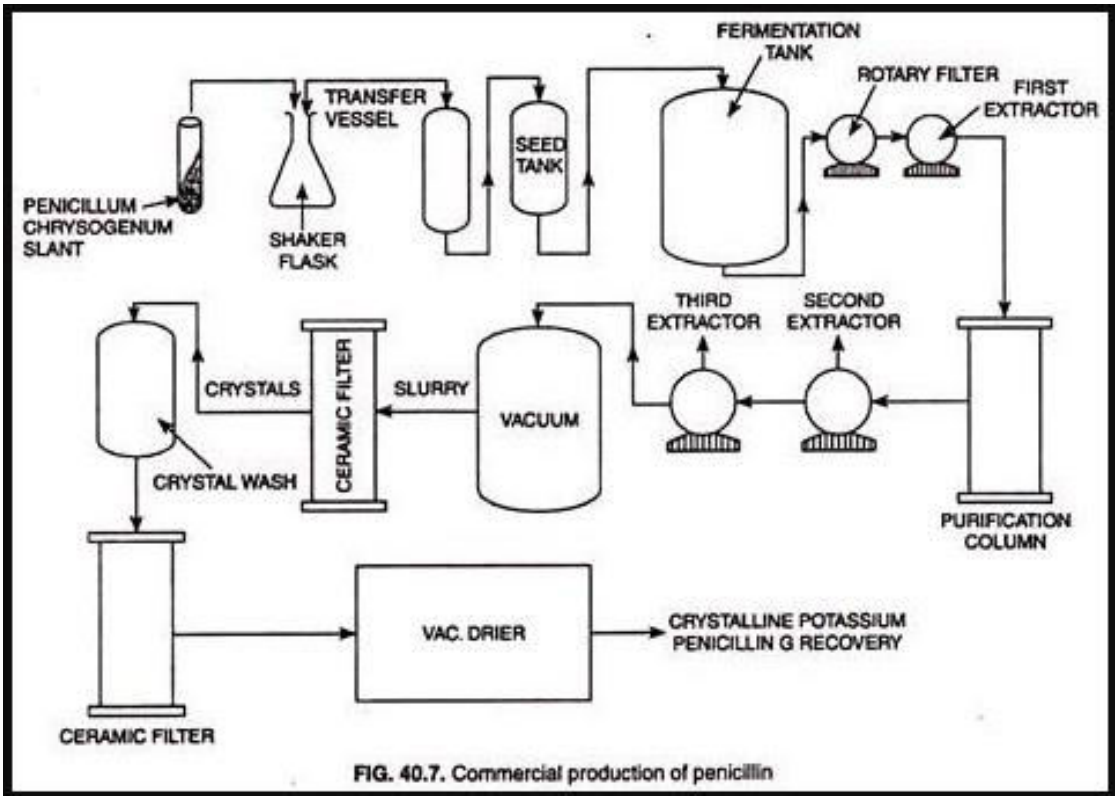


FIG. 40.7. Commercial production of penicillin

Lecture 4. Industrial Acid production

4.1 Citric Acid production

Citric acid is the most important organic acid produced in tonnage and is extensively used in food and pharmaceutical industries. It is produced mainly by submerged fermentation using *Aspergillusniger*or*Candida* sp. from different sources of carbohydrates, such as molasses and starch based media. However, other fermentation techniques, e.g. solid state fermentation and surface fermentation, and alternative sources of carbon such as agro-industrial residues have been intensively studied showing great perspective to its production.

Citric acid ($C_6H_8O_7$, 2 - hydroxy - 1,2,3 - propane tricarboxylic acid), a natural constituent and common metabolite of plants and animals, is the most versatile and widely used organic acid in the field of food (60%) and pharmaceuticals (10%). It has got several other applications in various other fields. Currently, the global production of citric acid is estimated to be around 736000 tones/year (Química e Derivados, 1997), and the entire production is carried out by fermentation. In Brazil, almost the entire demand of citric acid is met through imports. There is constant increase (3.5-4%) each year in its consumption, showing the need of finding new alternatives for its manufacture.

4.2. Production Techniques & Raw Materials

Several attempts have been made to produce citric acid using molasses, which is preferred due its low cost and high sugar content (40-55%). The composition of molasses depends on various factors, e.g. the kind of beet and cane, methods of cultivation of crops and fertilizers and pesticides applied during cultivation, conditions of storage and handling (e.g. transport, temperature variations), production procedures, etc. Both, cane and beet molasses are suitable for citric acid production. However, beet molasses is preferred due to its lower content of trace metals. Generally, cane molasses contains calcium, magnesium, manganese, iron and zinc, which have a retarding effect on the synthesis of citric acid. Consequently, some pre-treatment is required for the removal/reduction of trace metals. Despite that, cane molasses posses difficulties in achieving good fermentation yields.

Liquid fermentation

Submerged fermentation: The submerged fermentation (SmF) process is the commonly employed technique for citric acid production. It is estimated that about 80% of world production is obtained by SmF. Several advantages such as higher yields and productivity and lower labour costs are the main reasons for this. Two types of fermenters, conventional stirred fermenters and tower fermenters are employed, although the latter is preferred due to the advantages it offers on price, size and operation (Rohr et al., 1983). Preferentially, fermenters are made of high-grade steel and require provision of aeration system, which can maintain a high dissolved oxygen level. Fermenters for citric acid production do not have to be built as pressure vessels since sterilization is performed by simply steaming without applying pressure. Cooling can be done by an external water film over the entire outside wall of the fermenter.

In SmF, different kinds of media are employed such as sugar and starch based. Molasses and other raw materials demand pre-treatment, addition of nutrients and sterilization. Inoculation is performed either by adding a suspension of spores, or of pre-cultivated mycelia. When spores are used, a surfactant is added in order to disperse them in the

medium. For pre-cultivated mycelia, an inoculum size of 10% of fresh medium is generally required. Normally, submerged fermentation is concluded in 5 to 10 days depending on the process conditions. It can be carried out in batch, continuous or fed batch systems, although the batch mode more frequently used.

Surface fermentation: The first individual process for citric acid production was the liquid surface culture (LSC), which was introduced in 1919 by Société des ProduitsOrganiques in Belgium, and in 1923 by Chas Pfizer & Co. in US. After that, other methods of fermentation, such as submerged fermentation were developed. Although this technique is more sophisticated, surface method required less effort in operation and installation and energy cost (Grewal and Kalra, 1995).

In the classical process for citric acid manu-facture, the culture solution is held in shallow trays (capacity of 50-100 L) and the fungus develops as a mycelial mat on the surface of the medium. The trays are made of high purity aluminium or special grade steel and are mounted one over another in stable racks. The fermentation chambers are provided with an effective air circulation in order to control temperature and humidity. Fermentation chambers are always in aseptic conditions, which might be conserved principally during the first two days when spores germinate. Frequent contaminations are mainly caused by Penicilia, other Aspergilli, yeast and lactic bacteria (Rohr et al, 1983; Morgant, 1988). Refined or crude sucrose, cane syrup or beet molasses are generally used as sources of carbon. When applied, molasses is diluted to 15-20% and is treated with hexacyanoferrate (HFC).

Solid-state fermentation

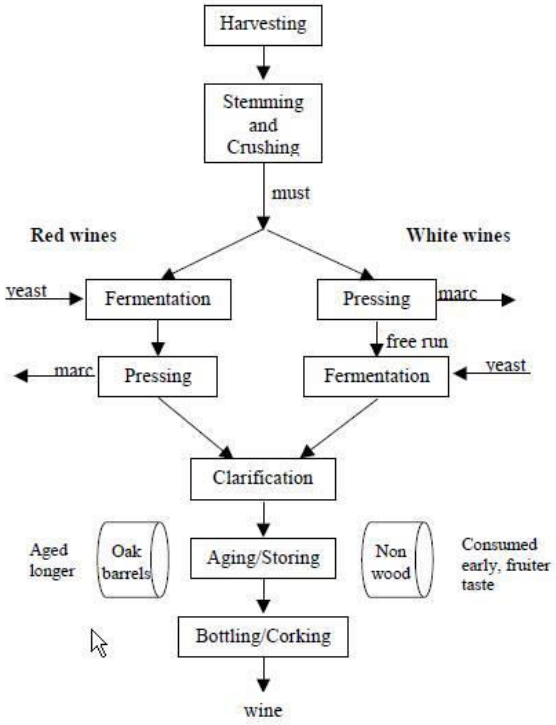
Solid-state fermentation (SSF) has been termed as an alternative method to produce citric acid from agro-industrial residues (Pandey 1991, 1992, 1994, Soccol 1994, Pandey and Soccol 1998). Citric acid production by SSF (the Koji process) was first developed in Japan and is as the simplest method for its production. SSF can be carried out using several raw material. Generally, the substrate is moistened to about 70% moisture depending on the substrate absorption capacity. The initial pH is normally adjusted to 4.5-6.0 and the temperature of incubation can vary from 28 to 30°C. The most commonly organism is *A. niger.* However there also have been reports with yeasts (Maddox and Kingston, 1983; Tisnadjaja et al., 1996). One of the important advantages of SSF process is that the presence of trace elements may not affect citric acid production so harmfully as it does in SmF. Consequently, substrate pre-treatment is not required.

Different types of fermenters such as conical flasks, glass incubators and trays, etc. have been used for citric acid fermentation in SSF. Vandenberghe et al. (1999a,b) used Erlen-meyer flasks and glass columns for the production of citric acid from gelatinized cassava bagasse. Higher yields were obtained in flasks without any aeration, and very little sporulation was observed. The same yields were found in column reactors only with variable aeration. This showed great perspective to use SSF process for citric acid production in simple tray type fermenters.

Lecture5. Industrial Alcoholic Beverage Production

5.1 Beer Production

5.2. Wine Production



Lecture 6.Production of Vitamins through Biochemical Processes

In addition to proteins (enzymes) and antibiotics, many other complex metabolites are produced by microbes; a few processes are of both commercial interest and competitive or better than straight chemical synthetic routes.

Of the microbially synthesized vitamins [B_{12}, riboflavin (B_2), thiamine, folic acid, pantothenic acid, pyridoxal], only B_{12} and B_2 are produced microbially, and the latter have been progressively displaced by synthetic routes. Vitamin B_{12}, also called cyanocobalamin, is synthesized exclusively by microbes in nature, yet is required by all animals. While microbial flora of the large intestine can synthesize B_{12}, its assimilation by humans does not occur, hence it must be obtained from food. Early 1980s world production was roughly ten tons, of which the pharmaceutical industries consumed about two-thirds (as cyanocobalamin, hydroxocobalamin, coenzyme B_{12}, and methylcobalamin), and the balance went to animal food (to supplement vegetable protein source B_{12} content).

Propionibacteria (freudenreichii and *shermanii*) are used primarily (Table 12.14) in a batch process employing two phases: a 2–4 day anaerobic operation followed by a 3–4 day aerobic stage. The anaerobic product (5′-deoxyadenosylcobinamide guanosine phosphate) is coupled with the aerobic product (5,6-dimethyl benzimidazole) to give, ultimately, the desired vitamin B_{12}. Recovery involves heating (to release cell-bound vitamin B_{12}), and chemical conversion to stable cyanocobalamin. A continuous, two-stage analog has also been effected. *Pseudomonas denitrificans* also produces B_{12} in a one stage process; cobalt and 5,6-dimethyl benzimidazole are also added. A dozen years of strain improvement have moved yields from 0.6 to 60 mg/L. Sugar beet molasses, providing inexpensive betaine, is used. Activated sludge in waste treatment is also a B_{12} source (4–10 mg/L), but recovery is difficult due to cost of resolution of many B_{12} analogs.

Lecture 7.Production of Single Cell Proteins

7.1. Introduction

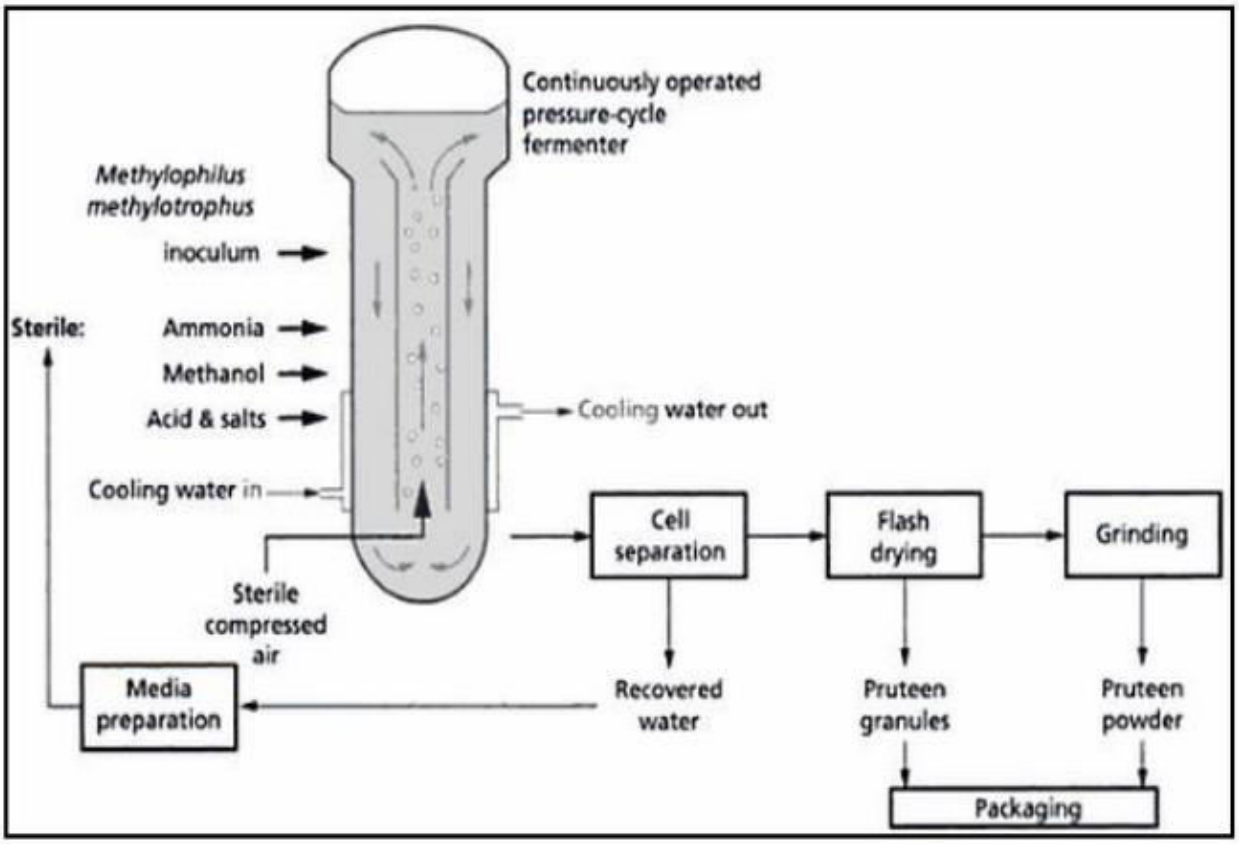
The increasing world deficiency of protein is becoming a main problem of humankind. Since the early fifties, intense efforts have been made to explore new, alternate and unconventional protein. For this reason, in 1996, new sources mainly yeast, fungi, bacteria and algae named Single Cell Protein (SCP) as coined to describe the protein production from biomass, originating from different microbial sources. Microbial biomass has been considered an alternative to conventional sources of food or feed.

