

# Biochemical Engineering

- Biological systems are very complex and beautifully constructed. They obey the rules of physics and chemistry.
- Living cells are predictable, and the processes to use them can be rationally constructed on commercial scale.
- Biochemical engineering is the application of engineering principles to design, develop and analyze processes using biocatalyst.

## Bioreactor

Microorganisms are able to use no. of carbon sources with a wide metabolic range. The use of inexpensive nutrients may create an opportunity for microbes to convert waste to useful products.

Fermentation is defined as the chemical transformation of organic compds. with the aid of enzymes.

Classes of organisms used in industrial process →  
Yeast  
Mold  
Single Bacteria  
Fungi

## Microbial Growth

Substrates + cell → Extracellular products + more cells



This growth is a result of variety of physical, chemical

and nutritional condit. As a result of nutrient utilization, microbial mass increases with time. It is an example of autocatalytic reaction. The rate of growth is directly proportional to cell conc.

### Ideal Reactors

- The phenomena occurring in reactor are:
- reaction
  - transfer of mass, heat and momentum (transport process)

The mechanisms by which A can enter or leave the vol. element are flow:

- When there is no mixing of streamlines (PFR)
- When the mixing is complete (CSTR)

PFR - Equal velocity along parallel

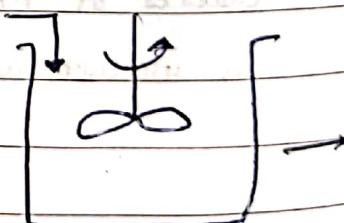
streamlines

Flat velocity profile

CSTR - No conc. gradient, feed is

inst. mixed and effluent

conc. is same everywhere in reactor



complete mixing only in transverse direction, rate is a fn. of axial direction.

The time the atoms have spent in the reactor is called residence time of atoms in the reactor

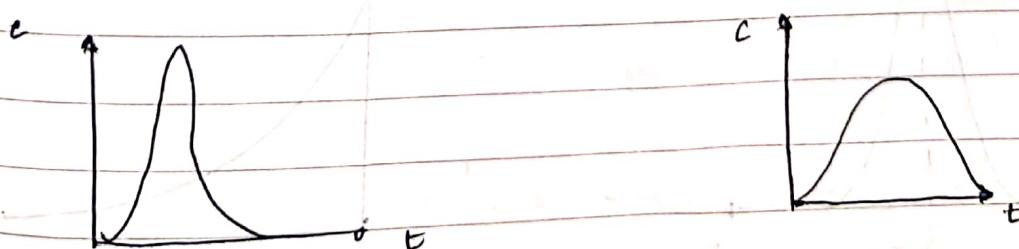
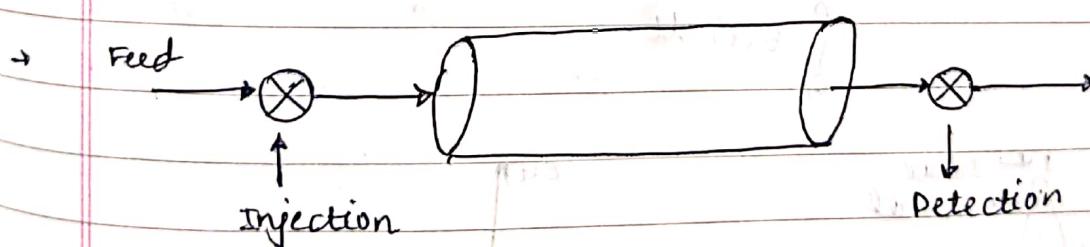
The residence time distribution (RTD) is a characteristic of mixing that occurs in chemical reactor.

- Ideal type reactors
  - Batch
  - PFR
  - CSTR

The primary cause for non-ideality is the imperfect distn' of material, energy within the reactor due to transport limitation of species in the reactor

The reasons for imperfect distn' of materials is due to altered performance which could be due to change in rxn rate, fouling and undesired rxn.

