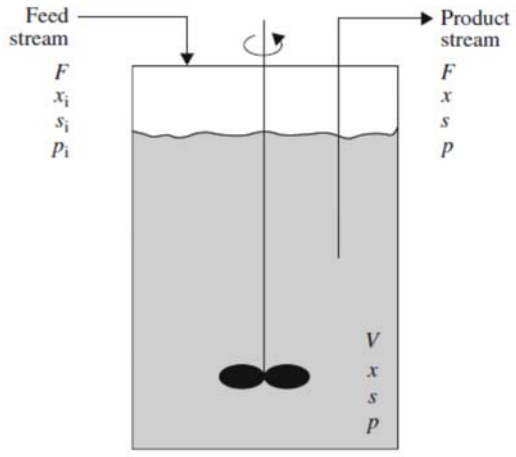
Debasis Sarkar (*Autumn 2022*)

Continuous Stirred Tank Reactor/Fermenter (Chemostat)

(Suspended Cell Culture)

Exponential growth in a batch culture may be prolonged by addition of fresh medium to the fermentation vessel. In a continuous culture, if the fresh medium is displaced by an equal volume of old culture, then continuous cell production can be achieved. Bioreactors are operated continuously in a few bioprocess industries such as brewing, production of bakers' yeast, and waste treatment. Enzyme conversions can also be carried out using continuous systems.



The primary form of continuous culture is a steady-state CSTR or *chemostat*. A chemostat ensures a time-invariant growth environment. The net growth rate is equal to the dilution rate, which is determined by the flow rate to the chemostat. Thus, the growth rate can be easily manipulated. A schematic diagram of a continuous stirred tank reactor (CSTR) is shown in above Figure. The mixing is done by an impeller, rising gas bubbles, or both. The purpose of mixing is to maintain homogeneity in the fermentation broth. In the steady-state condition, the concentration of any component in the vessel is independent of time.

Let us consider,

F = volumetric flow rate (L/h)
s_i = growth-limiting substrate concentration in the feed (g/L)
x_i = biomass (cell) concentration in the feed (g/L)

p_i = product concentration in the feed (g/L)
s = steady-state growth-limiting substrate concentration in product stream and in CSTR (g/L)
x = steady-state biomass concentration in product stream and in CSTR (g/L)
p = steady-state product concentration in product stream and in CSTR (g/L)

V = working or liquid volume of the reactor (L)

The steady-state mass balance equation for biomass is:

Cell accumulation = Cell in – Cell out + Cell growth – Cell death

Here, Cell accumulation = 0 (steady-state operation)

$$Fx_i - Fx + \mu xV - k_d xV = 0 \quad (1)$$

For sterile feed, $x_i = 0$

Additionally, if the rate of cell death is negligible compared with growth, $k_d << \mu$

$$\mu xV = Fx \quad (2)$$

Dividing throughout by V and defining dilution rate, $D = F/V$, we get from Eq. (2)

$$(D - \mu)x = 0$$

$$\mu = D \quad (3)$$

In a chemostat, cells are removed at a rate equal to their growth rate, and the growth rate of cells is equal to the dilution rate.

Combine μ = D (Eq. 3) with the Monod expression $\mu = \frac{\mu_{max} S}{K_S + S}$ to get

$$s = \frac{DK_S}{\mu_{max} - D} \quad (4)$$

This gives an equation for the steady-state concentration of limiting substrate in the reactor as a function of dilution rate.

The steady-state mass balance equation for growth-limiting substrate is:

$$Fs_i - Fs - \left(\frac{\mu}{Y_{XS}} + \frac{q_P}{Y_{PS}} + m_S\right)xV = 0 \tag{5}$$

where μ is the specific growth rate, Y_{XS} is the true biomass yield from substrate, q_P is the specific rate of product formation not directly linked with energy metabolism, Y_{PS} is the true product yield from substrate, and m_S is the maintenance coefficient.

Divide through (Eq. 5) by V, substitute the definition of dilution rate $D = F/V$, and replace μ by D (using Eq. 3) to get

$$x = \frac{D(s_i - s)}{\frac{D}{Y_{XS}} + \frac{q_P}{Y_{PS}} + m_S} \tag{6}$$

If there is no product synthesis or if production is directly linked with energy metabolism, we can drop q_P/Y_{PS}

$$x = \frac{D(s_i - s)}{\frac{D}{Y_{XS}} + m_S} \tag{7}$$

If, in addition, maintenance effects can be ignored,

$$x = (s_i - s)Y_{XS} \tag{8}$$

If we substitute s from (Eq. 4) in (Eq. 8) we obtain an expression for the steady-state biomass (cell) concentration in the CSTR:

$$x = \left(s_i - \frac{DK_S}{\mu_{max} - D}\right)Y_{XS} \tag{9}$$

NOTE: Eq. (9) is valid at steady state in the absence of maintenance requirements and when product synthesis is either absent or directly linked with energy metabolism.

The steady-state mass balance on fermentation product:

$$Fp_i - Fp + q_P xV = 0 \tag{10}$$

where q_P is the specific rate of formation for all classes of product. Dividing through by V, substituting the definition of the dilution rate ($D = F/V$) and rearranging gives an expression for the steady-state product concentration as a function of biomass concentration x:

$$p = p_i + \frac{q_P x}{D} \tag{11}$$

1. The biomass concentration x in (Eq. 11) can be evaluated from Eq. (6), (7), or (8).
2. Evaluation of q_P depends on the type of product formed.