A variety of microorganisms and substrate are used to produce single cell proteins. Yeast is suitable for single cell protein production because of its superior **nutritional quality**. The supplementation cereals with single cell proteins, especially yeast, make them as good as animal proteins (Huang and Kinsella, 1986). The necessary factor considered for use of SCP is the demonstration of the absence of toxic and carcinogenic compounds originated from the substrates, biosynthesized by the microorganisms or formed during processing. High nucleic acid content and low cell wall digestibility are two of the most important factors limiting nutritional and toxicological value of yeast for animal or human consumption (Alvarez and Enriquez, 1988). As constituents of nucleic acid, purine compounds in human diet mostly metabolized to yield **uric acid** whose high concentration may lead to gout or renal stones. However, nucleic acid is not a toxic component and it causes only physiological effects at higher levels like any other essential dietary ingredients taken in larger amounts. It has been calculated that 100 lbs of yeast will produce 250 tons of proteins in 24 h. Algae grown in ponds can produce 20 tons (dry weight) of protein, per acre, per year. Bacteria are usually high in protein (50 to 80%) and have a rapid growth rate.

7.2. Production procedure

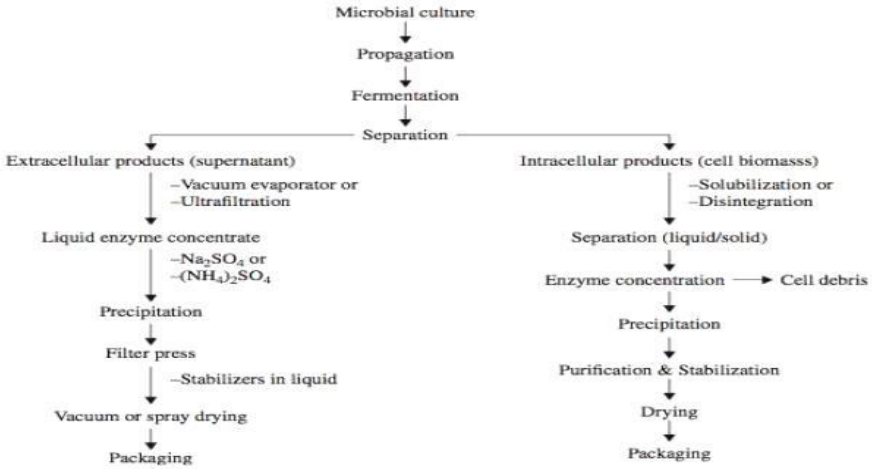
Single-cell proteins develop when microbes ferment waste materials (including wood, straw, cannery, and food-processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta).The problem with extracting single-cell proteins from the wastes is the dilution and cost. They are found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation, and filtration, or the use of semi-permeable membranes.

The single-cell protein must be dehydrated to approximately 10% moisture content and/or acidified to aid in storage and prevent spoilage. The methods to increase the concentrations to adequate levels and the de-watering process require equipment that is expensive and not always suitable for small-scale operations. It is economically prudent to feed the product locally and soon after it is produced.

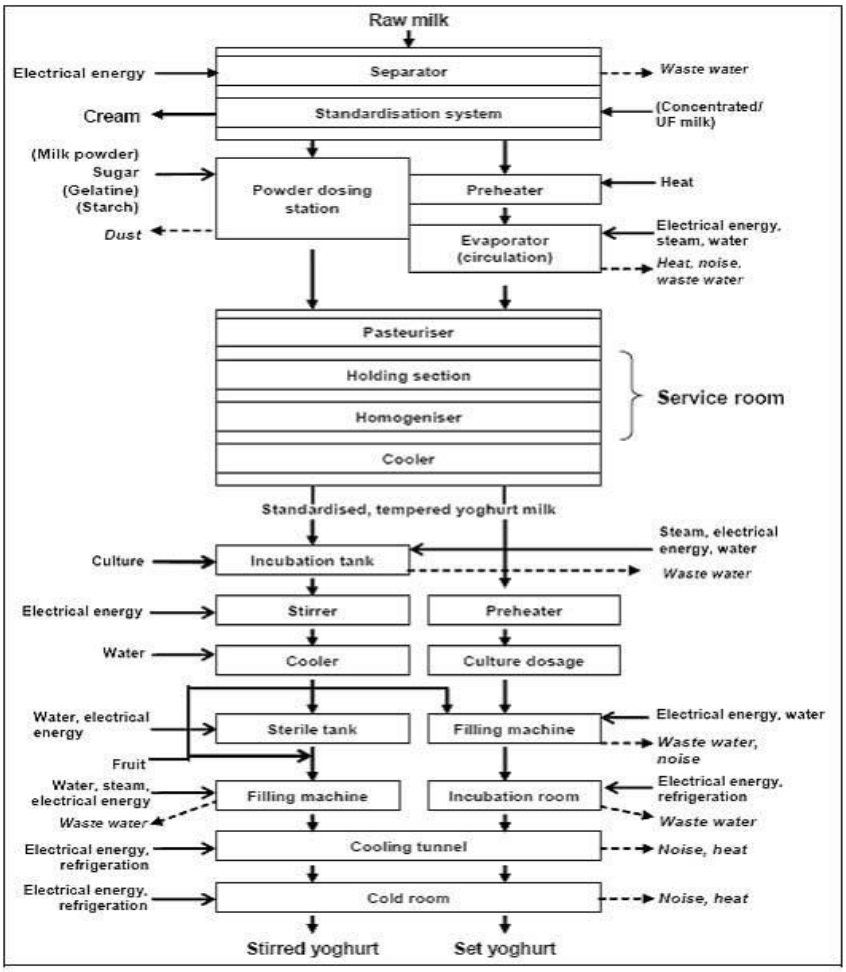

Continuously operated
pressure-cycle
fermenter
Methylophilus
methylotrophus
inoculum
Sterile:
Ammonia
Methanol
Acid & salts
Cooling water out
Cooling water in
Cell
separation
Flash
drying
Grinding
Sterile
compressed
air
Media
preparation
Recovered
water
Pruteen
granules
Pruteen
powder
Packaging

Lecture 8.Production of Enzymes and Dairy products

8.1. Enzyme production



8.2. Yoghurt production



MODULE II

Lecture 1.Mechanisms and kinetics in biochemical processes

1.1 Introduction

For microbes, growth is their most essential response to their physiochemical environment.Growth is a result of both replication and change in cell size. Microorganisms cangrow under a variety of physical, chemical, and nutritional conditions. In a suitable nutrientmedium, organisms extract nutrients from the medium and convert them into biologicalcompounds.

Partof these nutrients are used for energy production and part are usedfor biosynthesis and product formation. As a result of nutrient utilization, microbial massincreases with time and can be described simply by

$$\text{substrates} + \text{cells} \longrightarrow \text{extracellular products} + \text{more cells}$$

$$\Sigma S + X \longrightarrow \Sigma P + nX$$

Microbial growth is a good example of an autocatalytic reaction. The rate of growth is directlyrelated to cell concentration, and cellular reproduction is the normal outcome of thisreaction.The rate of microbial growth is characterized by the net *specific growth rate*, definedas

$$\mu_{net} \equiv \frac{1}{X}\frac{dX}{dt}$$

&

$$\mu_{net} = \mu_g - k_d$$

where X is cell mass concentration (g/l), t is time (h), and μ_{net} is net specific growth rate (h^{-1}). The net specific growth is the difference between a gross specific growth rate, μ_g (h^{-1}), and the rate of loss of cell mass due to cell death or endogenous metabolism, k_d (h^{-1}).

Microbial growth can also be described in terms of cell number concentration, N, as well as X. In that case

$$\mu_R \equiv \frac{1}{N}\frac{dN}{dt}$$

where μ_R is the net specific replication rate (h^{-1}). If we ignore cell death, k_d, then we use the symbol μ'_R; and in cases where cell death is unimportant, μ_R will equal μ'_R.

In this chapter we will discuss how the specific growth rate changes with its environment. First, we will consider growth in batch culture, where growth conditions are constantly changing.

Lecture 2.Introduction to Monod Kinetics and its validations

2.1. Substrate-limited growth

The relationshipof specific growth rate to substrate concentration often assumes the form of saturation kinetics.Here we assume that a single chemical species, S, is growth-rate limiting (i.e., anincrease in S influences growth rate, while changes in other nutrient concentrations haveno effect). These kinetics are similar to the Langmuir–Hinshelwood (or Hougen–Watson)kinetics in traditional chemical kinetics or Michaelis–Menten kinetics for enzyme reactions.

When applied to cellular systems, these kinetics can be described by the Monodequation:

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

where μ_m is the maximum specific growth rate when $S >> K_s$. If endogeneous metabolism is unimportant, then $\mu_{net} = \mu_g$. The constant K_s is known as the *saturation constant* or *half-velocity constant* and is equal to the concentration of the rate-limiting substrate when the specific rate of growth is equal to one-half of the maximum. That is, $K_s = S$ when $\mu_g = \frac{1}{2}\mu_{max}$. In general, $\mu_g = \mu_m$ for $S >> K_s$ and $\mu_g = (\mu_m/K_s)S$ for $S << K_s$. The Monod equation is semiempirical; it derives from the premise that a single enzyme system with Michaelis–Menten kinetics is responsible for uptake of S, and the amount of that enzyme or its catalytic activity is sufficiently low to be growth-rate limiting.

This simple premise is rarely, if ever, true; however, the Monod equation empiricallyfits a wide range of data satisfactorily and is the most commonly applied unstructured,nonsegregated model of microbial growth.The Monod equation describes substrate-limited growth only when growth is slowand population density is low. Under these circumstances, environmental conditions can be related simply to S. If the consumption of a carbon–energy substrate is rapid, then therelease of toxic waste products is more likely (due to energy-spilling reactions). At highpopulation levels, the buildup of toxic metabolic by-products becomes more important.The following rate expressions have been proposed for rapidly growing dense cultures:

$$\mu_g = \frac{\mu_m S}{K_{s0} S_0 + S}$$

where$S_0$is the initial concentration of the substrate and K_{s0} is dimensionless.

2.2. Other equations to describe the substrate-limited growth phase

Blackman equation:
$$\mu_g = \mu_m, \quad \text{iff } S \geq 2K_s$$
$$\mu_g = \frac{\mu_m}{2K_s} S, \quad \text{iff } S < 2K_s$$

Tessier equation:
$$\mu_g = \mu_m (1 - e^{-KS})$$

Moser equation:
$$\mu_g = \frac{\mu_m S^n}{K_s + S^n} = \mu_m (1 + K_s S^{-n})^{-1}$$

Contois equation:
$$\mu_g = \frac{\mu_m S}{K_{sx} X + S}$$

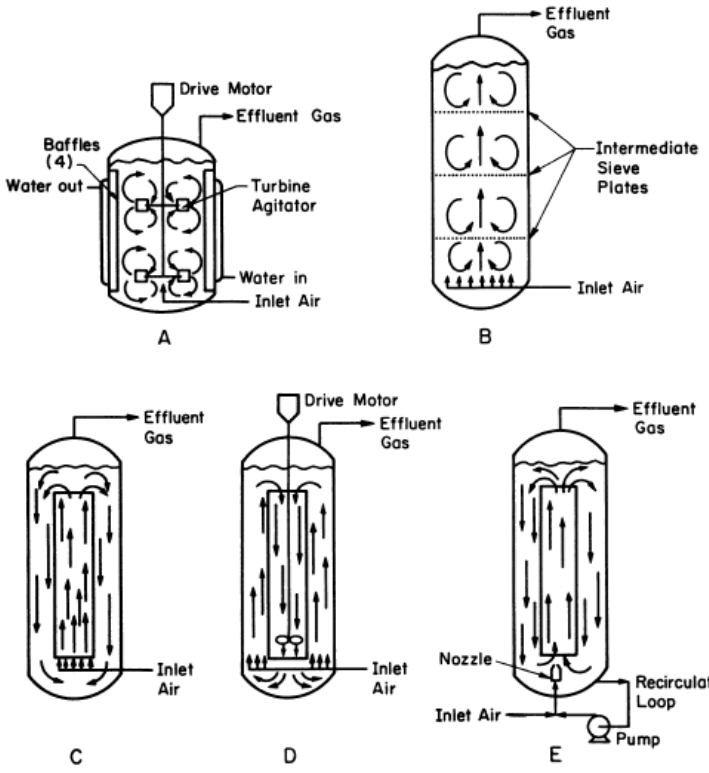
Lecture 3.Different types of fermenters

3.1. Introduction and types

On the basis of design and construction bioreactors may be

1. Stirred tank vessels with mechanical agitation. The most common example of this being the CSTR.
2. In many cases the agitation is done by air which reduces the shear damage of cells caused by mechanical agitators. These are grouped as Bubble Column Reactors.
3. In some Reactors not only do air is used for agitation but they follow a defined pathway inside the Reactors. These maybe classified as Airlift Reactors. Often they are subdivided as External Loop and Internal Loop Reactors.
4. Another type is where a packed bed is use. This mainly related with immobilized catalysts and are primarily associated with food and pharmaceutical industry.
5. A packed bed reactor with a decent up flow is termed as Fluidised Bed Reactor. Clogging issues which occur in a general Packed Bed Reactor may be avoided in this case.
6. When we are charging the packed bed from the top it gives us Trickling Bed Reactor. Most effluent treatment plants used this kind of reactor.

Apart from these a reactor may also be classified on the basis of its mode of operation and on the homogeneity of reactions.



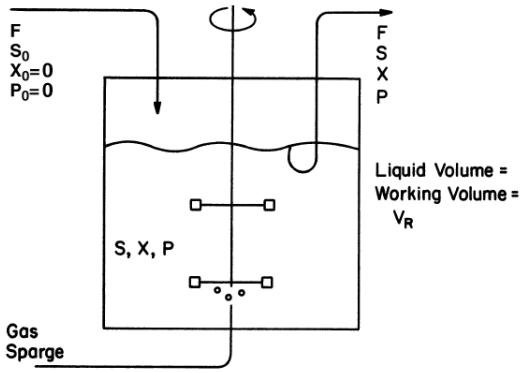
Bioreactor types: (a) Stirred-tank reactor, (b) bubble-column reactor,(c) airlift loop reactor with central draft tube, (d) propeller loop reactor, and (e) jet loop reactor. Arrows indicate fluid circulation patterns.

Lecture 4.Introduction to chemostat with recycle

4.1. The Ideal Chemostat

An ideal chemostat is the same as a perfectly mixed continuous-flow, stirred-tank reactor(CFSTR). Most chemostats require some control elements, such as pH and dissolvedoxygencontrol units, to be useful. Fresh sterile medium is fed to the completely mixedand aerated (if required) reactor, and cell suspension is removed at the same rate. Liquidvolume in the reactor is kept constant.A material balance on the cellconcentration around the chemostat yields is given below,

$$FX_0 - FX + V_R\mu_g X - V_R k_d X = V_R \frac{dX}{dt}$$



Simplified schematic of a chemostat.

where F is the flow rate of nutrient solution (l/h), V_R is the culture volume (l) (assumed constant), X is the cell concentration (g/l), and μ_g and k_d are growth and endogenous (or death) rate constants, respectively (h^{-1}). The reader should note that if cell mass is the primary parameter, it is difficult to differentiate cell death from endogenous metabolism. When we use k_d, we imply that endogenous metabolism is the primary mechanism for cell mass decrease. With k'_d, we imply that cell death and lysis are the primary mechanisms of decrease in mass.

