Microbial Growth Kinetics

Introduction: How cells grow

- In **enzymatic reactions,** enzymes require following conditions for growth and product formation
  - **a suitable substrate** and
  - **operating conditions** such as temperature, pH, etc.
  - a **proper medium** and

- The **liquid medium contains** the following ingredients:
  - **carbon** source,
  - **nitrogen** source,
  - **minerals**, and
  - **vitamins**.

Introduction: How cells grow

- The **carbon source** may be utilized for
  - cell growth,
  - product formation,
  - energy source, and
  - maintenance.

- The **nitrogen source** mostly contributes to the growth of cells.

- **Minerals** and **vitamins** are utilized mostly as cofactors in the metabolic pathways of living cells.

Introduction: How cells grow

- **In a suitable nutrient medium, the process associated with cell growth:**
  - Utilization of materials from the medium by the cells where organisms extract nutrients from the medium
  - and convert them into biological compounds / generation of metabolic end products in the medium

- Part of these nutrients are used for **energy production** and part are used for **biosynthesis** and **product formation**.

- As a result of nutrient utilization, microbial mass increases with time

Introduction: How cells grow

- For microbes, growth is their most essential response to their physiochemical environment.
- Growth is a result of both replication and change in cell size.
- Microorganisms can grow under a variety of physical, chemical, and nutritional conditions.

- The **growth of cells is monitored** in two ways:
  - In **unicellular organisms**,
  - the mass of living cells is proportional to the number of cells.
  - So the concentration of the cells may be expressed in either the number of viable cells/volume or the mass of cells/ volume.

- But in the case of **mold growth**,
  - the size and density of the cells are not necessarily proportional to their numbers.
  - So the concentration of mold-like cells is expressed only in mass/volume.

Kinetics of Microbial Cell Growth

- Microbial biomass and product formation can be given as:

Substrate + Cells Extra → extracellular cells + more cells

$\sum S + X \rightarrow \sum P + nX$

- Microbial growth is a good example of an **autocatalytic reaction**.
- The rate of growth is directly related to cell concentration, and cellular reproduction is the normal outcome of this reaction.
- The rate of microbial growth is characterized by the specific growth rate (μ_g):

$$\mu_{net} \equiv \frac{1}{X}\frac{dX}{dt}$$

- Where X is the cell mass concentration (g/l) ,
- t is time (h),
- μ_{net} is net specific growth rate (h^{-1})

Kinetics of Microbial Cell Growth

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

- Here μ_{net} (h^{-1}) is the difference between the gross specific growth rate of the cells (μ_g) and the rate of cell death K_d (μ_d)

- Microbial growth can also be described in terms of cell number / concentration (N/X). In that case

