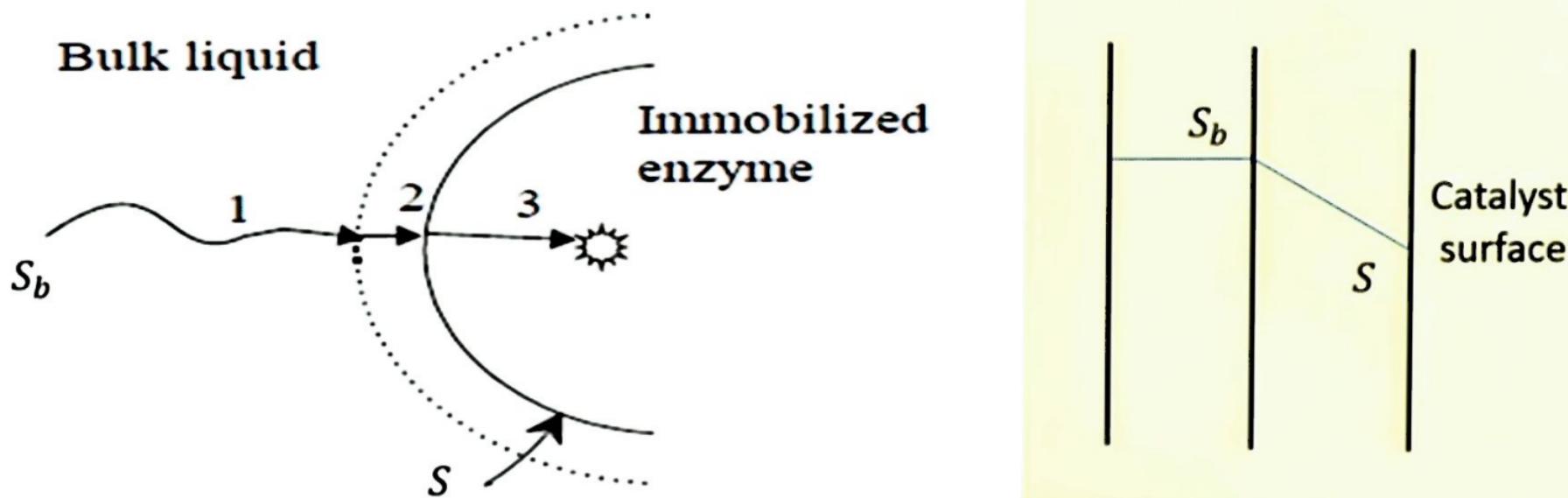


Effect of mass transfer resistance



Steps 1 and 2 are the external mass transfer resistance. Step 3 is the intra-particle mass transfer resistance



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Transportation of substrate in immobilized system

- The hypothetical path of a substrate from liquid to the reaction site in an immobilized enzyme
 - 1. Transfer from the bulk liquid to a relatively unmixed liquid layer surrounding the immobilized enzymes
 - 2. Diffusion through the relatively unmixed liquid layer
 - 3. Diffusion from the surface of the particle to the active site of the enzyme in an inner support



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External mass transfer resistance

- If an enzyme is immobilized on the surface of an insoluble particle, the path is only composed of the first and second steps, external mass transfer resistance
- The rate of mass transfer is given by

$$N_S = k_S a(S_b - S)$$

Where, S_b and S are substrate concentration in the bulk of solution and at the immobilized enzyme surface, respectively. k_S is the mass transfer coefficient (length/time). a is the total surface area of immobilized enzyme per unit volume



External mass transfer resistance

- ☐ At steady state rate of mass transfer is equal to that of substrate consumption

$$k_S a (S_b - S) = (-r_S) = \frac{v_{max} S}{K_m + S}$$

This shows the relationship between the substrate concentration in the bulk of solution and that at the surface of an immobilized enzyme



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Effectiveness factor (for external mass transfer)

To measure the extent which the reaction rate is lowered because of resistance to mass transfer, the effectiveness factor of an immobilized enzyme (η) can be defined as

$$\eta = \frac{\text{actual reaction rate}}{\text{rate if not slowed by mass transfer}}$$

$$\eta = \frac{\frac{v_{max} S}{K_m + S}}{\frac{v_{max} S_b}{K_m + S_b}} = \frac{\frac{\kappa x_S}{1 + \kappa x_S}}{\frac{\kappa}{1 + \kappa}}$$

Effectiveness factor:

Where effectiveness factor is a function of x_S and κ

$$\alpha_S = \frac{x_S}{S_D}$$

- If $x_S = 1$, the concentration at surface is equal to the bulk concentration, then $\eta = 1$, which indicates there is no mass transfer limitation
- If $\underline{x_S} \approx 0$, the mass transfer rate is very slow as compared to the reaction rate

Effectiveness factor (intra particle mass transfer)

To measure the extent which the reaction rate is lowered because of resistance to mass transfer, the effectiveness factor of an immobilized enzyme (η) can be defined as

$$\eta = \frac{\text{observable reaction rate}}{\text{rate if not slowed by mass transfer}}$$

$$= \frac{\text{performance of heterogeneous system}}{\text{performance of homogeneous system}}$$

$$\eta = 1$$

Problem

Glucose is converted to fructose by using immobilized glucose isomerase. Find out the height of the immobilized enzymes column? Following data are given:

Diameter of the column (D_T) = 5 cm

Particle size 30/40 mesh (about 0.71 mm average diameter, d_p),

Feed rate (F) = 500 mL/h

Glucose concentration in feed at $60^\circ C$ = 500 g/L,

Glucose conversion efficiency = 60%,

Feed viscosity (μ) = 3.6 c.p. at $60^\circ C$,

Feed density (ρ) = 1.23 g/mL at $60^\circ C$,

Substrate diffusivity (D) = 0.21×10^{-5} cm²/sec at $60^\circ C$

Void fraction (ε) = 0.35



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Solution

We know that

Z =height of the column

ε = void fraction

a_v = ratio of the particle surface area to volume

Y_2 = mole fraction of substrate in product

Y_1 = mole fraction of substrate in feed

Satterfield has suggested an expression for column height as follows

$$Z = \frac{\varepsilon (Re)^{\frac{2}{3}} (Sc)^{\frac{2}{3}}}{1.09 a_v} \ln\left(\frac{Y_1}{Y_2}\right)$$



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Solution

$$Re = \frac{D_T v \rho}{\mu}$$

$$v = \frac{\text{Volumetric Feed flow rate}}{\text{crosssectional Area of the column}}$$

$$\text{Volumetric Feed flow rate} = 500 \frac{mL}{h} = 0.139 \frac{mL}{s}$$



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Difference between enzymatic and microbial reactions

Enzymes	Microbes
✓ Globular proteins that catalyse a specific reaction	✓ Living organisms that carry out a broad spectrum of biochemical reactions
✓ Act on specific substrates	✓ Act on variety of substrates
✓ Perform only at a particular pH and temperature	✓ Function at an optimal range of pH and temperature
✓ No ability to adapt to changing conditions or substrate sources	✓ Can adapt to changing environmental conditions or substrate sources
✓ Only substrate is required to carry out the reaction	✓ A growth medium is required comprising of carbon source, nitrogen source, vitamins, minerals etc.
✓ Can't repair themselves or reproduce.	✓ Can reproduce and bounce back if damaged.



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Kinetics of microbial cell growth

- ✓ The microbial biomass and product formation can be given as:

Substrate + cells → extracellular products + more cells

$$\text{i.e. } \sum S + X \rightarrow \sum P + nX$$

- ✓ The rate of microbial growth is characterized by the **net specific growth rate (μ_{net})**:

$$\mu_{net} = \frac{1}{X} \frac{dX}{dt} = \frac{1}{N} \frac{dN}{dt}$$

where, X is cell mass concentration, N is number of cells and t is time

- ✓ μ_{net} is the difference between gross specific growth rate (μ_g) and the rate of cell death (k_d)

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



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Monod model

It is an unstructured-unsegregated model

$$\mu = \frac{\mu_{max} S}{K_S + S}$$

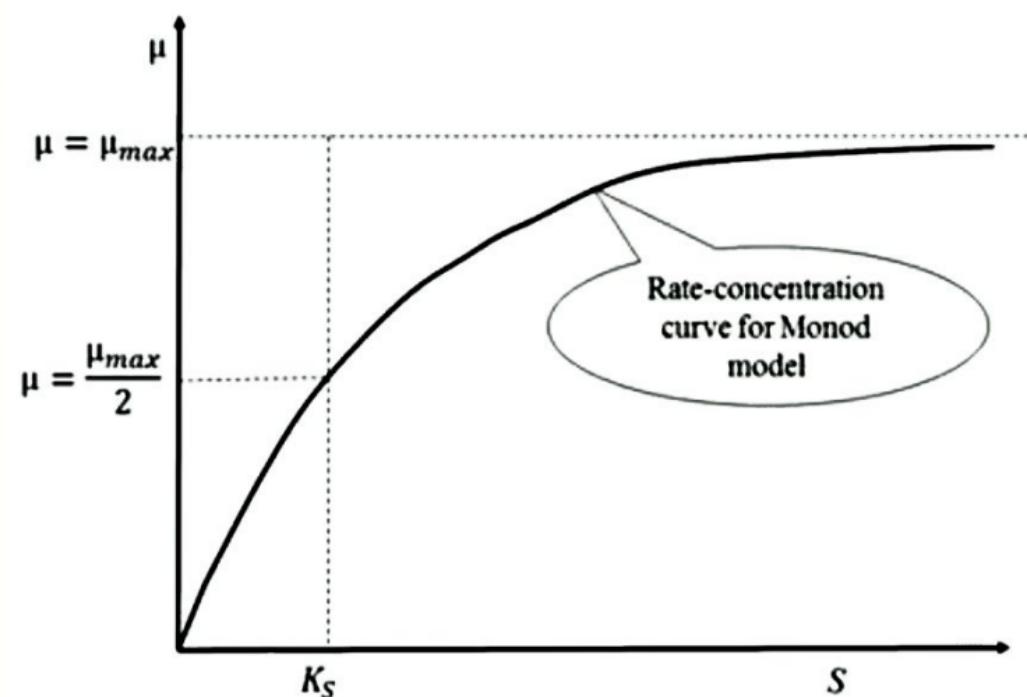
Where, μ = specific growth rate,

μ_{max} = maximum specific growth rate ($L h^{-1}$),

K_S = saturation constant and

S = limiting substrate concentration ($g L^{-1}$)

Medium \rightarrow A-G.

