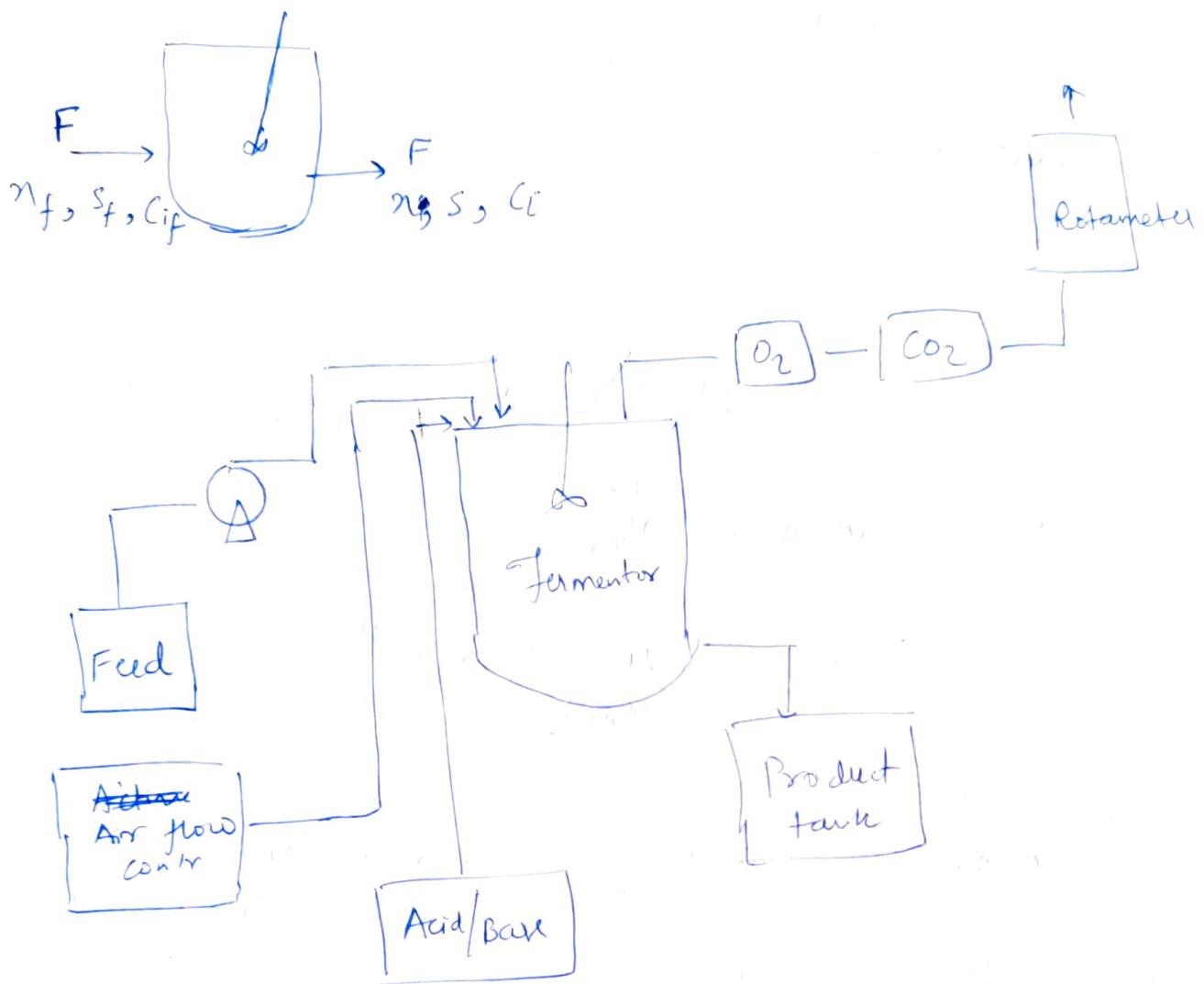


Continuous Stirred Tank Reactor



Mass balance -

$$F C_{if} - F c_i + V_R \dot{\gamma}_{fi} = \frac{d}{dt} (c_i V) \quad (\text{Steady state})$$

$$\Rightarrow \dot{\gamma}_{fi} = \frac{F}{V_R} (c_i - C_{if})$$

$$\Rightarrow \dot{\gamma}_{fi} = D (c_i - C_{if})$$

where $D = \frac{F}{V_R}$: dilution rate

Two operational parameters for continuous operation

1. Dilution rate, $D = \frac{F}{V_R}$ (unit - $\frac{1}{\text{Time}}$)

2. Average residence time, $\tau = \frac{V_R}{F} = \frac{1}{D}$ (time)

F - Volumetric flow rate (m^3/sec)

D : No. of liquid reactor volumes that pass through the reactor per unit time.

$$\dot{\eta}_f = \frac{dC_i}{dt} \quad \text{Batch reactor}$$

$$\dot{\eta}_f = D(C_i - C_{if}) \quad \text{CSTR}$$

relatively CSTR equations are easier to model however, Batch reactors are easier to implement i.e. perform experiments on.

Cell mass balance:

$$\frac{d(nV)}{dt} = F_{in}\eta_f - F_{out}\eta + (\mu - k_d)nV$$

Assumptions: we have sterile feed i.e. $\eta_f = 0$.

(i) Typically we have steady state

(steady state)

(ii) $F_{in} = F_{out}$

(steady state)

(iii) $\frac{d(nV)}{dt} = 0$

(steady state)

(iv) $k_d = 0$

$$\Rightarrow -Fa + \mu nV = 0$$

$$\Rightarrow \mu n - Dn = 0$$

$$\Rightarrow (\mu - D)n = 0 \rightarrow \begin{cases} n=0 \\ \mu=D \end{cases}$$

Non-zero cell mass can be obtained only for $\mu=D$.

Substrate mass balance.

$$\frac{d}{dt}(Sv) = F_{Sf} - F_S - \dot{S}_S v$$

$$Y_{XS} = \frac{\dot{S}_n}{\dot{S}_S} = \frac{\mu n}{\dot{S}_S}$$

$$\frac{d}{dt}(Sv) = F_{Sf} - F_S - \frac{\mu nv}{Y_{XS}}$$

— Directly coupled
no product formation.

$$\frac{d}{dt}(Sv) = F_{Sf} - F_S - \left(\frac{\mu}{Y_{XS}} + \frac{q_P}{Y_{PS}} + m_S \right) nv$$

— Indirectly coupled
with product formation.