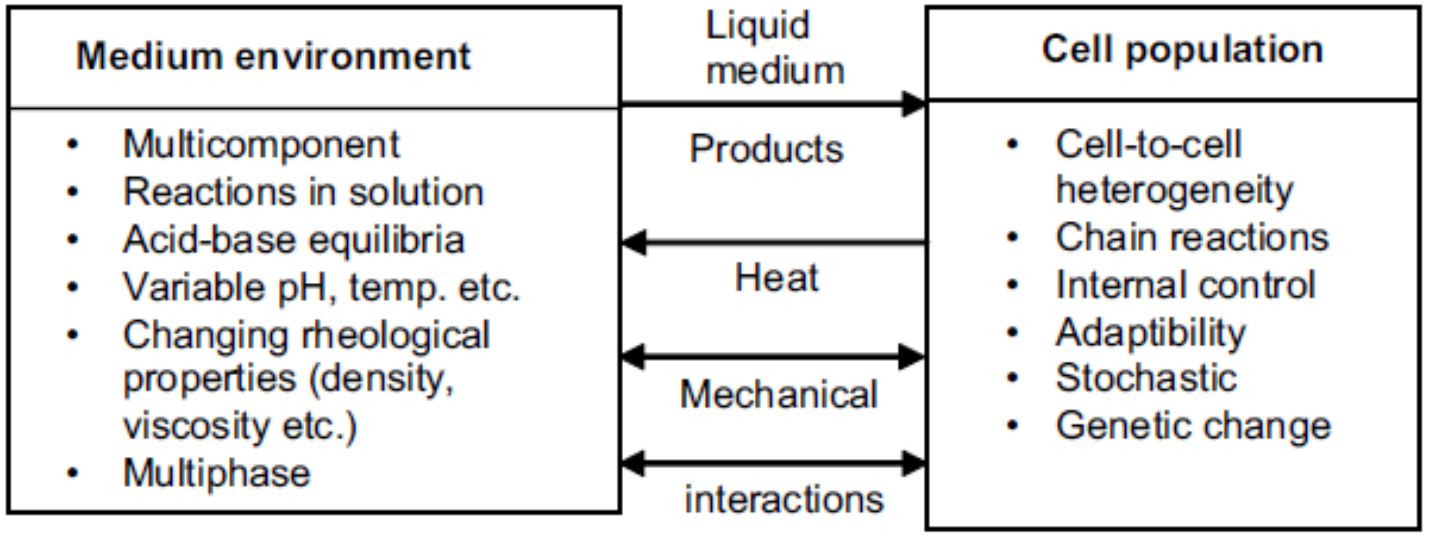
$$\mu_g = \frac{1}{X}\frac{dX}{dt} = \frac{1}{N}\frac{dN}{dt} \qquad \mu_R \equiv \frac{1}{N}\frac{dN}{dt}$$

- where μ_R is the net specific replication rate (h^{-1}).

- In this chapter we will discuss how the specific growth rate changes with its environment.

Introduction: How cells grow

- It is impractical to consider a kinetic model that has all the features mentioned in Fig.
- So it is necessary to simplify the model with some approximation and develop a useful representation of cell growth kinetics.



Summary of some of the important parameters, phenomena, and interactions that determine cell population kinetics.

