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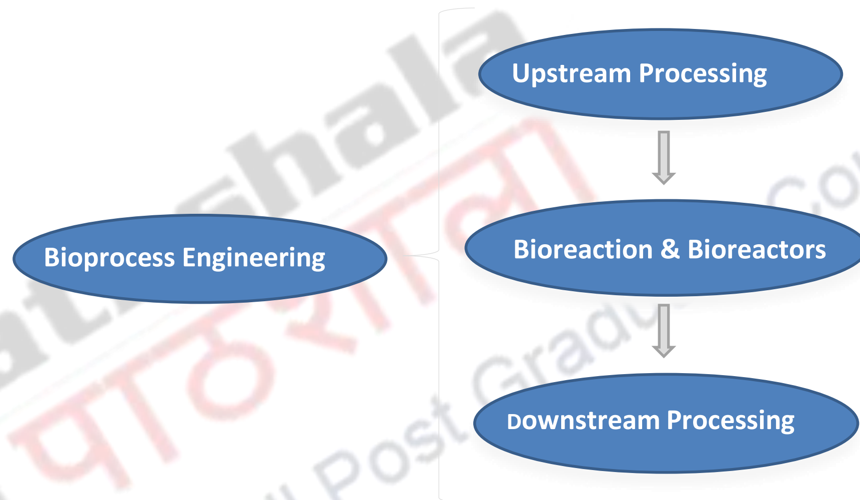
Description of Module	
Subject Name	
Paper Name	
Module Name/Title	Bioprocess Engineering

1. Objectives

- Understand the fundamental basics of microbial kinetics, metabolic stoichiometry and energetics.
- Understand the basics of bioreactor engineering with knowledge on design and operation of fermentation processes.
- Develop bioengineering skills for the production and purification of biochemical product using integrated biochemical processes

2. Concept Map



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3. Introduction

Bioprocess engineering encompasses the design, operation, control, and optimization of biochemical processes involving various biological pathways or reactions mediated by living cells of animals, plants and micro-organisms or enzymes under controlled conditions for the efficient biotransformation of raw material into a range of products at requisite scales. The product may be directly useful as food, medicine or the industrial compounds or indirectly in the way of bioprocess without any direct product formation as in the detoxification of industrial wastes or treatment of factory effluents. For production of fermented foods, micro-organisms have been used from the very past. Since then, new bioprocesses have been developed for production of wide variety of commercial products ranging from cheap to expensive specialty chemicals as antibiotics, therapeutic

proteins and vaccines. Bioprocess engineering is thus the backbone of the biotechnology industry that translates the research and development to the industries and is divided into three parts as per the processing-

1. Upstream processing
2. Bioreactor and bioreactions
3. Downstream processing

3.1 Upstream processing

The upstream processing of a bioprocess is the initial step in which the microorganisms or cell lines are grown in bioreactors using the basic steps as inoculum preparation , media formulation, improvement of inoculum by genetic engineering, growth kinetics optimization with an aim to increase the production levels along with the regulation of temperature, pH and pressure.

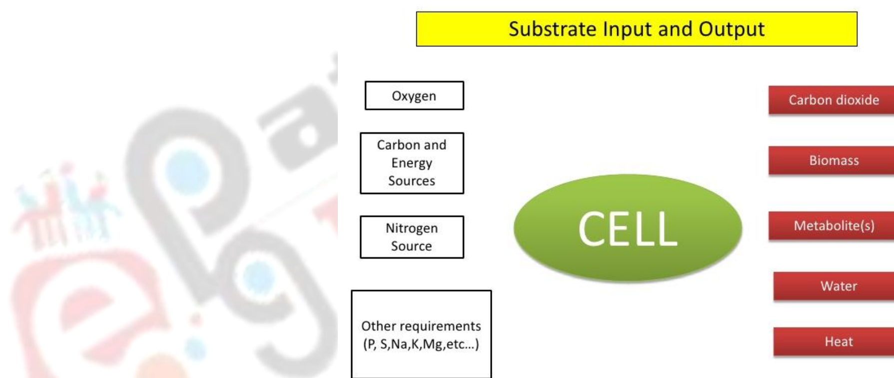


Fig 1: Substrate input and output of cell

3.1.1. Inoculum development:

Inoculum development refers to the increase in the density of viable number of micro-organisms from a dormant stock stage to a final productive stage. The criteria as given by Stanbury & Whitaker (1984) are important when considering a protocol for inoculum development.

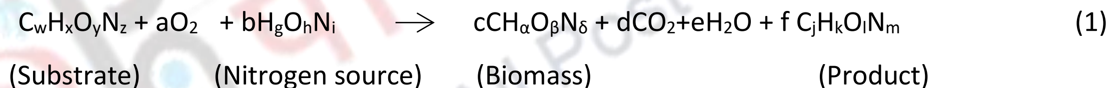
1. Active and healthy state inoculum is required to reduce the lag phase
2. Sufficient large volumes required with an optimum size of 0.1 – 10 % of the medium volume.

3. Suitable morphological form.
4. Contamination free.
5. Possess product forming capabilities.

3.1.2 Medium preparation:

All microorganisms require elements as carbon, nitrogen, mineral (possibly vitamins), water and oxygen (if aerobic). Hence, the formulation of the nutrients is the most important requisite for the optimized production of the desired product, cell biomass or a specific metabolite. The pH of the medium is controlled by acid and alkali additions or buffers. In some fermentation, precursor, inducer or inhibitor compounds are added during the course of fermentation for the targeted production of desired product. Media formulations and environmental conditions drastically affect the physiology, biochemistry and the morphological changes which further influence product yield and other fermentation properties.

First strategy for a rational design of fermentation media is according to the elementary composition of a specific micro-organism and of the main product that is produced. Second is to design according to the stoichiometry of growth and product formation which is represented by the chemical equation as



$$C_wH_xO_yN_z + aO_2 + bH_gO_hN_i \longrightarrow cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O + f\,C_jH_kO_lN_m \qquad (1)$$

(Substrate) (Nitrogen source) (Biomass) (Product)

Microbial growth stoichiometry involves the conversion of substrates into products and cellular materials and helps to determine the true yield coefficient based on substrate for biomass (Yx/s) and for product (Yp/s).

$$(Yx/s) = \frac{\text{gram cells produced}}{\text{g substrate consumed}} = \frac{c\ (\text{Mol. wt cells})}{\text{Mol.wt substrate}} \qquad (2)$$

$$(Yp/s) = \frac{\text{gram product produced}}{\text{gram substrate consumed}} = \frac{f\ (\text{Mol. wt product})}{\text{Mol. wt substrate}} \qquad (3)$$

Thus, complete knowledge of elemental composition of a specific industrial microorganism helps in the refinement of media formulation.