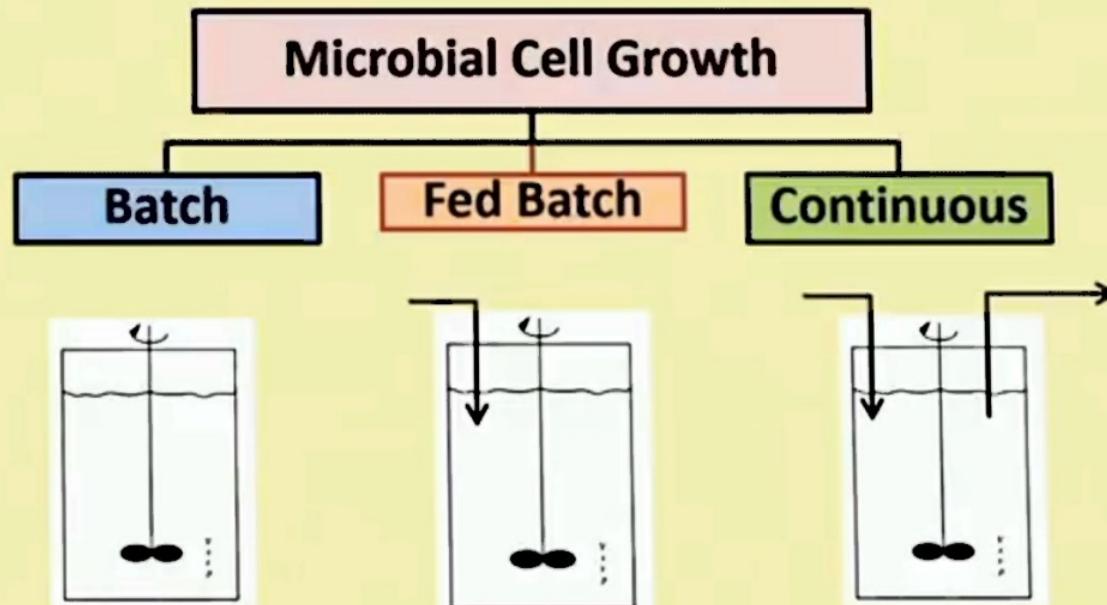


Limitations of Monod model

- ✓ When $S \rightarrow \infty, \mu \rightarrow \mu_{max}$
- ✓ It does not explain when, $S \rightarrow 0$
- ✓ Does not take care of the death phase
- ✓ Does not take care of inhibition effect

Microbial cell growth types

On the basis of mode of cultivation, microbial growth can be of three types:



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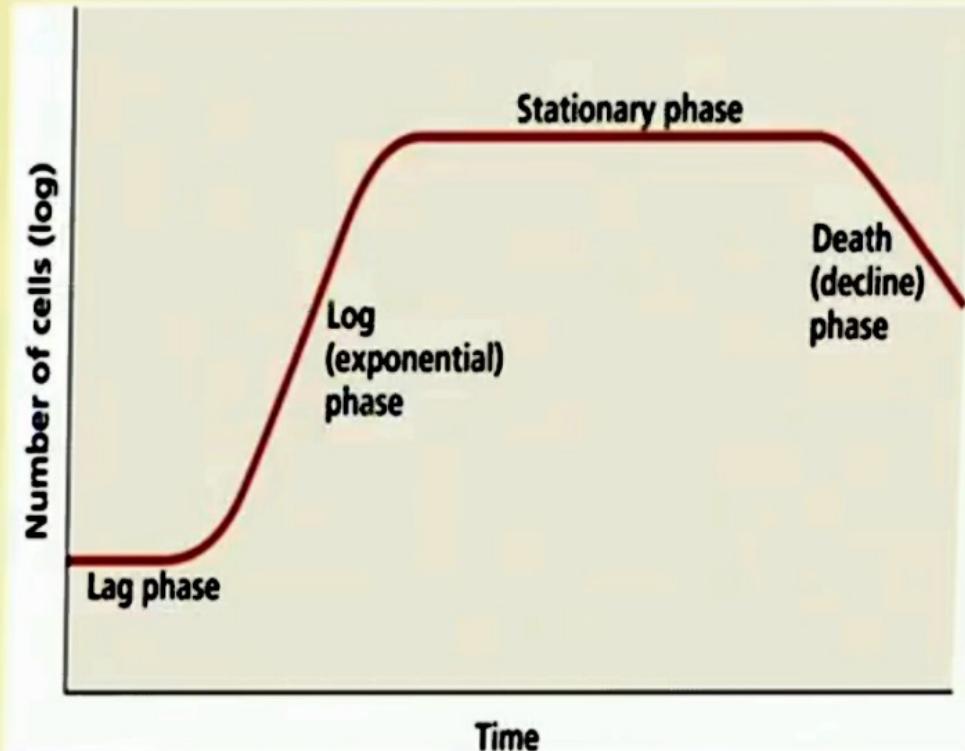
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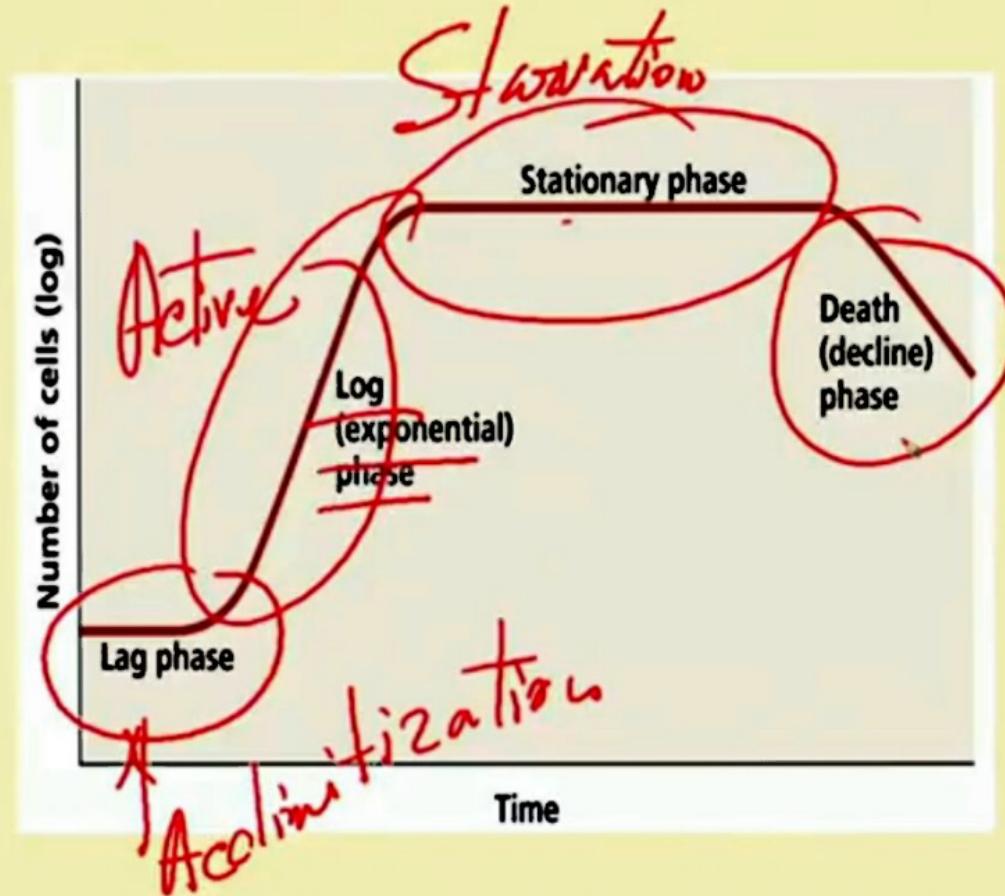
Batch growth

Phase	Description	Specific growth rate
Lag	Cell adapt to the new environment, no or very little growth	$\mu_{net} \sim 0$
Log	Growth achieves its maximum rate	$\mu_{net} \sim \mu_{max}$
Stationary	Growth ceases due to starvation	$\mu_{net} = 0$
Death	Cell losses viability and lyse	$\mu_{net} < 0$



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Log	Growth achieves its maximum rate	$\mu_{net} \sim \mu_{max}$
Stationary	Growth ceases due to starvation	$\mu_{net} = 0$
Death	Cell losses viability and lyse	$\mu_{net} < 0$



Batch cell growth Kinetics

Time required to double the microbial mass ($X = 2X_0$)

$$t_d = \frac{\ln 2}{\mu} \quad (\text{Doubling Time})$$

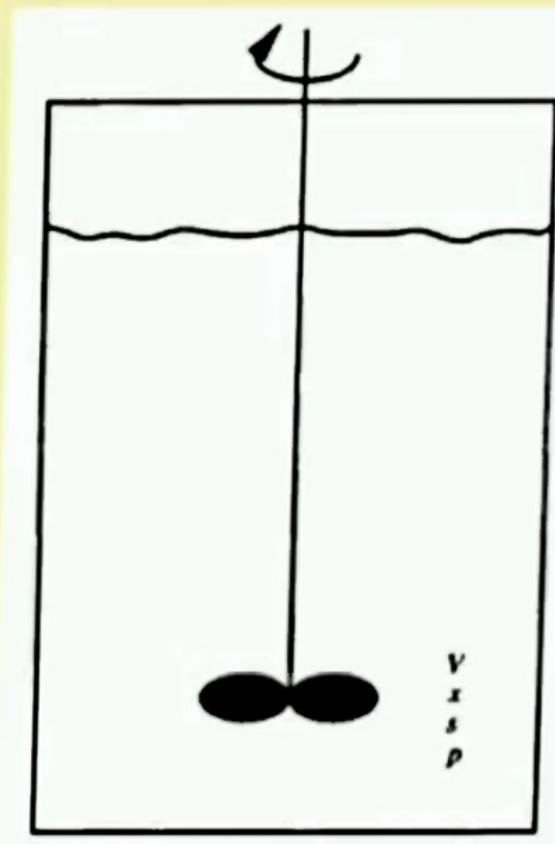
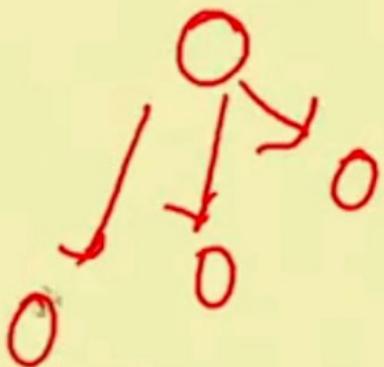
The minimum doubling time ($t_{d,mim}$) = $\frac{\ln 2}{\mu_{max}}$

Similarly for nth generation time (t_{gn}) (Generation Time)

$$\int_{X_0}^{X_n} \frac{dX}{X} = \int_0^{t_{gn}} \mu dt$$

$$\ln\left(\frac{X_n}{X_0}\right) = \mu(t_{gn})$$

$$t_{gn} = \frac{\ln\left(\frac{X_n}{X_0}\right)}{\mu}$$



Advantages and disadvantages of batch culture

Advantages	Disadvantages
✓ It is easier to set up and maintain	✓ Cannot hold the system in log phase for long duration
✓ Can be used to study the life cycle of the microbes	✓ Lower productivity
✓ Lower capital investment	✓ Requires high downtime for cleaning and sterilization
✓ Reduced risk of contamination or cell mutation as the growth period is short	✓ Safety problems when filling, emptying and cleaning
✓ Useful for the production of secondary metabolites	✓ Batch to batch variability

Models with cell growth inhibitors

- ✓ The inhibition pattern of microbial growth is analogous to enzyme inhibition.
- ✓ Often the **underlying mechanisms** are **complicated**
- ✓ The **kinetic constants** are obtained from **experimental data** by **curve fitting**.
- ✓ The inhibition can be of different types such as:
 - ❖ Substrate Inhibition
 - ❖ Product Inhibition
 - ❖ Inhibition by toxic compounds



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Substrate Inhibition

- ✓ At high substrate concentrations, microbial growth rate is inhibited by substrate.
- ✓ The substrate inhibition of growth may be competitive or non-competitive

- Non-competitive substrate inhibition: $\mu = \frac{\mu_{max}}{(1 + \frac{K_S}{S})(1 + \frac{S}{K_I})}$

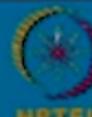
Or if K_I is $\gg K_s$, then $\mu = \frac{\mu_{max}S}{K_S + S + (\frac{S^2}{K_I})}$

- Competitive substrate inhibition: $\mu = \frac{\mu_{max}S}{K_S(1 + \frac{S}{K_I}) + S}$

- ✓ Substrate inhibition may be alleviated by slow, intermittent addition of substrate to the growth medium.