Also if the above equation is written in terms of cell number, then,k_d could only be a cell death rate. When balances are written in terms of cellnumber, the influence of endogenous metabolism can appear only in the substrate balanceequation. Since most experiments are done by measuring total cell mass rather than number,we write our examples based on X. However, the reader should be aware of the ambiguityintroduced when equations are written in terms of X.

$$\frac{dX}{dt} = DX_0 + (\mu_g - k_d - D)X$$

where D is *dilution rate* and $D = F/V_R$. D is the reciprocal of residence time.

Usually, the feed media are sterile, $X_0 = 0$, and if the endogenous metabolism or death rate is negligible compared to the growth rate ($k_d << \mu_g$) and if the system is at steady state ($dX/dt = 0$), then

$$\mu_g = D \quad (\text{if } k_d = 0)$$

In a chemostat, cells are removed at a rate equal to their growth rate, and the growth rateof cells is equal to the dilution rate. *This property allows the investigator to manipulategrowth rate as an independent parameter* and makes the chemostat a powerful experimentaltool.

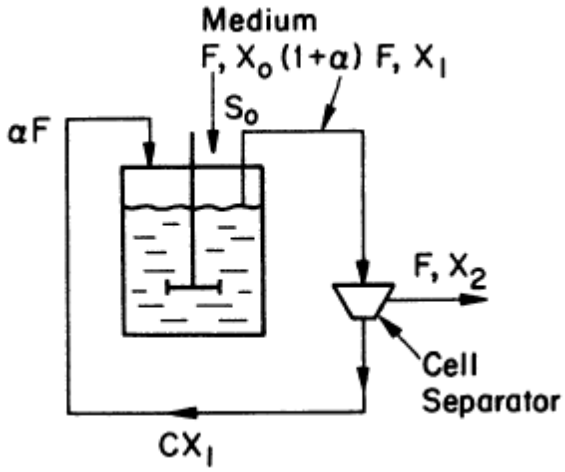
$$\mu_g = D = \frac{\mu_m S}{K_s + S}$$

4.2.Chemostat with Recycle

Microbial conversions are autocatalytic, and the rate of conversion increases with cell concentration. To keep the cell concentration higher than the normal steady-state level in a chemostat, cells in the effluent can be recycled back to the reactor. Cell recycle increases the rate of conversion (or productivity) and also increases the stability of some systems (e.g., waste-water treatment) by minimizing the effects of process perturbation. Cells in the effluent stream are either centrifuged, filtered, or settled in a conical tank for recycling. Consider the chemostat system with cell recycle as depicted in the figure below. A material balance on cell (biomass) concentration around the fermenter yields the following equation:

$$FX_0 + \alpha FCX_1 - (1+\alpha)FX_1 + V\mu_{net}X_1 = V\frac{dX_1}{dt}$$

where a is the recycle ratio based on volumetric flow rates, C is the concentration factor or ratio of cell concentration in the cell recycle stream to the cell concentration in the reactor effluent, F is nutrient flow rate, V is culture volume, X_0 and X_1 are cell concentrations in feed and recycle streams, and X_2 is cell concentration in effluent from the cell separator.



At steady state, and if $dX_1/dt = 0$ and $X_0 = 0$ (that is, sterile feed);

$$\mu_{net} = (1+\alpha-\alpha C)D = [1+\alpha(1-C)]D$$

Since $C > 1$ and $\alpha(1 - C) < 0$, then $\mu_{net} < D$. That is, *a chemostat can be operated at dilution rates higher than the specific growth rate when cell recycle is used.*

A material balance for growth-limiting substrate around the fermenter yields

$$FS_0 + \alpha FS - V\frac{\mu_g X_1}{Y^M_{X/S}} - (1+\alpha)FS = V\frac{dS}{dt}$$

At steady state, $dS/dt = 0$ and

$$X_1 = \frac{D}{\mu_g} Y^M_{X/S}(S_0 - S$$

Substitution of eq. 9.9 when $k_d = 0$ into eq. 9.11 yields

$$X_1 = \frac{Y^M_{X/S}(S_0 - S)}{(1+\alpha-\alpha C)}$$

Therefore, the steady-state cell concentration in a chemostat is increased by a factor of $1/(1 + \alpha - \alpha C)$ by cell recycle.

$$S = \frac{K_s D(1+\alpha-\alpha C)}{\mu_m - D(1+\alpha-\alpha C)}$$

Lecture 5.Introduction to air lift fermenter

5.1. Introduction

AirLift (or GasLift) bioreactors are an alternative to mechanically agitated systems that reduce shear stress and heat generation by eliminating the mechanical agitator. Airlift systems are similar to bubble column reactors where oxygen transfer, internal circulation, and mixing are achieved by bubbling air. They differ however in that air lift bioreactors contain an inner draft tube that creates the drafting force required for liquid circulation and possible improvement of bulk mixing. Utilization of an airlift system may also demonstrate cost savings in the elimination of a mechanical agitator and from the reduction of extra cooling requirements.

Our AL (AirLift) bioreactor systems are primarily based on customer's requirements and related demand in today's market. The volumetric mass transfer coefficient (kLa) and gas hold up very much depend on the physical properties of the system along with air & operating conditions. The below articles are great references for the characteristics and physical properties (fluid dynamics, mass transfer, heat transfer and scale up) of AirLift systems and their applications.

5.1. Types of ALBR (Air Lift Bioreactors)

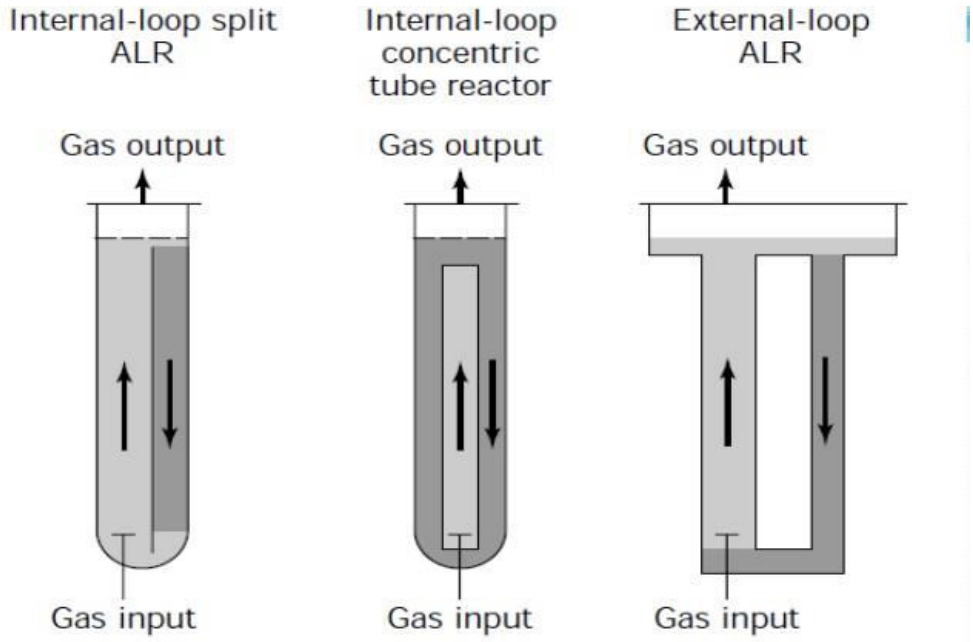
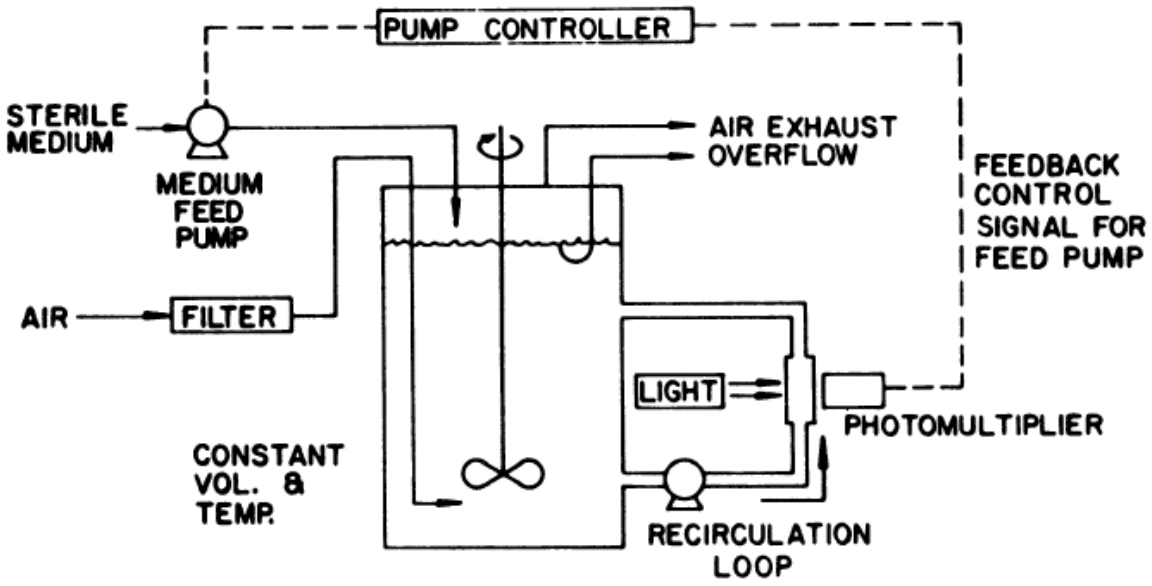


Figure 1. Different types of ALRs.

Lecture 6.Introduction to turbidostat, PFR, fluidized bed reactor

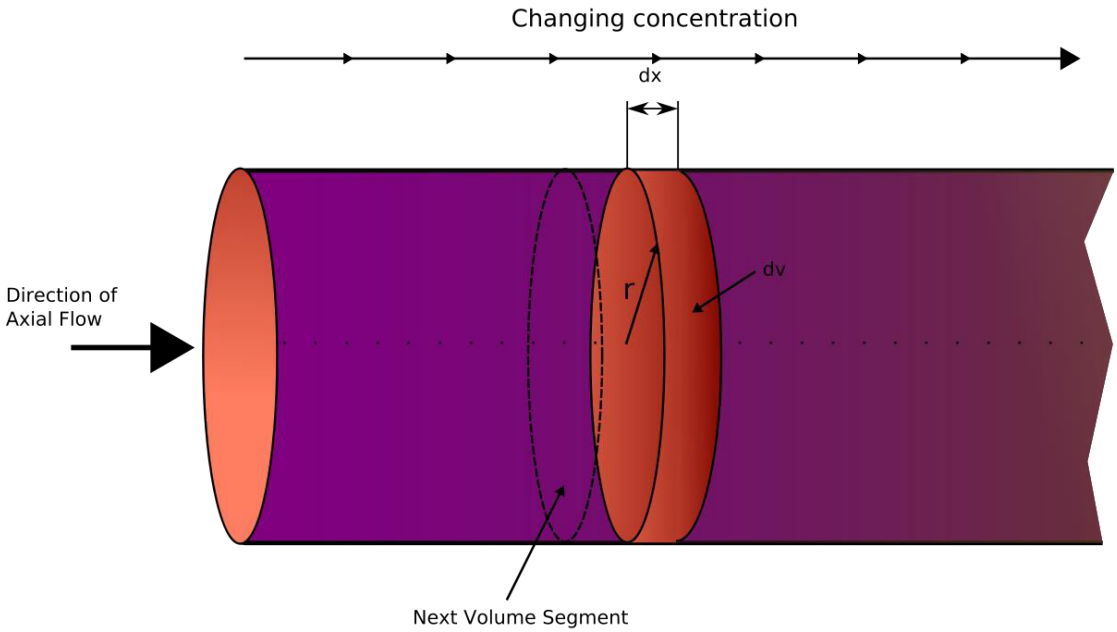
6.1. Introduction to turbidostat

In a turbidostat, the cell concentration in the culture vessel is maintained constant by monitoring the optical density of the culture and controlling the feed flow rate. When the turbidity of the medium exceeds the set point, a pump is activated and fresh medium is added. The culture volume is kept constant by removing an equal amount of culture fluid. The turbidostat is less used than the chemostat, since it is more elaborate than a chemostat and because the environment is dynamic. Turbidostats can be very useful in selecting subpopulations able to withstand a desiredenvironmental stress (for example, high ethanol concentrations), because the cell concentration is maintained constant. The selection of variants or mutants with desirable properties is very important



6.2. Introduction to PFR

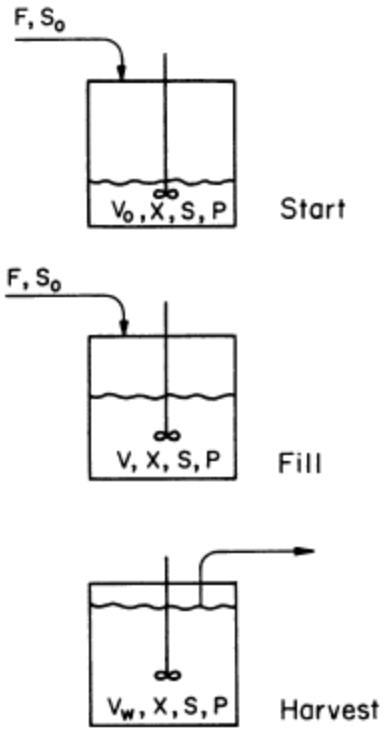
A plug flow reactor (PFR) can also be used for continuous cultivation purposes. Since there is no backmixing in an ideal PFR, fluid elements containing active cells cannot inoculate other fluid elements at different axial positions. Liquid recycle is required for continuous inoculation of nutrient media. In a PFR, substrate and cell concentrationsvary with axial position in the vessel. An ideal PFR resembles a batch reactor in which distance along the fermenter replaces incubation time in a batch reactor. In waste treatment, some units approach PFR behavior, and multistage chemostats tend to approach PFR dynamics if the number of stages is large (five or more).


Changing concentration
dx
Direction of
Axial Flow
r
dv
Next Volume Segment

Lecture7. Fed Batch reactions in biochemical processes

In fed-batch culture, nutrients are continuously or semicontinuously fed, while effluent isremoved discontinuously. Such a system is called a repeated fed-batch culture.Fed-batch culture is usually used to overcome substrate inhibition or catabolite repressionby intermittent feeding of the substrate. If the substrate is inhibitory, intermittent additionof the substrate improves the productivity of the fermentation by maintaining the substrateconcentration low. Fed-batch operation is also called the semicontinuous system orvariable-volume continuous culture.

SCHEMATIC OF A FED-BATCH CULTURE.