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Criteria for selection of a medium:

1. Maximum product or biomass yield per gram substrate consumed.
2. Maximum purity of product or biomass.
3. Maximum product formation rate.
4. Low unwanted product yield.
5. Cheap, consistent quality and availability throughout the year.
6. Minimal troubleshoots in upstream and downstream processings.
7. The composition of a fermentation medium may be simple to complex, depending on the particular microorganism and its fermentation.

Autotrophic microorganisms require only the simplest of inorganic media (inorganic salts, water, nitrogen source, carbon source is fulfilled by CO_2 or by carbonates) and are capable of synthesizing all the complex organic compounds required to sustain life.

Fastidious microorganisms on the other hand lack the ability to synthesize many of their sustenance and growth requirements. They require the presence of many simple to complex nutrients in the medium and must have an organic carbon supply to provide for synthesis of cell substances and release of metabolic energy.

Simple and complex media are further categorized as synthetic and crude respectively.

Synthetic medium:

- All the components are specifically defined and known compounds.
- Each component is relatively pure and the exact concentrations are known
- Concentration of one or several can be varied to determine the effect on cell growth and product yield.
- Individual components may be added or deleted as well.

However, these are expensive due to the relatively pure ingredients used and yields derived from these media are relatively low.

Crude media:

- Usually allows much higher yields.

- They contain crude or un-defined sources of nutrients and growth factors.

To make the process cost-effective, industrial-scale fermentations primarily focusses on the use of complex crude substrates mostly derived from natural sources as plants or animal, industrial by-products, where many carbon and nitrogen sources are almost undefined.

Criteria for selection of raw materials include:

1. Inexpensive, consistent quality and easy availability
2. Ease of transport in any form
3. Withstand sterilization procedures
4. Favourable properties that may influence agitation, aeration and foaming
5. Increased levels of desired product with respect to its production rate and yield per gram of substrate.
6. Low quantities of impurities
7. Overall health and safety implications

Animal cell culture media:

Animal cell culture media are normally based on complex media, such as Eagle's cell culture medium, containinig glucose, mineral salts, vitamins and amino acids. Mammalian cell medium usually contains serum as rich source of essential growth factors, including initiation and attachment factors and binding proteins, supply hormones, trace elements and protease inhibitors.

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Cultivation Media

Medium component	Defined Component	Un-defined Component
Carbon source	(Glucose,Fructose,Glycerol,xylose) (Sucrose, Starch)	Molasses, Meat extract, Peptone, Plant extracts and Materials (Cellulosic, lignoncellulosic and hemicellulosic materials, Starch complex, etc…)
Nitrogen source	Ammonium and Nitrate Salts	Yeast extract, Amino acid complex, Casein
Phosphate	Mono and di-phosphate salts	In traces of complex C- and N-sources
Sulphur	Ammonium and Magnesium sulphate	In traces of complex C- and N-sources
Magnesium	Mainly Magnesium sulphate	In traces of complex C- and N-sources
Mn, Mo, Fe, Zn, etc…	In form of Inorganic salts	In traces of complex C- and N-sources
Vitamin and Growth factors	Added in pure form of vitamin and growth factors preparation	Yeast extract, and may found also as traces in some C- and N-sources

Table 1: Cultivation media

Plant cell culture media:

Plant cell culture media are usually chemically defined. They contain an organic carbon source (as most plant cells are grown heterotrophically), a nitrogen source, mineral salts and growth hormones. Sucrose is frequently incorporated as the carbon source, particularly for secondary metabolite production, but glucose, fructose, maltose and even lactose have been used. Nitrate is the usual nitrogen source, often supplemented with ammonium salts. However, some species may require organic nitrogen, normally in the form of amino acids. The combination and concentration of plant hormones provided depend upon the specific fermentation.

3.1.3 Microbial Growth Kinetics and Specific Growth Rate:

Growth of microbes is an autocatalytic process i.e. the growth rate is proportional to the biomass. Thus, microbial growth can be described as a proportionate increase in all its cellular contents in the presence of appropriate medium and the culture conditions. Mathematically it is expressed as

$$\frac{dX}{dt} = \mu X \qquad (4)$$

where X is the concentration of microbial biomass, t is the time in hours and μ is the specific growth rate (hrs^{-1}). Integrating between time t_0 and time t when the concentrations of the cells are X_0 and X_t

$$\ln \frac{X_t}{X_o} = \mu t \quad (5)$$

$$X_t = X_0 e^{\mu t} \quad (6)$$

where X_0 is the original biomass concentration, X_t is the biomass concentration after a time interval t hours, e is natural logarithm base.

Eq. (6) can be written as:

$$\log X_t - \log X_0 = \mu t \text{ or,}$$

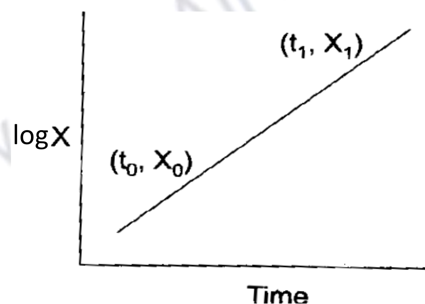
$$\log X_t = \log X_0 + \mu t \quad (7)$$

After taking natural logarithms,

$$2.303 \log X_t = 2.303 \log X_0 + \mu t$$

$$\log X_t = \log X_0 + 2.303\ \mu t \quad (8)$$

Thus a plot of log X_t vs time (t), results in a straight line with slope equal to μ / 2.303.



Bacteria divides by binary fission and the increase in bacterial population density is given by geometric progression. The time required to double its number is known as generation time and is given by the expression -

Let t_d be time taken for an initial cell population (N_o) to double ($N = 2N_o$) then,

substituting the values of N and t in equation (5)

$$\ln \frac{2N_o}{N_o} = \mu t_d$$

$$\mu t_d = \ln 2 \text{ or, } t_d = = \frac{\ln 2}{\mu} = \frac{0.69}{\mu} \qquad (9)$$

Differences lie in microorganisms with respect to their growth and production of products. Therefore, to get the desired product, microorganisms are grown as batch, fed-batch or continuous cultures. Batch and fed-batch fermentations are common than continuous fermentations. The mode of fermentation depends on the type of product being produced.

2.1 BATCH FERMENTATION

Batch fermentation system is a closed culture system containing an initial amount of nutrients and involves number of biochemical processes. In batch cultures, the cell properties such as size of cells, nutrients, metabolic function vary considerably during the various growth phases. A typical growth profile is given in the figure below.