The reasons for imperfect energy distn' (thermal distn') is due to change in rxn rate, autocatalytic rxn, exothermic rxn.



The RTD is determined by injecting a chemical called tracer

For pulse input

$$f(t) = \frac{c(t)}{\int_0^\infty c(t) dt}$$

$E(t)$  is called RTD fn. It is fn that describes in a quantitative manner how much time different fluid elements spent in the reactor.

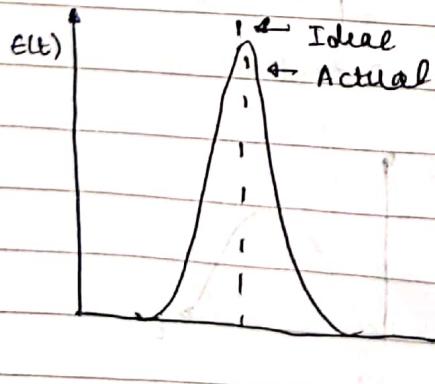
The fraction of material leaving the reactor that has resided in the reactor for times  $t_1$  to  $t_2$  is given by

$$\int_{t_1}^{t_2} E(t) dt$$

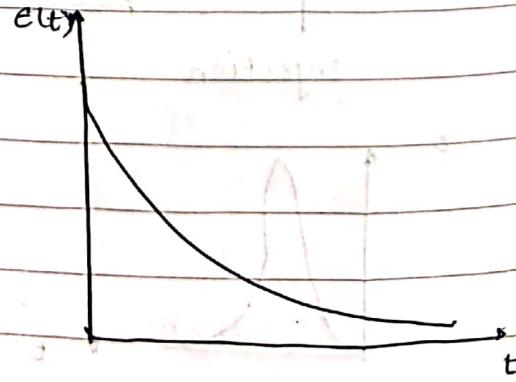
Note:  $\int E(t) dt = 1$

The mean value of the variable = to the first moment of RTD fn.