Consider a batch culture where the concentration of biomass at a certain time is given by

$$X = X_0 + Y^M_{X/S}(S_0 - S)$$

where S_0 is the initial substrate concentration, $Y^M_{X/S}$ is the yield coefficient, and X_0 is the initial biomass concentration. When biomass concentration reaches its maximum value (X_m), the substrate concentration is very low, $S << S_0$, and also $X_0 << X$. That is, $X_m \approx Y^M_{X/S}S_0$. Suppose that at $X_m \cong Y^M_{X/S}S_0$, a nutrient feed is started at a flow rate F, with the substrate concentration S_0. The total amount of biomass in the vessel is $X^t = VX$, where V is the culture volume at time t. The rate of increase in culture volume is

$$\frac{dV}{dt} = F$$

Integrating this equation we get

$$V = V_0 + Ft$$,where$V_0$is the initial culture volume

The biomass concentration in the vessel at any time t is

$$X = X^t / V$$

The rate of change in biomass concentration is

$$\frac{dX}{dt} = \frac{V(dX^t/dt) - X^t(dV/dt)}{V^2}$$

$$\Rightarrow \quad \frac{dX}{dt} = (\mu_{net} - D)X \quad , \quad \text{Since } dX^t/dt = \mu_{net}X^t,\ dV/dt = F, \text{ and } F/V = D.$$

When the substrate is totally consumed, $S \approx 0$ and $X = X_m = Y^M_{X/S}S_0$. Furthermore, since nearly all the substrate in a unit volume is consumed, then $dX/dt = 0$. This is an example of a quasi-steady state. A fed-batch system operates at quasi-steady state when nutrient consumption rate is nearly equal to nutrient feed rate. Since $dX/dt = 0$ at quasi-steady state, then

$$\mu_{net} = D$$

Lecture8.Problems on specific growth rate for microorganisms in a bioreactor

1.

In a fed-batch culture operating with intermittent addition of glucose solution, values of the following parameters are given at time $t = 2$ h, when the system is at quasi-steady state.

$V = 1000$ ml $\qquad F = \frac{dV}{dt} = 200$ ml/h

$S_0 = 100$ g glucose/l $\qquad \mu_m = 0.3\ h^{-1}$

$K_s = 0.1$ g glucose/l $\qquad Y^M_{X/S} = 0.5$ gdw cells/g glucose

$X^t_0 = 30$ g

a. Find V_0 (the initial volume of the culture).
b. Determine the concentration of growth-limiting substrate in the vessel at quasi-steady state.
c. Determine the concentration and total amount of biomass in the vessel at $t = 2$ h (at quasi-steady state).
d. If $q_P = 0.2$ g product/g cells, $P_0 = 0$, determine the concentration of product in the vessel at $t = 2$ h.

2.

In a chemostat with cell recycle, as shown in Fig. 9.1, the feed flow rate and culture volumes are $F = 100$ ml/h and $V = 1000$ ml, respectively. The system is operated under glucose limitation, and the yield coefficient, $Y^M_{X/S}$, is 0.5 gdw cells/g substrate. Glucose concentration in the feed is $S_0 = 10$ g glucose/l. The kinetic constants of the organisms are $\mu_m = 0.2\ h^{-1}$, $K_s = 1$ g glucose/l. The value of C is 1.5, and the recycle ratio is $\alpha = 0.7$. The system is at steady state.

a. Find the substrate concentration in the recycle stream (S).
b. Find the specific growth rate (μ_{net})of the organisms.
c. Find the cell (biomass) concentration in the recycle stream.
d. Find the cell concentration in the centrifuge effluent (X_2).

3.

Consider a 1000-l CSTR in which biomass is being produced with glucose as the substrate. The microbial system follows a Monod relationship with $\mu_m = 0.4\ h^{-1}$, $K_S = 1.5$ g/l (an unusually high value), and the yield factor $Y_{X/S} = 0.5$ g biomass/g substrate consumed. If normal operation is with a sterile feed containing 10 g/l glucose at a rate of 100 l/h:

a. What is the specific biomass production rate (g/l-h) at steady state?
b. If recycle is used with a recycle stream of 10 l/h and a recycle biomass concentration five times as large as that in the reactor exit, what would be the new specific biomass production rate?
c. Explain any difference between the values found in parts a and b.

MODULE III

Lecture1.Introduction to Downstream processing

Downstream processing refers to the recovery and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or fermentation broth, including the recycling of salvageable components and the proper treatment and disposal of waste. It is an essential step in the manufacture of pharmaceuticals such as antibiotics, hormones (e.g. insulin and humans growth hormone), antibodies (e.g. infliximab and abciximab) and vaccines; antibodies and enzymes used in diagnostics; industrial enzymes; and natural fragrance and flavor compounds. Downstream processing is usually considered a specialized field in biochemical engineering, itself a specialization within chemical engineering, though many of the key technologies were developed by chemists and biologists for laboratory-scale separation of biological products.

Downstream processing and analytical bioseparation both refer to the separation or purification of biological products, but at different scales of operation and for different purposes. Downstream processing implies manufacture of a purified product fit for a specific use, generally in marketable quantities, while analytical bioseparation refers to purification for the sole purpose of measuring a component or components of a mixture, and may deal with sample sizes as small as a single cell.

Let us look at flow sheet, the alcohol fermentation. I mean it has been there for almostfive thousand years, fermenting from sugars, fermenting from fruits, converting into alcohol. Once they convert into alcohol, it contains a large amount of a liquid where youralcohol, the percentage of alcohol is very little. So, the idea is to recover as much ofpossible the alcohol from this large quantity of liquid and then concentrate it to highdegree of purity. So, that is what is alcohol fermentation is all about. If you look at the entire flow sheet of alcohol manufacturing, you have thefermentation taking place here. That is where the sugars are getting converted into thefermentor alcohol, but if you look at the right hand side in this flow sheet, the entireportion is the called the downstream processing. This is where you are trying to isolateyour alcohol and then you are trying to purify your alcohol to a very large concentratorliquid form.

Once you collect the fermentation broth, you may be doing the distillation becausealcohol boils at lower temperature than rest of the mass. So, the distillation happens hereand then the product that is coming out is condensed here. This will be mostly alcoholand of course you are going to have water. If there are any other side products, which arealso going to be of lower boiling point is going to also condensed here. Now, the bottomsyou filter and then you collect the solids, you dry the solids. Finally, you may get somedried grainy material; you may also get dried soluble material. So, this can be used asanimal feed. It may be rich in protein. It may be very useful for animal feed.

So, once the alcohol is distilled out, which contains water and other low boilers, it isfurther distilled here. These are called purification and rectification columns. Finally,you get very pure alcohol on the extreme right hand side and you are going to have lot ofwater coming out as the bottom here. So, this particular operation of purification ofalcohol from fermentation contains several distillation columns and then filters anddriers.

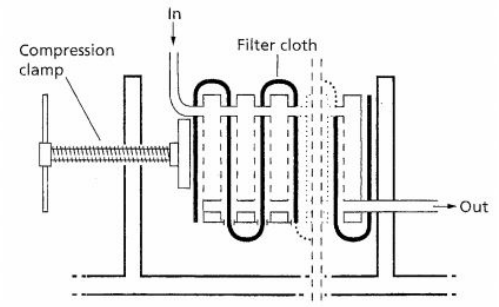
So, the downstream in alcohol fermentation are made of filters, driers and the distillation columns. It is quite a simple downstream operation if you consider alcohol fermentation,but if you go for other chemicals like bulk chemical or even specialty chemicals, evenproducts related to drugs or pharmaceutical, you may have very stringent requirement forpurity because a pharmaceutical product has to be extremely pure. You may go into steps like chromatography,different types of chromatography, separations like membranes and soon. This is because here it is a product, which can take, which can withstandlarge, high temperatures, you are resorting to distillation.But, you are going to have products, which are like protein or which are enzymes, whichmight not be able to withstand the temperature. You may not be able to use distillationthere. You may have to use some other downstream process. So, depending upon thetype of product, which you are trying to recover, the nature of the downstream variesquite a lot actually.

Lecture 2.Introduction to filtration

A filter medium constitutes the separating agent and retains the particles according to size while allowing the passage of the liquid through the filter. In cake filtration, the particles are retained as a cake on the filter medium. The flow through the filter layers is dependent on area of the filter &flow resistance provided by the filter medium &the cake. If the particles do not penetrate the filter medium, then its flow resistance will remain unchanged. However, the cake layer, as it grows thicker will provide increasing resistance. The cake layers, especially in biomass separation, are compressible & the changing effective pressure difference will influence the flow through the filter, e.g., of filter media are perforated sintered metal, cloth, synthetic fibres, cellulose, glass wool, ceramics & synthetic membranes. Many types of filtration equipment are available.

2.1. Plate and frame filters

These are cheap and versatile - the surface area can be adjusted by varying the number of plates. Not suitable for the removal of large quantities of solids from broths as the plates have to be dismantled for solids recovery. They are used as polishing devices to filter out low residual solids.



A PLATE AND FRAME FILTER

2.2. Filter Press

A filter press is built of a sequence of perforated plates alternating with hollow frames. The plates are covered with a suitable filter medium (cloths) that create a series of chambers through which the slurry can be forced. Solids are retained in the chambers and the filtrate discharges into the hollows on the plate surface and drain out.

2.3. Membrane Filter Press

The cake chambers are covered with a rubber membrane that is inflated using air or water, allowing in situ compacting of the cake. These allow higher yield and drier cake but involve a higher capital investment. Filtration using microporous membranes operated under pressure is a viable alternative to centrifugation.

2.4. Vacuum filters

Vacuum filters are used for clarification of fermentation broths (containing 10-40% solids by volume with particle sizes ranging between 0.5-10 μm) due to simplicity of operation and low cost. The best known vacuum filters are the rotary drum vacuum filters which are used for filtration of filamentous fungi and yeast cells. They are used to clarify large volumes of liquid with automatic solids discharge. Tangential flow (cross flow filtration) is an effective method to separate cells from liquid where high value product is involved. The parallel motion of fluid to the membrane helps reduce the thickness of the cell layer on the filter surface.

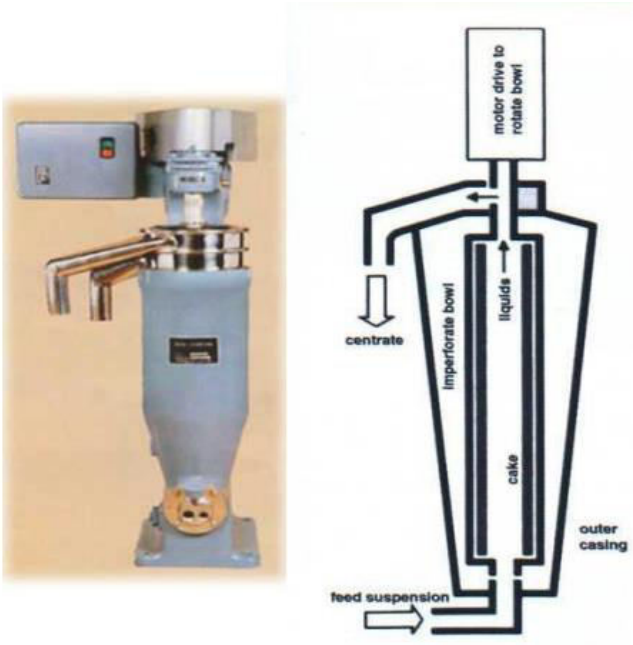
Lecture 3.Introduction to centrifugation

3.1. Introduction

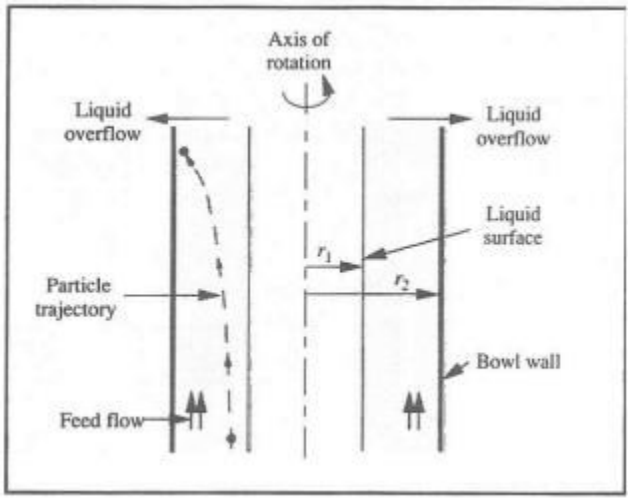
- ⇨ Separation by means of the accelerated gravitational force achieved by a rapid rotation.
- ⇨ Relies on the density difference between the particles and the surrounding medium, most effective when the particles to be separated are large, the liquid viscosity is low and the density difference between particles and fluid is great.
- ⇨ Batch centrifuge is common in the labs but the low processing capacity limits its use in large scale.
- ⇨ Continuous centrifuges are common in large-scale processing in which the deposited solids are removed continuously or intermittently.

3.2. Tubular bowl centrifuge

- Very commonly used in food and pharmaceutical industries.
- Feed enters under pressure through a nozzle at the bottom, and moves upwards through the cylindrical bowl.
- As the bowl rotates, particles traveling upward are spun out and collide with the walls of the bowl. Solids hitting the wall can form the cake.
- As the feed rate increases, the liquid layer moving up the wall becomes thicker thus reducing the performance of the centrifuge by increasing the distance a particle must travel to reach the bowl.
- This system lacks a provision of solids rejection, the solids can only be removed by stopping the machine, dismantling it and scraping or flushing the solids out manually.
- Typical range of centrifugal force: **13000-16000 x g**



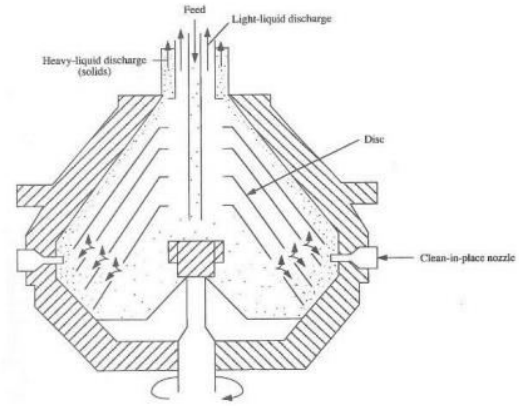
TUBULAR BOWL CENTRIFUGE

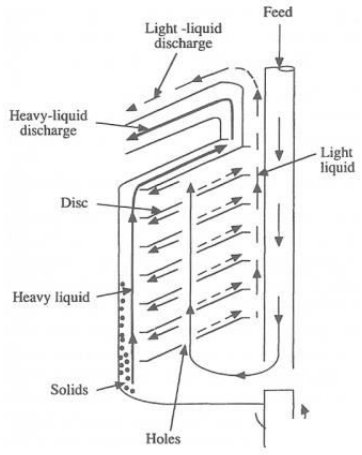


SEPERATION OF SOLIDS IN A TUBULAR BOWL CENTRIFUGE

3.3. Disc-stack bowl centrifuge

- Contain conical sheets of metal (discs) which are stacked with clearances as small as 0.3 mm. The discs rotate with the bowl to split the liquid into thin layers. Feed is released near the bottom of the centrifuge and travels upwards through matching holes in the discs.
- Between the discs, heavy components of the feed are thrown outward under the influence of centrifugal forces as lighter liquid is displaced towards the center of the bowl. As they are flung out, the solids strike the undersides of the discs and slide down to the bottom edge of the bowl. At the same time, the lighter liquid flows in and over the upper surfaces of the discs to be discharged from the top of the bowl.
- Heavier liquid containing solids can be discharged either at the top of the centrifuge or through nozzles around the periphery of the bowl.
- Typical range of centrifugal force: **5000-15000 x g**



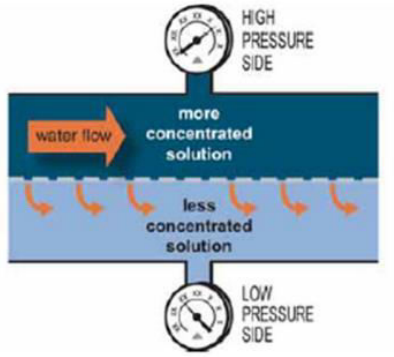


DISC-STACK BOWL CENTRIFUGE WITH CONTINUOUS DISCHARGE OF SOLIDS

Lecture 4.Introduction to membrane processes (reverse osmosis)

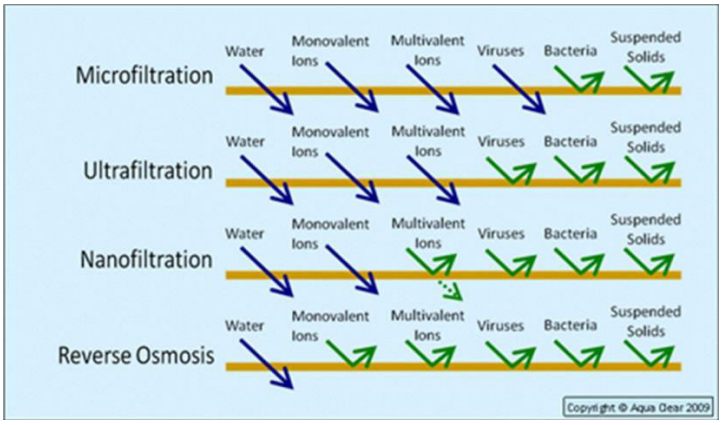
In order to understand the Reverse Osmosis (RO), it first requires understanding the concept of most common natural phenomena known as osmosis. Osmosis is general and important process in the nature, which can be easily defined by water passing from less concentrated solution towards the highly concentrated solution through semipermeable barrier or membrane. The transfer carries on occurring until the both solution gain equal concentration.