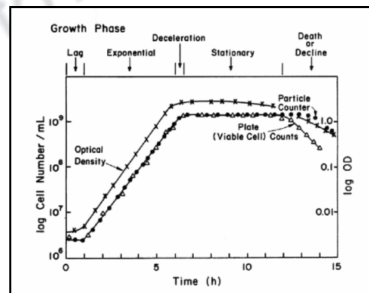


Fig 2 : A typical microbial culture growth in batch conditions

The Fig 2 shows the following growth phases:-

Lag phase:

- Considered as adaptation period for the cells to their new environment with no immediate increase in cell number. Commercially, its beneficial to reduce lag phase length by using a suitable inoculum.
- Synthesis of new enzymes, ATP, cofactor, ribosomes.
- Slight increase in cell mass and volume
- Prolonged by poor quality inoculum in terms of volume, condition (high % of dead cells), age, poor medium

Exponential phase:

- In this phase, the cells multiply rapidly (exponentially) as well adjusted to their new environment
- Balanced growth
- Growth is at maximum specific growth rate μ_{max} and is independent of nutrient concentration

Stationary Phase:

- Nutrients exhaust i.e. S =0
- Accumulation of undesired secondary metabolic products
- Growth rate becomes equal to death rate.
- No net growth.

Decline phase:

- Final stage of cell cultivation
- Number of cells decline exponentially due to lysis.
- Death rate follows the first-order kinetics ;

$$\frac{dN}{dt} = - \frac{K_d}{N} \qquad (10)$$

upon integration leads to

$$N = N_s e^{-K_d t} \qquad (11)$$

where N_s is the cell concentration at the end of the stationary phase; K_d is the first order death rate constant. Thus, in batch cultures, the cell properties such as size of cells, internal nutrients, metabolic function vary considerably during the various growth phases.

Substrate concentration effect on growth

Monod equation growth rate is a hyperbolic function of the concentration of the growth limiting nutrient(s).

$$\mu = \mu_m S / Ks + S \qquad (12)$$

where μ_m = Maximum specific growth rate t^{-1}, S = concentration of the limiting substrate (mg/l), Ks = half saturation constant (mg/l).

Monod equation is expressed in terms of cell number or cell mass (X) as:

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

$$\text{Thus, } \frac{dX}{dt} = \mu_m SX / Ks + S \qquad (13)$$

Monod equation has two limiting cases:

1. High substrate concentration: S >>> Ks $\longrightarrow \frac{dX}{dt} = \mu_m X$

   Under these conditions, growth occurs at maximum growth rate.

2. Low substrate concentration: S <<< Ks $\longrightarrow \frac{dX}{dt} = \mu_m S X / Ks$

This type of growth is typically found in batch flask systems at the end of the growth curve as the substrate is nearly consumed.

The Monod equation can also be expressed as a function of substrate utilization.

$$\frac{ds}{dt} = \frac{-1}{Y}\left[\frac{dx}{dt}\right] \longrightarrow \frac{ds}{dt} = \frac{-1}{Y}\left[\frac{\mu_m SX}{Ks + S}\right] \text{ where } Y = \text{biomass yield} = \frac{\text{(g) biomass produced}}{\text{(g) Substrate consumed}} \qquad (14)$$

Advantages of batch culture systems:

1. Reduced risk of contamination or cell mutation as the growth period is short.
2. Lower capital investment.
3. Production of wide varying product/biological systems.
4. High conversion rate of raw materials.

Disdvantages of batch culture systems:

1. Lower productivity levels related to more time consumption
2. Increased focus on instrumentation.
3. Requirement of several subculture levels.
4. High costs for labour and/or process control.
5. More susceptible to pathogenic microorganisms or toxins.

Applications of batch cultures:

1. Products involving low risk of contamination or mutation of microbes.
2. Production of small amounts of product required.
3. Employing proper product separation in batch or semi-continuous processes.

Batch growth Kinetics

Yield coefficient is based on the amount of consumption over other

Biomass yield : $Y_{x/s} = -\frac{\Delta X}{\Delta S}$

Product yield : $Y_{x/s} = \frac{\Delta P}{\Delta S}$

Growth yield based on consumption of oxygen : $Y_{x/s} = -\frac{\Delta X}{\Delta O_2}$

ΔS = ΔS assimilation into biomass + ΔS assimilation into an extracellular product + ΔS growth energy + ΔS maintenance energy

2.2. FED-BATCH FERMENTATION

A fed-batch is a batch process which is always at a quasi-steady state as it is based on the non-toxic level feeding of a growth limiting substrate to culture without removing the fermentation broth. It is designed to accommodate the increasing volumes with accelerated cell growth resulting in high cell density.

Advantages of fed batch systems:

- High cell yield
- Almost at stationary state

Disadvantages:

- Lower productivity levels due to consumption of time in preparation of fresh run in reactor
- Higher costs for labour or process control.

Applications

- Fed-batch culture is ideal where high concentration of substrate inhibits microbial growth.
- Production of intracellular metabolites in maximum amount.
- Increased biomass production if desired product is intracellular.

Ex: Two stage production of penicillin which is a secondary metabolite. In "idiophase" a production phaseof penicillin, the biomass is maintained at low levels by feeding phenyl acetic acid ,a precursor of penicillin, at levels below toxicity.

CONTINUOUS FERMENTATION

Continuous culture is an open process wherein exponential growth can be extended by the addition of fresh medium containing a limiting substrate. Fresh medium displaces an equal volume of spent medium along with the biomass. Thus, a steady state is reached.

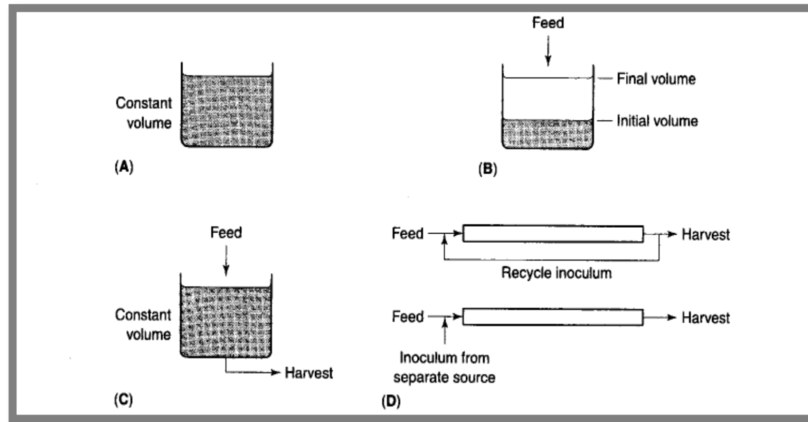


Fig 3: Fermentation methodologies (A) Batch (B) Fed-Batch (C) Continuous flow well-mixed (D) Continuous plug flow, with and without recycling of inoculum.