~~Monad~~ Monod Kinetics

$$\mu = \frac{\mu_{max} s}{K_s + s}$$

$\mu=D$ (piche wall approximation use Kuro bro)

$$D = \frac{\mu_{\max} s}{k_8 + s}$$

$$k_8 + s = \frac{\mu_{\max} s}{D}$$

$$k_8 = \left(\frac{\mu_{\max} - D}{D} \right) s$$

$$s = \frac{DK_8}{\mu_{\max} - D}$$

For steady state

$$\frac{F(s_f - s)}{V} - \left(\frac{\mu}{Y_{XS}} + \frac{q_p}{Y_{PS}} + m_g \right) n = 0$$

$$n = \frac{D(s_f - s)}{\frac{D}{Y_{XS}} + \frac{q_p}{Y_{PS}} + m_g}$$

For no product and directly coupled mechanism -

$$n = \frac{D(s_f - s)}{D/Y_{XS}}$$

$$n = Y_{XS} (s_f - s)$$

$$n = \left(s_f - \frac{D k_8}{\mu_{\max} - D} \right) Y_{XS}$$

Product mass balance

$$\frac{d}{dt}(\rho v) = F_{Pi} - F_p + q_p(\rho v)$$

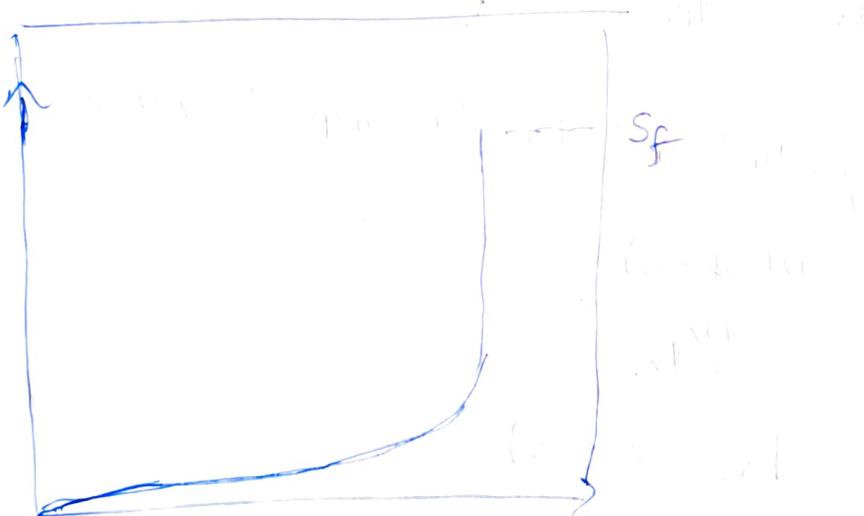
At steady state:

$$F_{Pi} - F_p + q_p(\rho v) = 0$$

$$\Rightarrow D(p_i - p) + q_p \cdot n = 0$$

Let's draw some graphs.

1. At low flow rate $F \rightarrow 0 \Rightarrow D \rightarrow 0$



$$D \rightarrow (1/\text{hr})$$

1) Chemostat with Immobilized cells 01/11/22
 Biomass balance

2) Substrate balance

Chemostat with immobilized cells -

At steady state, mass balance for substrate

$$F_{S_i} - F_S = \frac{\mu n_S}{Y_{XS}} V - \frac{n_T \mu n_{im}}{Y_{XS}} V = 0.$$

Dividing by V .

$$D(s_i - s) = \frac{\mu}{Y_{XS}} (n_S + n_T n_{im})$$

$$D = \mu \left(1 + \frac{n_T n_{im}}{n_S} \right)$$

$$\frac{\mu_{max S}}{K_S + s} = D$$

Chemostat with suspended cell only ($n_{im} = 0$)

At steady state $D = \mu$ and the max. op. dilution rate D_{crit} is limited by the maximum specific growth rate of the cells.

$$D_{crit} = \frac{\mu_{max} s_i}{K_S + s_i}$$

$$K_S < C s_i$$

$$D_{crit} \approx \mu_{max}$$

$$D = \mu \left(1 + \frac{n_I n_{im}}{n_s} \right)$$

$$\frac{n_I n_{im}}{n_s} > 0$$

$$\frac{\mu_{max} s}{K_s + s} = D(s_i - s)$$

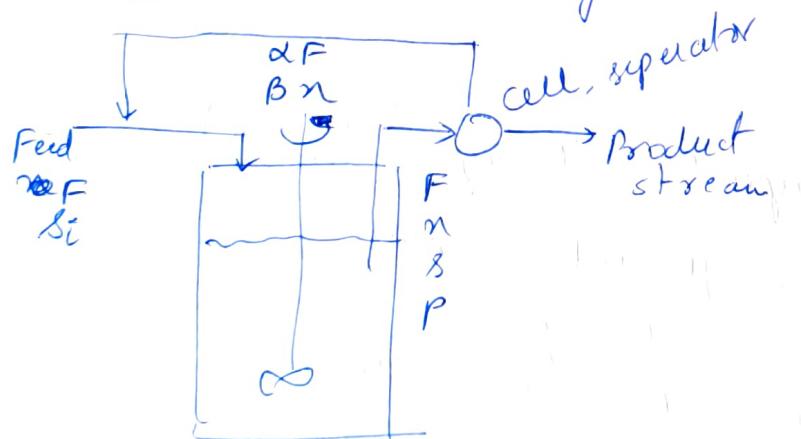
Two stage Chemostat with ~~recycle~~

$$\mu_2 = D_2 - D_1 \frac{x_1}{x_2} \frac{v_1}{v_2}$$

$$D_1 = \frac{F_1}{V_1}$$

$$D_2 = \frac{F_1 + F_2}{V_2}$$

Chemostat with recycle.



Fed-batch reactor -

Volm of soln is not const.