RO is completely alter process of osmosis. Osmosis occurs naturally without requiring external energy, whereas RO requires to apply external energy/pressure using a high pressure pump on the side of the highly concentrated solution. At osmotic pressure, the flow between two solutions will be ceased whereas applying pressure or energy greater than osmotic pressure reverse the flow form highly concentrated solution towards the less concentrated solution. The amount of pressure required depends on the salt concentration of the feed water. The higher the concentration of the feed water, the more pressure is required to overcome the osmotic pressure. A simple schematic of reverse osmosis is given below.



SCHEMATIC OF REVERSE OSMOSIS PROCESS

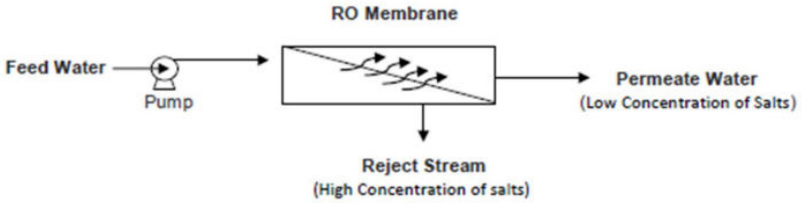
Reverse osmosis is membrane filtration technique. Within the membrane filtration, there are many other technologies are also well known along Reverse Osmosis :Nanofiltration (NF) , Ultrafiltration (UF) and Microfiltration (MF)



COMPARISON CHART OF DIFFERENT FILTRATION VARIOUS MEMBRANE FILTRATION TECHNOLOGIES

RO is very effective techniques for treating water. According to the literature, RO is capable of removing up to 99% of the dissolved salts (ions), particles, colloids, organics, bacteria and

pyrogens from the feed water. The membrane within the RO rejects contaminants based on their size and charge. Molecules with molecular weight greater than 200 are likely to be rejected by the fully functioning RO system. Besides, the greater the ionic charge of contaminants has higher chances of getting stuck in membrane. However, the gases are not removed by the RO because gases such as CO_2 is not highly ionized while in the solution and have very low molecular weight.



OPERATION OF RO WATER PURIFICATION SYSTEM

Lecture 5.Introduction to membrane processes (ultrafiltration)

Ultrafiltration (UF) is a pressure-driven membrane process used throughout downstream processing for: (1) protein concentration, (2) buffer exchange and desalting, (3) removal of small contaminants, (4) protein purification, and (5) virus clearance. This chapter will consider the first three applications—other chapters in this volume discuss the final two processes. Separation in UF is primarily owing to differences in solute size, with the larger species retained by the membrane whereas the solvent and smaller components pass into the filtrate through the membrane pores. Electrostatic (and other long-range) interactions can also affect the rate of solute transport, e.g., charged solutes are strongly excluded from the membrane pores during operation at low salt concentrations *(1)*.

UF membranes are cast from a wide range of polymers in both flat sheet and hollow fiber form. These membranes have an asymmetric structure with a very thin skin layer (approximately 0.5 μm thick), which provides the membrane its selectivity, and a more macroporous substructure which provides the required mechanical and structural integrity. UF membranes have mean pore size ranging from 10–500 Å. However, most manufacturers rate their membranes by the nominal molecular weight cutoff, which is defined as the molecular weight of a solute with a particular retention coefficient *(R)*:

$$R = 1 - C_p/C_F \quad (1)$$

where C_p and C_F are the solute concentrations in the permeate solution and feed stream, respectively. Data are typically obtained with different model proteins or with polydisperse dextrans *(2)*. Unfortunately, the procedures for

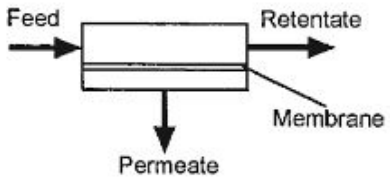


Fig. 1. Tangential flow filtration.

assigning molecular weight cutoffs, including the choice of solutes, the specific buffer and flow conditions, and the chosen retention value (e.g., $R = 0.9$) vary widely throughout the industry, making it difficult to use these classifications for actual process development.

Lecture 6.Introduction to membrane processes (chromatographic separation)

6.1. Introduction

Chromatography is an analytical technique commonly used for separating a mixture of chemical substances into its individual components, so that the individual components can be thoroughly analyzed. Chromatography techniques are also can be used in the final stages of purification of a number of products. There are many types of chromatography e.g., liquid chromatography, gas chromatography, ion-exchange chromatography, affinity chromatography, but all of these employ the same basic principles (Peter, et.al. 2016). Chromatography uses these two phases to separate the components in mixtures. One of the phases is immobilized, usually by chemically bonding it to a solid particle or the inside of a tube. This part is generally referred to as the stationary phase, but is also often called the packing, support, or column. The other phase, typically a liquid, gas or supercritical fluid, is passed across the stationary phase by applying pressure on the inlet of the system. This phase is called the mobile phase, or carrier. The samples are subjected to flow by mobile liquid onto or through the stable stationary phase. The sample components are separated into fractions based on their relative affinity towards the two phases during their travel. The fraction with a greater affinity to stationary layer travels slower and at a shorter distance, while that with a less affinity travels faster and longer. Chromatography has numerous applications in biological and chemical fields. It is widely used in biochemical research for the separation and identification of chemical compounds of biological origin. In the petroleum industry the technique is employed to analyze complex mixtures of hydrocarbons (Roy, et.al. 2016). As a separation method, chromatography has a number of advantages comparing with the conventional techniques—crystallization, solvent extraction, and distillation, for example. It can be handled simply and very pure products can be recovered. It is capable of separating all the components of a multicomponent chemical mixture in low temperature according to the size or chemical properties, without requiring an extensive foreknowledge of the identity, number, or relative amounts of the substances present. In many fermentation processes, chromatographic techniques are used to isolate and purify relatively low concentrations of metabolic products. In this report, chromatography will be concerned with the passage and separation of different solutes as liquid is passed through a column (Stanbury, et.al. 2003).

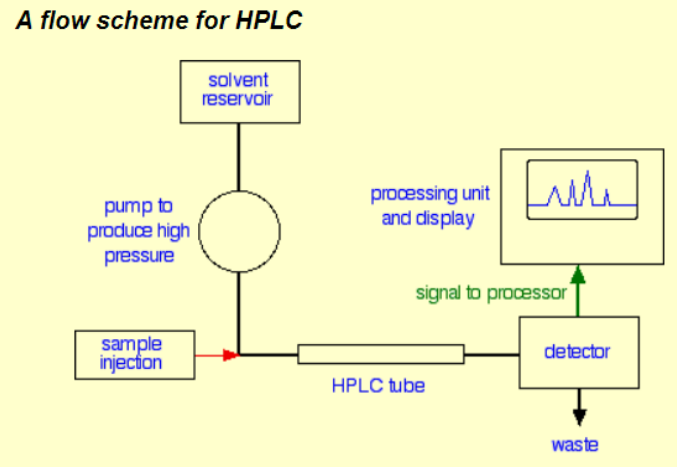
6.2. HPLC

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.

It also allows you to use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture.

The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.

Confusingly, there are two variants in use in HPLC depending on the relative polarity of the solvent and the stationary phase.



Retention time

The time taken for a particular compound to travel through the column to the detector is known as its ***retention time***. This time is measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound.

Different compounds have different retention times. For a particular compound, the retention time will vary depending on:

- the pressure used (because that affects the flow rate of the solvent)
- the nature of the stationary phase (not only what material it is made of, but also particle size)
- the exact composition of the solvent
- the temperature of the column

That means that conditions have to be carefully controlled if you are using retention times as a way of identifying compounds.

Lecture 7.Introduction to Extraction

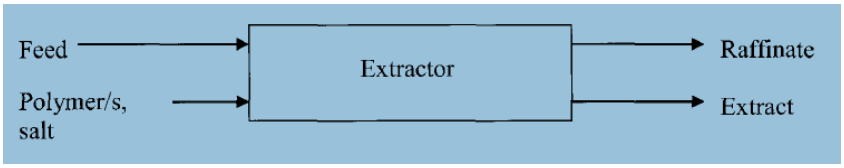
An extraction process makes use of the partitioning of a solute betweentwo immiscible or partially miscible phases. For example the antibioticpenicillin is more soluble in the organic solvent methyl isobutyl ketone(MIBK) than in its aqueous fermentation medium at acidic pH values.
This phenomenon is utilized for penicillin purification. When theextraction takes place from one liquid medium to another, the process isreferred to as liquid-liquid extraction. When a liquid is used to extract solutes from a solid material, the process is referred to as solid-liquidextraction or leaching. In this chapter we will mainly discuss liquidliquidextraction. When a supercritical fluid is used as an extracting solvent, the process is referred to as supercritical fluid extraction (SFE).

Liquid-liquid extraction involves transfer of solute from one liquidphase to another. The three basic steps common to all liquid-liquidextraction processes are:

Mixing or contacting
Transfer of solute between two partially or completely immiscibleliquids requires intimate contact of the two. This is usually achieved bydispersing one liquid (the dispersed phase) as tiny droplets in the otherliquid (i.e. the continuous phase). Solute transport rate depends on theinterfacial mass-transfer coefficient which depends on the hydrodynamicconditions in the system and on the available contact area. Vigorousmixing can achieve both these requirements.

Phase separation or settling
Once the desired extent of solute transport has been achieved (i.e.equilibrium has been reached), the next step is to allow the droplets ofthe dispersed phase to coalesce. This eventually leads to the separation of
the two liquids into distinct layers due to density difference. In a liquidliquidextraction process, it is important that the two phases (i.e. raffinateand the extract) should have sufficient density difference to facilitatesegregation of phases.

Collection of phases
After settling (or phase separation), the extract and raffinate phases arecollected as separate streams by appropriate means.



AQUEOUS TWO-PHASE EXTRACTION

Lecture 8.Problems on downstream processing

1.

100 litres of an aqueous solution of citric acid (concentration = 1 g/l) is contacted with 10 litres of an organic solvent. The equilibrium relationship is given by $C_E = 100\ C_R^2$, where C_R and C_E are the citric acid concentrations in the raffinate and extract respectively and are expressed in g/l. Calculate:

a) The concentration of citric acid in the raffinate and the extract.
b) The fraction of citric acid extracted.

If the extract thus obtained is then contacted with a further 100 litres of aqueous solution of citric acid (concentration = 1 g/l) calculate:

c) The concentration of citric acid in the raffinate and extract phases of the second extraction. Comment on these results.

2.

A plasmid was found to have a retention time of 10 minutes in a chromatographic column having a volume of 0.01 m^3 and a voidage fraction of 0.3. The distribution coefficient (K) of the plasmid is known to be equal to 2. Calculate the mobile phase retention time and flow rate at which the above separation was carried out. We would like to use the same column-mobile phase system to separate the plasmid from RNA (which has a capacity factor of 4.66). Comment on the feasibility.

3.

The following data was obtained from a constant pressure cake filtration experiment:

Time (s)	5	10	20	30
$V(t)$ (litres)	0.040	0.055	0.080	0.095

The following additional information is given:
$A = 0.1$ ft^2, $C_s = 0.015$ kg/l, $\mu = 1.1$ centipoise, $\Delta P = 10$ N/m^2.

a) Determine R_M
b) Determine the specific cake resistance

4.

50 litres of filtrate is collected in 30 minutes when an inorganic suspension is filtered through a sintered glass filter using a pressure drop of 50 kPa. How much filtrate will be collected in 30 minutes at a pressure drop of 100 kPa? Assume that R_M is negligible.

MODULE IV

Lecture1. Design parameters associated with bioreactors

1.1. Introduction

The design and mode of operation of a fermenter mainly depends on the production organism, the optimal operating condition required for target product formation, product value and scale of production. The design also takes into consideration the capital investment and running cost.

• Large volume and low value products like alcoholic beverages need simple fermenter and do not need aseptic condition.

• High value and low volume products require more elaborate system of operation and aseptic condition. Bioreactors differ from conventional chemical reactors in that they support and control biological entities.

As such, bioreactor systems must be designed to provide a higher degree of control over process upsets and contaminations, since the organisms are more sensitive and less stable than Chemicals. Biological organisms, by their nature, will mutate, which may alter the biochemistry of the bioreaction or the physical properties of the organism. Analogous to heterogeneous catalysis, deactivation or mortality occur and promoters or coenzymes influence the kinetics of the bioreaction. Although the majority of fundamental bioreactor engineering and design issues are similar, maintaining the desired biological activity and eliminating or minimizing undesired activities often presents a greater challenge than traditional chemical reactors typically require. Other key differences between chemical reactors and bioreactors are selectivity and rate. In bioreactors, higher selectivity — that is, the measure of the system's capability for producing the preferred product (over other outcomes) — is of primary importance. In fact, selectivity is especially important in the production of relatively complex molecules such as antibiotics, steroids, vitamins, proteins and certain sugars and organic acids. Frequently, the activity and desired selectivity occur in a substantially smaller range of conditions than are present inconventional chemical reactors. Further, deactivation of the biomass often poses more severe consequences than a chemical upset.

1.2. Requirement for designing of a bioreactor

Due to above mentioned demands made by biological systems on their environment, there is no universal bioreactor. However, the general requirements of the bioreactor are as follows:
1. The vessel should be robust and strong enough to withstand the various treatments required such as exposure to high heat, pressure and strong chemicals and washings and cleanings.
2. The vessel should be able to be sterilized and to maintain stringent aseptic conditions over long periods of the actual fermentation process.
3. The vessel should be equipped with stirrers or mixers to ensure mass transfer processes occur efficiently.
4. It should have sensors to monitor and control the fermentation process.
5. It should be provided with inoculation point for aseptic transfer in inoculum.
6. Sampling valve for withdrawing a sample for different tests.
7. Baffles should be provided in case of stirred fermenter to prevent vertex formation.