$$t_m = \frac{\int_0^\infty t E(t) dt}{\int_0^\infty E(t) dt}$$

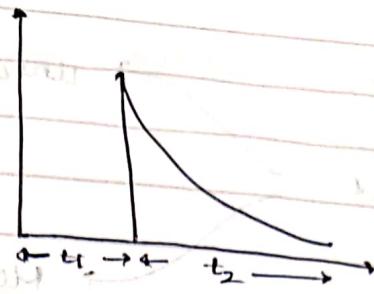


RTD of a near PFR

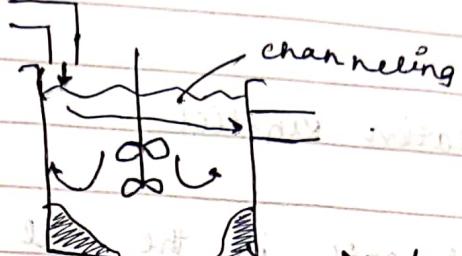


RTD of a perfectly mixed

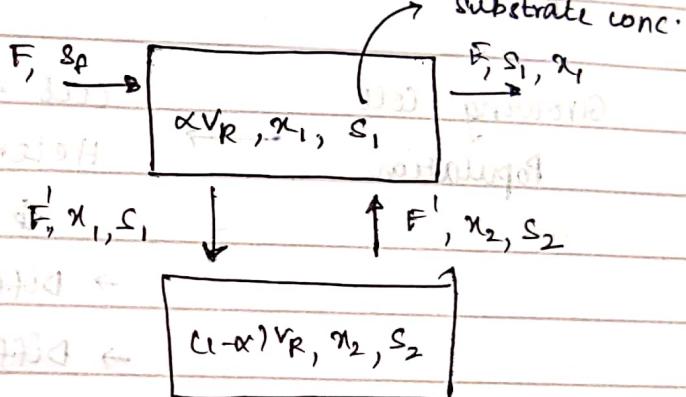
PFR followed by  
CSTR



Non-ideal CSTR



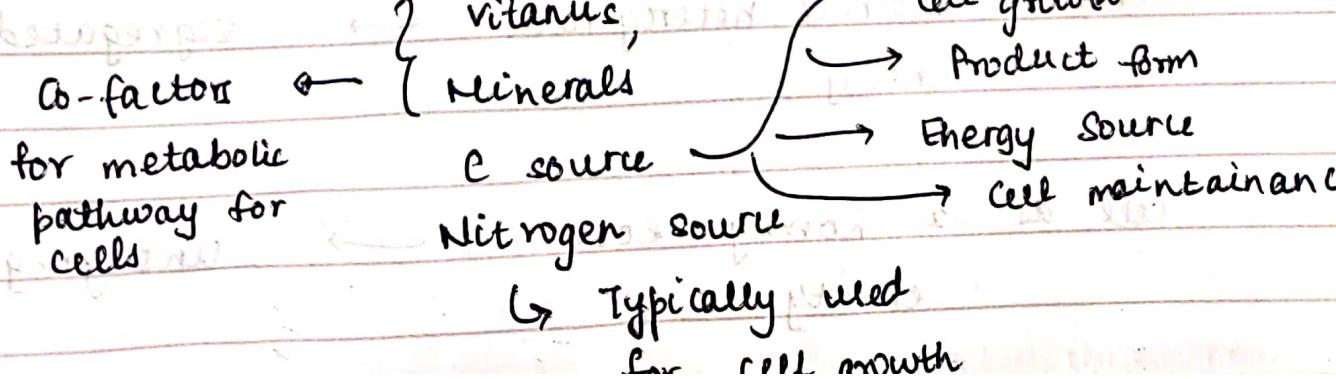
To analyse the above  
we make the following  
arrangement



Cells + Substrate  $\rightarrow$  More Cells + Products

The cells require appropriate environment and operating conditions for cell growth

Environment  $\rightarrow$  Culture Media



Growth of cell

→ unicellular → Mass/vol  
or No./vol

Moldy → Mass/vol.

cell population kinetics

The components in the cell are DNA, RNA, Proteins, Polysaccharides etc.

Growing Cell

Population

cell to cell

Heterogeneity

→ Differ in age

→ Differ in chem activity

Consider

Viewpoint

cell populn as one single component → unstructured

cell populn as a multi component → structured

cell as discrete heterogenous entity → segregated

cell as a homogeneous entity → unsegregated

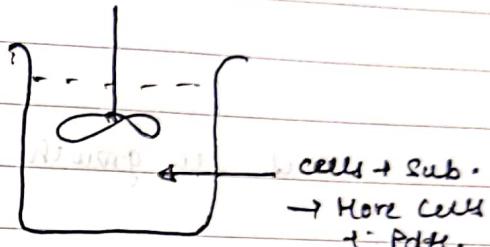
Well Mixed Bioreactors for Kinetic measurement (Ideal)

→ Avoid spatially non-uniform conditions

## ① Batch Reactor (Unsteady)

Let  $c_i$  be the conc. of  $i^{th}$  component and  $v_R$  be the vol.

Inoculation <sup>um</sup> is inclusion of some live cell, so that the run starts.



$$[\text{Rate In}] - [\text{Rate Out}] + [\text{Rate Form.}] = [\text{Accumulation}]$$

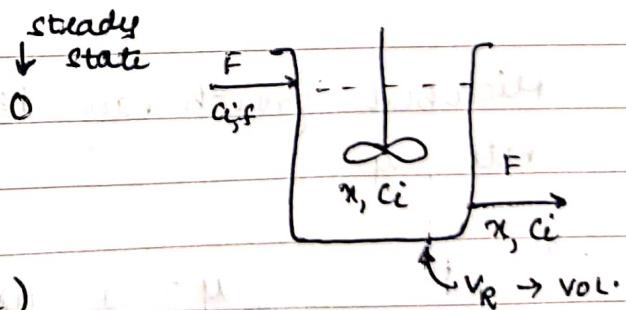
$$v_R r_{fi} = \frac{d}{dt} [v_R c_i]$$

(mass/vol-time)