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Inhibition by toxic compounds

It is analogous to enzyme inhibition

Non-competitive inhibition: $\mu = \frac{\mu_{max}}{(1 + \frac{K_S}{S})(1 + \frac{I}{K_I})}$

Competitive inhibition:

$$\mu = \frac{\mu_{max}S}{K_S \left(1 + \frac{I}{K_I}\right) + S}$$

Uncompetitive inhibition:

$$\mu = \frac{\mu_{max}S}{\left(\frac{K_S}{(1 + \frac{I}{K_I})} + S\right)\left(I + \frac{I}{K_I}\right)}$$



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Logistic Equation

- ✓ It characterizes cell growth in terms of **carrying capacity** i.e. the maximum cell mass that can be obtained (X_m)
- ✓ The rate expression can be given as:

$$\frac{dX}{dt} = kX(1 - \frac{X}{X_m})$$

Where k is the logistic rate constant (h^{-1}), X_m is the maximum biomass concentration at the end (gl^{-1}) and X is the biomass concentration at any time (gl^{-1})

- ✓ The integral form of above equation can be given as:

$$X = \frac{X_0 e^{kt}}{\{1 - X_0/X_m\}(1 - e^{kt})}$$



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Logistic Equation vs. Monod Equation

- ✓ In **Monod kinetics**, microbial growth is related to **biomass concentration** and **limiting substrate concentration**
- ✓ The **logistic equation** is **independent of substrate concentration** and is only related to **biomass concentration**.
- ✓ In the logistic equation growth is **directly proportional** to **biomass concentration** and the **carrying capacity ($X_m - X$)**.

Growth models for filamentous organisms

- ✓ Filamentous organisms such as **molds** form **microbial pellets** at high cell densities in suspension culture
- ✓ Cells growing inside pellets are subjected to **diffusional limitations**
- ✓ In the absence of mass transfer limitations, the radius of pellet in the submerged culture increases linearly with time such as:

$$\frac{dR}{dt} = k_p \dots (1)$$

Where, R is the pellet radius.

Growth models for filamentous organisms

The biomass 'M' can be given as:

$$M = \rho \frac{4}{3} \pi R^3 \quad .. (2)$$

From Eq. (1) and (2), the growth rate can be expressed as:

$$\frac{dM}{dt} = \underbrace{\rho 4\pi R^2}_{\text{Surface Area}} \frac{dR}{dt} = k_p \rho 4\pi R^2$$

$$\text{Or, } \frac{dM}{dt} = \gamma M^{2/3}$$

$$\text{Where, } \gamma = k_p (36\pi\rho)^{1/3}$$

Diagram of a cylinder with radius R and height l .

$$V = \frac{1}{3} \pi R^3$$

~~Volume~~ mass $\propto l$



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Production kinetics in cell culture

Luedeking - Piret Model

It combines growth associated and non-growth associated product formation

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X$$
$$\frac{1}{X} \frac{dP}{dt} = \alpha \frac{1}{X} \frac{dX}{dt} + \beta$$
$$\frac{dp}{dt} = \alpha \mu + \beta$$

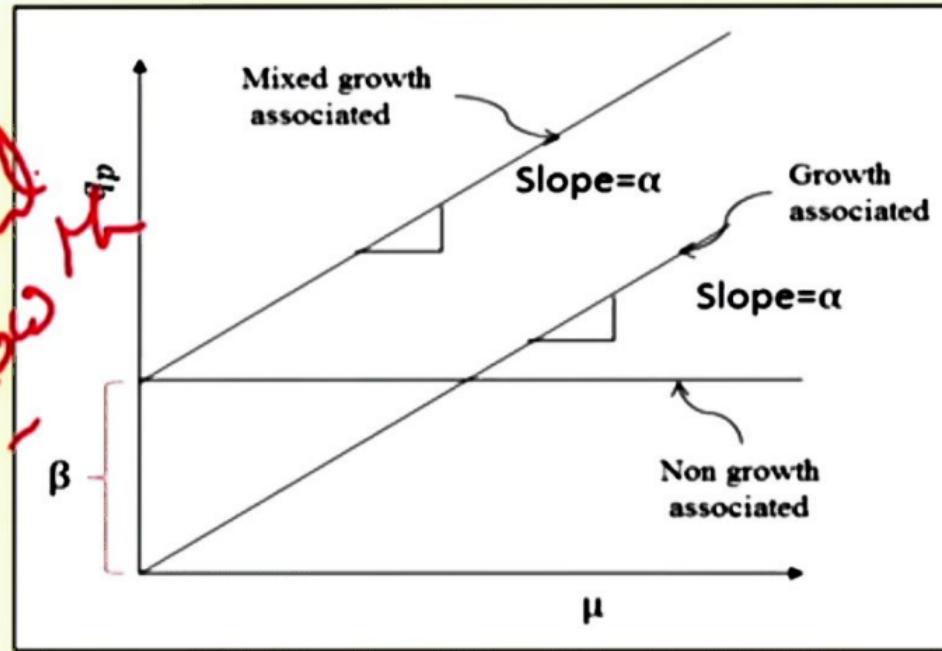
$\frac{dp}{dt}$ = rate of product formation

$\frac{dX}{dt}$ = biomass growth rate

$\alpha \frac{dp}{dt}$ → growth associated product formation

βX → non growth associated product formation

*J = growth associated
↓ = non growth*



α and β are Luedeking-piret constant

- $\alpha = 0 \rightarrow$ non growth associated production
- $\beta = 0 \rightarrow$ growth associated production
- $\alpha \neq 0, \beta \neq 0 \rightarrow$ mixed growth associated production

Continuous culture



- ✓ Usually performed in CSTR/Chemostat or PFR
- ✓ Fresh medium is continuously introduced at a constant rate
- ✓ The culture volume is kept constant by continuous removal of culture at the same rate,
- ✓ supply of a single nutrient controls growth rate (Limiting Substrate).
- ✓ the dilution rate (that is, the rate of addition of fresh medium) determines the specific

$$\text{growth rate of the culture } (D = \frac{F}{V})$$

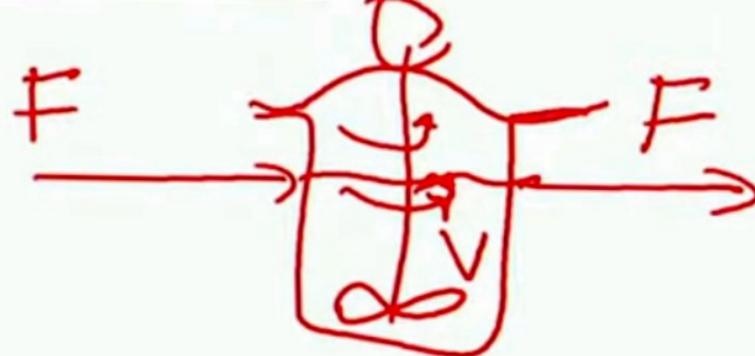
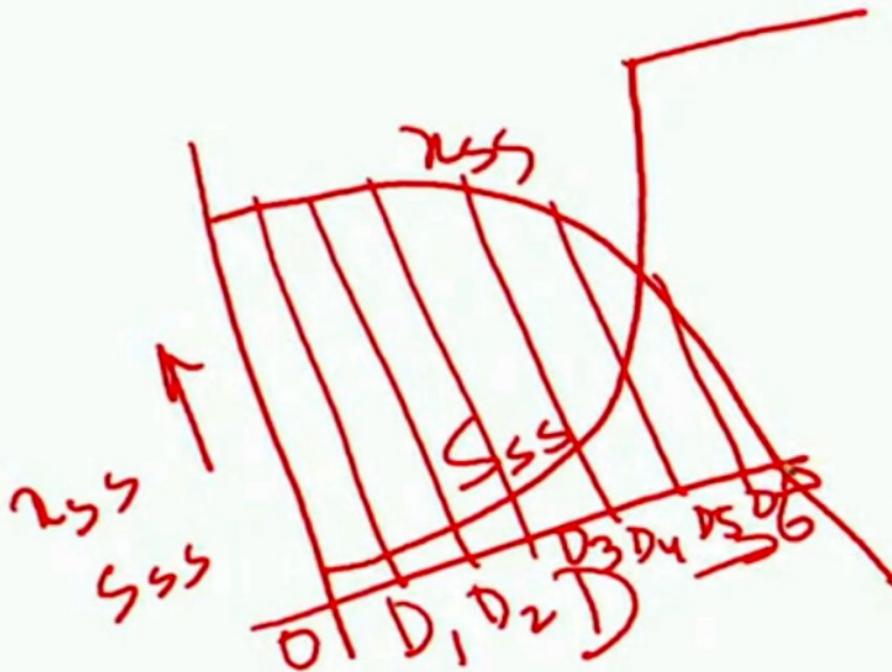


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V = Working liquid

$$\frac{F}{V} = \frac{\text{Vol}}{\text{Time}} = \text{time}$$

$= \text{Dilution rate}$

generation time for
time per division
= Cell division

$$\frac{1}{D} = HRT$$

H = hydraulic
 R = Retention

Advantages and disadvantages of Chemostat

Advantages:

- ✓ Log phase can be operated for the infinite period of time.
- ✓ Effect of growth limiting substrate on the cell growth and morphology of the cells can be easily monitored.
- ✓ Several plant metabolites produced during the transition of phases which can be operated very easily in a chemostat
- ✓ Results obtained are reliable and reproducible

Disadvantages :

- ✓ Cell washout problem
- ✓ Growth over long periods can cause mutation or contamination



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Chemostat with cell mass recycle

- ✓ Chemostat recycle is performed to keep the cell concentration higher than the normal steady-state level in a chemostat
- ✓ Cell recycle increases the rate of conversion (or productivity)
- ✓ Increases critical dilution rate for washout thereby increases operating flexibility
- ✓ Can be performed using a centrifuge or settling tank to concentrate biomass leaving the reactor.