Unsteady-state Biomass Balance:

$$d(xV)/dt = \mu xV - Fx \tag{12}$$

Unsteady-state Substrate Balance:

$$d(sV)/dt = Fs_i - Fs - \left(\frac{\mu}{Y_{XS}} + \frac{q_P}{Y_{PS}} + m_S\right)xV \tag{13}$$

Unsteady-state Product Balance:

$$d(pV)/dt = Fp_i - Fp + q_P xV \tag{14}$$

Critical Dilution Rate:

Recall the biomass balance (x = biomass concentration):

$$d(xV)/dt = \mu xV - Fx$$

$$dx/dt = \mu x - Dx$$

At steady-state, $dx/dt = 0$ and $x(\mu - D) = 0$

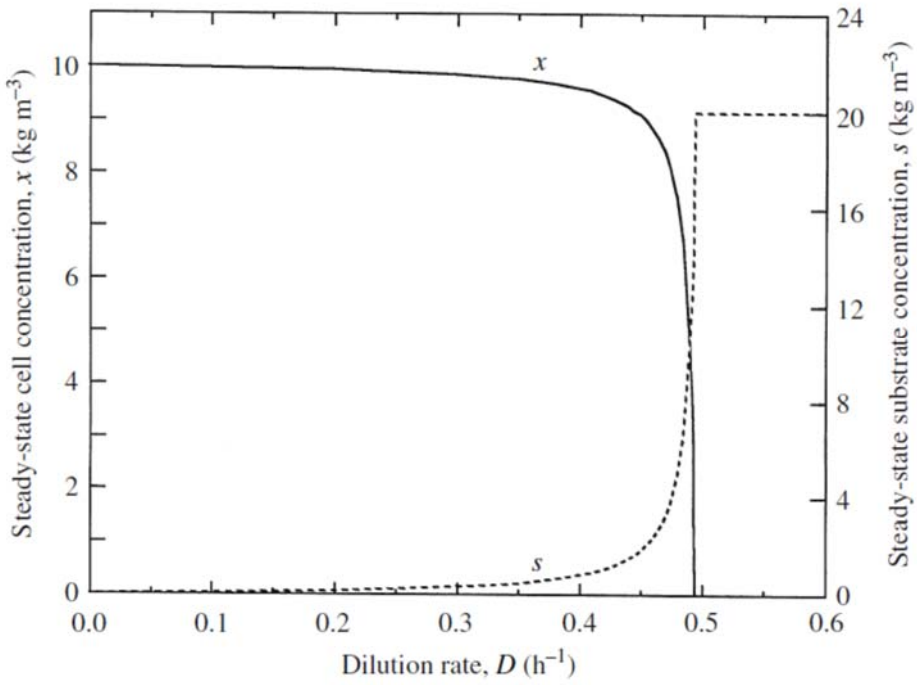
Then, $\mu = D$. Such condition is valid only at steady condition. As the fresh medium flow rate increases, cell concentration gradually decreases because of high dilution rate. There is a specific dilution rate known as critical dilution rate where washout of cells takes place. At the critical dilution rate there is not enough time for the microorganisms to replicate.

Use (Eq. 4) and (Eq. 9) to plot steady-state (D vs x) and (D vs s).

Simply vary D and find s and x. You need values for s_i, K_S and μ_{max} for a given system.

$$s = \frac{DK_S}{\mu_{max} - D} \qquad x = \left(s_i - \frac{DK_S}{\mu_{max} - D}\right) Y_{XS}$$

NOTE: $D_{max} < \mu_{max}$



At low feed rates ($D \rightarrow 0$) nearly all the substrate is consumed at steady state.
Thus, from Eq. (8),

$$x \approx s_i Y_{XS}.$$

As D increases, s increases slowly at first and then more rapidly as D approaches μ_{max}. Correspondingly, x decreases so that $x \rightarrow 0$ as $D \rightarrow \mu_{max}$. The condition at high dilution rate whereby x reduces to zero is known as washout. Washout of cells occurs when the rate of cell removal in the reactor outlet stream is greater than the rate of generation by growth.

The critical dilution rate D_{crit} is the dilution rate at which the steady-state biomass concentration becomes zero. This can be estimated by substituting $x = 0$ in Eq. (9) and solving for D:

$$D_{crit} = \frac{\mu_{max} s_i}{K_S + s_i} \tag{15}$$

For most cell cultures $K_S << s_i$, thus $D_{crit} \approx \mu_{max}$.

To avoid washout of cells from the chemostat, the operating dilution rate must always be less than D_{crit}. Near washout, the system is very sensitive to small changes in dilution rate D that cause relatively large change in x and s.

NOTE:

$$D_{max} < D_{washout} \leq \mu_{max}.$$

Optimum Dilution Rate to Maximize Volumetric Biomass Productivity:

Volumetric biomass productivity is defined as, $Q_X = Dx$ g/(l h)

Recall Eq. (9) for expression of x: $$x = \left(s_i - \frac{D K_S}{\mu_{max} - D} \right) Y_{XS}$$

Thus, Dx is

$$Dx = D\left(s_i - \frac{D K_S}{\mu_{max} - D} \right) Y_{XS} \qquad (16)$$

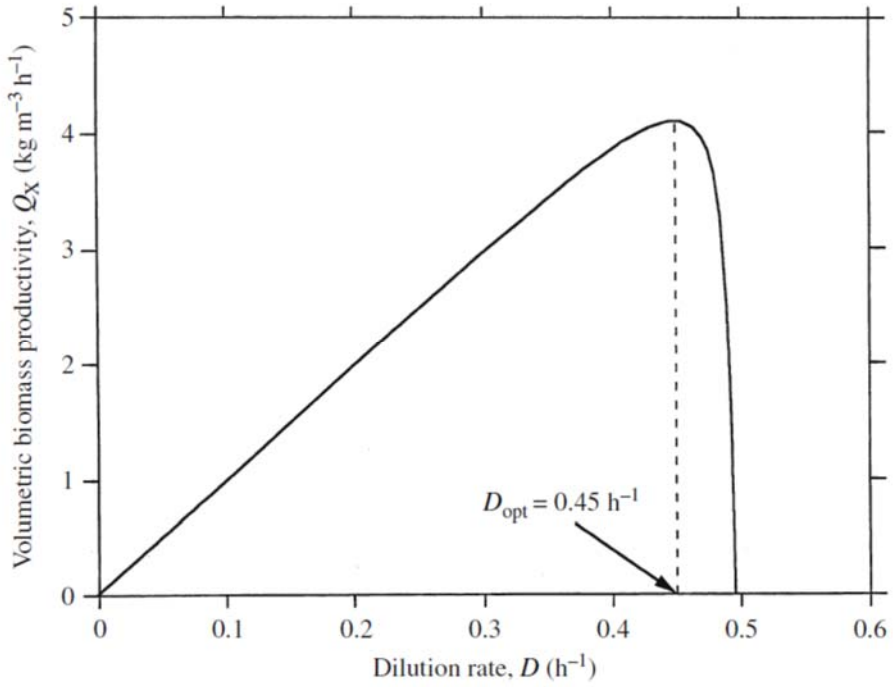
To find optimum dilution rate (D_{opt}) that maximize biomass productivity Dx, set