In continuous fermentation, the flow of medium is related to the volume of the vessel by the dilution rate (D) as follows:

$D = F/V$ where F is the flow rate (l/h) and V is the volume (l). (15)

The net change in cell concentration over time is expressed as:

$dX/dt = \text{growth} - \text{output}$

or $dX/dt = \mu X - DX$ (16)

Under steady state conditions, the cell concentration remains constant, therefore $dx/dt = 0$ and $\mu x = dx$ and $\mu = D$.

At steady state, $\mu = D$ (17)

Thus under steady – state conditions the specific growth rate is controlled by the dilution rate.

Substituting $\mu = (\mu_{max}\, s)/ (Ks + s)$ in equation (16)

$$\frac{dX}{dt} = X\left[\frac{(\mu_{max}\, s)}{Ks + s} - D\right] \qquad (18)$$

The net change in the residual growth limiting substrate concentration is given as

$$\frac{ds}{dt} = \text{Input of substrate} - \text{output of substrate} - \text{consumption by cells}$$

$$\frac{ds}{dt} = DS_R - Ds - \mu_{max} \frac{X}{Y} \left[\frac{s}{Ks + s} \right] \quad (19)$$

At steady state both ds/dt and dX/dt equal zero.

Equating equations (18) and (19),

$$\overline{X} = Y (S_R - \overline{s})$$

$$\overline{S} = \frac{Ks\, D}{\mu_{max} - D} \quad (20)$$

where $\overline{X}$ and $\overline{s}$ are the steady state cell concentration and residual substrate concentration respectively.

Thus, D controls μ.

- If the dilution rate is increased above μmax, there is complete wash out of the cells and the cells don't get sufficient time to double. The stage just below this situation is called as critical dilution rate (D_{crit}).

Homogenously mixed bioreactor can be of two types –

(a) Chemostat: Chemostat is a self-balancing culture system where the growth rate of the culture is controlled by its chemical environment, that is the availability of one limiting component in the medium.

(b) Turbidostat:

It is an another type of continuous culture where the concentration of cells and hence the turbidity in the culture is kept constant by controlling the flow of medium.

Advantages of continuous culture

- Accurate information regarding kinetic constants, maintenance energy and growth yields.

- Regulation of growth rates for extended periods by varying the dilution rates.
- Enhances selectivity for thermophiles, osmotolerant or mutant organisms.
- Results in higher productivity per unit volume

Disadvantages:

- Control of some non-growth associated products is not easy.
- Wash-out phenomenon caused by growth on walls and hence cell accumulation results in unsteady-state.
- Loss of original product strain over time if a faster growing one overtakes it.
- Difficult to maintain filamentous organisms due to viscosity and heterogenous nature of broth
- Elongated growth periods increase the risk of contamination

1.3. Microbial Products

Microbial products can be classified into three major categories:

- Growth-associated products
- Non-growth-associated products
- Mixed-growth-associated products

- **Growth-associated products**
- Produced along with microbial growth
- Specific product formation rate is proportional to specific growth rate, μ_g
- The rate expression for product formation in growth-associated production is:

$$q_p = \frac{1.\ dP}{x\ .\ dt} = Yp/x\ .\ \mu_g$$

where q_p is product formation rate (h^{-1})

- Production of a constitutive enzyme is an example

- **Non-growth-associated products**
- Produced during stationary phase

- Specific product formation rate is constant: $q_p = \beta$ = constant
- Secondary metabolite production as most antibiotics (e.g. penicillin) are examples

- **Mixed-growth-associated product**
- Produced during deceleration and stationary phases
- The specific product formation rate is given by the Luedeking-Piret equation:

$$q_p = \alpha \mu_g + \beta$$

- $\alpha = 0$ signifies that the product is completely non-growth associated while $\beta = 0$, signifies that it is completely growth-associated
- Examples: secondary metabolites, production of lactic acid and xanthan gum

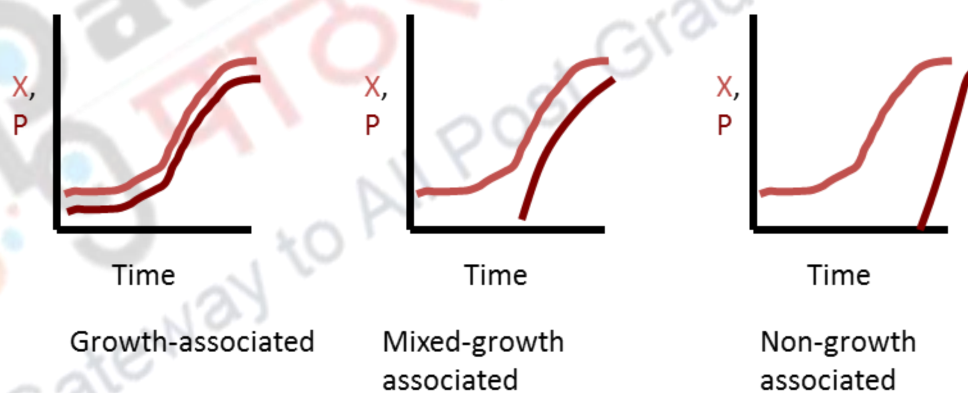


Fig 4: Microbial production profiles

1.4. Bioreactor and Bioreactions

Bioreactors are specially designed cylindrical vessels with hemispherical top and/or bottom built to provide suitable optimum environment conditions that allow growth of the micro-organisms for efficiently producing target product—cell biomass, metabolite and bioconversion.

The design and operation of a bioreactor depends on the organism, operating conditions for desired product formation, value and scale of product.