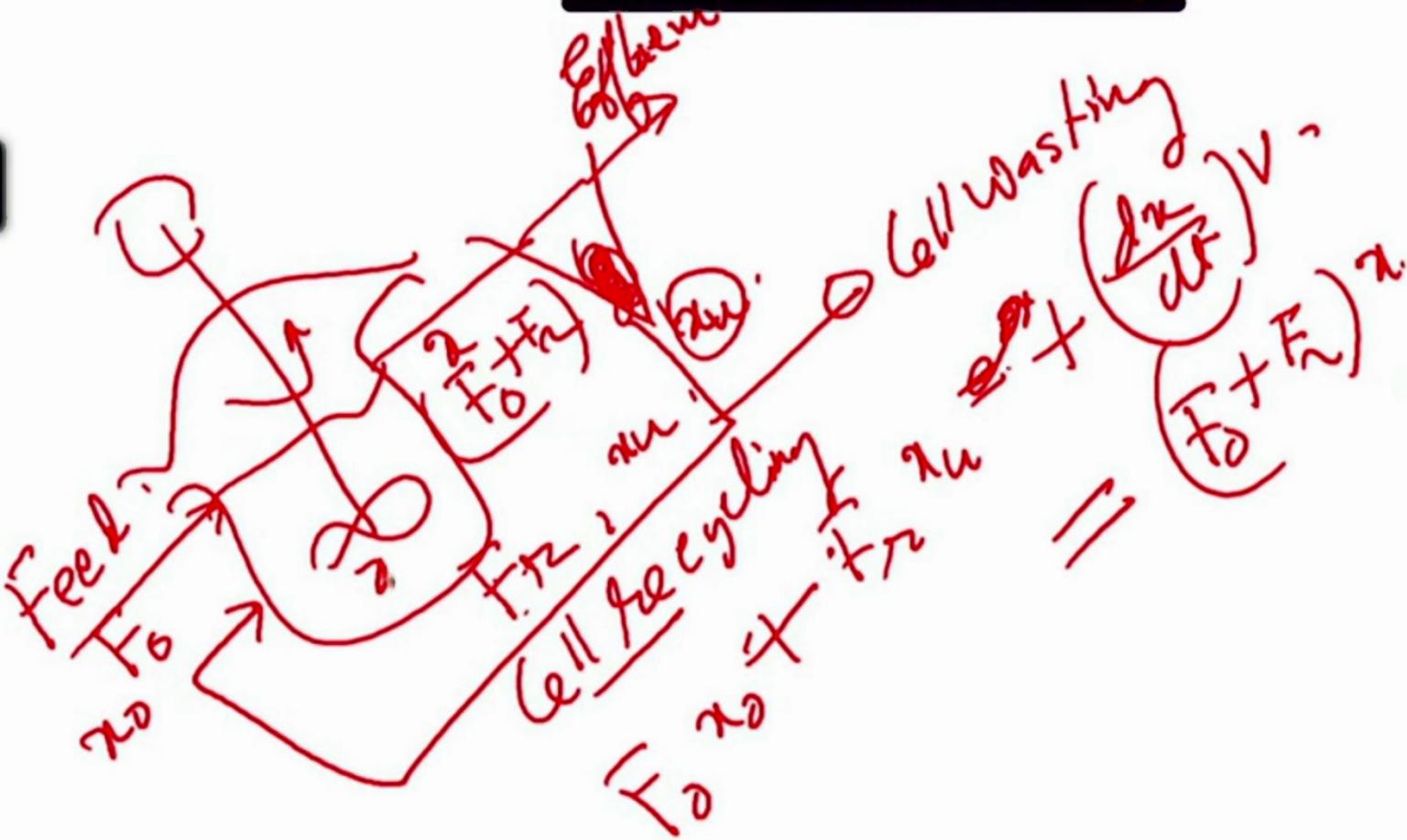


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Chemostat with cell mass recycle

The figure shows schematic of a chemostat operated in recycle mode where:

F_0 : Input Feed flow rate

S_0 : Initial substrate concentration

X_0 : Initial cell mass concentration

F_a : Output Feed flow rate

S : Output substrate concentration

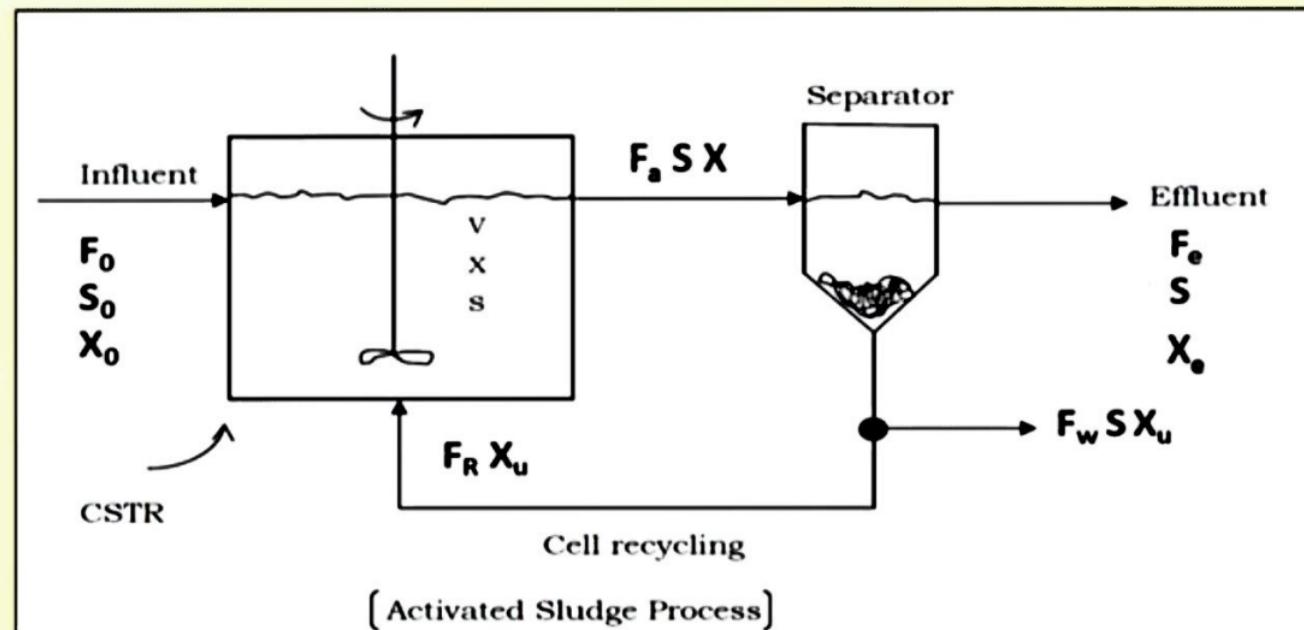
X : Steady state cell mass concentration

F_R : Recycle Feed flow rate

X_u : Recycle cell mass concentration

F_e : Effluent Feed flow rate

X_e : Effluent cell mass concentration



Chemostat with cell mass recycle

At steady state, the cell mass balance across the chemostat can be given as:

Input + Generation = Output + Accumulation + Cell death

$$(F_0 X_0 + F_R X_u) + V \cdot \frac{dX}{dt} = F_a \cdot X + 0 + 0 \dots (1)$$

Now, it should be noted that

$$\alpha = \frac{F_R}{F_0}; \text{ where } \alpha \text{ is the recycle ratio}$$

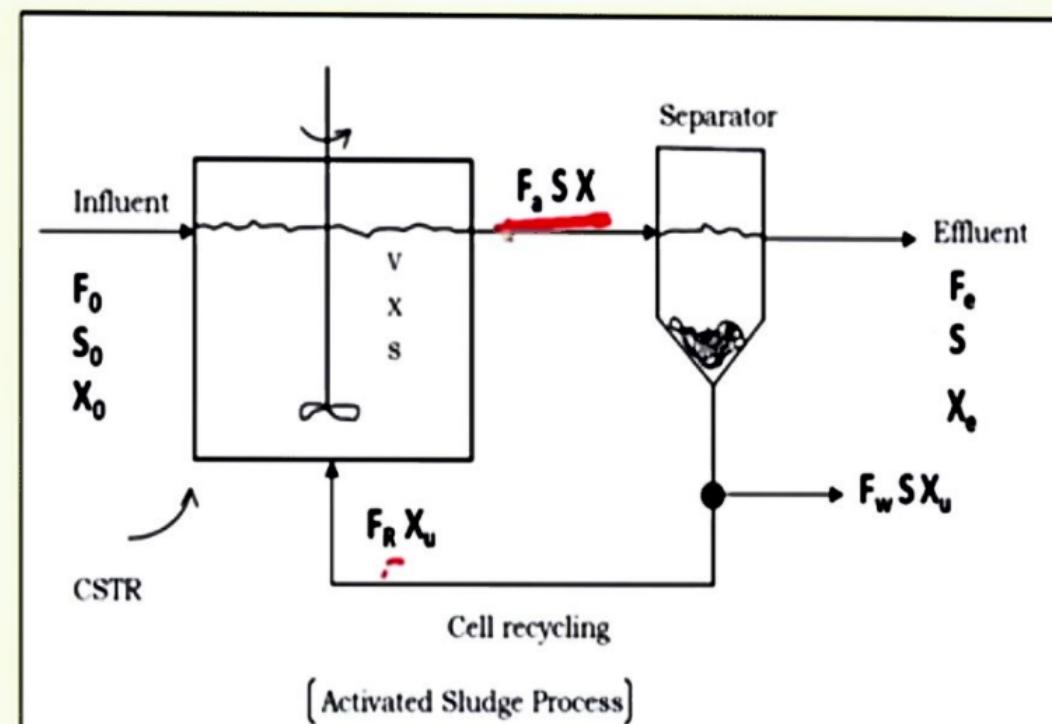
$$\text{So, } F_R = \alpha F_0 \dots (2)$$

$$\text{Also, } F_a = F_0 + F_R$$

$$\text{Therefore, } F_a = F_0 + \alpha F_0 \rightarrow F_a = F_0 (1 + \alpha) \dots (3)$$

From Eq. (2) and (3), Eq. (1) can be written as:

$$(F_0 X_0 + \alpha F_0 X_u) + V \cdot \frac{dX}{dt} = F_0 (1 + \alpha) \cdot X + 0 + 0 \dots (4)$$



Chemostat with cell mass recycle

At steady state, the cell mass balance across the chemostat can be given as:

$$\text{Input} + \text{Generation} = \text{Output} + \text{Accumulation} + \text{Cell death}$$

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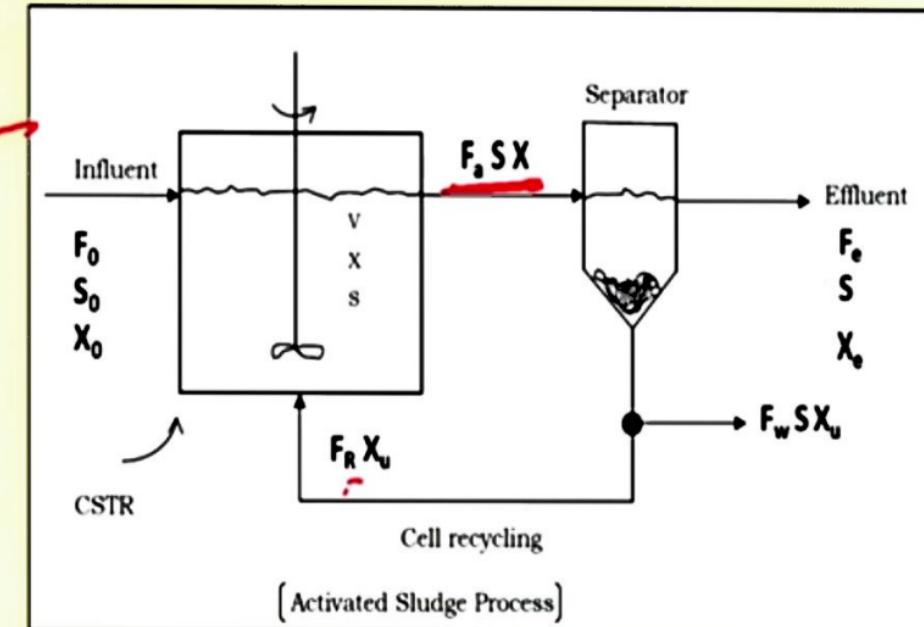
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Chemostat with cell mass recycle

For sterile feed $X_0 = 0$; So Eq. (4) becomes

$$\alpha F_0 X_u + V \cdot \mu X = F_0 (1 + \alpha) \cdot X \quad (\text{since } \frac{dX}{dt} = \mu X)$$

Dividing above equation by V , we get

$$\begin{aligned} \alpha \frac{F_0}{V} X_u + \mu X &= \frac{F_0}{V} (1 + \alpha) \cdot X \\ \alpha D X_u + \mu X &= D (1 + \alpha) \cdot X \quad (\text{Since } \frac{F_0}{V} = D) \quad \dots (5) \end{aligned}$$

Now, $C = \frac{X_u}{X}$ where C is the **concentration ratio**.