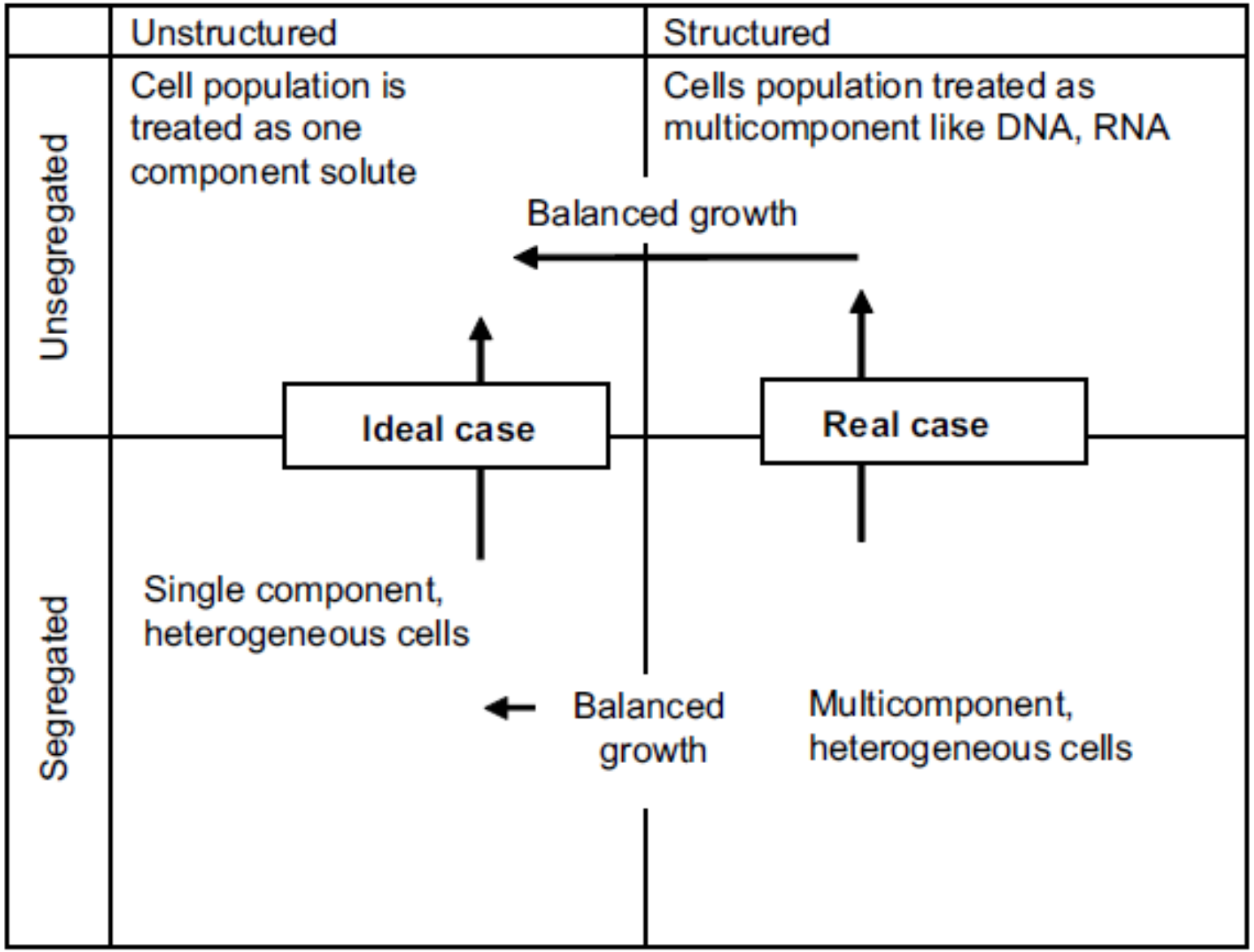
Kinetic Models for microbial cell Growth

- Model classifications for mathematical representation of cell populations is required
- Arnold Fredrickson and Henry Tsuchiya classified the microbial systems according to the number of components used in the cellular representation
- The term '**structured**' indicates a model representing cell material that consists of multiple chemical components.
- The **structured** model usually deals with the kinetics of the change in individual components present in the cells, such as RNA, DNA, proteins, etc.
- The **unstructured** model is considered a single component system assuming the kinetics of change of all components is same.

Introduction: How cells grow

- The term '**segregated**' designates a model describing the presence of individual cells in a heterogeneous population.
- In the **segregated** model, the kinetics of individual cells is taken into consideration separately.
- In the **unsegregated** model, the growth characteristics of individual cells are assumed to be same.
- The **real condition** of the living system is a **structured, segregated** one.
- In **ideal condition**, the cell growth kinetics is assumed to be in the **unsegregated, unstructured** mode.