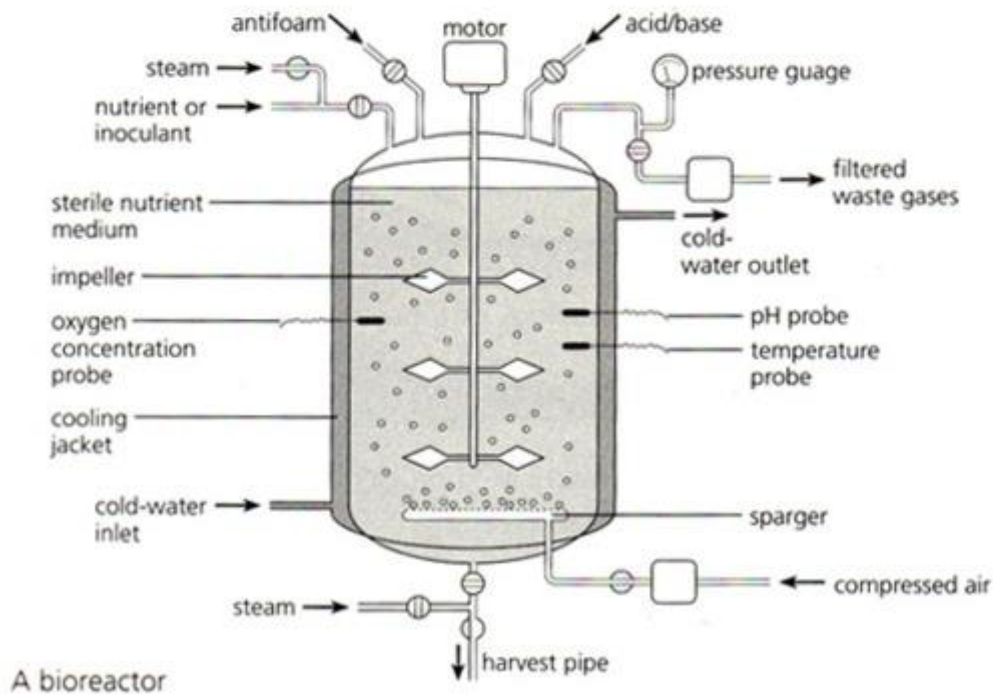


Fig 5: A Bioreactor

The design also takes into consideration the unique aspects of biological processes, capital investment and operation cost.

• Large volume but low value products require simple fermenter with no aseptic conditions.

• High value but low volume products require sophisticated operation and proper maintenance of aseptic conditions.

Criteria of Bioreactor Design:

A good bioreactor design should provide improved productivity, validation of desired parameters in a cost effective manner for achieving consistent and higher quality products. The criteria for bioreactor design is as follows:

1. Microbiological and biochemical characteristics
2. Hydrodynamic characteristics
3. Mass and heat characteristics
4. Kinetics of cell growth and product formation
5. Genetic stability
6. Design related to sterilization
7. Control of bioreactor conditions
8. Design of downstream product separation
9. Capital and operating costs
10. Scale-up.

Principle Types of Bioreactor (Fermenter)

- Batch fermenter
- Continuous stirred-tank fermenter (CSTR)
- Tubular fermenter
- Fluidized bed fermenter

1.5. Oxygen transfer rate in microbial processes

In aerobic bioprocesses, oxygen being sparingly soluble is supplied continuously in broths. The gas–liquid mass transfer in a bioprocess is strongly influenced by the hydrodynamic conditions in the bioreactors, physicochemical properties of the culture, geometrical parameters of the bioreactor and the presence of oxygen consuming cells.

The transport of oxygen from air bubbles to the cells has been represented by a number of steps and resistances in **Fig 6 .**

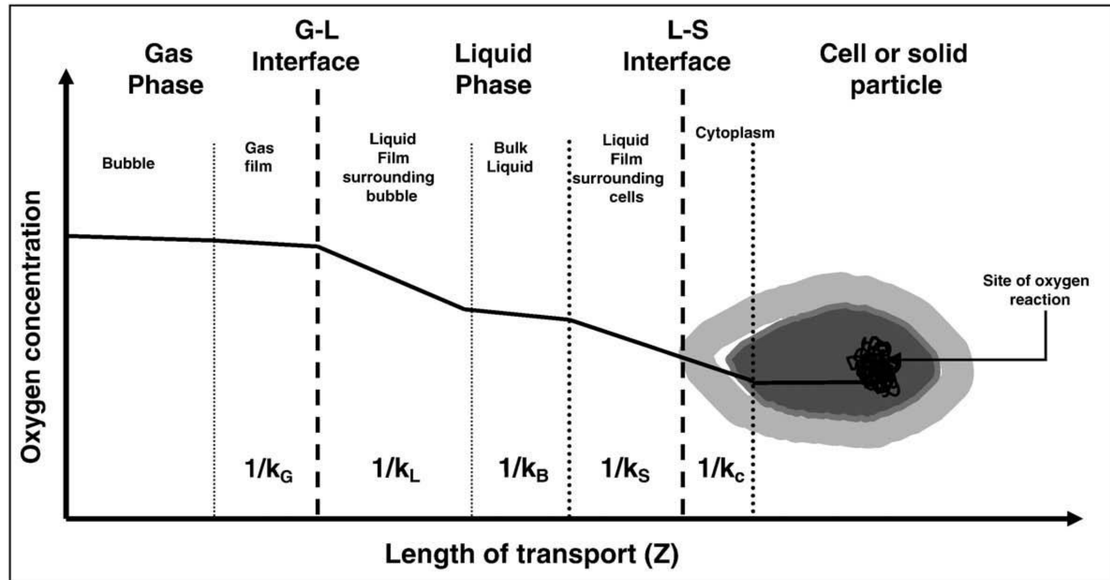


Fig 6. Steps and resistances for oxygen transfer from gas bubble to cell.

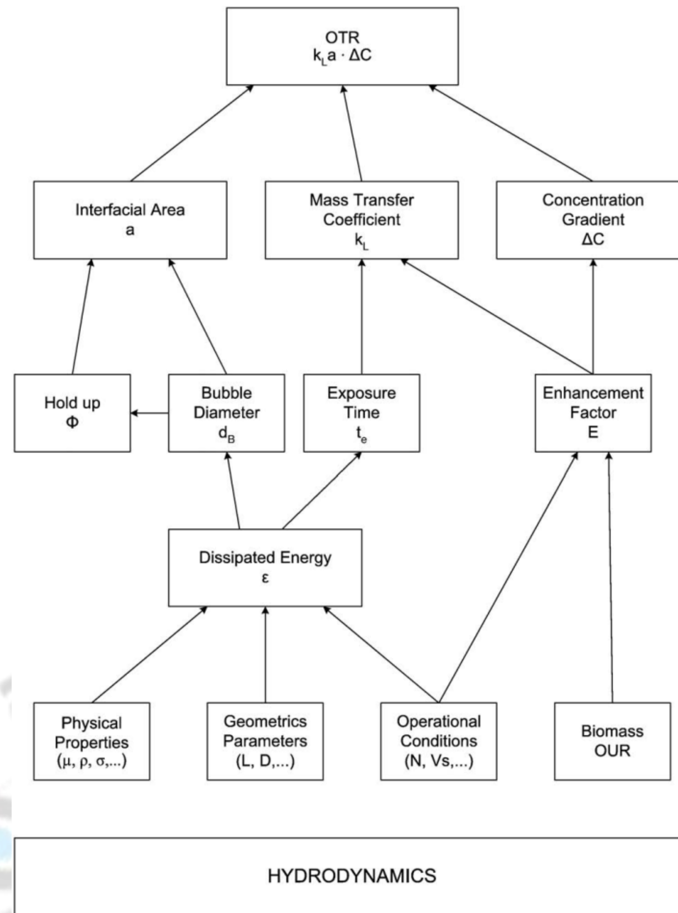


Fig7. Relationship between OTR, volumetric mass transfer coefficient and hydrodynamic parameters in bioreactors at several levels.