$$\text{If } v_R \text{ is const., } r_{fi} = \frac{dc_i}{dt}$$

## ② CSTR / chemostat (Steady)

$$F(c_{if} - c_i) + v_R r_f = 0$$



$$v_R r_{fi} = F(c_i - c_{if})$$

$$\Rightarrow r_{fi} = \frac{F}{v} (c_i - c_{if})$$

$\rightarrow$  Dilution rate

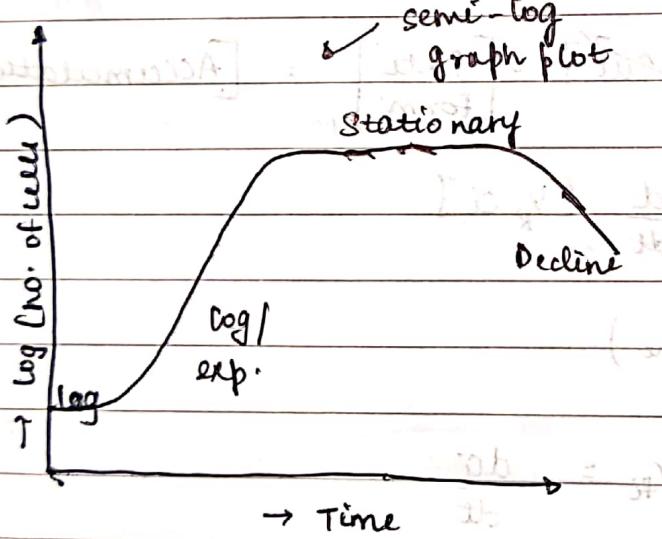
Assumption about cell popn (structured, Unstructured ...)  
- limit over.  $\rightarrow$  limiting growth rate substrate

Rate of microbial growth is characterised by specific growth rate ( $\mu$ )

$$\mu = \frac{1}{x} \cdot \frac{dx}{dt} = \frac{1}{N} \frac{dN}{dt}$$

$$\frac{dx}{dt} \Rightarrow \mu x \Rightarrow \mu = \frac{dx}{dt} \approx \frac{dx}{dt} \approx \frac{dx}{dt}$$

Thus cell growth is first order auto catalytic rxn.



Lag phase - Cells are preparing their cell machinery for growth

log phase - Growth app. an exp. curve

Stationary Phase - Cells stop growing

Microbial growth can be characterized by specific growth rate,  $\mu$

$$\mu = \frac{1}{x} \frac{dx}{dt} \quad \mu = \frac{1}{t} \frac{dx}{dt} \quad \mu = \frac{1}{t} \frac{dx}{dt}$$

$$\mu = \frac{1}{t} \frac{dx}{dt} \quad t = \text{time (s)}$$

$$\text{In terms of no. of cells, } \mu = \frac{1}{N} \frac{dN}{dt}$$

When the two cell comp. ie same i.e balanced growth condition, then

$$\mu = \nu \text{ i.e } \frac{dN}{dt} = \frac{dx}{dt} \quad \begin{matrix} \text{Substrate conc.} \\ \text{mass/vol} \end{matrix}$$

specific substrate uptake (consumption) rate,  $q_s = \frac{1}{\lambda} \frac{ds}{dt}$

specific product formation rate,  $a_p = \frac{1}{\lambda} \frac{dp}{dt}$  product rate mass/vol

### Mass Balance Eqn:



Rate of Output + Gen. = Accumulation  
Input - Death

$$\mu(xv) - k_d(xv) = \frac{dx}{dt}(xv)$$

↑  
death  
const.