$$\frac{d(Dx)}{dD} = 0$$

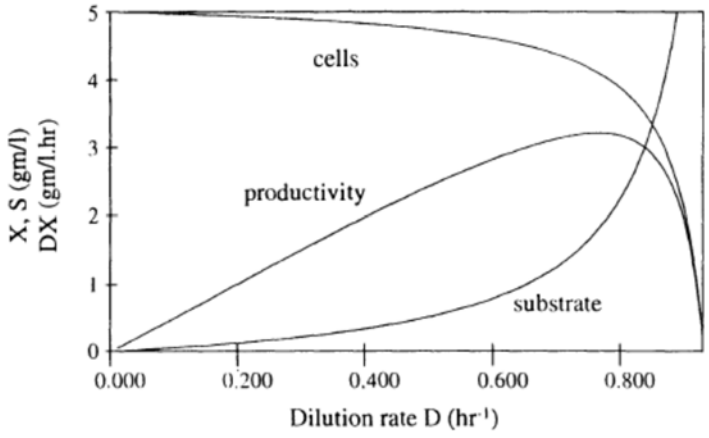
and solve for D. We thus get,

$$D_{opt} = \mu_{max}\left(1 - \sqrt{\frac{K_S}{K_S + s_i}} \right) \qquad (17)$$

Operation of a chemostat at D_{opt} gives the maximum rate of biomass production from the reactor. However, because D_{opt} is usually very close to D_{crit}, it may not be practical to operate at D_{opt}. Small variations of dilution rate in this region can cause large fluctuations in x and s and, unless the dilution rate is controlled very precisely, washout may occur.



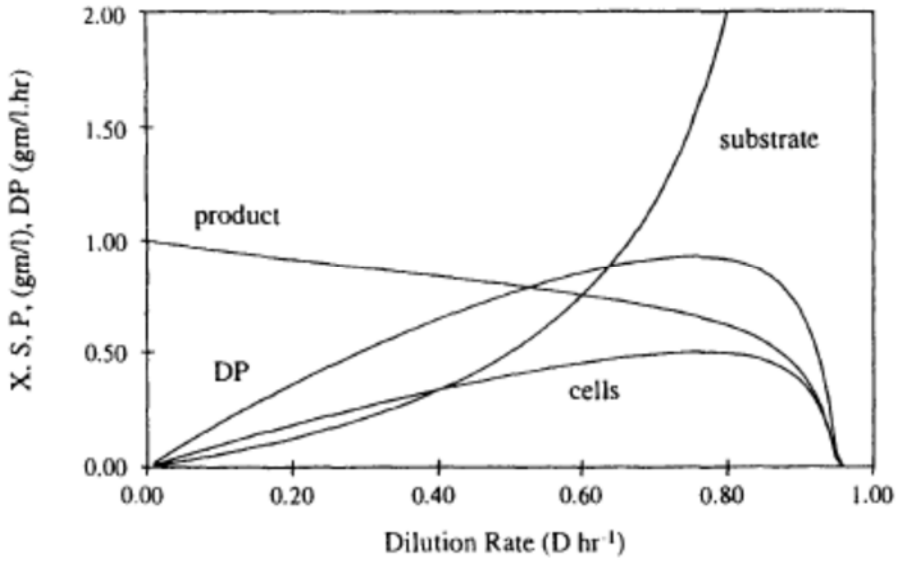
NOTE: The above figure can be drawn using Eq. 16 for given values of μ_{max}, s_i, and Ks. For this system, D_{opt} = 0.45 h^{-1} and Dcrit ≈ 0.5 h^{-1}.



Steady-state biomass concentration, substrate concentration, and volumetric biomass productivity as a function of dilution rate

NOTE:

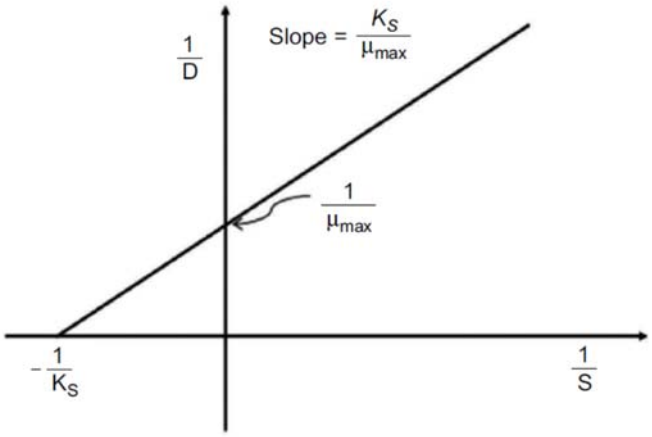
Similarly, we can define Q_P = DP as volumetric productivity of product.



Determination of Kinetic Constants in Chemostat:

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

μ = D



Advantages of Chemostat:

1. Growth rate can be controlled and maintained indefinitely. So one can operate the log phase of growth for the maximum cell mass production for the infinite period of time.

2. Effect of growth-limiting substrate can be easily monitored. The chemostat can be used to study the period of unbalanced growth, which occurs during the transition period between steady states at different specific growth rates. The formation of some plant metabolite is found to increase during the transition of phases.

3. Results obtained are reliable and reproducible.

Disadvantages of Chemostat:

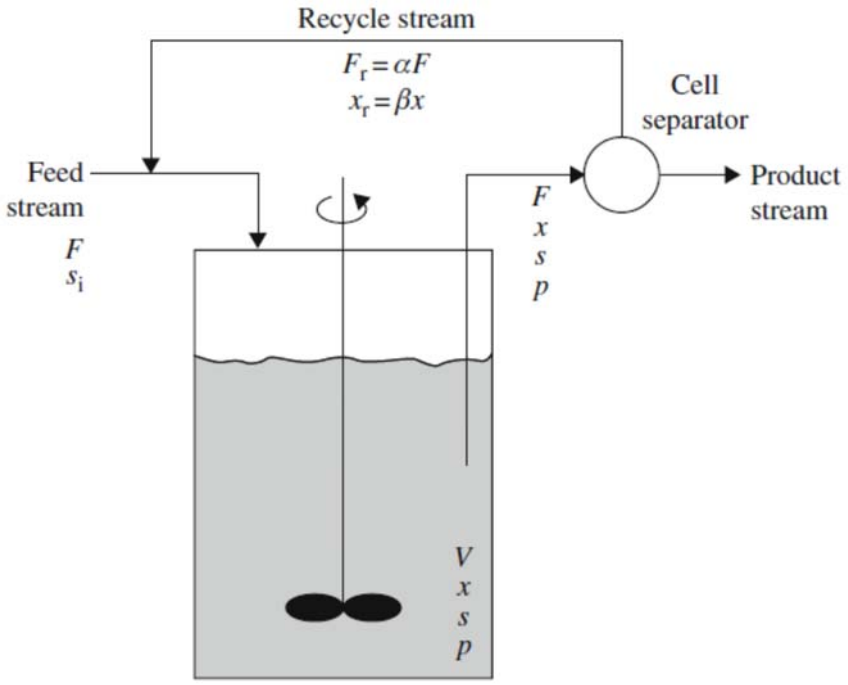
1. The major problem of a chemostat is cell washout. It is difficult to operate at $D_{\max}$ because it is very close to D_{washout}.

2. Cell growth over long periods can cause mutation or contamination.

The problem of cell washout of a chemostat can be overcome by the following methods:

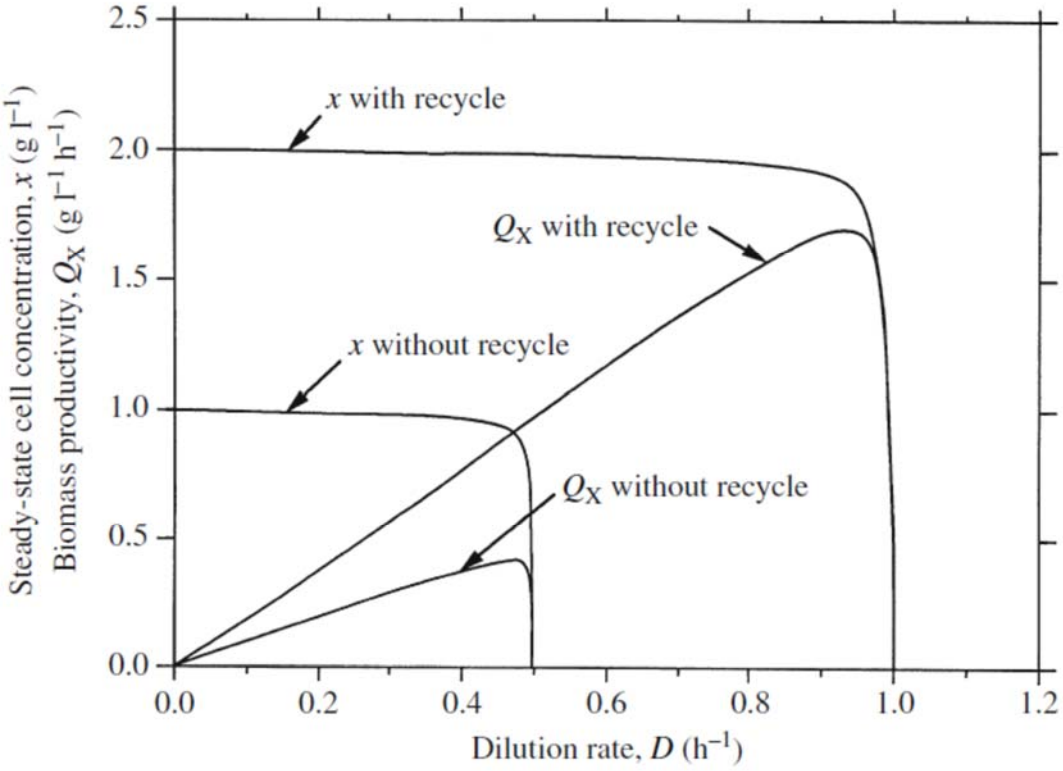
1. Chemostat with cell recycling
2. Whole-cell immobilization

Single-Stage CSTR (Chemostat) with Recycle:

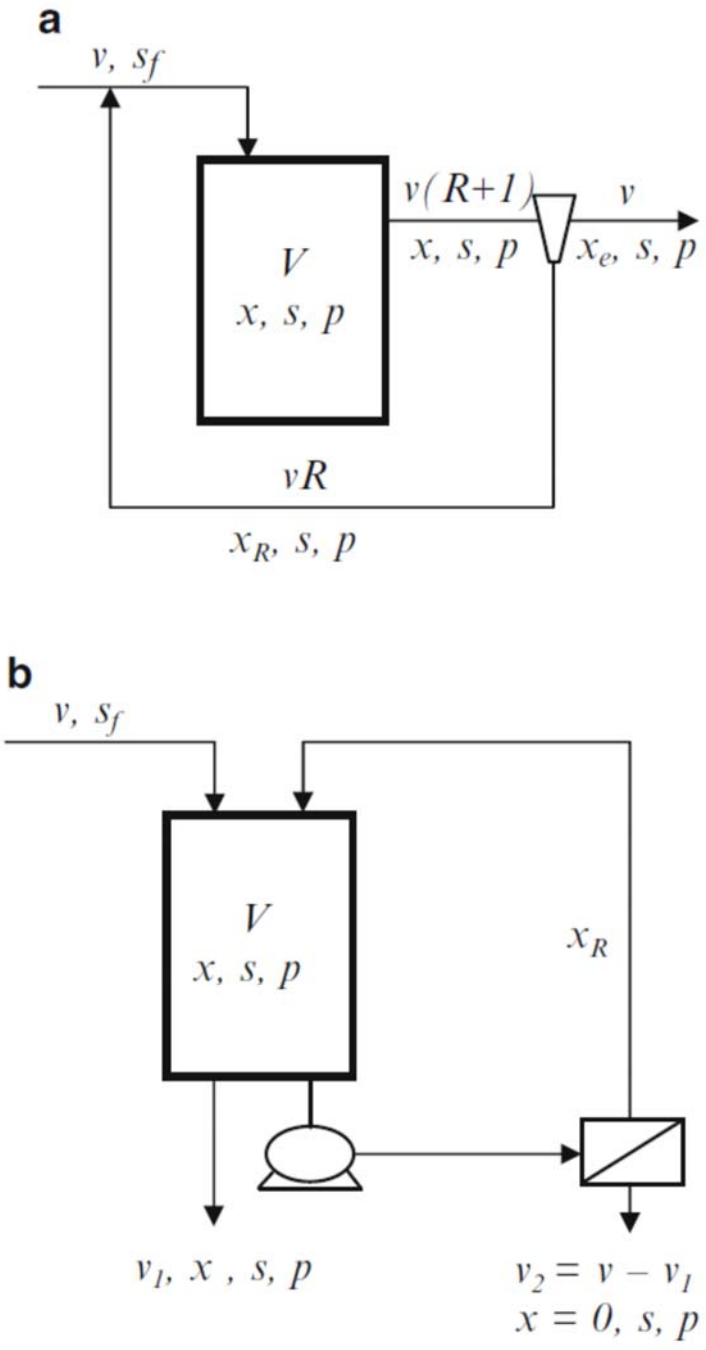


Advantages of using a chemostat with cell recycling are as follows:

1. Cell recycling is carried out to keep the cell concentration higher than the normal steady-state level in a chemostat.
2. Cell recycle increases the rate of conversion (or productivity).
3. Increases critical dilution rate for washout, thereby increasing operating flexibility.
4. Can be operated by using a centrifuge or settling tank to concentrate biomass and recycling a portion of the settled biomass to the reactor for maintaining cell mass concentration constant.



Critical dilution rate for washout is inreased ($Q_X = Dx$ = biomass productivity)



Biomass recirculation using a hydro-cyclone (a), and an ultrafilter (b).

In (b), the permeate $v_2 = v - v_1$ is cell free. The bleed stream v_1 is taken from the reactor.

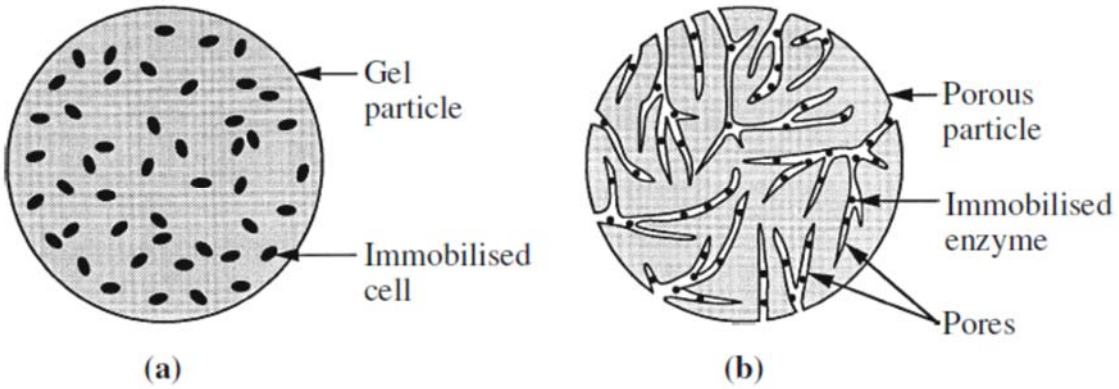
Chemostat with Immobilised Cells:

Immobilization of cells as biocatalysts is almost as common as enzyme immobilization. Immobilization is the restriction of cell mobility within a defined space. It is the entrapment or binding of cells by physical or chemical forces.

Physical entrapment within porous matrices is the most widely used method of cell immobilization. Various matrices can be used for the immobilization of cells. Among these are porous polymers (agar, alginate, k-carrageenan, polyacrylamide, chitosan, gelatin, collagen), porous metal screens, polyurethane, silica gel, polystyrene, and cellulose triacetate. Polymer beads should be porous enough to allow the transport of substrates and products in and out of the bead.

Immobilization by polymerization is a simple method. The polymerizing solution is mixed with the cell suspension, and polymerization takes place to form a polymeric block, which is pressed through a sieve plate to obtain regular-shaped particles. Suspension or emulsion polymerization can also be used to form polymeric beads for cell entrapment.

Encapsulation is another method of cell entrapment. Microcapsules are hollow, spherical particles bound by semipermeable membranes. Cells are entrapped within the hollow capsule volume. The transport of nutrients and products in and out of the capsule takes place through the capsule membrane. Microcapsules have certain advantages over gel beads. More cells can be packed per unit volume of support material into capsules, and intra-particle diffusion limitations are less severe in capsules due to the presence of liquid cell suspension in the intra-capsule space. Various polymers can be used as capsule membranes. Among these are nylon, collodion, polystyrene, acrylate, polylysine–alginate hydrogel, cellulose acetate–ethyl cellulose, and polyester membranes.



(a) cells and enzymes can be immobilized by entrapment within gels such as alginate, agarose, and carrageenan, (b) an alternative to gel immobilization is entrapment within porous solids such as ceramics, porous glass, and resin beads.

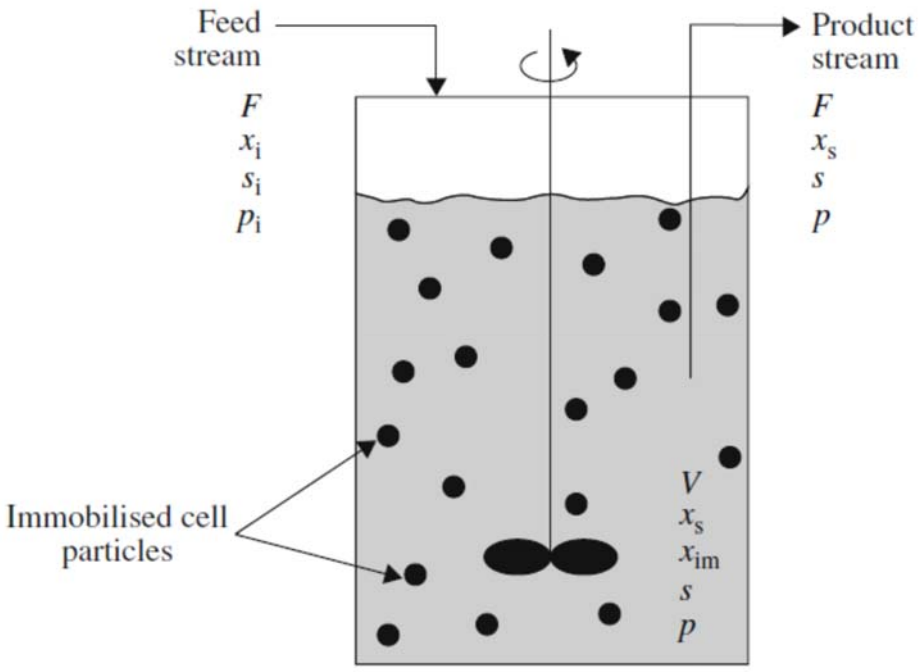
Immobilized cell cultures have the following potential advantages over suspension cultures.