The two film theory proposed by Whitman (1923) on gas–liquid mass transfer describes the flux through each film as the product of the driving force by the mass transfer coefficient.

Thus $$N_{AG} = k_G (P_g - P_i) = k_L (C_i - C_L) \qquad (21)$$

Where, k_G and k_L, are the local mass transfer coefficients; P_g is the oxygen partial pressure in the gas bubble; and C_L, the dissolved oxygen concentration in the bulk liquid; index i refers to values at the gas–liquid interface.

Since it is not easy to directly measure the interfacial concentrations the overall mass transfer coefficient can be rewritten:

$$N_{AL} = K_G (P_g - P^*) = K_L (C^* - C_L) \quad \text{as } (p^*=HC^* \text{ - Henry's law}) \qquad (22)$$

where P* is the oxygen pressure in equilibrium with liquid phase; C* is the oxygen saturation concentration in the bulk liquid in equilibrium to the bulk gas phase,; K_G and K_L are the overall mass transfer coefficients. Combining Eqs. (21) and (22), the following relationship is obtained

$$\frac{1}{K_L} = \frac{1}{Hk_G} + \frac{1}{k_L}$$

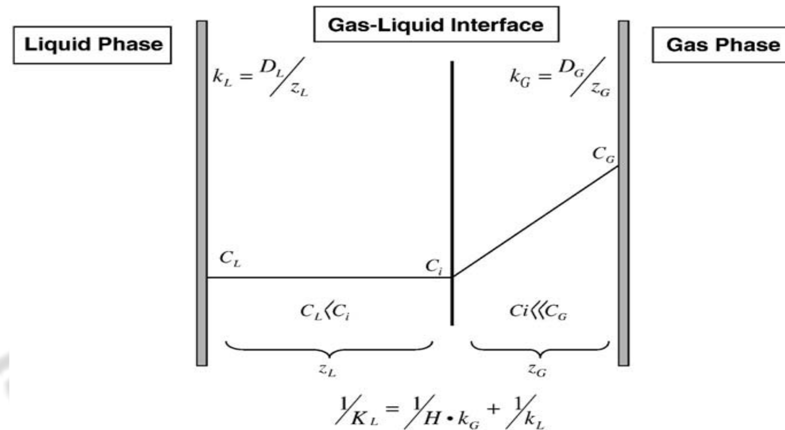


Fig 8. Schematic representation of the gas–liquid interface

Overall mass transport coefficient is equal to the local coefficient: $K_L = k_L$ as the greatest resistance for mass transfer is on the liquid side of the interface. Hence, gas phase resistance can be neglected.

The oxygen mass transfer rate per unit of reactor volume, N_A , is given as the product of overall flux and the gas–liquid interfacial area per unit of liquid volume, a:

$$N_A = a\, N_{AL} = K_L a\, (C^* - C_L)$$

Since K_L and a cannot be measured separately, the product K_La is measured and is called as **volumetric mass transfer coefficient** characterizing the transport from gas to liquid.

Maximum Oxygen transfer rate (OTR_{max})

At steady state, oxygen transfer rate from bubbles equals the oxygen consumption rate by the cells.

Therefore, $K_La\,(C^* - C_L) = q_0x$ where q_0 is specific oxygen uptake rate

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When $C_{L} = 0$ (max. OTR), the bioreactor can withstand maximum biomass concentration

$$X_{max} = \frac{K_L a\, C^*}{q_0}$$

$$(K_L a)_{critical} = q_0 x / (C^* - C_{critical})$$

Generally, the critical oxygen concentration is 5-25 % of the oxygen saturation values in cultures. In Newtonian suspensions, such as of yeast and bacterial fermentations, the critical oxygen concentration is constant and is not affected by fermentation conditions. However, in non-newtonian suspensions containing filamentous micro-organisms, the critical oxygen demand is found to be dependent on fermentation conditions.

1.6. Factors affecting volumetric mass transfer coefficient –

Various factors affects mass transfer rate, which in turn show effect on $k_L a$

(i) Bubble size .

- Small bubbles have high interfacial area and result in increased $K_L a$.

(ii) Gas Hold-up

It is the volume fraction of the gas held up in the total volume comprising the liquid and the held-up gas together.

$$\varepsilon = \frac{V_G}{V_G + V_L} = \frac{V_G}{V}$$

Higher values of ε indicates higher amount of gas held up in the system. Smaller bubbles stay for longer times in the liquid and result in higher ε values and higher mass transfer rates. Smaller bubbles (d_B<<1mm) create nuisance in fermenters.

(iii) Gas Velocity

The Superficial gas velocity (U_G) is the linear velocity of gas obtained on basis of volumetric flow rate of the gas per cross-sectional area of bioreactor.

$$K_La = 2 \times 10^{-3} (P/V)^{0.7} U_G^{0.2}$$

Mass transfer rates increases with U_G as:

- If gas flow rate is very high and the stirrer speed is low, the gas does not get dispersed properly. It rises up and results in impeller flooding. So flow rate is either reduced or agitation speed in increased to increase K_La.
- If flow rate is too low compared to agitator speed, then gas bubbles rise simply recirculate. This increases gas hold up but mass transfer coefficient and hence the mass transfer rate reduces considerably.

(iv) Type of sparger

Not much information on the effect of spargers on mass transfer as reported by Hassan and Robinson (1977).

(v) Type of Agitator

Type of agitator and agitator design for effective mixing shows great effect on mass transfer rates.