Volm balance : $F = \frac{dV}{dt}$

Biomass Balance : $\frac{d(nV)}{dt} = F n_i + \mu nV - k d nV$

$$n \frac{dV}{dt} + V \frac{dn}{dt} = F n_i$$

CLASS 2

Date 1/1/

End Sem

Saathi

- Steady state cell & substrate conc. as f. of diln. rate in a chemostat:

$$\rightarrow D = \text{diln. rate} = \frac{\text{Vol. flowrate}}{\text{Vol. of chemostat}}$$

- Critical D : feed rate so high that cells in the reactor get washed out \therefore substrate conc. in Chemostat same as inlet conc.

$$\rightarrow S_{\text{sterile feed}} = D K_s$$

$$X_{\text{sterile feed}} = Y_{XS} \left(S_f - \frac{D K_s}{Y_{\text{max}} - D} \right)$$

(Qx)

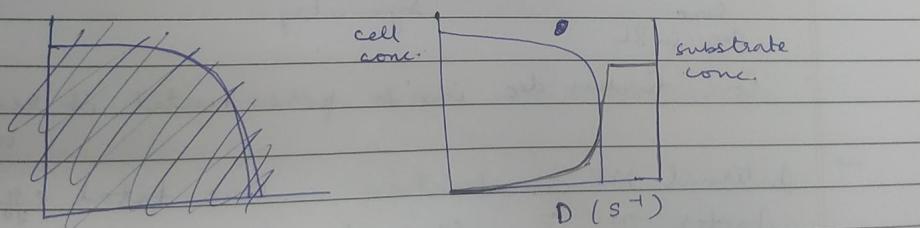
* Biomass productivity $= D \times \text{cell mass conc.}$

$$= \cancel{D} X \quad [D \uparrow \Rightarrow X \downarrow]$$

Inc \uparrow desired.

\therefore optimum]

\rightarrow



$$Q_x = D \left[S_f - \frac{D K_s}{Y_{\text{max}} - D} \right] Y_{XS}$$

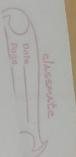
Find optimal D by setting $\frac{d Q_x}{d D} = 0$

$\boxed{\text{critical } D < \text{critical } D}$



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Page No.



Saathi

Date

M

- Date 1/1 D ref'd for optimal operation
 N. tight control on D \Rightarrow productivity ≈ 0
 \Rightarrow opt. D w.r.t. size to exit D \Rightarrow productivity ≈ 0

* Experimental validation of Monod's model \checkmark

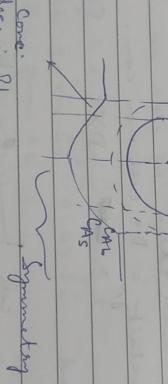
\Rightarrow Chemostat with immobilized cells

- Cells move out, we lose bioactivity \Rightarrow imp. to retain them.
- 2 types of cells:
- Immobilized cells retain suspended cells move out with perf. stream.

Segment

BL

CAB



core

dec in BL

Cone. further dec. inside pores until it reaches minimum

- \rightarrow Internal MT consideration: use total effectiveness factor $\eta_T = \frac{\text{Desired rate of conversion}}{\text{Rate of conversion if cone everywhere is CAB}}$

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Date / /

10th

Saath

Mass vol :

$$\text{Stressed} \rightarrow F_{ns} \rightarrow S_p$$

$$\text{Gol. Law. : } F_{pi} - F_{rs} + M \times s \sqrt{M \gamma_{min} v} = 0$$

(@ s_s is constant & independent of time)

Forwards and backwards : γ_{min} & v are constant.

$$\Rightarrow D \times s = M \times s + \gamma_T M \times v_{in}$$

$$= 4 C \times s + \gamma_T M \times v_{in}$$

$$\Rightarrow D \times s = M \times s \left[1 + \gamma_T \frac{v_{in}}{s} \right]$$

$$\therefore \gamma = 1 \quad \therefore M \times s = 4 C$$

$$\therefore D = M \left[1 + \gamma_T \frac{v_{in}}{s} \right] \quad \text{Ans}$$

For only unsheathed particles, $D = M$.

$$(v_{in}=0)$$

3. Unsheathed particles : $v_{in} = 0$ & $v_{out} = 0$

Unsheathed particles have zero velocity.

Sheathed particles have zero velocity.

Sheath voltage is applied to sheathed particles.

Page No.

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CLASS 3

A projector is mounted on a ceiling track, projecting a presentation slide onto a whiteboard.

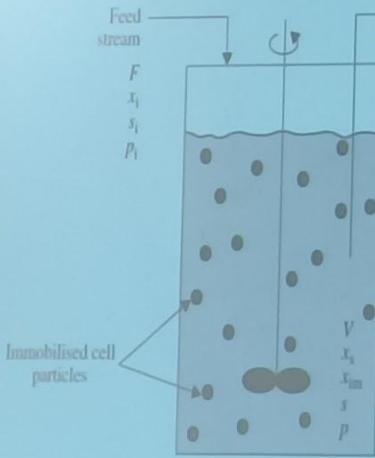
Chemostat with Immobilized Cells: Biomass Balance

x_i is zero for sterile feed and cell death is assumed to be negligible.

At steady state, the mass balance equation for cell:

$$-F x_s + \mu x_s V + \mu x_{im} V = 0$$

If diffusional limitations affect the growth rate of the immobilized cells, replace μx_{im} by $\eta_T \mu x_{im}$
 $(\eta_T = \text{effectiveness factor})$



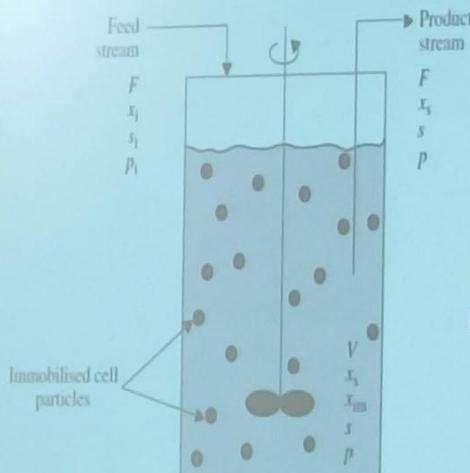
Dividing through by V and applying dilution rate $D = F/V$:

$$D x_s = \mu (x_s + \eta_T x_{im}) \quad D = \mu \left(1 + \frac{\eta_T x_{im}}{x_s} \right)$$

Chemostat with Immobilized Cells: Biomass Balance

x_f is zero for sterile feed and cell death is assumed to be negligible.