1. Immobilization provides high cell concentrations.
2. Immobilization provides cell reuse and eliminates the costly processes of cell recovery and cell recycle.
3. Immobilization eliminates cell washout problems at high dilution rates.
4. The combination of high cell concentrations and high flow rates (no washout restrictions) allows high volumetric productivities.
5. Immobilization may also provide favourable micro-environmental conditions (i.e., cell–cell contact, nutrient–product gradients, pH gradients) for cells, resulting in better performance of the biocatalysts (e.g., higher product yields and rates).
6. In some cases, immobilization improves genetic stability.
7. For some cells, protection against shear damage is important.

Disadvantage:

The major limitation on immobilization is that the product of interest should be excreted by the cells. A further complication is that immobilization often leads to systems for which diffusional limitations are important. In such cases the control of micro-environmental conditions is difficult, owing to the resulting heterogeneity in the system. With living cells, growth and gas evolution present significant problems in some systems and can lead to significant mechanical disruption of the immobilizing matrix.

The following figure schematically shows a chemostat with immobilized cells. Suspended cells are removed from the reactor in the product stream; immobilised cells are retained inside the vessel. Let us assume that the concentration of immobilised cells per unit volume of liquid in the reactor (x_{im}) is constant. This is achieved if all particles are retained in the vessel and any cells produced by immobilised cell growth are released into the medium.



Let us consider,

The concentration of immobilised cells per unit volume of liquid in the reactor = x_{im}

The concentration of suspended cells = x_s

The specific growth rates of suspended and immobilised cells are the same and equal to μ.

The steady-state mass balance on suspended cells:

(Compare with the equation for chemostat with suspended cell)

As suspended cells are produced by growth of both the suspended and immobilised cell populations, the equation for the immobilised cell fermenter must contain two cell generation terms:

$$-Fx_s + \mu x_s V + \mu x_{im} V = 0 \tag{18}$$

If diffusional limitations affect the growth rate of the immobilised cells, μx_{im} must be replaced by $\eta_T \mu x_{im}$ where η_T is the total effectiveness factor. Dividing through by V and applying the definition of the dilution rate ($D = F/V$) gives:

$$D x_s = \mu (x_s + \eta_T x_{im}) \tag{19}$$

or

$$D = \mu \left(1 + \frac{\eta_T x_{im}}{x_s}\right) \tag{20}$$

For $x_{im} = 0$, the above Eq. (19) reduces to equation for a chemostat containing suspended cells only: $\mu = D$.

The steady-state mass balance on substrate:

(Compare with the equation for chemostat with suspended cell)

$$Fs_i - Fs - \frac{\mu x_s}{Y_{XS}} V - \frac{\eta_T \mu x_{im}}{Y_{XS}} V = 0 \tag{21}$$

Dividing through by V, applying the definition of the dilution rate ($D = F/V$) and rearranging gives:

$$D(s_i - s) = \frac{\mu}{Y_{XS}}(x_s + \eta_T x_{im}) \tag{22}$$

Combining Eq. (22) with Eq (20) $D = \mu\left(1 + \frac{\eta_T x_{im}}{x_s}\right)$

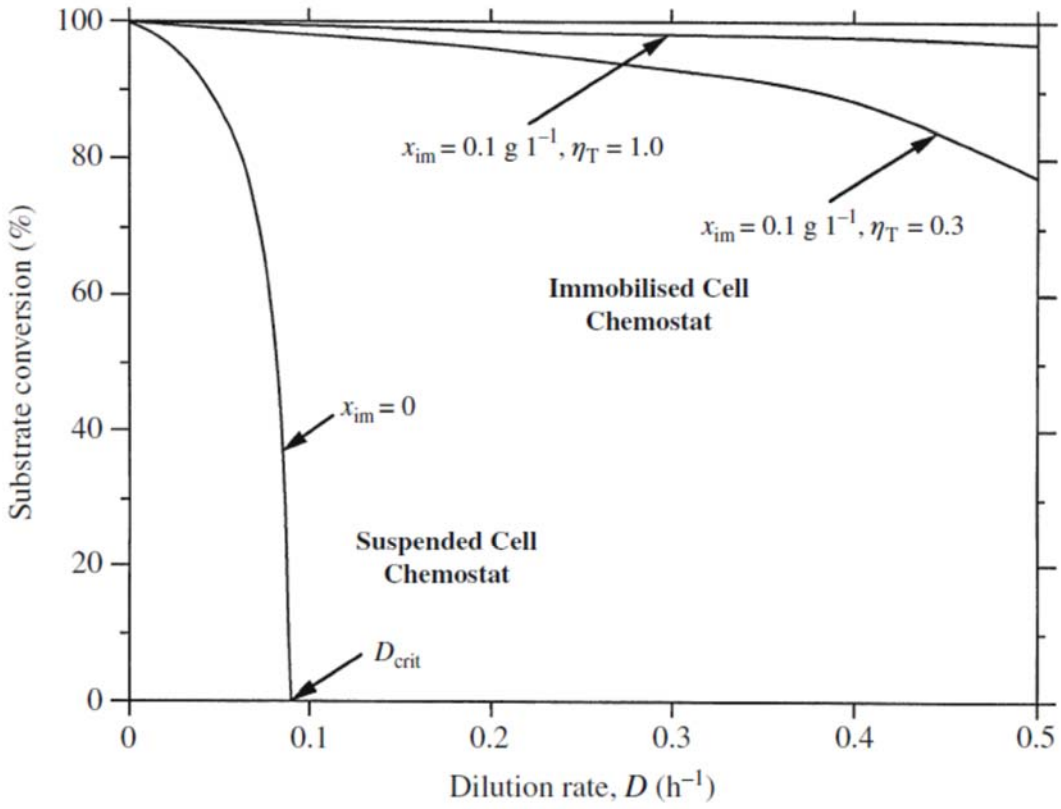
and Monod expression $\mu = \frac{\mu_{max} S}{K_S + S}$

we get,

$$\frac{\mu_{max} s}{K_S + s} = \frac{D(s_i - s)Y_{XS}}{(s_i - s)Y_{XS} + \eta_T x_{im}} \tag{23}$$