(vi) Power input to agitator

The effect of power input on interfacial area (a) is as -

$$a = 1.44 \left[\frac{(P/V)^{0.4} \rho_L^{0.2}}{\sigma_L^{0.6}}\right]\left[\frac{u}{u_t}\right]^{0.5}$$

Where ρ_L = Density of liquid broth

σ_L = Surface tension of liquid broth

u_t = Terminal velocity

The interfacial area varies as 0.4 power of the power input per unit volume (P/V). The power input is also related to agitator speed through the Power number (N_P)

$N_p = P/\rho\, n^3 di^5$ or $P = N_p\, \rho\, n^3 di^5$ where di is the bubble diameter

The dependence of Power no. on the agitator speed varies based on the type of flow as

$P \propto n^2$ for laminar regime

$P \propto n^3$ for turbulent regime

(vii) Temperature

The temperature has two effects on mass transfer:

1. It increases the diffusivity of the gas into the liquid.

2. Increases the value of K_La.

However, increase in the temperature decreases the solubility of gas and hence reduces $C^*O_{2,L}$

So, ($C^*O_{2,L} - CO_{2,L}$) will reduce and thereby it reduces mass transfer. In the range of 10-40^{0}C, temperature rise increases the mass transfer. But temperature > 40^{0}C, the mass transfer will decreases

(viii) Pressure

Pressure affects the mass transfer by increasing the solubility of the gas in the liquid phase, which is given by Henry's law:

$$P_{O2,G} = H \times C^*O_{2,L}$$

The partial pressure and total pressure of the system are related by:

$$P_{O2,G} = P_T \text{ (Total Pressure)} \times Y_{O2} \text{ (mole fraction of } O_2)$$

Thus, a total pressure P_T increases, $p_{O2,G}$ increases, and hence $C^*_{O2,L}$ increases, which in turn will increase driving force ($C^* - C_L$).

(ix) Antifoaming agents

Most of the culture broths contain proteins which causes foaming. Use of silicon based antifoaming agents effects the surface chemistry of bubbles by reducing surface tension. This reduces coalescence of smaller bubbles into larger bubbles thereby increasing 'a'. However, these surface active agents reduce the motility of gas-liquid interface and reduce mass transfer coefficient. Thus the increase in 'a' is countered by reduction of K_L which being larger, reduces the overall K_La

(x) Existence of cells

Oxygen transfer in culture broths is greatly influenced by the presence of cells by two ways:

1. **Physical Influence**: Cells interfere in the break-up and formation of the gas bubbles by influencing their surface properties. These get absorbed on the gas-liquid interface decreasing the mass transfer coefficient. Cells do not allow smaller bubbles to coalesce into bigger bubbles, hence the increase in interfacial area is maintained and this in turn results in overall mass transfer.

2. **Quantitative influence**: In quantitative influence, the cells absorb oxygen during the process which increases the driving force and hence oxygen transfer. The influence of cells in enhancing the mass transfer depends on:

1. Type of cells
2. Morphology of cells
3. Concentration of cells

1.7. Optimization of nutritional and physical conditions in bioreactor

The objective of process optimization is in maintaining optimum and homogenous reaction conditions to minimize microbial stress and in enhancing volumetric productivity using several nutritional (e.g. suitable carbon and energy sources, nitrogen sources, other key macro-nutrients, micro-nutrients, and the effects of inducers/repressors of enzyme production) and environmental parameters (temperature and process variables such as pH, dissolved oxygen, Pressure, agitation).

Scale-Up

Design of a production-scale system is based on the performance of a model system characteristics as-

- thermodynamics e.g. C_L^*, C_P (scale independent)
- kinetics e.g. specific growth rate, μ (scale independent)
- transport-phenomena e.g. k_La (scale independent)

One of the fundamental problems is incomplete mixing on scale-up, concentration gradients, cell growth on walls etc.

Similarity between model and prototype systems

For similarity in two mixing systems, it is (ideally) important to achieve:

1. **Geometric similarity:** Both parameters as the shape and linear dimensions should be related by a constant factor

i.e. $\left(\frac{D}{T}\right)_M = \left(\frac{D}{T}\right)_F$ and $\left(\frac{H}{T}\right)_M = \left(\frac{H}{T}\right)_F$ etc.

2. **Kinematic similarity:** Similar flow patterns with a constant ratio of velocities at same points

2. **Dynamic similarity:** All forces at same points in two geometrically similar systems have a constant ratio, i.e.

$$\left(\frac{P_0}{\rho N^3 D^5}\right)_M = \left(\frac{P_0}{\rho N^3 D^5}\right)_F$$

$$\left(\frac{\rho N D^2}{\mu}\right)_M = \left(\frac{\rho N D^2}{\mu}\right)_F$$

$$\left(\frac{N^2 D}{g}\right)_M = \left(\frac{N^2 D}{g}\right)_F$$

However it is very difficult to achieve dynamic similarity in presence of more than one dimensionless group

Common criteria for scale-up:

i. Constant P/V – Most commonly used criterion for scale-up

For **non-gassed system** at constant P/V

$P/V = KN_i^3D^2$

$N_1^3D_1^2 = N_2^3D_2^2$

$N_1/N_2 = \left[D_2/D_1\right]^{2/3}$

For **gassed system** at constant P/V

$$P_g / V = \frac{KN_i^{7/2} D_i^{7/2}}{Q^{1/4}}$$

$$\frac{N_1^{7/2} D_1^{7/2}}{Q^{1/4}} = \frac{KN_2^{7/2} D_2^{7/2}}{Q_2^{1/4}}$$

$$N_1/N_2 = \left[(D_2/D_1)^{7/2} (Q_1/Q_2)^{1/4} \right]^{2/7}$$

$$N_1/N_2 = (D_2/D_1) (Q_1/Q_2)^{1/14}$$

ii. Constant kLa – Desirable for biological processes

$$N_1/N_2 = (D_2/D_1)^{13/21} (Q_1/Q_2)^{5/42}$$

iii. Constant mixing time – In otherwords indicates constant Reynold's no.

$$N_{Re} = \text{Constant}$$

$$t_2 = t_1 (D_2/D_1)^{11/18}$$

iv. Constant impeller tip speed

$$N_1 = N_2 (D_2/D_1)$$

3. Downstream processing

- It refers to separation and purification processes of fermentation/enzyme reaction into desired products.
- Accounts for 60% of the total production costs

Points to be considered while developing a downstream process:

1. Nature of material to be processed

2. Location of the target product: e.g. intracellular, extracellular, inclusion bodies

3. Concentration of product

4. Relative properties of the product with respect to contaminants

5. Sensitivity to high shear rates, organic solvents, pH and temperature

6. Desired physical form of the final product, e.g. lyophilized powder, sterile solution, suspension

7. Percentage purity,

8. Process economics

Operation of downstream processing on basis of properties:

1. Filtration, membrane separation, centrifugation - Size
2. Centrifugation, sedimentation, floatation - Density
3. Membrane separation - Diffusivity
4. Extraction, chromatography, adsorption- Polarity
5. Extraction, precipitation, crystallization - Solubility
6. Adsorption, membrane separation, Electrophoresis - Electrostatic charge
7. Distillation, membrane distillation, pervaporation- Volatility

A RIPP (Recovery, Isolation, Purification and Polishing) strategy involves initial use of low resolution techniques as precipitation, filtration, centrifugation, and crystallization to significantly reduce the volume and increase the overall concentration followed by high resolution techniques to obtain pure and polished finished products.