At steady state, the mass balance equation for cell



$$-Fx_s + \mu x_s V + \mu x_{im} V = 0$$

If diffusional limitations affect the growth rate of the immobilized cells, replace μx_{im} by $\eta_T \mu x_{im}$ (η_T = effectiveness factor)

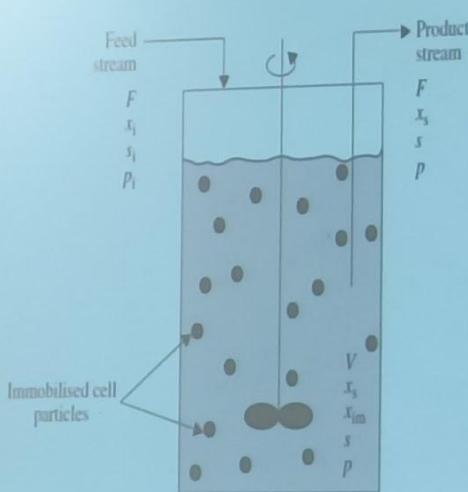
Dividing through by V and applying dilution rate $D = F/V$:

$$Dx_s = \mu (x_s + \eta_T x_{im}) \quad D = \mu \left(1 + \frac{\eta_T x_{im}}{x_s} \right)$$

Note: For $x_{im} = 0$, $\mu = D$.

Chemostat with Immobilized Cells: Substrate Balance

At steady state, the mass balance equation for substrate:
(Assuming same Y_{XS} for both cell populations)



$$F_{S_i} - F_S - \frac{\mu x_s}{Y_{XS}} V - \frac{\eta_T \mu x_{im}}{Y_{XS}} V = 0$$

Dividing through by V and applying dilution rate $D = F/V$:

$$D(s_i - s) = \frac{\mu}{Y_{XS}} (x_s + \eta_T x_{im})$$

Using $D = \mu \left(1 + \frac{\eta_T x_{im}}{x_s} \right)$ we can write:

$$\frac{\mu_{max} s}{K_S + s} = \frac{D(s_i - s) Y_{XS}}{(s_i - s) Y_{XS} + \eta_T x_{im}}$$

This equation relates steady-state substrate concentration s , dilution rate D , and immobilized cell concentration x_{im}

Chemostat with Immobilized Cells

Chemostat with Immobilized Cell:

$$D = \mu \left(1 + \frac{\eta_T x_{im}}{X_s} \right) > 0$$
$$\frac{\mu_{max} s}{K_S + s} = \frac{D(s_i - s) Y_{XS}}{(s_i - s) Y_{XS} + \eta_T x_{im}} < 1$$

Chemostat with Suspended Cell only ($X_{im} = 0$):

At steady state $D = \mu$ and the maximum operating dilution rate D_{crit} is limited by the maximum specific growth rate of the cells.

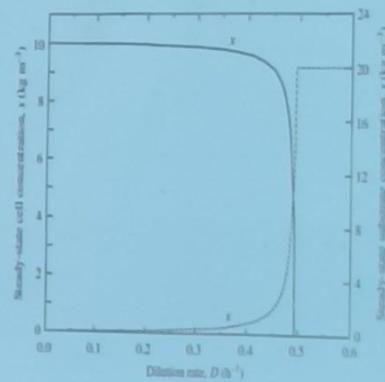
$$D_{crit} = \frac{\mu_{max} s_i}{K_S + s_i}$$

For most cell cultures, $K_S \ll s_i$

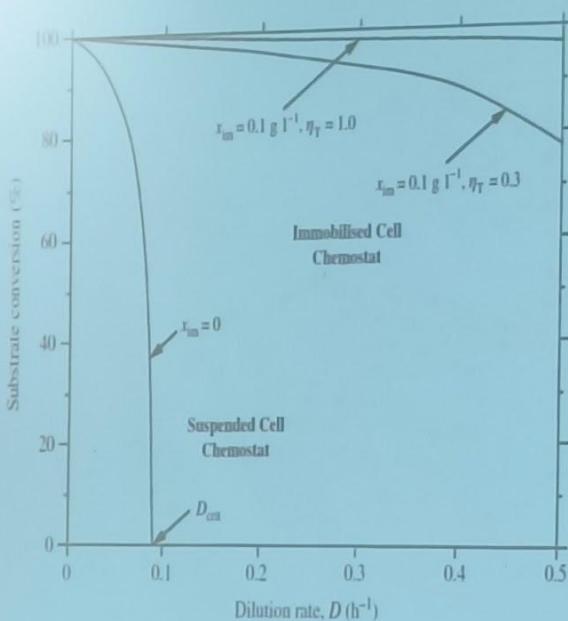
Thus, $D_{crit} \approx \mu_{max}$

For any $x_{im} > 0$, $D > \mu$ at steady state in the immobilized cell reactor.

Accordingly, the dilution rate is no longer limited by the maximum specific growth rate of the cells.



Chemostat with Immobilized Cells



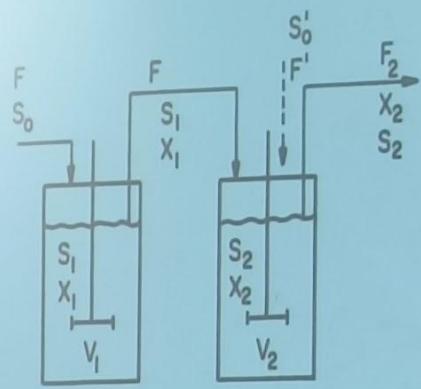
Lower values of η_T means that mass transfer limitations are significant

Steady-state substrate conversion in a chemostat as a function of dilution rate with and without immobilised cells. The curves were calculated using the following parameter values: $\mu_{max} = 0.1 \text{ h}^{-1}$, $K_S = 10^{-3} \text{ g l}^{-1}$, $Y_{XS} = 0.5 \text{ g g}^{-1}$, and $s_i = 8 \times 10^{-3} \text{ g l}^{-1}$.

Immobilized cell chemostats can be operated at D considerably greater than D_{crit} without washout.

At a given dilution rate, the presence of immobilized cells also improves substrate conversion and reduces the amount of substrate lost in the product stream.