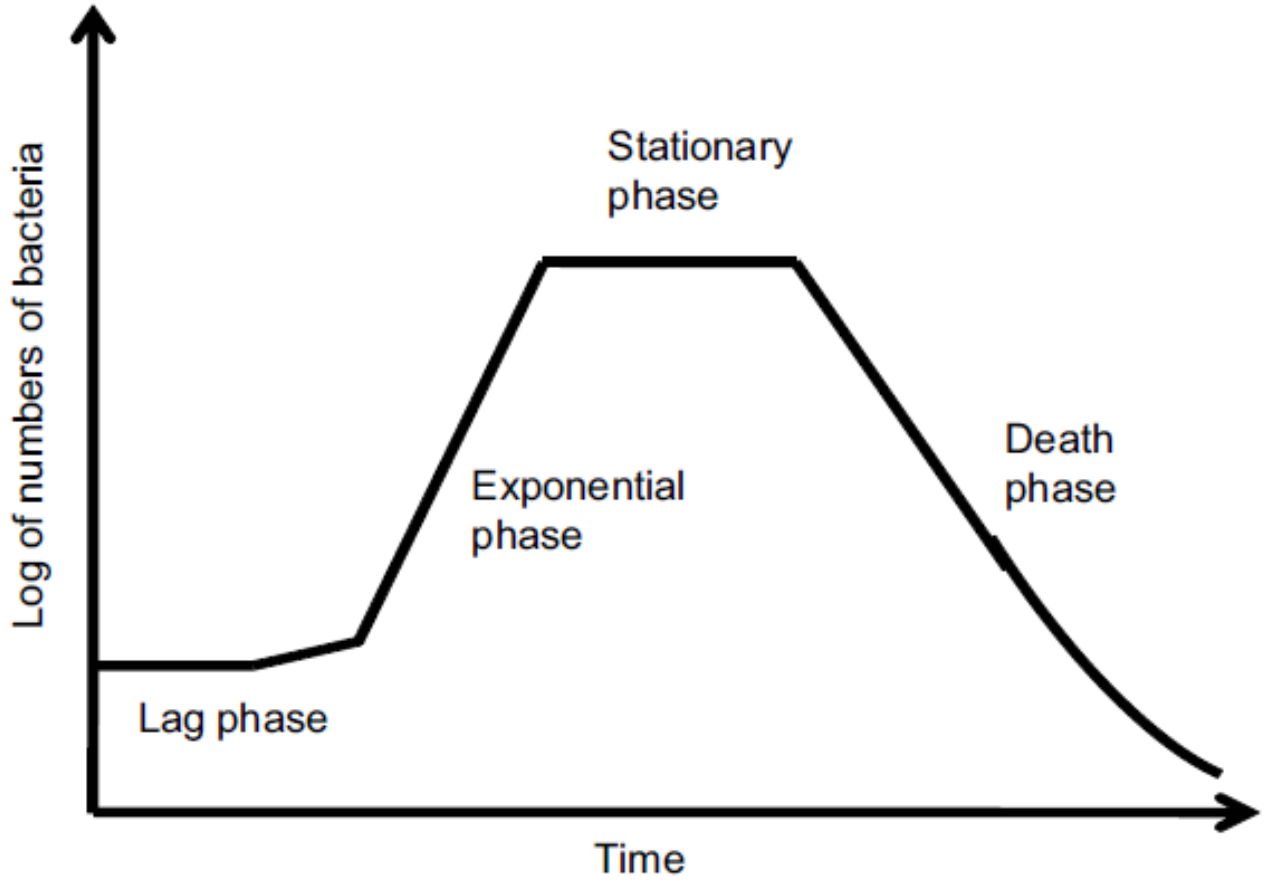
Kinetic Models for microbial cell Growth



Microbial Cell Growth Cycle

- A typical batch growth curve includes the following phases as shown in Fig:

- lag phase,
- log phase / exponential growth phase
- stationery phase, and
- death phase



Growth cycle of the bacteria

Microbial Cell Growth Cycle

- During the **lag phase**,
- occurs immediately after inoculation
- Microorganisms reorganize their molecular constituents when they are transferred to a new medium.
- Depending on the composition of nutrients, new enzymes are synthesized.
- The internal machinery of cells is adapted to the new environmental conditions.
- Is a period of adaptation of cells to a new environment / bacteria acclimatize themselves to the environment.

Microbial Cell Growth Cycle

- During the **lag phase**,
- These changes reflect the intracellular mechanisms for the regulation of the metabolic processes
- In this period, the individual bacteria become mature and divide a little without increase in cell number density.
- In this phase, RNA, enzymes, and other molecules undergo synthesis.
- The lag phase can last for 1 h to several days.
- It is also known as the acclimatization phase.