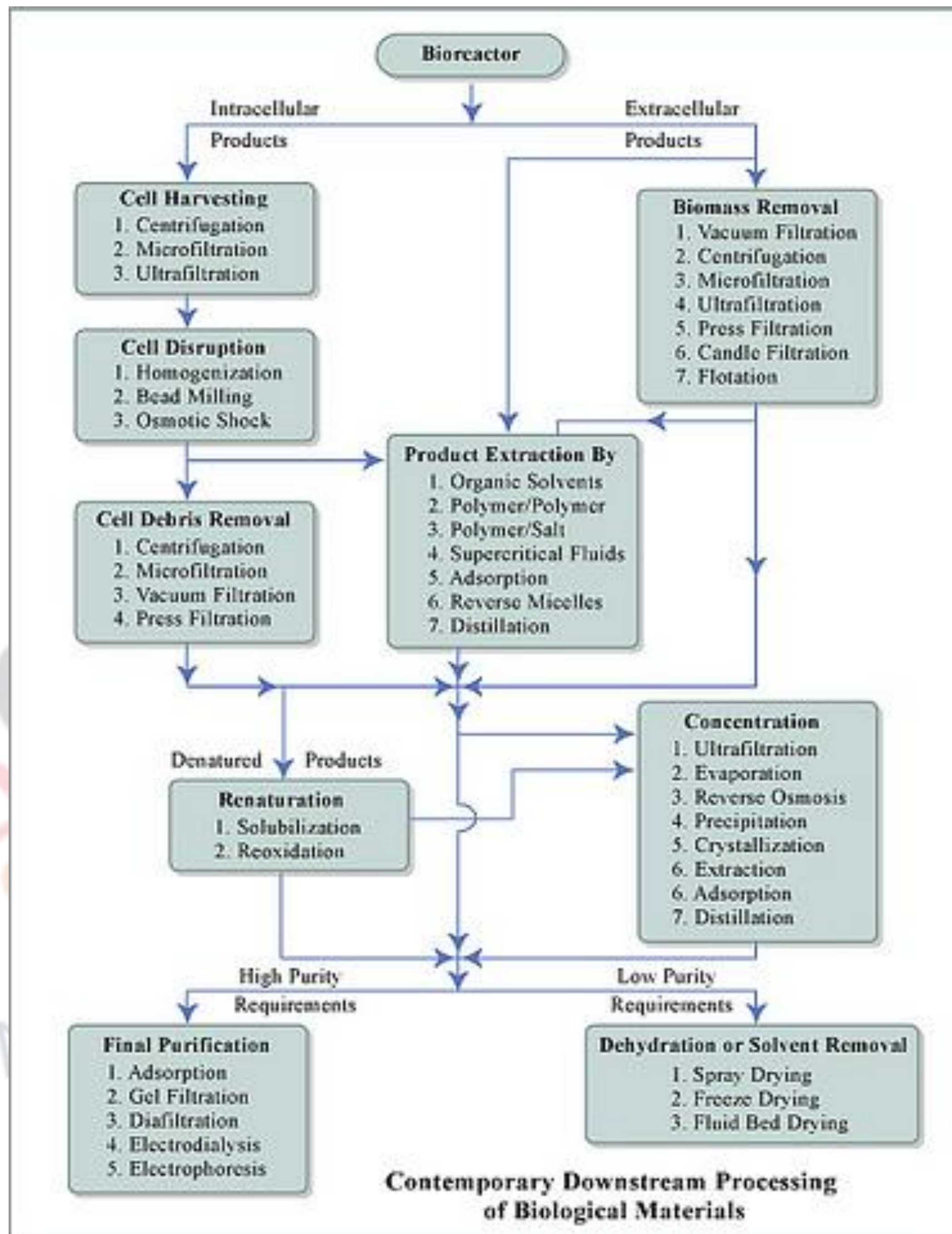

Fig 9. Flow diagram of Downstream processing of biological materials

Manufacturing Cost in Bioprocess Industries

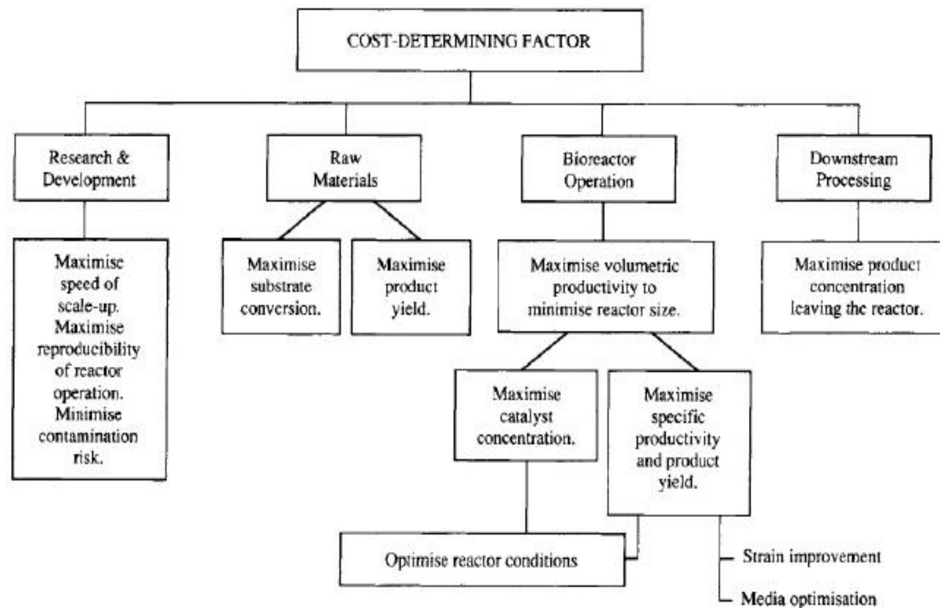

Fig 10: Flow chart of manufacturing cost in bioprocess industries

3. Summary

In this lecture we learnt about fundamental basics of microbial kinetics, metabolic stoichiometry and energetics, the basics of bioreactor engineering with knowledge on design and operation of fermentation processes. Also it had helped to understand bioengineering skills for the production and purification of biochemical product using integrated biochemical processes