Two-Stage Chemostat System



Conditions such as pH, temperature, and medium composition can be varied in each reactor.

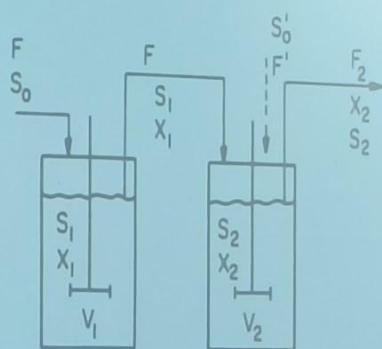
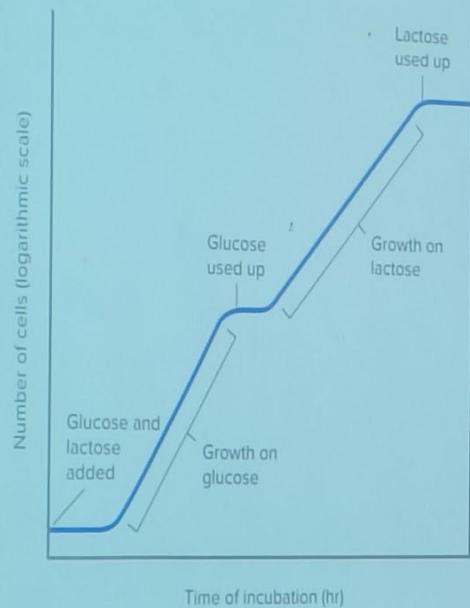
This is advantageous if the reactor conditions required for growth are different from those required for product synthesis.

Example: the production of recombinant proteins and many metabolites not directly linked with energy metabolism.

Substrate leaving the first reactor at concentration s_1 is converted in the second tank. Thus, $s_2 < s_1$ and $P_2 > P_1$

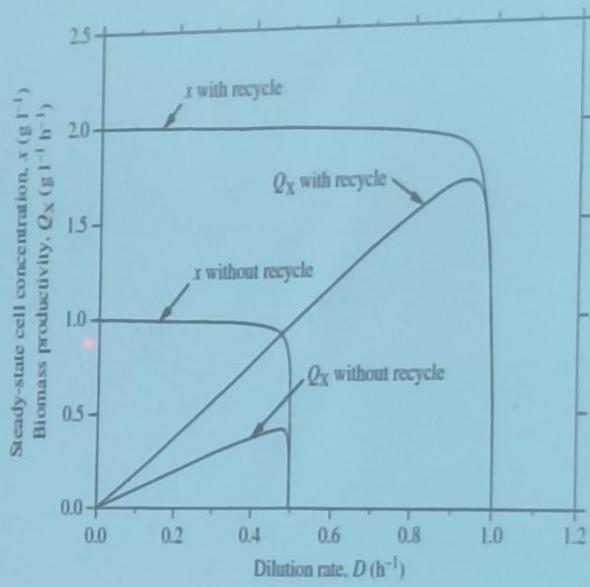
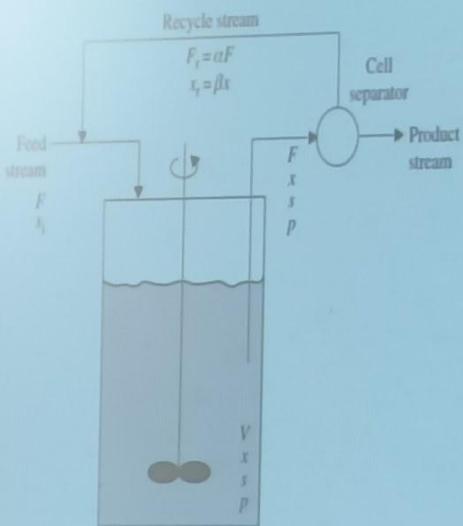
In some applications, the second CSTR is supplemented with fresh medium containing nutrients, inducers, or inhibitors for optimal product formation.