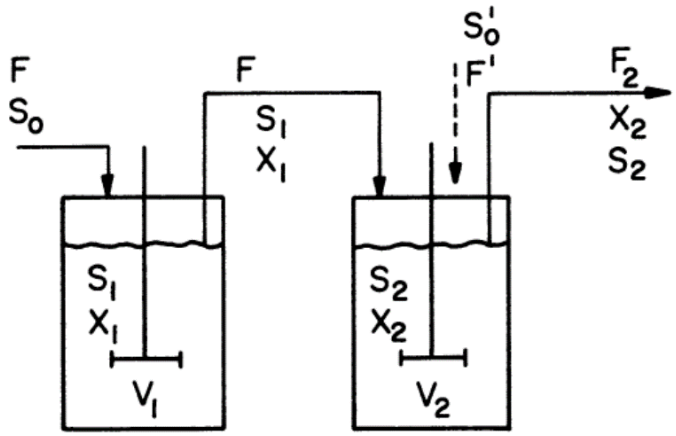
1. For a chemostat with suspended cells only (i.e., $x_{im} = 0$), at steady state $D = \mu$ and the maximum operating dilution rate D_{crit} is limited by the maximum specific growth rate of the cells.

2. From Eq. (22) we see that for any $x_{im} > 0$, the dilution rate D at steady state in the immobilised cell reactor is greater than μ. Therefore, the dilution rate is no longer limited by the maximum specific growth rate of the cells. Thus chemostat with immobilised cell can be operated at D considerably greater than D_{crit} without washout.



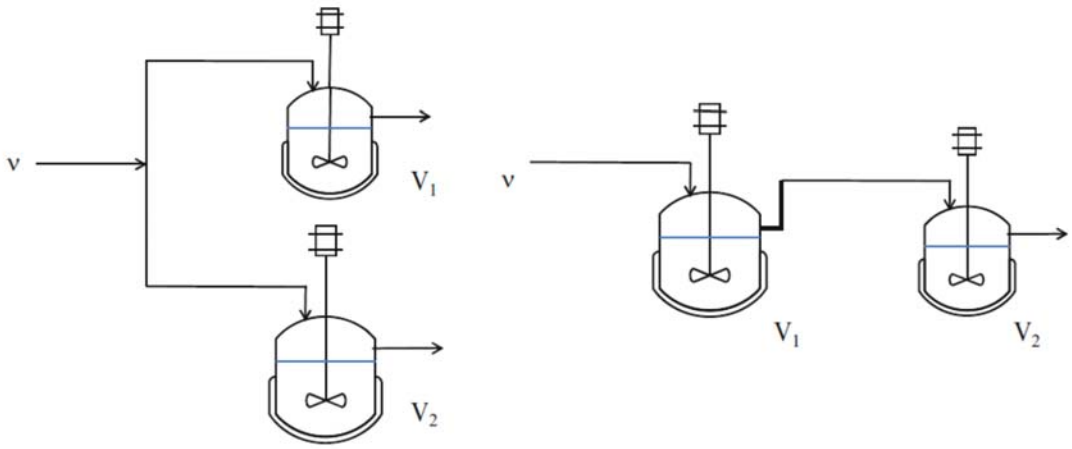
However, reaction rates with immobilised cells can be reduced significantly by the effects of mass transfer in and around the particles. As illustrated in above figure, at the same concentration of immobilised cells, the substrate conversion at $\eta_T = 1$ is greater than at lower values of η_T when mass transfer limitations are significant.

Chemostat Cascade (Chemostat in Series):



The joining together of two or more CSTR in series produces a multistage process in which 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, for example, in the production of recombinant proteins and many metabolites not directly linked with energy metabolism.

In some fermentations, particularly for secondary metabolite production, the growth and product-formation steps need to be separated, since optimal conditions for each step are different. Conditions such as temperature, pH, and limiting nutrients may be varied in each stage, resulting in different cell physiology and cellular products in multistage systems.



Fed-Batch Operation:

A batch culture of microbes fed continuously with culture medium is described as a “fed batch culture”. When a portion of a “fed batch culture” is withdrawn at intervals the system is described as a “repeated fed batch culture”. In fed-batch culture, nutrients are continuously or semi-continuously fed, while products are removed at the end of the run. Fed-batch culture is usually used to overcome substrate inhibition or catabolite repression by intermittent feeding of the substrate. If the substrate is inhibitory, intermittent addition of the substrate improves the productivity of the fermentation by maintaining the substrate concentration low. Fed-batch operation is also called the semi-continuous system or variable-volume continuous culture.

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. This is important, for example, in cultures

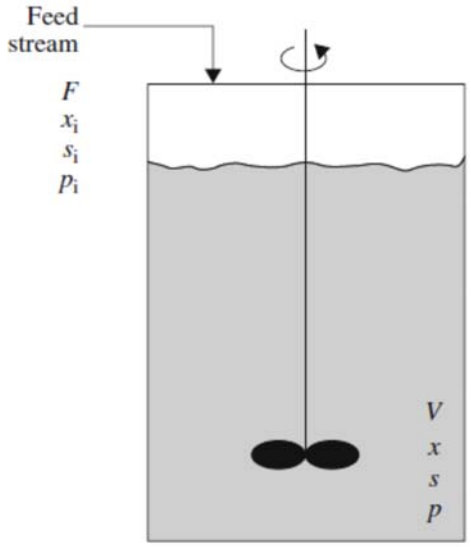
1. where the oxygen demand during fast growth is too high for the mass transfer capabilities of the reactor, or
2. when high substrate concentrations are inhibitory, or
3. when high substrate concentrations switch on undesirable metabolic pathways

Fed-batch culture is used extensively in the production of bakers’ yeast to overcome catabolite repression and control oxygen demand. It is also used routinely for penicillin production. Enzyme reactions are rarely carried out as fed-batch operations. Space must be allowed in fed-batch reactors for the addition of fresh medium; in some cases, a portion of the broth may be removed before injection of additional material. The flow rate and timing of the feed are often determined by monitoring parameters such as the dissolved oxygen tension or exhaust gas composition.