So, $X_u = CX$; putting in Eq. (5) we get

$$\alpha DCX + \mu X = D (1 + \alpha) \cdot X$$

By rearranging we get; $\mu = D [1 + \alpha(1 - C)] \quad \dots (6)$



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Chemostat with cell mass recycle

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$$\alpha F_0 X_u + V \cdot \mu X = F_0 (1 + \alpha) \cdot X \quad (\text{since } \frac{dX}{dt} = \mu X)$$

$$L = \frac{F_u}{F_0} L$$

Dividing above equation by V, we get

$$\alpha \frac{F_0}{V} X_u + \mu X = \frac{F_0}{V} (1 + \alpha) \cdot X$$

$$\alpha D X_u + \mu X = D (1 + \alpha) \cdot X \quad (\text{Since } \frac{F_0}{V} = D) \quad \dots (5)$$

$$\begin{aligned} C &= \frac{X_u}{X} \\ \mu &= \frac{\mu}{D} = 1 + \alpha (1 - C) \end{aligned}$$

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$$\alpha D C X + \mu X = D (1 + \alpha) \cdot X$$

By rearranging we get;

$$\mu = D [1 + \alpha(1 - C)] \quad \dots (6)$$

$$\begin{aligned} \mu &= 0.2 \\ D &= 1 = u \\ D &= \frac{1}{0.25} \\ \mu &= D \cancel{\times u} \end{aligned}$$



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Chemostat with cell mass recycle

For sterile feed $X_0 = 0$; So Eq. (4) becomes

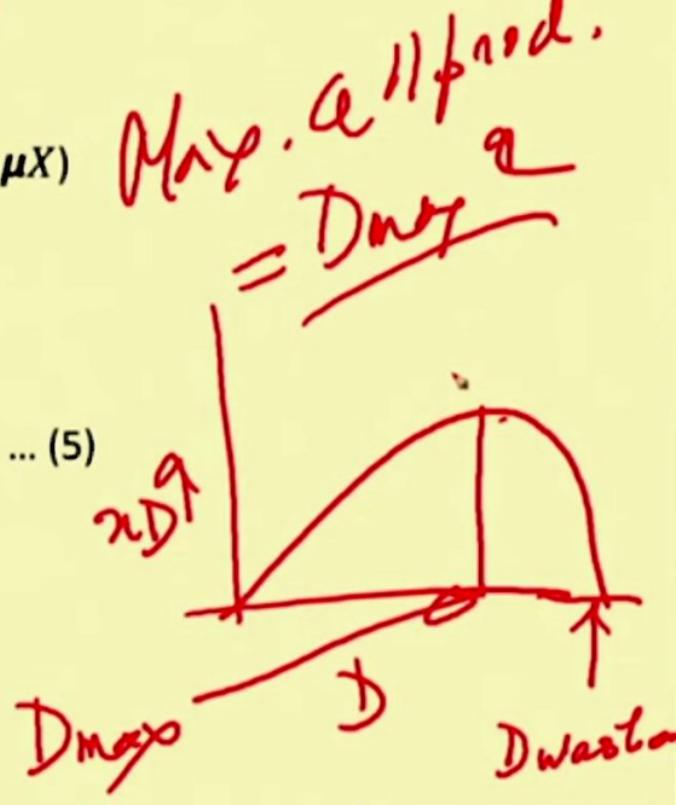
$$\alpha F_0 X_u + V \cdot \mu X = F_0 (1 + \alpha) \cdot X \quad (\text{since } \frac{dX}{dt} = \mu X)$$

May. a 11th std.
= D_{max}

Dividing above equation by V, we get

$$\alpha \frac{F_0}{V} X_u + \mu X = \frac{F_0}{V} (1 + \alpha) \cdot X$$

$$\alpha D X_u + \mu X = D (1 + \alpha) \cdot X \quad (\text{Since } \frac{F_0}{V} = D) \quad \dots (5)$$



Now, $C = \frac{X_u}{X}$ where C is the **concentration ratio**.

So, $X_u = CX$; putting in Eq. (5) we get

$$\alpha DCX + \mu X = D (1 + \alpha) \cdot X$$

$$\mu = D [1 + \alpha(1 - C)] \quad \dots (6)$$

By rearranging we get;



Chemostat with cell mass recycle

At steady state, the substrate mass balance across the chemostat can be given as:

Input + Generation = Output + Consumption + Accumulation

$$(F_0 S_0 + \cancel{F_R S}) + 0 = F_a \cdot S + V \cdot \frac{dS}{dt} + 0 \dots (7)$$

From Eq. (2) and (3), Eq. (1) can be written as:

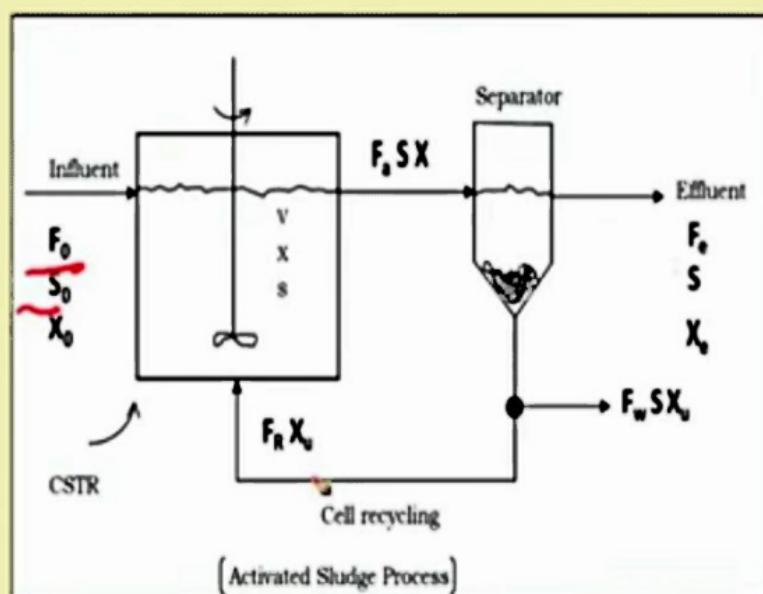
$$F_0 S_0 + \alpha F_0 S = F_0 (1 + \alpha) \cdot S + V \cdot \left(\frac{dS}{dX} \frac{dX}{dt} \right)$$

$$F_0 S_0 + \cancel{\alpha F_0 S} - F_0 S - \cancel{\alpha F_0 S} = V \cdot \left(\frac{1}{Y_{X/S}} \mu X \right) \quad (\text{Since } \frac{dX}{dS} = Y_{X/S}; \frac{dX}{dt} = \mu X)$$

$$F_0 (S_0 - S) = V \cdot \left(\frac{1}{Y_{X/S}} \mu X \right)$$

$$D(S_0 - S) = \frac{1}{Y_{X/S}} \mu X \quad (\text{Since } D = \frac{F_0}{V})$$

$$X = \frac{D(S_0 - S)}{\mu} \cdot Y_{X/S}$$



Chemostat with cell mass recycle

Putting value of μ From Eq. (6), we get

$$X = \frac{(S_0 - S)}{1 + \alpha(1 - C)} \cdot Y_{X/S} \quad \dots (8)$$

Thus, the biomass increases by a factor of $\frac{1}{1 + \alpha(1 - C)}$ as compared to chemostat without recycle.

The substrate concentration 'S' can be obtained by applying Monod kinetics to Eq. (6)

$$\frac{\mu_{max}S}{K_S + S} = D [1 + \alpha(1 - C)]$$

$$\frac{\mu_{max}}{D [1 + \alpha(1 - C)]} = \frac{K_S + S}{S}$$

$$S = \frac{K_S D [1 + \alpha(1 - C)]}{\mu_{max} - D [1 + \alpha(1 - C)]} \dots (9)$$

Putting S value in Eq. 8, we get: $X = \frac{Y_{X/S}}{[1 + \alpha(1 - C)]} [S_0 - \frac{K_S [1 + \alpha(1 - C)] D}{\mu_{max} - [1 + \alpha(1 - C)] D}]$



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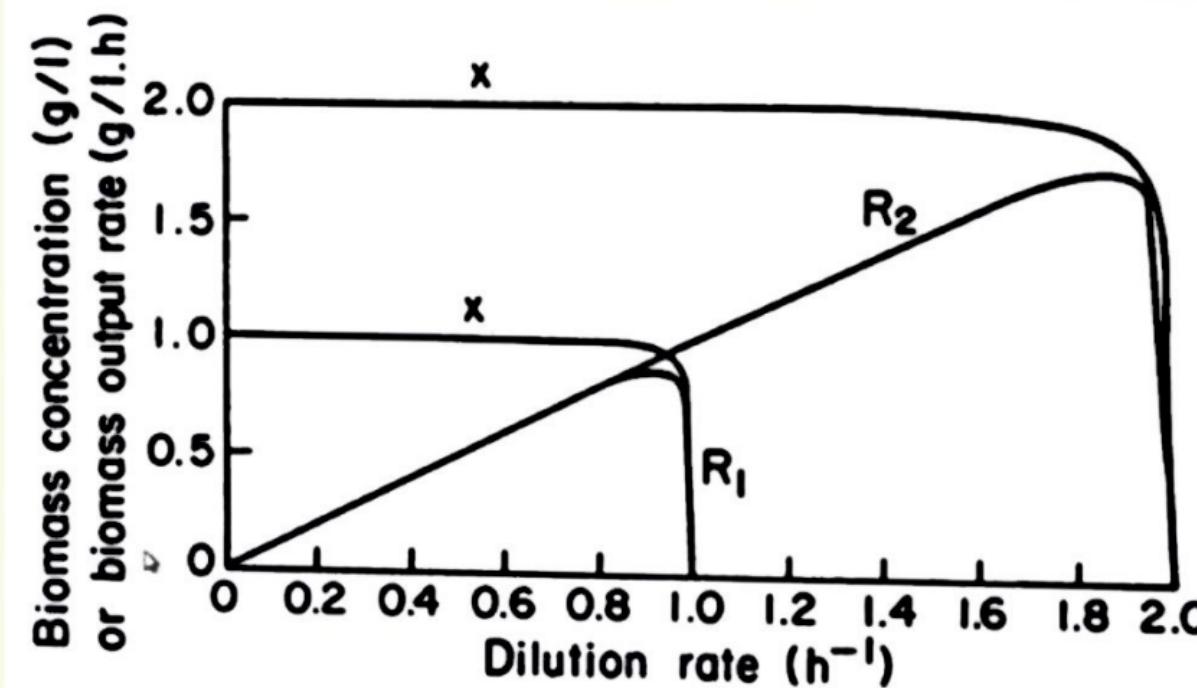


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Chemostat with cell mass recycle



R_1 - cell mass output rate per volume without recycle

R_2 - cell mass output rate per volume with recycle

Continuous operation using Plug-Flow Reactor

- ✓ Analysis of plug flow reactor for cell culture follows same procedure as for enzymatic reaction.
- ✓ Material balance for cell mass in small section (Δz) can be given as