Considering const. vol.,  $\frac{dx}{dt} = (\mu - k_d)x$   
and  ~~$\mu = k_d$~~   $\mu - k_d \approx \mu$

$$\text{If } k_d \neq 0, \frac{dx}{dt} = (\mu - k_d)x$$

$$\text{Integrating (at } t=0, x=x_0) \Rightarrow x = x_0 e^{-kt}$$

## Doubling Time

It is the time at which  $\lambda = 2\lambda_0$

$$\lambda = \frac{\ln 2}{t_{d}}$$

$$2\lambda_0 > \lambda_0 e^{\lambda_0 t_{d}}$$

$$\frac{t_{d}}{\lambda_0} = \ln 2$$

$$\text{or taking log both sides } \therefore t_{d} = \frac{\ln 2}{\lambda_0}$$

## Cell Growth Kinetics

### ① Monod Kinetics

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

$$K_s + S$$

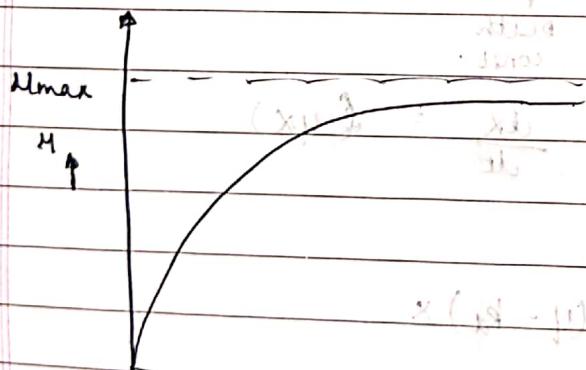
$K_s$  is called saturation const.

Two

$$\textcircled{1} \quad S > K_s \Rightarrow \mu = \mu_{\max}$$

zero order dependence on  $S$

$$\textcircled{2} \quad S \ll K_s \Rightarrow \mu \propto S \quad \text{1st order dependence on } S$$



Saturation const. is that value of  $S$ , that gives  $\mu$  as  $\mu_{\max}/2$

$$\frac{1}{\mu} = \frac{K_s + S}{\mu_{\max} S} \Leftrightarrow \frac{1}{\mu} = \frac{K_s}{\mu_{\max} S} + \frac{1}{\mu_{\max}}$$

Yield coefficient / factors in cell culture

$$\gamma_{F/G_i} = \frac{\Delta F}{\Delta G_i} \text{ producing}$$

$\Delta G_i$  - consuming

$$\gamma_{X/S} = \frac{\text{Mass of cells}}{\text{Mass of sub. consumed}}$$

How much cell produced per mass of sub. consumed

$$\gamma_{P/S} = \frac{\text{Mass of product formed}}{\text{Mass of substrate consumed}}$$

$$\gamma_{X/O_2} = \frac{\text{Mass of cell}}{\text{Mass of } O_2 \text{ consumed}}$$

The defn: of yield co-efficients can be generalised as

$$\gamma_{JK} = \frac{\Delta J}{\Delta K} \text{ depend on time on the period over which they are measured}$$

$\Delta K$  for a consumed sub. is -ve in value

If  $r_J$  and  $r_K$  are the volumetric rates of production and consumed of J and K, in a closed, const. vol. reactor, the inlet yield can be calculated by

$$r_{KS} = \lim_{\Delta t \rightarrow 0} \frac{\Delta K}{\Delta S} = - \frac{dx}{ds} = \frac{r_K}{r_S}$$

Observed biomass yield based on total substrate consumed

$$\gamma'_{KS} = \frac{-\Delta X}{\Delta S_f} = \frac{-\Delta X}{\Delta S_i + \Delta S_p}$$

$\gamma_{xs}$  → Biomass yield from substrate

$S_T$  → Total mass of substrate consumed

$S_G$  → Substrate used for growth

$S_R$  → Remaining mass of substrate

$$\left( -\frac{ds}{dt} \right)_{\text{overall}} = \left( -\frac{ds}{dt} \right)_g + \left( -\frac{ds}{dt} \right)_{\text{maintenance}}$$

$$\frac{\gamma_X}{\gamma_{xs}(\text{overall})} = \frac{1}{\gamma'_{xs}/(\text{sc growth})} + \frac{m}{M} \leftarrow \begin{array}{l} \text{maintenance coeff} \\ \text{specific growth} \\ \text{rate of cell} \end{array}$$

$\uparrow$   $\gamma_{xs}$  is  $\gamma_{xs}$  true cell yield

The slope of the curve  $1/\gamma_{xs}$  vs  $1/\gamma_X$  gives  $m$ .