8. It should be provided with facility for intermittent addition of an antifoam agent.
9. In case of aerobic submerged fermentation, the tank should be equipped with the aerating device.
10. Provision for controlling temperature and pH of fermentation medium.
11. Man hole should be provided at the top for access inside the fermenter for different purposes.

1.3. Height-to-diameter ratio (Aspect ratio).

The height-to-diameter ratio is also a critical factor in vessel design. Although a symmetrical vessel maximizes the volume per material used and results in a height-to-diameter ratio of one, most vessels are designed with higher ratio. The range of 2-3:1 is more appropriate and in some situation, where stratification of the tank content is not an issue or a mixer is used, will allow still higher ratio to be used in design.

The vessels for microbiological work should have an aspect ratio of 2.5-3:1, while vessels for animal cell culture tend to have an aspect ratio closer to 1. The basic configuration of stirred tankbioreactors for mammalian cell culture is similar to that of microbial fermenter but the major difference is there in aspect ratio, which is usually smaller in mammalian cell culture bioreactor. In stirred tank bioreactor (STR), height to diameter aspect ratio is 3:1 or 4:1 while in the case of CSTR, the aspect ratio is maintained more than 1, to ensure high residence time of gas phase, increase the transfer efficiency and to ensure less power input on introduction of gas, uniform power dissipation. In miniature bioreactor, aspect ratio is kept equal to large bioreactor in order to predict hydrostatic pressure and therefore oxygen solubility at the different scale of operation.

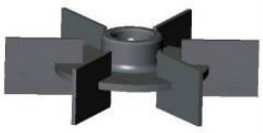
1.4. The agitator (impeller).

The agitator is required to achieve a number of mixing objective.

- Bulk fluid and gas-phase mixing,
- Air dispersion,
- Oxygen transfer,
- Heat transfer,
- Suspension of solid particles and maintain a uniform environment throughout the vessel contents.
- Enhancement of mass transfer between dispersed phases.

Large pitch blade impeller

Marine propeller



45° Flat blade disk turbine

Lecture 2.Mass transfer in microbial reactors

2.1. Gas-liquid mass transfer in bioreactors

Microbial cells often grown aerobically in stirred tank reactors -oxygen supply is often limiting in such conditions.

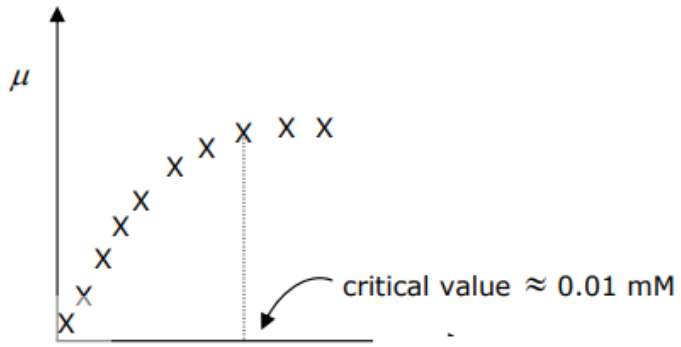


Figure 1. μ vs dissolved oxygen.

D.O. = dissolved oxygen

Equilibrium solubility of O_2 ≈ 1 mM

2.2. Oxygen pathway in a bioreactor

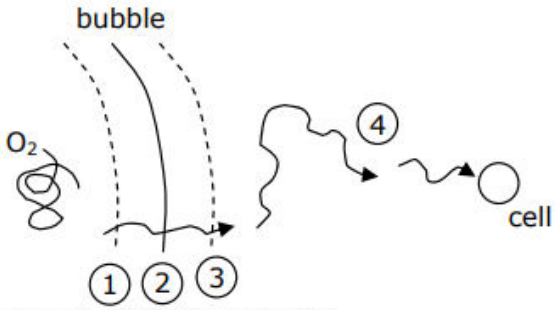


Figure 2. Oxygen pathway.

1) Diffusion across stagnate gas film
2) Absorption
3) Stagnate liquid layer (rate-limiting step)
4) Diffusion and convection

2.3. Diffusional Limitations in ImmobilizedEnzyme Systems

Diffusional resistances may be observed at different levels in immobilized enzymes. Theseresistances vary depending on the nature of the support material (porous, nonporous), hydrodynamicalconditions surrounding the support material, and distribution of the enzymeinside or on the surface of the support material. Whether diffusion resistance has a significanteffect on

the rate of enzymatic reaction rate depends on the relative rate of the reaction rate and diffusion rate, which is characterized by the Damköhler number (Da).

$$\mathrm{Da} = \frac{\text{maximum rate of reaction}}{\text{maximum rate of diffusion}} = \frac{V_{m'}}{k_L[S_b]}$$

where $[S_b]$ is substrate concentration in bulk liquid (g/cm^3) and k_L is the mass-transfer coefficient (cm/s).

The rate of enzymatic conversion may be limited by diffusion of the substrate or reaction, depending on the value of the Damköhler number. If Da >> 1, the diffusion rate is limiting. For Da << 1, the reaction rate is limiting, and for Da ≈ 1, the diffusion and reaction resistances are comparable. Diffusion and enzymatic reactions may be simultaneous, with enzymes entrapped in a solid matrix, or may be two consecutive phenomena for adsorbed enzymes.

2.4.Impeller Reynold's Number

It has been found out that the Aeration Efficiency (k_la) is proportional to the power consumption per unit volume of fluid

$$k_la = (\text{Power Consumed / Unit volume of liquid})^{0.95}$$

The turbulent motion of the fluid and air bubbles can be defined by Impeller Reynold's Number

$$L^2 N \rho / \mu$$

where, L = Diameter of the impeller
N = Rotational Speed of the Impeller
ρ = Density of fluid
μ = Viscosity

In an aditated and fully baffled system,

$(L^2 N \rho / \mu) > 10^5$

Lecture 3.Oxygen Uptake Rate and related considerations

3.1. An introduction to dissolved oxygen

Dissolved oxygen (DO) is an important substrate in aerobic fermentations and maybe a limiting substrate, since oxygen gas is sparingly soluble in water. At high cell concentrations,the rate of oxygen consumption may exceed the rate of oxygen supply, leadingto oxygen limitations. When oxygen is the rate-limiting factor, specific growth ratevaries with dissolved-oxygen concentration according to saturation kinetics; below a criticalconcentration, growth or respiration approaches a first-order rate dependence on thedissolved-oxygen concentration.

Above a *critical oxygen concentration*, the growth rate becomes independent of thedissolved-oxygen concentration.Oxygen is a growth-rate-limiting factor when theDO level is below the critical DO concentration. In this case, another medium component(e.g., glucose, ammonium) becomes growth-extent limiting. For example, with *Azotobactervinelandii*at a DO = 0.05 mg/l, the growth rate is about 50% of maximum even if alarge amount of glucose is present. However, the maximum amount of cells formed is notdetermined by the DO, as oxygen is continually resupplied. If glucose were totally consumed,growth would cease even if DO = 0.05 mg/l. Thus, the extent of growth (mass ofcells formed) would depend on glucose, while the growth rate for most of the culture periodwould depend on the value of DO.The critical oxygen concentration is about 5% to 10% of the saturated DO concentrationfor bacteria and yeast and about 10% to 50% of the saturated DO concentration formold cultures, depending on the pellet size of molds. Saturated DO concentration in waterat 25 degree Celcius and 1 atm pressure is about 7 ppm. The presence of dissolved salts and organicscan alter the saturation value, while increasingly high temperatures decrease the saturationvalue.Oxygen is usually introduced to fermentation broth by sparging air through thebroth. Oxygen transfer from gas bubbles to cells is usually limited by oxygen transferthrough the liquid film surrounding the gas bubbles. The rate of oxygen transfer from thegas to liquid phase is given by

$$N_{O_2} = k_L a(C^* - C_L) = \text{OTR}$$

where k_L is the oxygen transfer coefficient (cm/h), a is the gas–liquid interfacial area (cm^2/cm^3), $k_L a$ is the volumetric oxygen transfer coefficient (h^{-1}), C^* is saturated DO concentration (mg/l), C_L is the actual DO concentration in the broth (mg/l), and the N_{O_2} is the rate of oxygen transfer (mg O_2/l·h). Also, the term *oxygen transfer rate* (OTR) is used.

The rate of oxygen uptake is denoted as *OUR* (*oxygen uptake rate*) and

$$\text{OUR} = q_{O_2} X = \frac{\mu_g X}{Y_{X/O_2}}$$

where q_{O_2} is the specific rate of oxygen consumption (mg O_2/g dw cells·h), Y_{X/O_2} is the yield coefficient on oxygen (g dw cells/g O_2), and X is cell concentration (g dw cells/l).

When oxygen transfer is the rate-limiting step, the rate of oxygen consumption isequal to the rate of oxygen transfer. If the maintenance requirement of O_2 is negligiblecompared to growth, then

$$\frac{\mu_g X}{Y_{X/O_2}} = k_L a(C^* - C_L)$$

The preceding correlations are not very good for Newtonian systems and are evenworse for non-Newtonian or highly viscous systems. They also neglect the effects ofmedium components on $k_L a$. The presence of salts and surfactants can significantly alterbubble size and liquid film resistance around the gas bubble. These factors also can affect*oxygen solubility* (C^*). Temperature and pressure also affect $k_L a$andC^*. Finally, on thesupply side, it should be noted that C_L is maintained at a value above the critical oxygenconcentration (see Chapter 6) but at a low enough

value to provide good oxygen transfer.For many bacterial fermentations, a $C_L$of about 1 mg/l provides a good margin of safety ifmixing is incomplete and yet allows a good rate of oxygen transfer.

3.2. Measurement of k_La in a bioreactor

Although $k_La$is difficult to predict, it is a measurable parameter. Four approachesare commonly used: unsteady state, steady state, dynamic, and sulfite test. The way inwhich these methods are applied depends on whether the test is being made on the systemin the presence or absence of cells.

A new reactor prior to operation can be filled with pure water or a medium in whichC^* can be accurately measured. Oxygen is removed from the system by sparging with N_2.With the *unsteady-state method*, air is then introduced and the change in dissolved oxygen(DO) is monitored until the solution is nearly saturated. In this case,

$$\frac{dC_L}{dt} = k_L a(C^* - C_L)$$

or

$$\frac{-d(C^* - C_L)}{dt/(C^* - C_L)} = k_L a$$

or

$$\ln(C^* - C_L) = -k_L at$$

Another approach is the *sulfite method*. In the presence of Cu^{2+}, the sulfur in sulfite (SO_3^{2-}) is oxidized to sulfate (SO_4^{2-}) in a zero-order reaction. This reaction is very rapid, and consequently C_L approaches zero.

The rate of sulfate formation is monitored and is proportional to the rate of oxygen consumption ($\frac{1}{2}$ mol of O_2 is consumed to produce 1 mol of SO_4^{2-}). Thus,

$$\tfrac{1}{2} dC_{SO_4}/dt = k_L aC^*$$

where C_{SO_4} is the concentration of SO_4^{2-}. The factor, $\frac{1}{2}$, requires that C_{SO_4} and C^* be expressed in terms of moles. Oxygen solubility, C^*, is a constant dependent on medium composition, temperature, and pressure and can be measured separately. Thus,

$$k_L a = \frac{1}{2}\frac{dC_{SO_4}/dt}{C^*}$$

Perhaps the best way to determine *kLa*is the *steady-state* method, in which thewhole reactor is used as a respirometer. Such an approach requires the accurate measurementof oxygen concentration in all gas exit streams and a reliable measurement of *CL*. Amass balance on O2 in the gas allows the rate of O2 uptake, OUR, to be calculated:

$$k_L a = \frac{\text{OUR}}{C^* - C_L}$$

OUR could also be estimated with off-line measurements of a sample of the culture in a respirometer, but using information from the actual fermenter is ideal. One complication, however, in this (and other methods) is the value of C^* to use. In a large fermenter, gas is sparged under significant pressure (due at least to the liquid height in an industrial fermenter). C^* is proportional to pO_2, which depends on total pressure as well as the fraction of the gas that is oxygen. At the sparger point, pO_2 will be significantly higher than at the exit, due to both higher pressure and the decrease in the fraction of the gas that is O_2 because of consumption by respiration. In a bubble column, the log mean value of C^* based on pO_2 at the entrance and exit would be a justifiable choice. In a perfectly mixed vessel, the composition of gas in the exit stream should be the same as bubbles dispersed anywhere in the tank, and consequently C^* would be based on pO_2 at the exit. In an actual tank, a knowledge of the residence time distribution of gas bubbles is necessary to estimate a volume averaged value of C^*.

The *dynamic* method shares similarities with the steady-state method in that it usesa fermenter with active cells. It is simpler in that it requires only a dissolved oxygen (DO)probe and a chart recorder rather than off-gas analyzers as well as the DO probe requiredin the steady-state method. The governing equation for DO levels is:

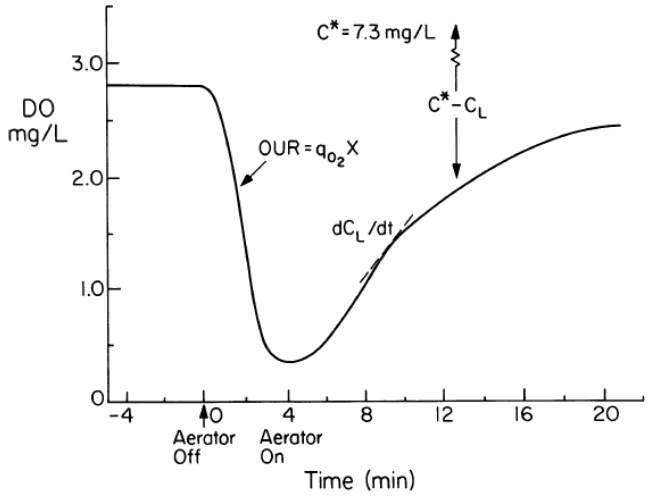
$$dC_L/dt = \text{OTR} - \text{OUR}$$

$$dC_L/dt = k_L a\,(C^* - C_L) - q_{O_2} X$$

The dynamic method requires that the air supply be shut off for a short period (eq< 5min) and then turned back on.Since there is no gas bubbles when the gas is off, $k_La$will be zero. Hence,

$$dC_L/dt = -q_{O_2} X$$

and the slope of the descending curve will give the OUR or $-q_{o2}X$. The lowest value of $C_L$obtained in the experiment must be above the critical oxygen concentration so that q_{O2} is independent of C_L.



Example of response of DO in a fermenter when stopping and then restartingair flow.

The dynamic method can be used to estimate OUR and k_La.