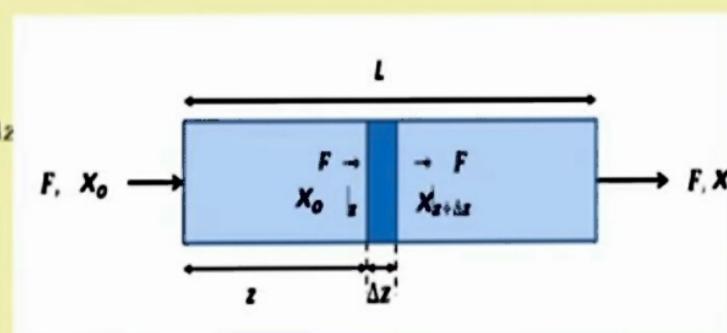
Input + generation = Output + Consumption + Accumulation

$$FX_{0z} + \mu X \cdot A\Delta z = FX_{z+\Delta z}$$
$$F(X_{z+\Delta z} - X_{0z}) = \mu X \cdot A\Delta z$$

$$u \frac{X_{z+\Delta z} - X_{0z}}{\Delta z} = \mu X \quad (u = \frac{F}{A}; \text{Superficial velocity})$$

Applying limit $z \rightarrow 0$ to above equation we get

$$u \left(\lim_{z \rightarrow 0} \frac{X_{z+\Delta z} - X_{0z}}{\Delta z} \right) = \mu X \quad \text{or, } u \left(\frac{dX}{dz} \right) = \mu X$$



A- Cross-sectional area of the reaction
F- Volumetric flow rate

$$dV = A\Delta z$$



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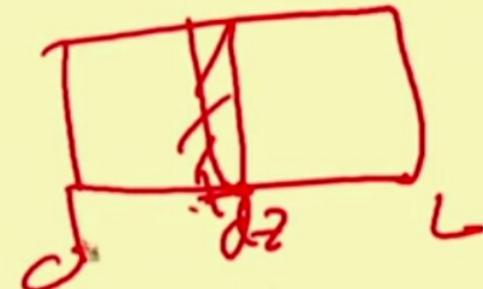
Continuous operation using Plug-Flow Reactor

Rearranging and integrating above equation we get

$$u \int_{X_0}^X \frac{dX}{X} = \mu \int_0^L dz$$

$$\ln \frac{X}{X_0} = \mu \cdot \frac{L}{u}$$

$$\text{So, } \ln \frac{X}{X_0} = \mu \frac{V/A}{F/A} = \ln \frac{X}{X_0} = \mu \tau$$



$$\text{Therefore, } \tau = \frac{1}{\mu} \ln \frac{X}{X_0}$$

The above equation suggests that $\tau_{PFR} = \tau_{Batch}$



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Drawbacks of Plug-Flow Reactor

- ✓ Not suitable for the growth of **suspended cells**
- ✓ Can be used using **recycle** or **immobilized cell reactions** however the inherent operational problems cannot be avoided
- ✓ Difficult to control due to **temperature and composition variations**
- ✓ PFR maintenance is also more **expensive**
- ✓ Rarely employed in **Industrial fermentations**



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Comparison between major modes of cultivation



- ✓ Kinetic characteristics of PFRs are same as the Batch reactors
- ✓ When large number of CSTRs are connected in series, the conversion characteristics approach those of Batch and PFRs
- ✓ Rates of conversion in chemostat operated at D_{max} are **10-20 times greater than PFR or Batch.**
- ✓ For most fermentations, CSTRs offer significant theoretical advantages over other nodes of reactor operation.
- ✓ However, despite the benefits of CSTR, the majority of commercial fermentations are conducted in Batch
- ✓ Batch fermentations have lower risk of contamination as compared to CSTR
- ✓ Equipment and control failures during long term operation are the associated problems with CSTR

Bioprocess Engineering Principles by Pauline M. Doran



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Whole cell immobilization

- Immobilization of whole cells is an alternative to enzyme immobilization and it is a well-developed method **for the utilization of enzyme (inside the cell) from microbes**
- Immobilization of whole cells become particularly effective when the **individual enzymes become inactive during direct immobilization, or the isolation and purification of enzyme is not cost effective**
- The greatest advantage of whole cell immobilization is that here **the enzymes inside the cell or whole cell will be active and stable for long period of time**

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- The greatest advantage of whole cell immobilization is that here the enzymes inside the cell or whole cell will be active and stable for long period of time

Advantages of whole cell immobilization

- Multiple enzymes can be introduced to a single step
- Extraction and purification of cells are not required
- Cells are stable for long time
- Cost effective method
- The immobilized whole cell reactor can be operated at a dilution rate that is higher than the maximum specific growth rate of the microorganism



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Purpose of whole cell immobilization

- ✓ Increase the volumetric productivity
- ✓ Increase the product concentration in the outlet stream
- ✓ Decrease the substrate concentration in the outlet stream.



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Methods of whole cell immobilization

- Methods of whole cell immobilization are same as that described for the enzyme immobilization and they include

- Adsorption
- Covalent bonding
- Cell to cell cross linking
- Encapsulation
- Entrapment



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Immobilization of whole cell

Method	Support Material	Cells	Reaction
Adsorption	Gelatin	<i>Lactobacilli</i>	Lactose \Rightarrow lactic acid
	Porous glass	<i>Saccharomyces</i>	Glucose \Rightarrow ethanol
	Cotton fibers	<i>Zymomonas</i>	Glucose \Rightarrow ethanol
	DEAE Cellulose	<i>Nocardia</i>	Steroid conversion
Covalent bonding	Cellulose + cyanuric chloride	<i>S. cerevisiae</i>	Glucose \Rightarrow ethanol
	Titanium oxide	<i>Acetobacter</i>	Vinegar
Cross linking	Glutaraldehyde	<i>E. coli</i>	Fumaric acid
Entrapment	Aluminium alginate	<i>Candida tropicalis</i>	Phenol degradation
	Calcium alginate	<i>S. cerevisiae</i>	Glucose \Rightarrow ethanol
Encapsulation	Polyester	<i>Streptomyces sps.</i>	Glucose \Rightarrow fructose
	Alginate polylysine	Hybridoma cells	Monoclonal antibodies

<http://www.easybiologyclass.com/enzyme-cell-immobilization-techniques>



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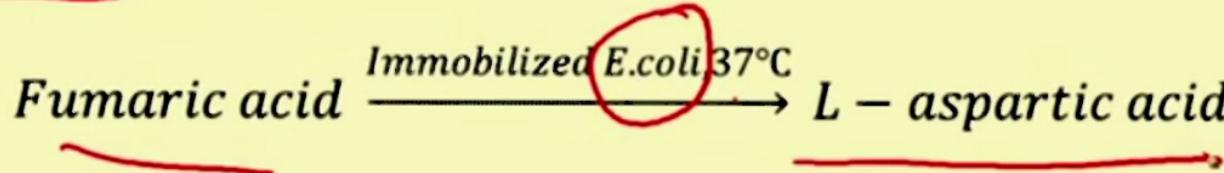


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Industrial application of Immobilized whole cell

Amino acid synthesis



- 95 % conversion of Fumaric acid was observed at a flow rate of 0.8 mL/mL bed vol/h.
- Cell is usually entrapped in polyacrylamide gel



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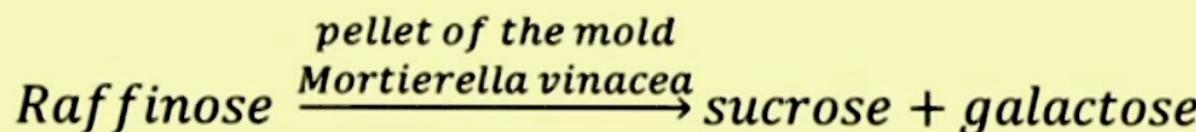


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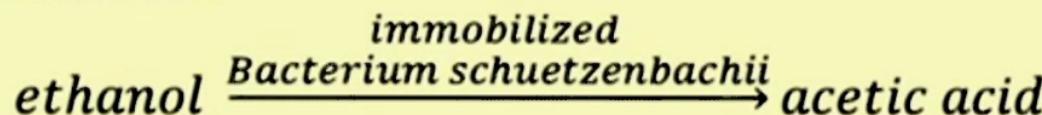
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Industrial application of Immobilized whole cell

Carbohydrate transformation



Organic acid production



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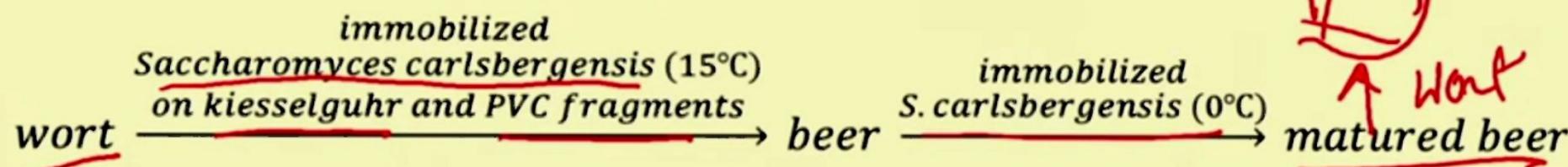
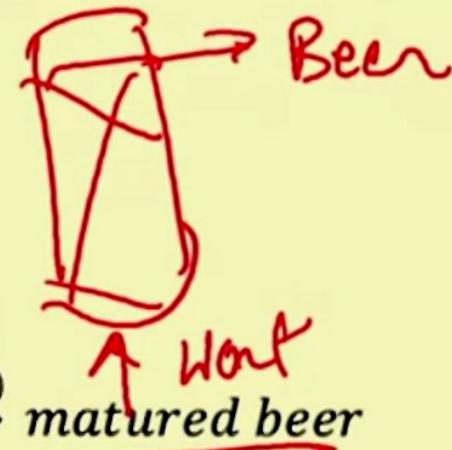


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Industrial application of Immobilized whole cell



- ✓ The support material was packed into a column 2 m long and 0.2 m in diameter.
- ✓ The wort was passed through the column at 3 L/h.
- ✓ The column operated 3 months without contamination



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Activity of immobilized whole cells

- ✓ Expressed in two ways
 - **Relative activity (r_1):** comparing the activity of immobilized cell with the same number of free cells
 - **Absolute specific activity (r_2):** the of reaction based on unit weight or unit volume of the whole catalyst.



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Activity of immobilized whole cells



- ✓ Adsorption and entrapment methods give r_1 value close to 100%, while the r_2 value in the case of the former is less because of limits in cell loading



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Spore immobilization

- ✓ For the filamentous fungi, homogeneous immobilization is difficult without breakage of the hyphae.
- ✓ Fragmentation of mycelia again cause reduced activity
- ✓ Commercial plants exploiting spore immobilization techniques are in operation for steroid biotransformation by *Curvularia*, *Fusarium* etc.



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$$F / A_S = q_{max} \times S / [(K_S + S) (S_o - S)]$$

Where F is the volumetric flow rate of wastewater

$$A_S = F [(K_S + S) (S_o - S)] / q_{max} \times S$$

Total surface area of 'n' number of discs

$$A_S = F [(K_S + S_i) (S_{i-1} - S_i)] / q_{max} \times S_i$$

Where I = 1, 2, 3, , n



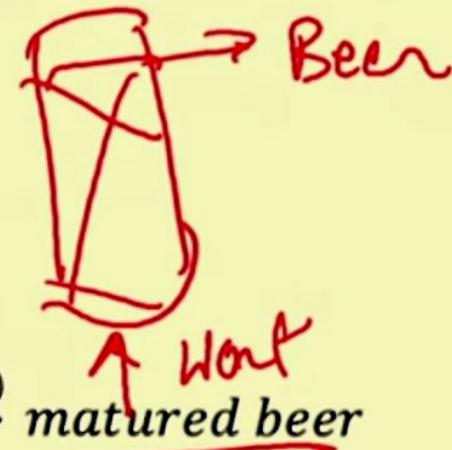
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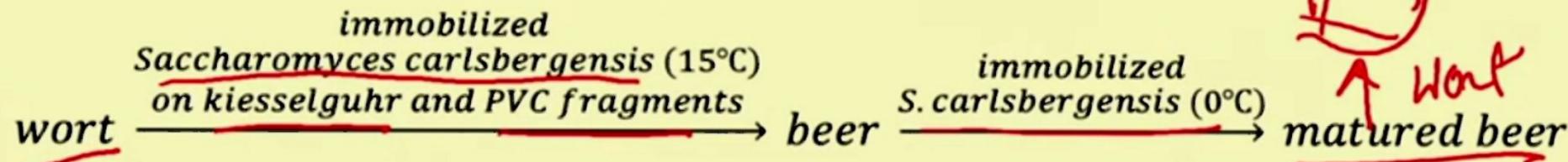
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Industrial application of Immobilized whole cell



Other product



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Factors affecting immobilization

✓ Cell matrix interaction

- When using reticulated polyurethane foam, in order to any immobilized cells to function well, the volumetric fraction of the foam has to be sufficient enough for all the cells and the reticulated pores of the foam is large enough to contain the cells.