In a typical fed-batch process for protein synthesis, the reactor is started as a batch, and a suitably large biomass concentration is obtained by consumption of the initial substrate. The rate of product formation is typically zero during the batch cultivation, since product is only synthesized when an inducer is added. When the biomass concentration has increased to a

desired level and correspondingly the substrate level has decreased to a low level, a feed of (usually very concentrated) substrate is started. At the same time, the inducer is added to switch on the metabolic pathways that lead to the desired product. During the whole fed-batch period, no product is withdrawn from the reactor and the medium volume keeps increasing. At the end of the fed-batch period, the whole or a portion of the medium is harvested and sent to downstream processing. New substrate is added, and (possibly after a batch period) a second fed-batch period is started. This is *repeated fed-batch* operation.

A well-mixed fed-batch fermenter is shown schematically in the following figure. The volumetric flow rate of entering feed is F. Although F can be function of time, we will assume here that F is constant. Owing to input of the feed, the liquid volume V is not constant. Equations for fed-batch culture are derived by carrying out unsteady-state mass balances.



F = volumetric flow rate (L/h)
s_i = growth-limiting substrate concentration in the feed (g/L)
x_i = biomass (cell) concentration in the feed (g/L)
p_i = product concentration in the feed (g/L)

s = growth-limiting substrate concentration in the reactor (g/L)
x = biomass concentration in the reactor (g/L)
p = product concentration in the reactor (g/L)

V = volume of the reactor (L)

Total Mass Balance:

$$\frac{d(\rho V)}{dt} = F_i \rho_i - F_o \rho_o$$

where ρ is the density of the reactor contents, V is the liquid volume in the reactor, F_i and F_o are the input and output mass flow rates, and ρ_i and ρ_o are the densities of the input and output streams, respectively. For the fed-batch reactor shown in above figure, $F_o = 0$ and $F_i = F$. With dilute solutions such as those used in bioprocessing, we can assume that ρ is constant and that $\rho_i = \rho$; density can then be taken outside of the differential and cancelled through the equation. Therefore, above equation for fed-batch fermenters becomes:

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

Mass Balance on Biomass:

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

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

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

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

Defining, dilution rate $D = F/V$:

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

Usually the feed material is sterile so that $x_i = 0$. If, in addition, the rate of cell death is negligible compared with growth so that $k_d << \mu$, above equation becomes:

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

Mass Balance on Growth-Limiting Substrate:

$$\frac{d(sV)}{dt} = Fs_i - \left(\frac{\mu}{Y_{XS}} + \frac{q_P}{Y_{PS}} + m_S\right)xV$$

$$\frac{ds}{dt} = D(s_i - s) - \left(\frac{\mu}{Y_{XS}} + \frac{q_P}{Y_{PS}} + m_S\right)x$$

Final Form of Mass Balance Equations:

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

$$\frac{ds}{dt} = D(s_i - s) - \left(\frac{\mu}{Y_{XS}} + \frac{q_P}{Y_{PS}} + m_S\right)x$$

The above differential equations describe the rates of change of the cell and substrate concentrations in fed-batch reactors and these equations can be solved for a given dilution rate *D*. Because *D* is a function of time, integration of these equations is more complicated than for batch reactors.

Simplification (Quasi Steady-State Approximation):

The mathematical form of the fed-batch model is similar to that of the chemostat equations. Thus, it can be concluded that the fed batch will behave analogously. A dynamic steady state will be achieved for sufficiently low flow rates such that the specific growth rate is maintained exactly equal to the dilution rate $D = F/V$. This dynamic steady state has been termed a "quasi-steady state." It is characterized by a constant value of biomass concentration.

Let us examine the situation where 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 $dx/dt \approx 0$.

The mass balance of biomass in fed-batch reactor is, $$\frac{dx}{dt} = x(\mu - D)$$

Thus, if $dx/dt \approx 0$, we have $\mu \approx D$.

Therefore, substituting $\mu \approx D$ into the Monod expression $$\mu = \frac{\mu_{max} S}{K_S + S}$$ we get,

$$D \approx \frac{\mu_{max} s}{K_S + s}$$

Rearrange above equation to get the following expression for the substrate concentration as a function of dilution rate, D:

$$s \approx \frac{D K_S}{\mu_{max} - D}$$

Let us assume that the culture does not produce product or, if there is product formation, that it is directly linked with energy generation. If maintenance requirements can also be neglected, then the substrate balance equation

$$\frac{ds}{dt} = D(s_i - s) - \left(\frac{\mu}{Y_{XS}} + \frac{q_P}{Y_{PS}} + m_S\right)x$$

can be simplified to: $$\frac{ds}{dt} = D(s_i - s) - \frac{\mu x}{Y_{XS}}$$

When the cell density in the reactor is high, virtually all substrate entering the vessel is consumed immediately; therefore, $s << s_i$ and $ds/dt \approx 0$. By applying these relationships with $\mu \approx D$ to above substrate balance equation, we get: $$x \approx Y_{XS}\, s_i$$

For product synthesis directly coupled with energy metabolism, the above equation for biomass

$$x \approx Y_{XS}\, s_i$$

allows us to derive an approximate expression for the product concentration in fed-batch reactors. Assuming that the feed does not contain product:

$$p \approx Y_{PS}\, s_i$$

Even though the cell concentration remains virtually unchanged with $dx/dt \approx 0$, because the liquid volume increases with time in fed-batch reactors, the total mass of cells also increases.

The rate of increase of total biomass in the reactor = dX/dt, where $X = xV$ = total biomass

Using $dV/dt = F$, $dx/dt \approx 0$, and $x \approx Y_{XS}\, s_i$

we can write

$$\frac{dX}{dt} = \frac{d(xV)}{dt} = x\frac{dV}{dt} + V\frac{dx}{dt} = Y_{XS}\, s_i\, F$$

The above equation can now be integrated with initial condition $X = X_0$ at the start of liquid flow to give:

$$X = X_0 + (Y_{XS}\, s_i\, F)\, t_{fb}$$

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

At quasi-steady state, the specific growth rate μ and the dilution rate F/V are approximately equal; therefore as V increases, the growth rate decreases. Note that under quasi-steady-state conditions, x, s, and p are almost constant, but μ, V, D, and X are changing.