Lecture 4. Rheological properties of biological broths

Cells and biological tissues are complex materials. They are made of multiple elements or sub–elements whose complexity is still far from being understood. Most of our knowledge concerning the rheological properties of cells and tissues comes from previous studies on soft matter, i.e. materials whose structure gives rise to intriguing properties such as the ones of polymeric systems, suspensions, gels, emulsions, foams, pastes, etc. Such materials possess uncommon rheological properties (rheology is the science of flowing matter and deals with the study of the forces needed to achieve particular deformations or velocities) because their components contain both solid particles (or other types of inclusions like polymers, etc.) and fluids. Therefore one may say that they are viscoelastic but they may also be viscoplastic or elasto–visco–plastic. By plasticity, we generally mean that a given material can undergo very large deformations once the stress becomes higher than a certain yield limit. Cellular materials are different from usual soft materials due to the fact that they can develop an active response when submitted to stresses. This response is due to mechanotransduction, which is the ability of cells to transform mechanical external stresses into biochemical signals (and vice versa) in order to transfer information to and from the nucleus in order to achieve a particularcell function. This can lead to particular behaviours such as migration, adhesion, reaction to mechanical stress, etc.

The yield stress is a very important quantity in rheology, associated with the existence of interactions (weak or strong) causing the impossibility for a fluid to flow at small shear stresses. Therefore, below the yield stress, the material usually behaves as an elastic solid, and above it, can flow. The most commonly used model for describing such fluids exhibiting yield stresses $t_s$is the Bingham model (equivalent to Casson's model at low and high shear rates)

Roels et al has developed a model for the rheological behavior of filamentous suspension using CassionEqualtion and introducing a morphology factor (T_o) and the micellial constant 'x'. The Cassson Equation may be written as torque 'μ' and the rotational speed 'n' for turbine impeller and the equation becomes

$$(\mu)^{1/2} = (\mu_O)^{1/2} [1+(k_C / (\mu_O)^{1/2}) n^{1/2}]$$

Roels et al used data on *Penicilliumchrysogenum* and developed the relation

$$(d^x) = (T_O / x^{2.5})$$

Generally, the morphology factor 'd^x' was found to decrease with fermentation time.

Lecture 5.Introduction to scale up operations

5.1. Scale up Operations

Generally, fermenters maintain a height-to-diameter ratio of 2 to 1 or 3 to 1. If theheight-to-diameter ratio remains constant, then the surface-to-volume ratio decreases dramaticallyduring scale-up. This change decreases the relative contribution of surface aerationto oxygen supply and dissolved-carbon-dioxide removal in comparison to thecontribution from sparging. For traditional bacterial fermentations, surface aeration isunimportant, but for shear-sensitive cultures (e.g., animal cells) it can be critical becauseof restrictions on stirring and sparging. More important in bacterial and fungal fermentations is *wall growth*. If cells adhereto surfaces, and if such adherent cells have altered metabolism (e.g., due to mass transferlimitations), then data obtained in a small fermenter may be unreliable in predicting cultureresponse in a larger fermenter.

Perhaps even more importantly, it can be shown that the physical conditions in alarge fermenter can never exactly duplicate those in a smaller fermenter if *geometric similarity*is maintained.

In general, two criteria to ensure similarity during scale-up:
– Geometric similarity of the physical boundary
– Dynamic similarity of the flow fields

In the case described in the bottom table, a stirred-tank diameter has beenincreased by a factor of 5, resulting in a 125-fold increase in volume, since the height-to-diameterratio was maintained constant. Four cases are treated in the table: scale-upbased on constant power input (P_0/V), constant liquid circulation rate inside the vessel(pumping rate of impeller per unit volume, Q/V), constant shear at impeller tip (ND_i), andconstant Reynolds number ($ND_i^2\rho/\mu$).Sincethese quantities have different dependencies on N and D_i, a change of scale *must result* inchanges in the physical environment that the cells experience. When these changes alterthe distribution of chemical species in the reactor, or they destroy or injure cells, the metabolicresponse of the culture will differ from one scale to another. In some cases cells respondto modest changes in mechanical stress by changing physiological functions evenwhen there is no visible cell injury or cell lysis. Thus, different scale-up rules (constantP/V implies constant OTR, constant Re implies geometrically similar flow patterns, constant$N$ to give constant mixing times, and constant tip speed to give constant shear) cangive very different results.

Scale-up criterion	Designation	Small fermenter, 80 l	Production fermenter, 10,000:l			
			Constant, P_0/V	Constant, N	Constant, $N \cdot D_i$	Constant, Re
Energy input	P_0	1.0	125	3125	25	0.2
Energy input/volume	P_0/V	1.0	1.0	25	0.2	0.0016
Impeller rotation number	N	1.0	0.34	1.0	0.2	0.04
Impeller diameter	D_i	1.0	5.0	5.0	5.0	5.0
Pump rate of impeller	Q	1.0	42.5	125	25	5.0
Pump rate of impeller/volume	Q/V	1.0	0.34	1.0	0.2	0.04
Maximum impeller speed (max. shearing rate)	$N \cdot D_i$	1.0	1.7	5.0	1.0	0.2
Reynolds number	$ND_i^2\rho/\mu$	1.0	8.5	25.0	5.0	1.0

Traditional scale-up is highly empirical and makes sense only if there is no changein the controlling regime during scale-up, particularly if the system is only reaction oronly transport controlled. Common scale-up rules are the maintenance of constant power-to-volume ratios, constant k_La, constant tip speed, a combination of mixing time and

Reynold'snumber, and the maintenance of a constant substrate or product level (usuallydissolved-oxygen concentration).Results are more favorable with Newtonian broths than with non-Newtonian systems.The failure of any of these rules is related to changes in the controlling regime uponscale-up. No empirical rule can satisfactorily address such situations. Advances are beingmade in models that predict flow distribution, mixing times, and gas dispersion in fermenters,as well as models to predict explicitly cellular responses to changes in the localenvironment. If these models can be integrated, they may provide a much more fundamentallysound basis for scale-up. With the advent of improved supercomputers, the computationaldemands of such sophisticated models can be met.

5.2. Scale down Operations

Although scale-up models and the use of characteristic time analysis are potentially attractive,a more immediate approach to the rational scaling of reactors is *scale-down*. The basicconcept is to provide at a smaller scale an experimental system that duplicates exactly thesame heterogeneity in environment that exists at the larger scale. In many cases, scale-upwill require using existing production facilities, so it is important to mimic those productionfacilities at a smaller scale. At the smaller scale, many parameters can be tested more quicklyand inexpensively than at the production scale. Also, such a small-scale system can be usedto evaluate proposed process changes for an existing operating process.Construction of scale-down apparatus can be a powerful complement to mathematicalmodels, scale-up rules, and traditional pilot-plant operation.

Lecture 6.Cost analysis of alcohol production plant and fermentation plant design project

In this chapter, we examine the continuing role which economics plays in bioprocess research, development, and commercialization. The first section introduces general development phases, then illustrates them with a reasonably complete fermentation example which begins with a process flow concept, works through process equipment sizing, materials and utility needs, costs of initial plant and of operations, and an estimate of profitability (return on investment). Subsequently, characteristic features of particular fermentation processes are discussed, including fine chemicals, bulk chemicals, and single cell protein. In these examples, the relative cost importance of substrate feedstocks, equipment, utilities, and bioreactor vs. recovery sections will be examined, since identification of major cost areas frequently pinpoints process economic weaknesses and thereby indicates whether an engineering process improvement and/or strain development would be most logical to pursue in process optimization.

Circumstances peculiar to each general product area will also be noted. For example, the expenses for new drug development (fine chemical) must also include costs of obtaining clinical evidence for and sales approval by the Food and Drug Administration. Bulk chemicals from fermentation involve sufficiently large scale operation that a market for the incidental biomass must be found for by-product credit, or a waste disposal cost is incurred. Ethanol from biomass processes include not only fermentation and recovery sections but may also require a substantial pretreatment process to hydrolyze and solubilize the biomass components. Moreover, different countries (including the United States) have developed various forms of tax subsidies or credits for ethanol plants: such devices have a clear impact on process economics. Biological waste treatment provides the main process example for which substrate conversion (rather than biomass production or product formation) is the operating goal; here, biomass (cell sludge) disposal is responsible for a major fraction of plant operating costs.

Process Economics

Economic considerations play a continuing role at nearly every stage of a plant design project (Table 12.1). For a project which eventually achieves commercialization, these stages include inception of process or product idea, a preliminary evaluation of economics and markets, the development of any additional data needed for final detailed design, a final complete economic evaluation in light of all data, creation of a detailed engineering design, procurement of site and equipment, construction of buildings and process, process start-up and trial runs, and regular production operation.

Inception may arise from any source: a sales department suggestion, emergence of a competing product, a customer request, an offshoot of a current process, or a new idea from research and development. The idea may come internally from within the (eventual) production company and may be patented by the producer, or it may arise through outside patents and achieve realization through royalty and licensing agreements.

In the long run, each project is expected to recover its costs and return an appropriate profit. Thus, an immediate need arises, given a process or product inception, to carry out a *preliminary evaluation of economics and market*. The latter determines the potential market size for various assumed product prices. The profitability, or lack thereof, is determined from the economics evaluation which includes estimates of costs incurred to meet the assumed market size and required price.

If the preliminary evaluations are promising, the *development of data necessary for final design* normally follows. This development includes both market

Table 12.1 Stages in plant design project

1. Inception
2. Preliminary evaluation of economics and market†
3. Development of data necessary for final design†
4. Final economic analysis
5. Detailed engineering design
6. Procurement
7. Construction
8. Start-up and trial runs
9. Production

and cost refinement. A full market analsyis is made, and prospective customers often receive final product samples to determine if the product is satisfactory and thus if the sales potential assumed is realistic. The cost studies include development of a complete plant process flowsheet, determination of corresponding capital and operating cost, and estimation of process profit each year over the assumed lifetime or pay-out period. These latter results would also include a simple sensitivity analysis to establish the key assumed cost and performance measures which most strongly affect the profitability calculations, since costs may change significantly before a process is realized.

Prior to a final detailed design, a management review provides a *final economic analysis*, in which the project is set within a larger picture for consideration. Questions asked here often include the following: How does the estimated profitability compare with similar estimates for other possible projects which are also competing for a limited pool of corporate investment capital? Does the proposed process build upon existing corporate technical manpower and marketing strengths, or is a new path indicated, requiring new personnel? Is the process in an area which is likely to develop related processes, or is the area singular? Will the proposed process have the flexibility to be used in other related projects being considered or anticipated, or is the process (and the personnel) singular? What are the anticipated growth directions for the company as a whole, and does the process continue an established strength, develop technological experience and products in an anticipated growth direction, or spread activities too thinly? If this review is positive, then funds are committed for the next several steps.

The *detailed engineering design* phase provides complete determinations of all information needed for subsequent plant construction: equipment sizing and specifications, controls, services, piping configurations, and final price quotations. The detailed design includes production of a complete construction design: elevation drawings, plant-layout arrangements, and any other information needed for actual plant construction.

Procurement includes purchase and receipt of land and equipment not previously on hand, and arrangement of permits for all anticipated activities: construction, operation, utility installment and hookups, discharges (air, water), sewer hookups, etc. Note that appreciable lead times will be necessary, especially for specialty or custom fabricated equipment.

The *construction* phase involves erection of the complete plant. Following this phase, *start-up and trial runs* are undertaken, including break-in and trials of the plant and its units, operator training, and establishment of start-up, operation, shutdown, and safety procedures. Full *production* is the final subsequent phase.

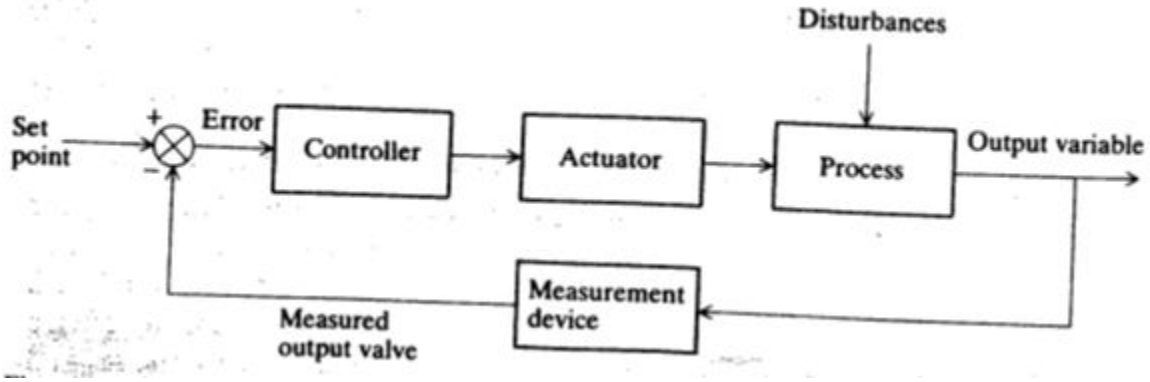
Repeatedly throughout all phases, economic assumptions are revisited and examined to see if the anticipated market, available raw materials, corporate goals, FDA or USDA approvals, or other important factors have shifted in a manner which significantly alters the economic prospects of the project. Since such evaluations may be demanding and complex, a standard objective method is typically used, e.g., a return-on-investment or related criterion. Objectivity aids in

obtaining the clearest picture: a pessimistic or excessively conservative approach may cause abandonment of a project which may be the key to future corporate development; an overly optimistic approach can lead to expenditures which, in the form of a complete final plant, could be disastrous.

Lecture 7.Process Control of bioprocess

Obtaining satisfactory process performance requires maintenance of operating conditions at design values. Because of unpredictable upsets which invariably enter a process due to fluctuations in pumping rates, flow patterns, mixer speed and other operating conditions, and because of chemical changes within the process, control action is usually required to maintain specified conditions. Sometimes we can improve batch reactor performance by varying process conditions such as pH or temperature in a predetermined fashion as the reaction occurs. Here, too, controls are required in order to carry out the desired batch operation program. In this section we first examine controls to maintain desired values of measured variables. Then, we consider control based on estimated process conditions.

Using control of a bioreactor as an example, we frequently wish to control pH, temperature, aeration rate, agitation speed, and perhaps dissolved oxygen partial pressure at specified values. Since all these quantities can be measured on-line, regulation of each of these process state variables can be accomplished using a conventional *feedback controller*, the basic components of which are summarized in Fig. 10.20. Here, the controlled or output variable, say pH, is measured, and the analyzer output is sent to a controller where the measured output value is compared to the desired, or set point, value. Based on the deviation between desired and measured value, control action is determined by some control algorithm.



Lecture 8.Problems on bioreactor scale up and mass transfer in bioreactor.

1.

After a batch fermentation, the system is dismantled and approximately 75% of the cell mass is suspended in the liquid phase (2 l), while 25% is attached to the reactor walls and internals in a thick film (ca. 0.3 cm). Work with radioactive tracers shows that 50% of the target product (intracellular) is associated with each cell fraction. The productivity of this reactor is 2 g product/l at the 2 l scale. What would be the productivity at 20,000 l scale if both reactors had a height-to-diameter ratio of 2 to 1?

2.

Consider the scale-up of a fermentation from a 10 l to 10,000 l vessel. The small fermenter has a height-to-diameter ratio of 3. The impeller diameter is 30% of the tank diameter. Agitator speed is 500 rpm and three Rushton impellers are used. Determine the dimensions of the large fermenter and agitator speed for:

a. Constant *P/V*

b. Constant impeller tip speed

c. Constant Reynolds number

3.