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Factors affecting immobilization

✓ Light

- Metabolism can be affected by periodic exposure to light, and the quality and intensity of the light are significant.
- Only the outer cell layers of the cultures in the immobilized matrix may receive some light.
- This may be advantageous in the case where some precursors are formed in light and some in dark condition
- The supply of light to the interior of the immobilized cell matrix may be possible by the use of optical fibers



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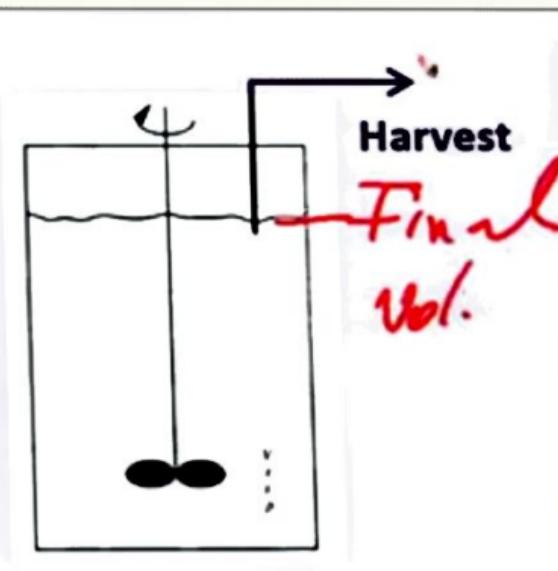
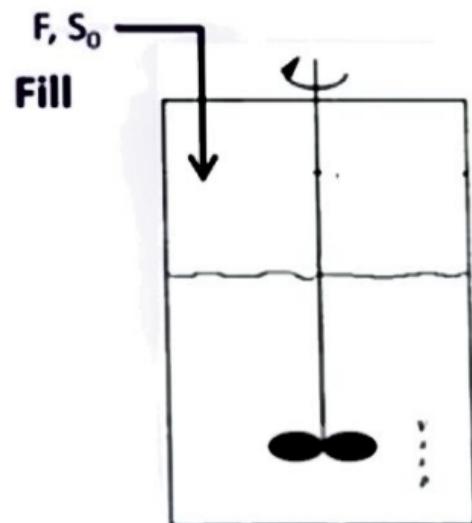
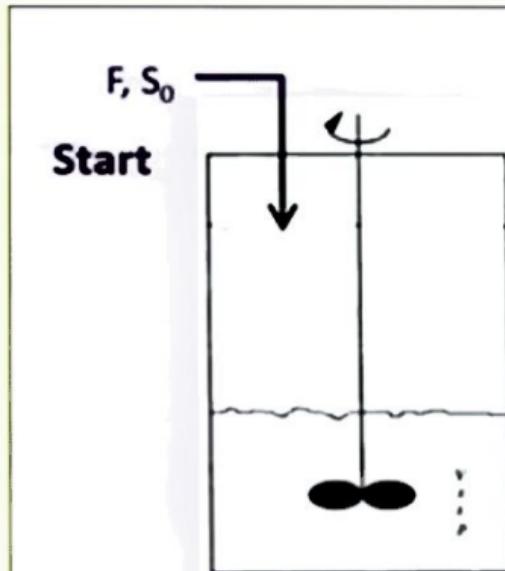
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Kinetics of Fed batch cell growth

Variable volume fed batch

In this system, a solution of **limiting substrate** at the **same concentration** as that in the initial medium is added resulting in an **increase in volume**.



Kinetics of Fed batch cell growth

Variable volume fed batch

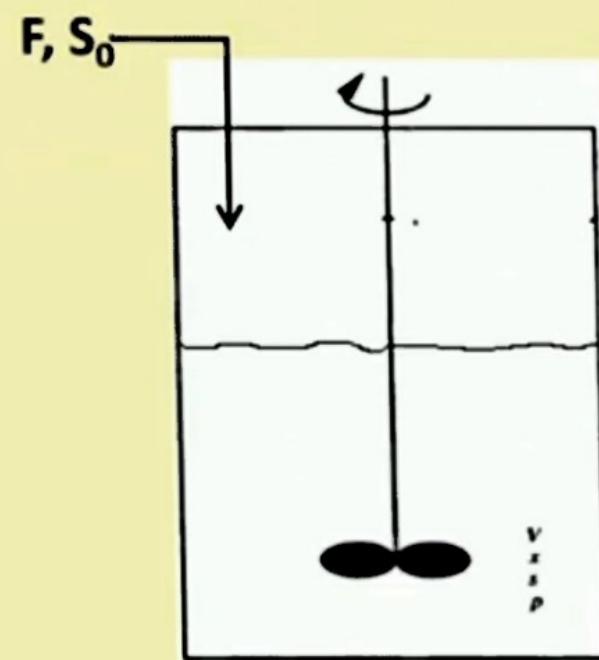
As the substrate is added continuously at a constant flow rate F , the rate of change in volume can be given as:

$$\frac{dV}{dt} = F \quad \dots (1)$$

Rearranging and integrating above equation we get,

$$\int_{V_0}^V dV = \int_0^t F dt \rightarrow V = V_0 + Ft \quad \dots (2)$$

Where, V is the volume of the reactor at time t and V_0 is the initial volume of the reactor (time $t = 0$)



Kinetics of Fed batch cell growth

Variable volume fed batch

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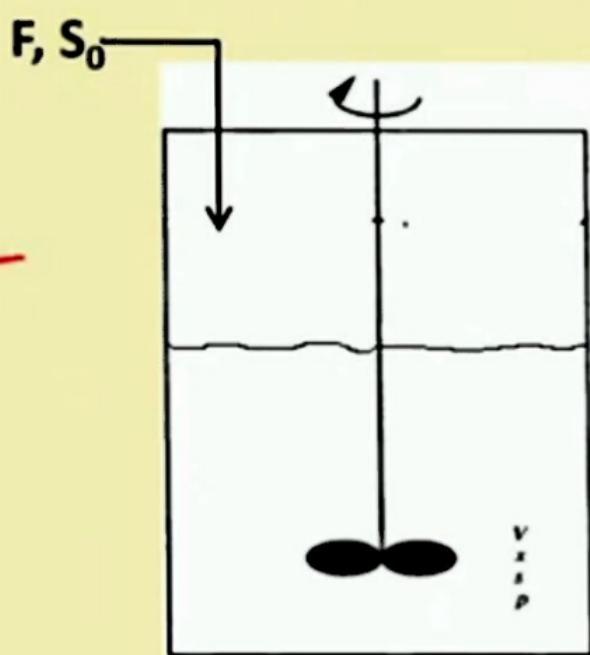
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Where, V is the volume of the reactor at time t and V_0 is the initial volume of the reactor (time $t = 0$)

$$F = \frac{V_0}{\text{Time}}$$



Kinetics of Fed batch cell growth

Variable volume fed batch

At quasi steady state, $S_{\text{added}} = S_{\text{consumed}}$ and X is constant

The cell mass balance can be given as

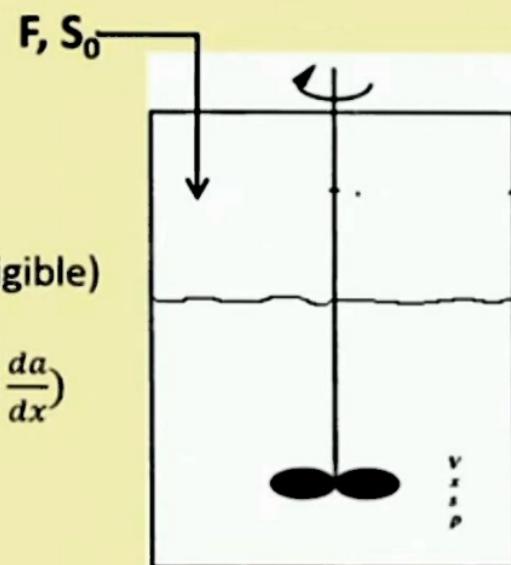
Input + cell generation = Output + accumulation + cell death

$$FX_0 + \mu X V = 0 + \frac{dXV}{dt} + 0 \quad (\text{Assuming cell death is negligible})$$

$$FX_0 + \mu X V = X \frac{dV}{dt} + V \frac{dX}{dt} \quad (\text{Since } \frac{d}{dx}(ab) = a \frac{db}{dx} + b \frac{da}{dx})$$

At steady state, $X_0 = 0; \frac{dX}{dt} = 0$

$$\text{Therefore, } \mu X V = X \frac{dV}{dt} \rightarrow \mu = \frac{1}{V} \frac{dV}{dt} = \frac{F}{V} \dots (3) \quad (\text{From Eq. 1})$$



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Kinetics of Fed batch cell growth

Variable volume fed batch

The flow rate (F) can be related to the volume (V) by dilution rate (D) as:

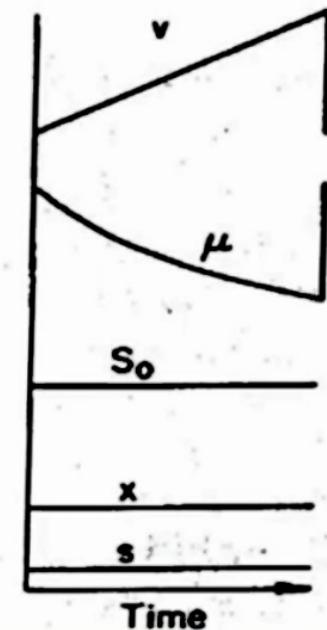
$$D = \frac{F}{V} \dots (4)$$

Thus, From eq. (2), (3) and (4)

$$\mu = D = \frac{F}{V_0 + Ft} \dots (5)$$

Applying Monod Kinetics, $\mu = D = \frac{\mu_{max}S}{K_s + S} = \frac{F}{V_0 + Ft}$

By rearranging, $S = \frac{K_s D}{\mu_{max} - D}$



Behavior of X, S, V and μ over time

P.F. Stanbury, A. Whittaker and S.J. Hall. Principles of Fermentation technology



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Kinetics of Fed batch cell growth

Variable volume fed batch

Now, the biomass concentration at time t can be given as:

$$X = \frac{X_t}{V}, \text{ where } X_t \text{ is the total biomass concentration}$$