### Theoretical and Observed Yields

Relationship between theoretical and observed yield

at no waste or losses  $\gamma_{xs} = \gamma_{xT}$

with some losses  $\gamma_{xs} < \gamma_{xT}$

losses due to

waste

other losses

water loss

relationship to other conditions like pH or temperature

theoretical yield is not the same as the observed yield

theoretical yield is not the same as the observed yield

$$\frac{\gamma_{xT}}{\gamma_{xs}} = \frac{\gamma_{xT}}{\gamma_{xT} - \gamma_{xT} \frac{\gamma_{xT}}{\gamma_{xT}} \text{ loss}} = \frac{\gamma_{xT}}{\gamma_{xT} - \gamma_{xT} \text{ loss}}$$

theoretical yield is based on ideal conditions

$$\frac{\gamma_{xT}}{\gamma_{xs}} = \frac{\gamma_{xT}}{\gamma_{xT} - \gamma_{xT} \frac{\gamma_{xT}}{\gamma_{xT}} \text{ loss}}$$

## → Production Formation Kinetics

specific growth rate of cell ( $\mu$ ) =  $\frac{1}{\kappa} \frac{dx}{dt}$

specific substrate consumption rate ( $q_s$ ) =  $\frac{1}{x} \frac{ds}{dt}$

specific product formation rate ( $q_p$ ) =  $\frac{1}{x} \frac{dp}{dt}$

→ Fermentation products can be classified acc. to the relationship b/w product synthesis and energy generation in the cell.

① Products directly associated with gen. of energy

Eg:- ethanol, acetic acid, acetone

② Products indirectly associated with gen. of energy

Eg:- Amino acids and their pdts.

③ Products for which there is no clear direct or indirect coupling to gen. of energy

Eg :- Penicillin, Vitamins

→ The rate of pdt. formation in cell culture can be expressed as a fn: of biomass concn

$$r_p = q_p x$$

↑ specific rate of pdt.  
↓ substrate formation

→ The rate of pdt. formation must account for growth-associated and maintenance associated production as follows

$$m_p \rightarrow \text{kg pdt. (kg biomass)}^{-1} \cdot \text{c}^{-1}$$

$$r_p = Y_{px} r_x + m_p K_{dissimilat.} \xrightarrow{\text{biomass conc.}} \text{maintenance}$$

↳ vol. rate  
 of biomass formation  
 True yield

$$\text{W.R.T., } r_x = \mu x$$

$$r_p = (Y_{px} \mu + m_p) x$$

$$r_p = Y_{px} \mu + m_p$$

### → Substrate update kinetics

Refers to change in time instantaneous product concentration

$$\frac{ds}{dt} = \frac{1}{V} \frac{ds}{dt} \text{ (in c.d.)}$$

Substrate in c.d.  $\frac{ds}{dt}$   $\rightarrow$  specific substrate conc. rate

$$r_s = q_{xs} x \text{ (in c.d.)}$$

↳ vol. rate of substrate consumption

transition to steady state in absence of inhibition

$$\Rightarrow r_s = \frac{r_x + m_s x}{Y_{xe}} \quad \left[ Y_{xs} = \frac{r_x}{r_s} \right]$$

in absence of extra cellular pdt. synthesis

Using Monod Eqn:

$$\mu = \frac{\mu_m s}{K_S + s}$$

$$r_s = \frac{\mu_m s}{\mu_m s + m_s} x$$