Microbial Cell Growth Cycle

- **lag phase depends on**,

- **The age of the inoculum culture:**
- age refers to how long a culture has been maintained in a batch culture
- the lag period increases with the age of the inoculum.
- there is an optimal inoculum age resulting in minimum lag period.

- **To minimize the duration of the lag phase**,
- cells should be adapted to the growth medium and conditions before inoculation,
- and cells should be young and active
- the inoculum size should be large (5% to 10% by volume).
- The nutrient medium may need to be optimized and
- certain growth factors included to minimize the lag phase.

Microbial Cell Growth Cycle

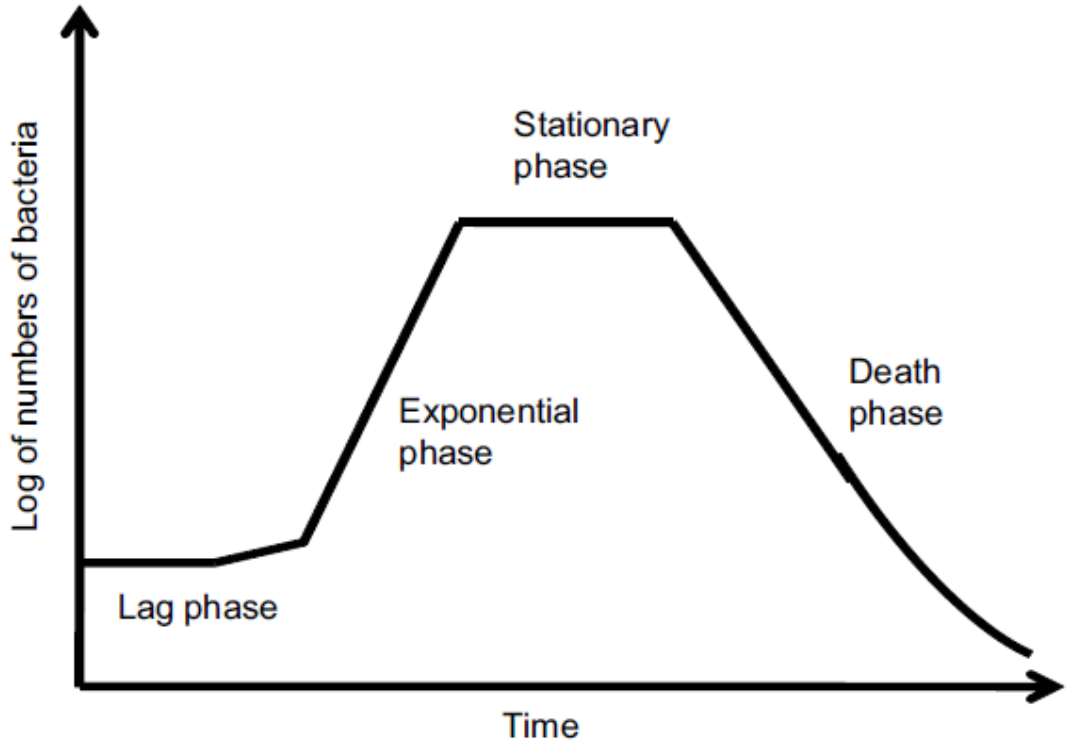
- The **log phase** is also termed as **the logarithmic growth phase** or the **exponential phase**.

- the cells have adjusted to their new environment

- After this adaptation period, cells can multiply rapidly, and cell mass and cell number density increase exponentially with time.

- This is a period of balanced growth, in which all components of a cell grow at the same rate.

- That is, the average composition of a single cell remains approximately constant during this phase of growth.



Growth cycle of the bacteria

Microbial Cell Growth Cycle

- The **log phase** is also termed as **the logarithmic growth phase** or the **exponential phase**.
- That is, the average composition of a single cell remains approximately constant during this phase of growth.
- During balanced growth, the net specific growth rate determined from either cell number or cell mass would be the same.
- Since the nutrient concentrations are large in this phase, the growth rate is independent of nutrient concentration.
- The exponential growth is characterized by a straight line on a semi logarithm plot of ln X versus time
- slope of this line gives the specific growth rate of the organism.