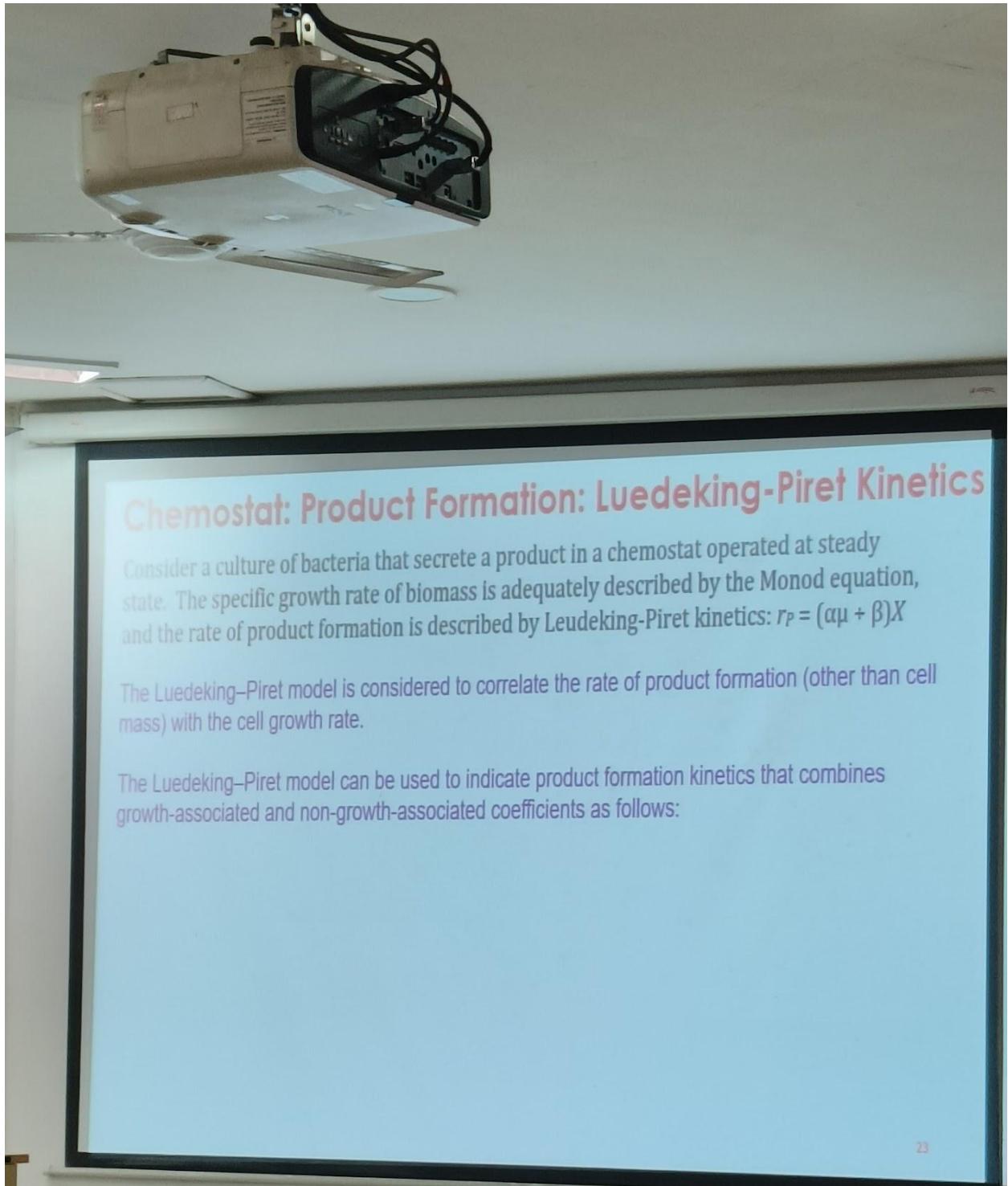
Two-Stage Chemostat System



Diauxic Growth of *E. coli*

Chemostat with Recycle







Chemostat: Product Formation: Luedeking-Piret Kinetics

Consider a culture of bacteria that secrete a product in a chemostat operated at steady state. The specific growth rate of biomass is adequately described by the Monod equation, and the rate of product formation is described by Leudeking-Piret kinetics: $r_P = (\alpha\mu + \beta)X$

The Luedeking-Piret model is considered to correlate the rate of product formation (other than cell mass) with the cell growth rate.

The Luedeking-Piret model can be used to indicate product formation kinetics that combines growth-associated and non-growth-associated coefficients as follows:

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

where dP/dt is the rate of product formation, dX/dt is the biomass growth rate, α is the growth-associated coefficient, and β is the nongrowth-associated coefficient.

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

Chemostat: Product Formation: Luedeking-Piret Kinetics

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where dP/dt is the rate of product formation, dX/dt is the biomass growth rate, α is the growth-associated coefficient, and β is the nongrowth-associated coefficient.

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

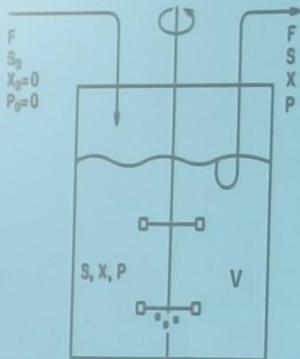
If $\alpha = 0$, the product is non-growth associated (Ethanol, Enzymes)

$$q_P = \alpha\mu + \beta$$

If $\beta = 0$, the product is growth associated (All antibiotics)

Chemostat: Product Formation: Luedeking-Piret Kinetics

Consider a culture of bacteria that secrete a product in a chemostat operated at steady state. The specific growth rate of biomass is adequately described by the Monod equation, and the rate of product formation is described by Leudeking-Piret kinetics: $r_P = (\alpha\mu + \beta)X$



$$r_X = \mu X$$

$$r_S = q_S X$$

$$r_P = q_P X$$

$$\text{Luedeking-Piret equation: } \frac{1}{X} \frac{dp}{dt} = q_p = \alpha \mu + \beta$$

In cultures where product synthesis is only indirectly coupled to energy metabolism, the rate of substrate consumption is a function of three factors: the growth rate, the rate of product formation, and the rate of substrate uptake for maintenance.

$$r_S = \frac{r_X}{Y_{XS}} + \frac{r_P}{Y_{PS}} + m_S X$$

$$r_S = \left(\frac{\mu}{Y_{XS}} + \frac{q_P}{Y_{PS}} + m_S \right) X$$

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Chemostat: Mass Balances

Consider a culture of bacteria that secrete a product in a chemostat operated at steady state. The specific growth rate of biomass is adequately described by the Monod equation, and the rate of product formation is described by Leudeking-Piret kinetics: $r_P = (\alpha\mu + \beta)X$

$$X \text{ balance: } 0 = -FX + \mu X V \text{ or } DX = \mu X \text{ or } 0 = -FX + \frac{\mu_{max} S}{S + K_s} X V$$

$$S \text{ balance: } 0 = F(S_0 - S) + r_S V \text{ or } 0 = F(S_0 - S) - V \left(\frac{1}{Y_{X/S}} \frac{\mu_{max} S}{S + K_s} X + \frac{\alpha \left(\frac{\mu_{max} S}{S + K_s} \right) X + \beta X}{Y_{P/S}} \right)$$

Chemostat: Mass Balances

Consider a culture of bacteria that secrete a product in a chemostat operated at steady state. The specific growth rate of biomass is adequately described by the Monod equation, and the rate of product formation is described by Leudeking-Piret kinetics: $r_p = (\alpha\mu + \beta)X$

$$X \text{ balance: } 0 = -FX + \mu X V \text{ or } DX = \mu X \text{ or } 0 = -FX + \frac{\mu_{\max} S}{S + K_s} X V$$