At quasi steady state, $\frac{dx}{dt} = 0$ i.e. $\frac{d(\frac{X_t}{V})}{dt} = 0$

$$\frac{V\left(\frac{dX_t}{dt}\right) - X_t\left(\frac{dV}{dt}\right)}{V^2} = 0 \quad (\text{Since } \frac{d}{dx}(a/b) = \frac{b\frac{da}{dx} - a\frac{db}{dx}}{b^2})$$

$$\frac{dX_t}{dt} = \frac{X_t}{V} \frac{dV}{dt} = FX \quad \dots (6)$$



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Kinetics of Fed batch cell growth

Variable volume fed batch

The total biomass concentration (X_t) can be given as :

$$X_t = X_0 + (X_t - X_0)$$

$$X_t = X_0 + Y_{X/S}(S_0 - S) \quad (\text{Since } Y_{X/S} = \frac{X_t - X_0}{S_0 - S})$$

Now, when $S = 0$, and $X_0 \ll X_t$; the above equation can be written as

$$X_t = Y_{X/S} S_0 \dots (7)$$

From Eq. (6) and (7);

$$\frac{dX_t}{dt} = FY_{X/S} S_0$$

Integrating above equation we get $\int_{X_0}^{X_t} dX = FY_{X/S} S_0 \int_0^t dt \rightarrow X_t = X_0 + FY_{X/S} S_0 t$

It can be seen that at $t = 0$, $X_t = X_0$



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$$\frac{dX_t}{dt} = \frac{X_t}{V} \frac{dV}{dt} = FX \quad \dots (6)$$

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Variable volume fed batch

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$$X_t = X_0 + (X_t - X_0)$$

$$X_t = X_0 + Y_{X/S} (S_0 - S) \quad (\text{Since } Y_{X_t/S} = \frac{X_t - X_0}{S_0 - S})$$

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$$X_t = Y_{X/S} S_0 \dots (7)$$

From Eq. (6) and (7);

$$\frac{dX_t}{dt} = F Y_{X/S} S_0$$

Integrating above equation we get $\int_{X_0}^{X_t} dX = F Y_{X/S} S_0 \int_0^t dt \rightarrow X_t = X_0 + F Y_{X/S} S_0 t$

It can be seen that at $t = 0$, $X_t = X_0$



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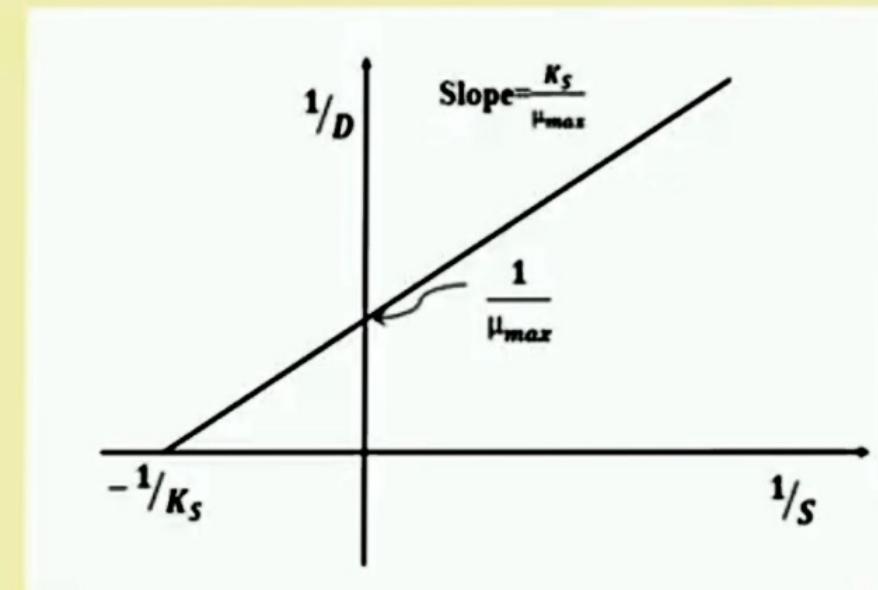


Kinetics of Fed batch cell growth

Variable volume fed batch

The kinetic parameters (μ_{max} and K_S) can be estimated by plotting $\frac{1}{D}$ vs. $\frac{1}{S}$ using Lineweaver-Burk Plot

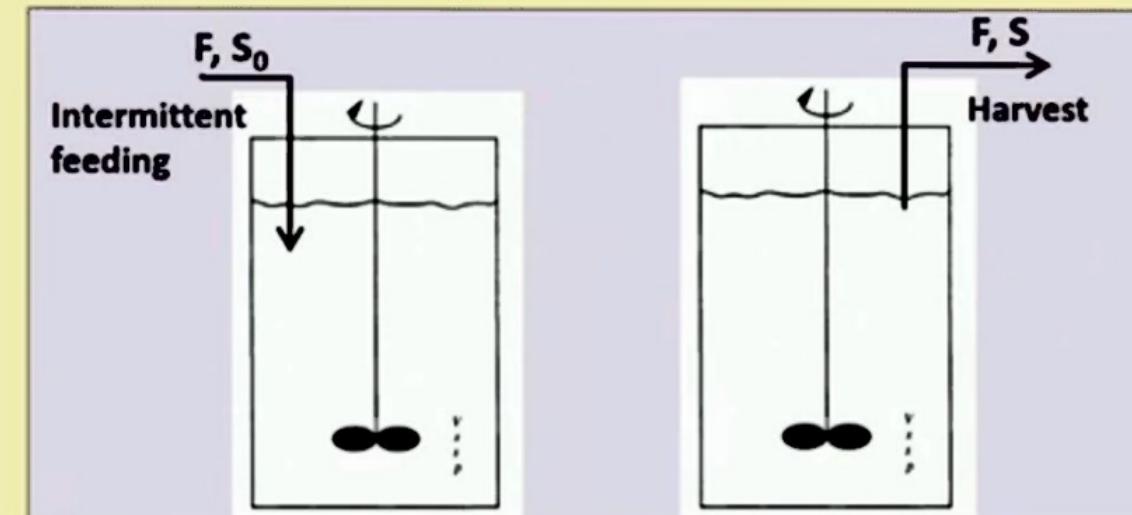
$$\frac{1}{D} = \frac{1}{\mu_{max}} + \frac{K_S}{\mu_{max}} \frac{1}{[S]}$$



Kinetics of Fed batch cell growth

Constant volume fed batch

In this system, a **very concentrated solution of the limiting substrate is added intermittently at very low flow rate** (lower than variable fed batch), resulting in **insignificant increase in volume**



Kinetics of Fed batch cell growth

Constant volume fed batch

Since the limiting substrate is added intermittently, the rate of change in cell mass is dependent on the flow rate such that:

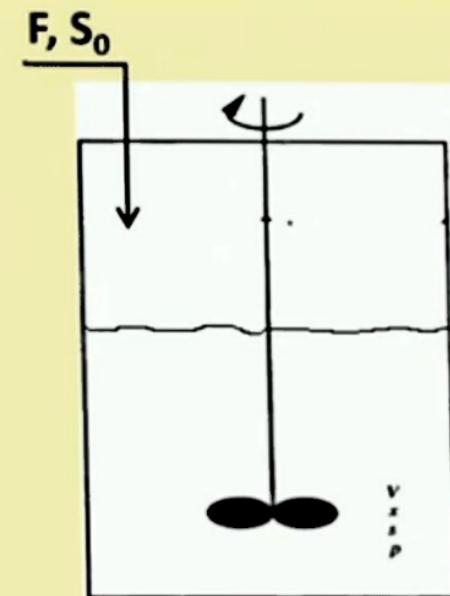
$$\frac{dx}{dt} = G \cdot \frac{dx}{ds} = G \cdot Y_{X/S} \dots (1)$$

Where G is substrate feed rate in g/L.h

The cell mass balance can be given as

Input + cell generation = Output + accumulation + cell death

$$FX_0 + \mu X V = 0 + \frac{dXV}{dt} + 0 \quad (\text{Assuming cell death is negligible})$$



Kinetics of Fed batch cell growth

Constant volume fed batch

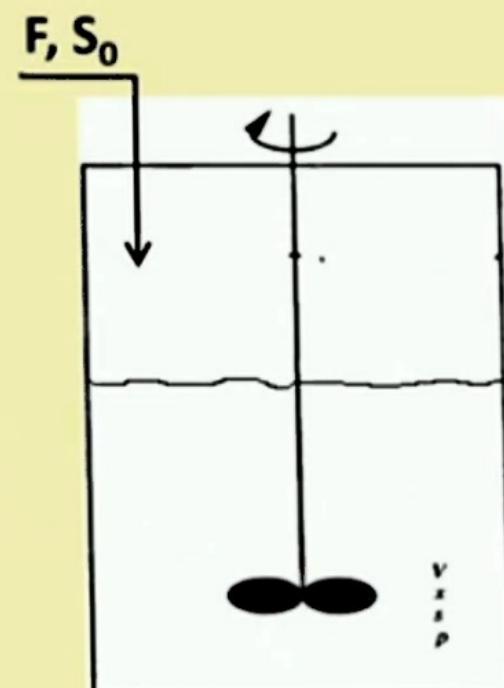
Since volume is constant, at $X_0 = 0$; the above equation can be written as

$$\mu X = \frac{dX}{dt} = G \cdot Y_{X/S} \dots (2) \text{ (From Eq. 1)}$$

Therefore, $\underline{\mu = \frac{1}{X} G \cdot Y_{X/S}} \dots (3)$

From the above equation, If $\frac{1}{X} G \cdot Y_{X/S}$ is less than μ_{max} , the limiting substrate is

consumed as soon as it enters the fermenter and thus $\frac{ds}{dt} = 0$



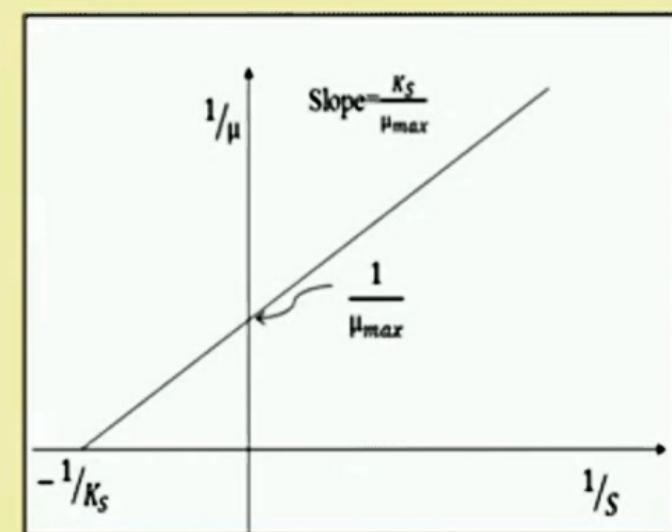
Kinetics of Fed batch growth

Constant volume fed batch

The kinetic parameters (μ_{max} and K_S) can be estimated by applying Monod kinetics in Eq. 3 and plotting $\frac{1}{\mu}$ vs. $\frac{1}{S}$ using Lineweaver-Burk Plot like in Batch system

$$\mu = \frac{\mu_{max} S}{K_s + S} = \frac{1}{X} G \cdot Y_{X/S}$$

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



Lineweaver-Burk plot

Advantages and disadvantages of Fed-batch culture

Advantages:

- ✓ **Higher yield**, resulting from a well-defined cultivation period
- ✓ Low level of limiting substrate concentration helps in **avoiding substrate inhibition**
- ✓ relieve **catabolite repression** when this is an issue in a production process
- ✓ avoid toxic effects of some medium components.

Disadvantages :

- ✓ **Lower productivity** levels due to **high downtime** for filling, heating, sterilization, cooling, emptying and cleaning the reactor.
- ✓ **Higher costs** in labour and/or dynamic process control for the process.



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