Microbial Cell Growth Cycle

- The **log phase** is also termed as **the logarithmic phase** or the **exponential phase**.

- The exponential growth rate is first order:

$$\frac{dX}{dt} = \mu_{net} X, \qquad X=X_0 \text{ at } t=0$$

- Integration yields:

$$\ln \frac{X}{X_0} = \mu_{net} t \qquad X = X_0 e^{\mu_{net} t}$$

- where X and X_0 are cell concentrations at time t and t = 0
- The time required to double the microbial mass is given by eq

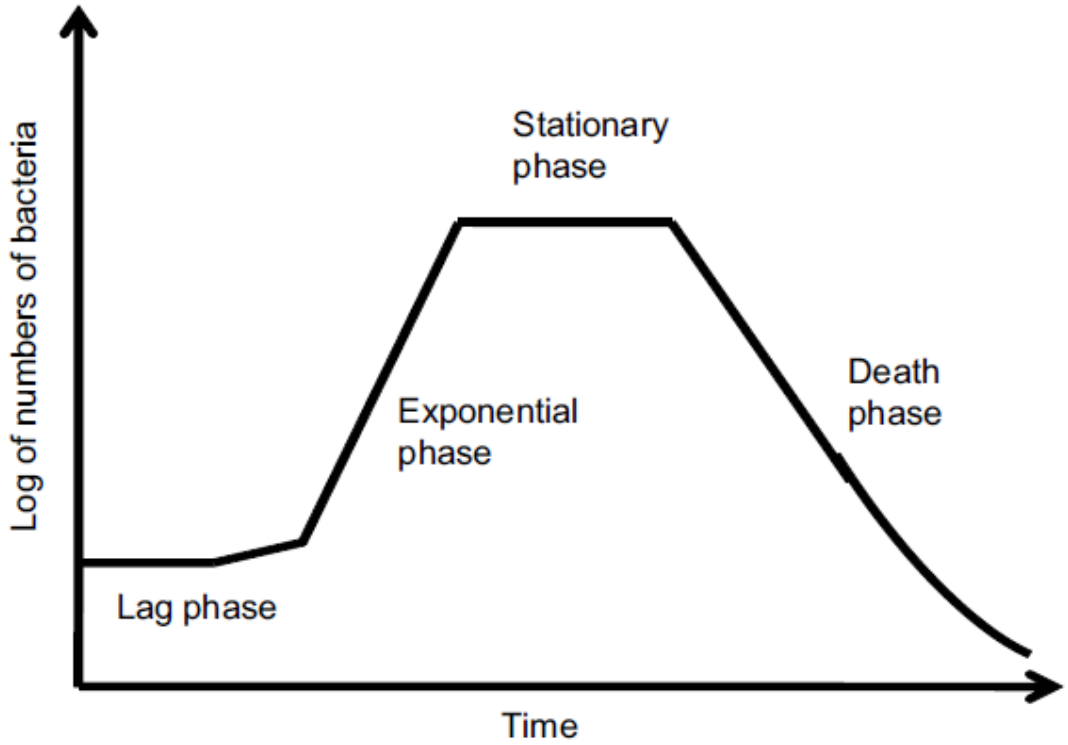
$$\tau_d = \frac{\ln 2}{\mu_{net}} = \frac{0.693}{\mu_{net}}$$

Microbial Cell Growth Cycle

- The **log phase** is also termed as **the logarithmic phase** or the **exponential phase**.

- The specific growth rates of different microorganisms are not uniform.