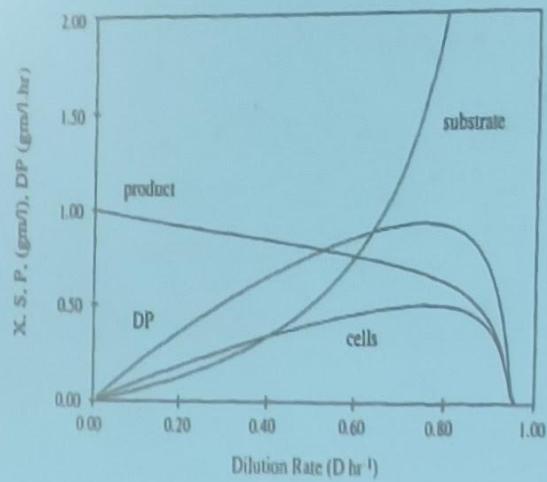
$$S \text{ balance: } 0 = F(S_o - S) + r_s V \text{ or } 0 = F(S_o - S) - V \left(\frac{1}{Y_{X/S}} \frac{\mu_{\max} S}{S + K_s} X + \frac{\alpha \left(\frac{\mu_{\max} S}{S + K_s} X + \beta X \right)}{Y_{P/S}} \right)$$

$$P \text{ balance: } 0 = -FP + r_p V \text{ or } 0 = -FP + (\alpha\mu X + \beta X) V \text{ or } 0 = -FP + \left(\alpha \frac{\mu_{\max} S}{S + K_s} X + \beta X \right) V$$

$$\text{or } DP = (\alpha\mu + \beta)X$$

Chemostat: Steady State

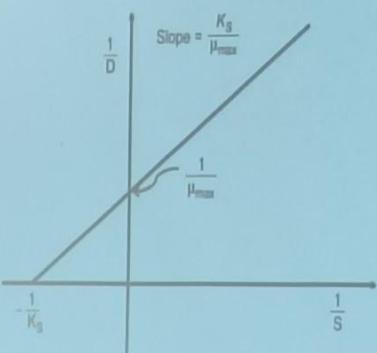
Consider a culture of bacteria that secrete a product in a chemostat operated at steady state. The specific growth rate of biomass is adequately described by the Monod equation, and the rate of product formation is described by Leudeking-Piret kinetics: $r_P = (\alpha\mu + \beta)X$



Determination of Kinetic Constants in Chemostat

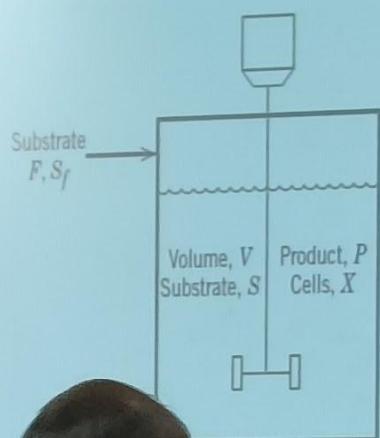
The kinetic parameters (μ_{\max} and K_S) can be estimated by plotting $1/D$ versus $1/S$.

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

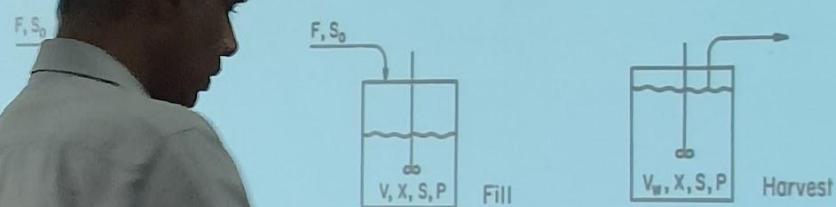


From the intercept, the μ_{\max} value can be found out, and from the slope, K_S can be determined.

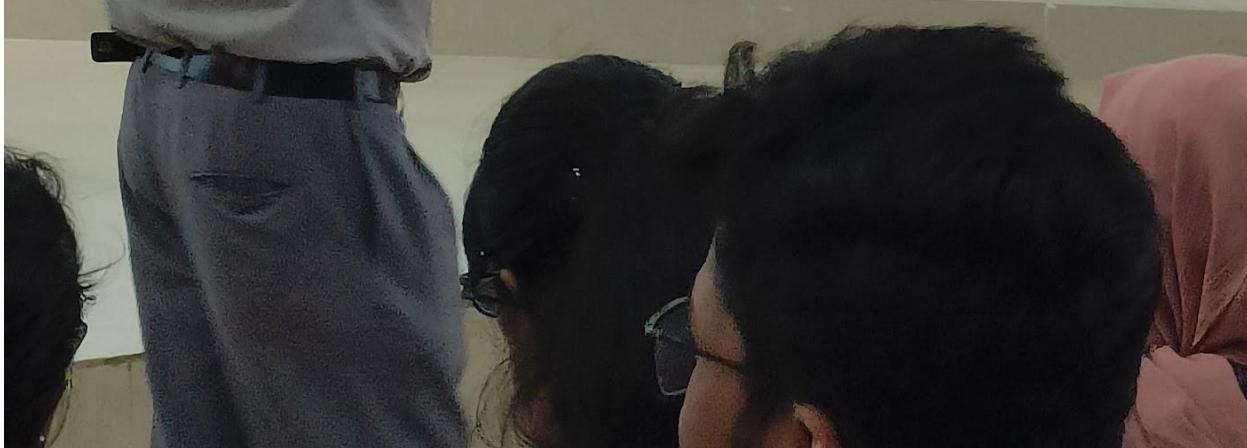
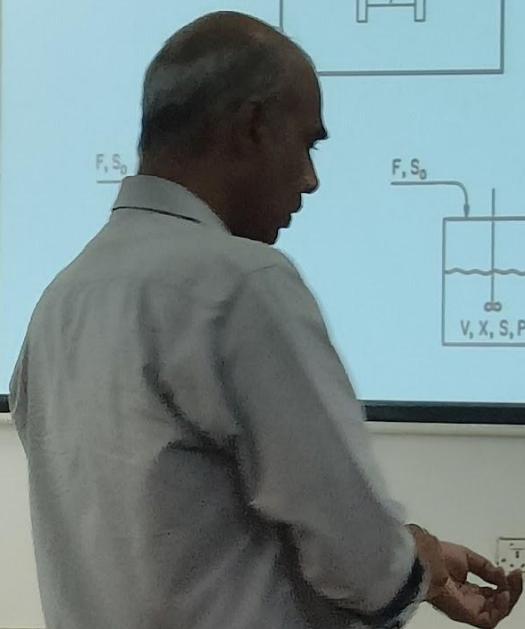
Fed-Batch Bioreactor