A $20m^3$ working volume bioreactor with the following dimensions is available in a plant for the production of penicillin

Tank diameter = 24m
Impeller diameter = 0.8m and 3 nos.
Impeller speed = 2.5 rps
No. of blades = 8
Type of impeller = turbine
Viscosity of broth = 1 mPa-s
Density of broth= $1.2x10^3$ kg/m^3
Ratio of gassed to ungassed power = 0.4
Aeration rate = 1 vvm
Volumetric mass transfer coefficient = k_La

$$= 2x10^{-3} (P_g/V)^{0.6} (V_S)^{0.667}$$

Where P_g/V in HP/m^3 and superficial gas velocity V_S in cm/min. The unit of k_La is sec^{-1}.

Calculate the value of impeller's Reynolds no. and k_La for the above bioreactor.

4.

The following data are available from a transient response technique of Dissolved Oxygen measurement in a glucose-gluconic acid fermentation in 5 lit. fermenter.

C_L (ppm)	3.5	3.5	3.5	3.5	3.5	3.5	3.0	2.5	2	1.5	1.0	1.4	1.85	2.25	2.55	2.75	3	3.25	3.3	3.4	3.45
Time (s)	10	15	25	30	35	40 (air – off)	45	50	55	60	60 (air-on)	68	73	80	88	95	103	111	117	122	130

Calculate the oxygen transfer co-efficient, oxygen uptake rate (OUR) and the saturated level of oxygen for the culture.

SAMPLE QUESTIONS

GROUP A

1. **Multiple Choice Question (MCQ) (Answer any ten) 10X1=10**

i. Corn steep liquor contains

(a) Lysine (b) Phenyalanine (c) Valine (d) None of these.

ii. Malting is done for the following reason

(a) To break down of starch (b) To produce sucrose (c) To produce flavor (d) All of these.

iii. Wine is prepared from

(a) Barley (b) Mollasses (c) Fruit juice (d) None of these.

iv. Vinegar contains

(a) 10 % acetic acid (b) 4 % acetic acid (c) 10 % alcohol (d) 4 % alcohol

v. Power no. for a 6 blade turbine is

(a) 4 (b) 6 (c) 8 (d) 10

vi. For turbulent flow during mixing with agitator the impeller's Reynold's no. should be

(a) 2100 (b) 10^5 (c) 10^3 (d) 4000.

vii. The broth characteristic of yeast suspension is best explained as

(a) Power law fluid (b) Casson fluid (c) Bingham plastic fluid (d) None of these.

viii. Streptomycin is produced by

(a) *Streptomyces fradiae* (b) *Streptomyces griseus*(c) *Streptomyces nodosus*(d) *Streptomyces erythraeus*

ix. Enzymes produced by microorganisms are

(a) intracellular (b) extra cellular (c) both (a) and (b) type (d) none of these.

x. For the purification of extra cellular enzyme the step should be omitted is

(a) fermentation (b) cell disruption (c) protein precipitation (d) enzyme purification

xi. Microbial cells can be disrupted by

(a) grinding with abrasives (b) treating with detergent (c) ultra sonication (d) all of these.

xii. Method not used for insoluble product separation is

(a) Centrifugation (b) filtration (c) Coagulation (d) Chromatography

xiii. Precursor is used in Penicillin formation _____

(a) To produce a particular type of penicillin
(b) To improve yeild
(c) To help in the extraction
(d) None of these

xiv. Phenoxyacetic acid is the precursor for which of the following type of penicillin ?

(a) Pen V
(b) Pen X
(c) Pen G
(d) None of these

xv. Why are baffles used in a bioreactor?

(a) To help in keeping the reactor sterilized
(b) To help in mixing
(c) To maintain temperature
(d) It helps during the product recovery

xvi. At high air flow which of the following type of sparger gives better aeration efficiency?

(a) Single Bubble Sparger
(b) Multiple Bubble Sparger
(c) Both (a) and (b)
(d) None of these

GROUP B

Short Question (SQ) (answer any three) 5 X3=15

2. What are the different resistances involved in transferring oxygen from the gas phase to a respiring cell?
3. How does the type of sparger selected for the fermenter affects it's aeration efficiency?
4. What are the cultural conditions that affect the O_2 demand of a reactor?
5. If the specific growth rate (μ) of an organism is constant when the oxygen transfer rate is equal to the oxygen uptake rate, show how the cell concentration varies with the dissolved oxygen concentration.
6. A strain of *Azotobacter sp.* is cultured in a 15 m^3 stirred fermenter for alginate production. Under current operating conditions, k_La is 0.17 s^{-1}. Oxygen solubility in the broth is approximately 8 X 10^{-3} Kg m^{-3}. The specific rate of oxygen uptake is 12.5 mmol g^{-1} h^{-1}. What is the maximum possible cell concentration?
7. How agitation improves the aeration efficiency of a fermenter? What do you understand by the term "Critical Oxygen Concentration"?
8. The diameter of a bubble generated at an orifice during aeration is independent of gas flow rate but depend on the orifice diameter- explain
9. How can we measure the 'volumetric Oxygen transfer co efficient' in a fermenter?
10. What do you understand by the term 'volumetric Oxygen transfer co efficient' of a fermenter? What is interfacial area?
11. What is Impeller's Reynolds's no.? What is Power no.?
12. How power no. is related to Impeller's Reynolds's no.?
13. Why is the Impeller power requirement decreased in aeration?
14. What is aeration no.?
15. What is scale up? What are the different methods of scale up of fermenter?
16. What is power law? Give example of power law fluid.
17. Discuss the glucose oxidize method of k_La measurement.

18. Discuss the sulfite oxidation method of k_L a measurement.
19. What is dissolved oxygen probe? How the sensitivity of probe can be affected?
20. Give the flow diagram of Gist Brocade's penicillin production . What is the chief substrate used for penicillin production.
21. What do you mean by active dry yeast?
22. Discuss the process of production of alcohol from molasses.
23. What is beer? What is the purpose of malting of barley? What are hops?
24. What are enzymes? Give example of enzymes used in food industry. What do you understand by immobilization of enzymes?
25. Write down the basic structure of penicillin. How different derivatives of penicillin can be produced?
26. Discuss the different phases of streptomycin production by *S.fradiae.*
27. How streptomycin is recovered from fermentation broth?
28. What are the basic raw materials for the production of baker's yeast?
29. What is single cell protein? What types of raw materials are suitable for the production of single cell protein?
30. What is the role of viscosity in the performance of a bioreactor?
31. In hydrocarbon fermentation by *Candida tropicalis* the following data are given. Maximum O2 uptake rate = 12 mmole/hr/gm of cell. Cell concentration at the time of maximum oxygen uptake rate = 10 gm/l (for 1% hydrocarbon source).Critical oxygen concentration for this organism is 0.032 mmole/l. Saturation oxygen concentration in the broth is 0.544 mmole/l. From the data determine the value of the minimum k_La required to supply sufficient D.O.
32. Write a short note on Damkoholar Number.
33. Discuss the specific function of an Ultracentrifuge and mention its operating rpm.
34. What are the filter mediums that are used for separation of biomolecules?
35. What are Flat Sheet Membranes and Hollow Fiber Membranes? What are the main materials with which organic and inorganic membranes are made?
36. Explain the van Deemeter equation for theoretical plate height in chromatographic column.
37. 50 litres of filtrate is collected in 20 minutes when an inorganic suspension is filtered through a sintered glass filter using a pressure drop of 50 KPa. How much filtrate will be collected in 20 minutes at a pressure drop of 100 Kpa? Assume that resistance offered by the media is negligible.
38. Egg white proteins are being separated by isocratic chromatography using 10 cm long column. The distribution co-efficient for the proteins are given below:

Protein	Distribution Co-efficient
Ovalbumin	0
Conalbumin	1
Lysozyme	5

If the voidage fraction of the column is 0.45 and the mobile phase retention time is 10 mins, predict the retention time of the 3 proteins.

GROUP C

Long answer type questions (LQ) (answer any three) 15 X3=45

1. What is power no.? How power no. is related to Impeller's Reynolds's no. for different types of impeller systems? Derive a mathematical model for correlating the overall mass transfer co efficient with the operating variables of a bioreactor provided with aeration and agitation devices.
2. Explain the different resistances that are involved in transferring oxygen from the gas phase to a respiring cell. How does the cell concentration vary with the dissolved oxygen concentration if the specific growth rate is constant?
3. What role does the agitator and the type of sparger play in determining the aeration efficiency of a reactor? How is the height of the liquid in the fermenter affecting the aeration efficiency?
4. Discuss the rheological properties of the fermenting broth. Discuss the morphology factor for mycelial broth in this perspective.
5. What is scale up? Discus the equal power per unit volume concept of scale up of bioreactor?
6. Give a general process flow sheet of a fermentation plant. How would proceed to determine the approximate production cost of a fermentation process?
7. With a flow diagram discuss the various steps involved in the recovery of penicillin from the fermentation broth and obtain purified procaine penicillin for clinical use.
8. Discuss the process for microbial production of lactic acid. How lactic acid is recovered from fermentation broth? Mention some uses of lactic acid.
9. With a suitable flow sheet explain the production of Baker's Yeast. Comment on the type of fortification and pretreatment which the substrate undergoes in the above mentioned process.
10. What are amylases? Discuss the process for microbial production of amylases. Mention its uses.
11. What is wine? Discuss the process for microbial production of red and white wine. What are rose wine and Champagne?
12. Discuss the process for manufacture of beer from barley malt with a flow diagram. What are malt adjuncts?
39. Define the oxygen transfer rate in the light of convective mass transfer. What is interfacial area? Discuss the dynamic method used for measuring the 'volumetric oxygen transfer co efficient' in a fermenter.
13. The content of a fermenter are to be mixed with a turbine impeller having six curved blades. The diameter of the turbine is 6 ft. If the temperature is 30^0c and the impeller is rotated at 60 rpm, estimate the Reynolds no. and compute the power requirement for mixing the fluid. The physical properties of the fluid may be considered that of water. The value of the power no. may be taken as 4.8 for the above impeller. Discuss the equal mixing time concept of scale up.
14. Discuss how the Monod model for microbial growth satisfies the shifting order kinetics which exists in the substrate limited environment. What do you mean by doubling time? Explain the Lineweaver – Burke plot.
15. Derive a mathematical equation for constant pressure flow cake filtration explaining the different aspects of the resistance offered by the cake and the media.
16. A plasmid was found to have a retention time of 10 minutes in a chromatographic column, having a volume of 0.01 m^3 and a voidage fraction of 0.3. The distribution coefficient (k) of the plasmid is known to

be equal to 2. Calculate the mobile phase retention time. Can we use the same column-mobile phase system to separate the plasmid from RNA (which has a capacity of 4.66)?

17. The following data was obtained from a constant pressure cake filtration experiment:

Time (s)	5	10	20	30
Volume (l)	0.04	0.055	0.080	0.095

Given information:

Area of filter medium (A) = 9.29×10^{-3} m^2

Mass of cake solids per unit volume of filtrate (C_S) = 0.015kg/l

Viscosity (μ) = 1.1×10^{-3} kg/m s

Pressure drop across the filter medium (ΔP) = 10 N/m^2

Calculate the specific cake resistance and the media resistance from the above date.

18. A bacterial fermentation was carried out in a reactor containing a medium having a density of 1.2×10^3 kg / m^3 and a viscosity of 0.02 Ns/m^2 .The broths was agitated with the help of an impeller having a speed of 90 rpm and air was introduced through the Spurger at the rate of 0.4 vvm. The fermenter equipped with two sets of flat blade turbines and baffle plates was having the following dimensions.

Diameter of fermenter = 4m; Diameter of impeller =2 m; baffle plate width =0.4 m; liquid depth in fermenter=6.5 m

For designing the overall fermentation process it is required to determine

a) Power requirement P , for ungassed system

b) Power consumption Pg , for gassed system

c) Volumetric oxygen transfer coefficient k_La and Impeller Reynolds no. the following relation may be used –

Pg/P=0.76; N_p = 6.0 (for turbine)

$k_La = 0.0635 (g/ v)^{0.95}(v_s)^{0.67}$

Where k_La is in kg mole/m^3 h.atm, P and Pg HP and $V_s$in m /hr :V in m^3 .

19. What are proteases? Discuss the production of microbial renin by fungi *Endothiaparasitica* and *Mucormichei*.

20. "A chemostat can be operated at dilution rates (D) higher than the specific growth rate (μ) when cell recycle is used" – Discuss.

21. Consider the scale up of a fermentation process from 10l to 10,000l vessel. The small fermenter has a height to diameter ratio of 3. The impeller diameter is 30% of the tank diameter. Agitator speed is 500 rpm and 3 impeller are used. Determine the dimensions of the larger fermenter and agitator speed for

(i) constant P/V

(ii) constant impeller tip speed

(iii) constant Reynolds no.

Assume vessels are cylindrical and geometrically similar.

22. A 20m^3 working volume bioreactor with the following dimensions is available in a plant for the production of penicillin

Tank diameter = 24m

Impeller diameter = 0.8m and 3 nos.

Impeller speed = 2.5 rps

No. of blades = 8

Type of impeller = turbine

Viscosity of broth = 1 mPa-s

Density of broth= $1.2x10^3$ kg/m^3

Ratio of gassed to ungassed power = 0.4

Aeration rate = 1 vvm

Volumetric mass transfer coefficient = k_La

$$= 2x10^{-3} (P_g/V)^{0.6} (V_S)^{0.667}$$

Where P_g/V in HP/m^3 and superficial gas velocity V_S in cm/min. The unit of k_La is sec^{-1}.

Calculate the value of impeller's Reynolds no. and k_La for the above bioreactor.

23. The following data were obtained in a constant pressure filtration unit for filtration of a yeast suspension.

t (min)	V(l filtrate)
4	115
20	365
48	680
76	850
120	1130

Characteristics of the filter are as follows:

A = 0.28 m^2, C = 1920 kg/m^3, $\mu = 2.9 \times 10^{-3}$ kg/m-s, α = 4m/kg

Determine

i) Pressure drop across the filter.

ii) Filter medium resistance (r_m)

iii) Determine the size of filter for the same pressure drop to process 4000 l of cell suspension in 20 mins.

24. "For a given power input, the magnitude of k_La is about the same irrespective of the mixing technique" – Discuss.

25. Discuss the Akita & Yoshida model to correlate the k_La with fermenter variables in a bubble column Bioreactor.

26. Discuss the operation of an ideal plug flow tubular reactor.

27. With a neat diagram discuss the operation of an airlift fermenter.

28. How you can determine the settling velocity of a single particle in gravitational and centrifugal field?

29. What is Ruth Equation for constant pressure filtration process?

30. What is meant by dilution rate? By performing mass balance on an ideal chemostat show how is it related to the specific growth rate of an organism? What do you mean by the critical dilution rate?

31. What are the different working modes of a bioreactor? What is meant by a sequencing batch reactor?

32. The following data are available from a transient response technique of Dissolved Oxygen measurement in a glucose-gluconic acid fermentation in 5 lit. fermenter.

C_L (ppm)	3.5	3.5	3.5	3.5	3.5	3.5	3.0	2.5	2	1.5	1.0	1.4	1.85	2.25	2.55	2.75	3	3.25	3.3	3.4	3.45
Time (s)	10	15	25	30	35	40 (air – off)	45	50	55	60	60 (air-on)	68	73	80	88	95	103	111	117	122	130

Calculate the oxygen transfer co-efficient, oxygen uptake rate (OUR) and the saturated level of oxygen for the culture.