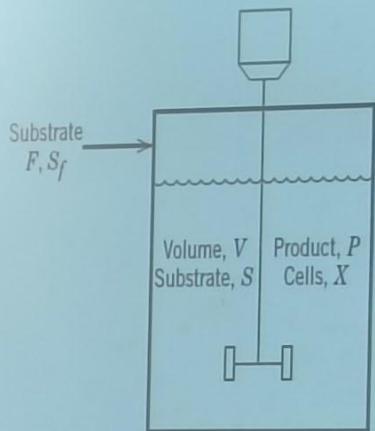
- Necessary nutrients are fed intermittently or continuously
- Dynamic operation
- The culture is harvested usually at the end – either fully or partially – repeated fed-batch
- Limiting nutrient's concentration can be controlled by manipulating $F(t)$ to maximize X and/or P



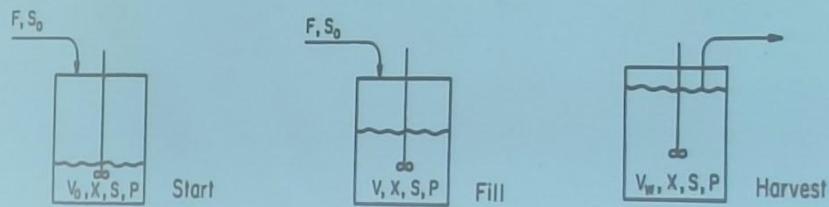
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Fed-Batch Bioreactor



- Necessary nutrients are fed intermittently or continuously
- Dynamic operation
- The culture is harvested usually at the end – either fully or partially – repeated fed-batch
- Limiting nutrient's concentration can be controlled by manipulating $F(t)$ to maximize X and/or P



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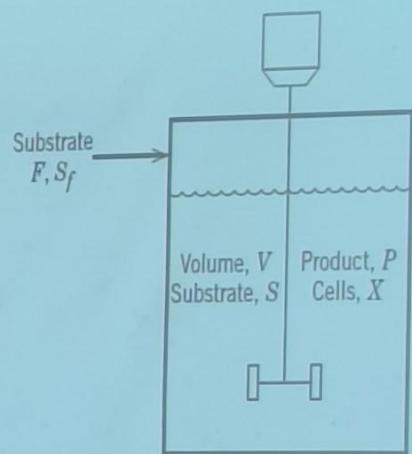
Fed-Batch Bioreactor

In fed-batch operations, intermittent or continuous feeding of nutrients is used to supplement the reactor contents and provide control over the substrate concentration.

By starting with a relatively dilute solution of substrate and adding more nutrients as the conversion proceeds, high growth rates are avoided.

Fed-batch operation is preferred

- where the oxygen demand during fast growth is too high for the mass transfer capabilities of the reactor, or
- when high substrate concentrations are inhibitory, or
- when high substrate concentration switch on undesirable metabolic pathways



Fed-batch culture is used extensively for production of

- Bakers' yeast
- Penicillin
- Citric acid
- Amylase enzyme
- Vinegar

Fed-Batch Bioreactor

Volume Balance

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

Biomass Balance

$$\frac{d(xV)}{dt} = Fx_i + \mu xV - k_d xV$$

Diagram of a Fed-Batch Bioreactor:

Feed stream

$xF + V \frac{dx}{dt} = Fx_i + (\mu - k_d) xV$

$$\frac{dx}{dt} = \frac{F}{V} x_i + x \left(\mu - k_d - \frac{F}{V} \right)$$

$$\frac{dx}{dt} = D x_i + x (\mu - k_d - D)$$

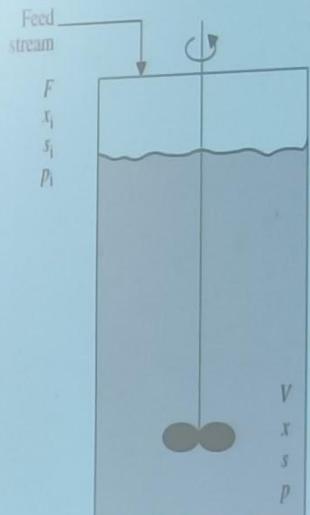
$$\frac{dx}{dt} = x (\mu - D)$$

Assuming the rate of cell death is negligible compared with growth

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Fed-Batch Bioreactor

$$\frac{d(sV)}{dt} = F s_i - \left(\frac{\mu}{Y_{XS}} + \frac{q_p}{Y_{PS}} + m_s \right) x V \quad \text{Substrate Balance}$$



$$\frac{ds}{dt} = D(s_i - s) - \left(\frac{\mu}{Y_{XS}} + \frac{q_p}{Y_{PS}} + m_s \right) x \quad \text{Substrate Balance}$$

$$\frac{dV}{dt} = F \quad \text{Volume Balance}$$

$$\frac{dx}{dt} = x(\mu - D) \quad \text{Cell Growth}$$

$$\frac{ds}{dt} = D(s_i - s) - \left(\frac{\mu}{Y_{XS}} + \frac{q_p}{Y_{PS}} + m_s \right) x \quad \text{Substrate Balance}$$

$$\frac{d(pV)}{dt} = q_p x V \quad \text{Product Formation}$$

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Fed-Batch Bioreactor

$$\frac{dV}{dt} = F \quad \frac{dx}{dt} = x(\mu - D) \quad \frac{d(pV)}{dt} = q_P x V \quad \frac{ds}{dt} = D(s_i - s) - \left(\frac{\mu}{Y_{XS}} + \frac{q_P}{Y_{PS}} + m_S \right)x$$

Because D is a function of time, integration of these equations is more complicated than for batch reactors.

Simplification:
The reactor is operated first in batch until a high cell density is achieved and the substrate is virtually exhausted. When this condition is reached, fed-batch operation is started with medium flow rate F .

As a result, the cell concentration x is maintained high and approximately constant so that $dx/dt \approx 0$.

When the cell density in the reactor is high, virtually all substrate entering the vessel is consumed immediately; therefore, $s \ll s_i$ and $ds/dt \approx 0$.

$$\frac{dX}{dt} = \frac{d(xV)}{dt} = x \frac{dV}{dt} + V \frac{dx}{dt} = Y_{XS} s_i F \quad X = X_0 + (Y_{XS} s_i F) t_{fb}$$

where t_{fb} is the fed-batch time after commencement of feeding. For Y_{XS} , s_i , and F constant, the total biomass in fed-batch fermenters increases as a linear function of time.