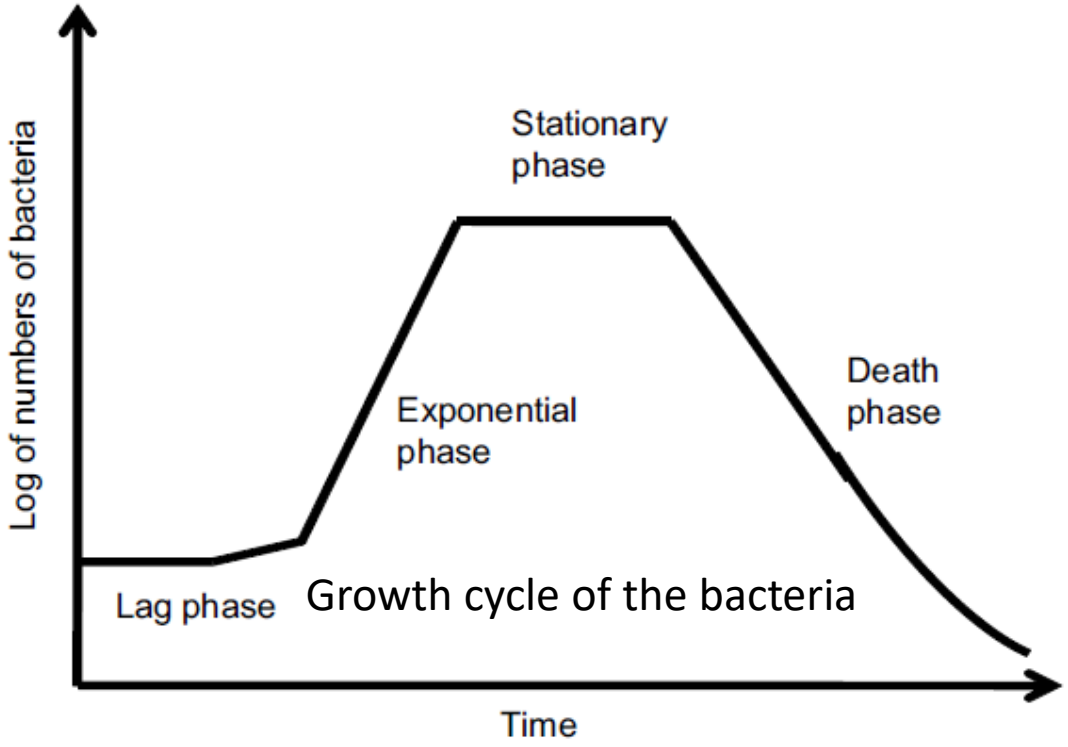
- Usually microbial cells in between the mid-log phase and the late log phase are most suitable for fermentation.



Growth cycle of the bacteria

Microbial Cell Growth Cycle

- **In the stationary phase**, the rate of cell growth is equal to the rate of cell death.
- Or the net growth rate is zero (no cell division)
- The stationary phase is considered the starvation phase due to the depletion of an essential nutrient, and/or the formation of an inhibitory product such as organic acids /toxic compounds.



- If an inhibitory product is produced and accumulates in the medium, the growth rate will slow down, depending on inhibitor production, and at a certain level of inhibitor concentration, growth will stop.

Microbial Cell Growth Cycle

- **In the stationary phase**, During the course of the stationary phase, one or more of the following phenomena may take place:

- Total cell mass concentration may stay constant, but the number of viable cells may decrease.

- Cell lysis may occur and viable cell mass may drop. A second growth phase may occur and cells may grow on lysis products of lysed cells

- Cells may not be growing but may have active metabolism to produce secondary metabolites.

- Cellular regulation changes when concentrations of certain metabolites (carbon, nitrogen, phosphate) are low.

Microbial Cell Growth Cycle

- **In the stationary phase**,

- The appropriate equation to describe the conversion of cell mass into maintenance energy or the loss of cell mass due to cell lysis during the stationary phase is

$$\frac{dX}{dt} = -k_d X \qquad\qquad X = X_{so} e^{-k_d t}$$

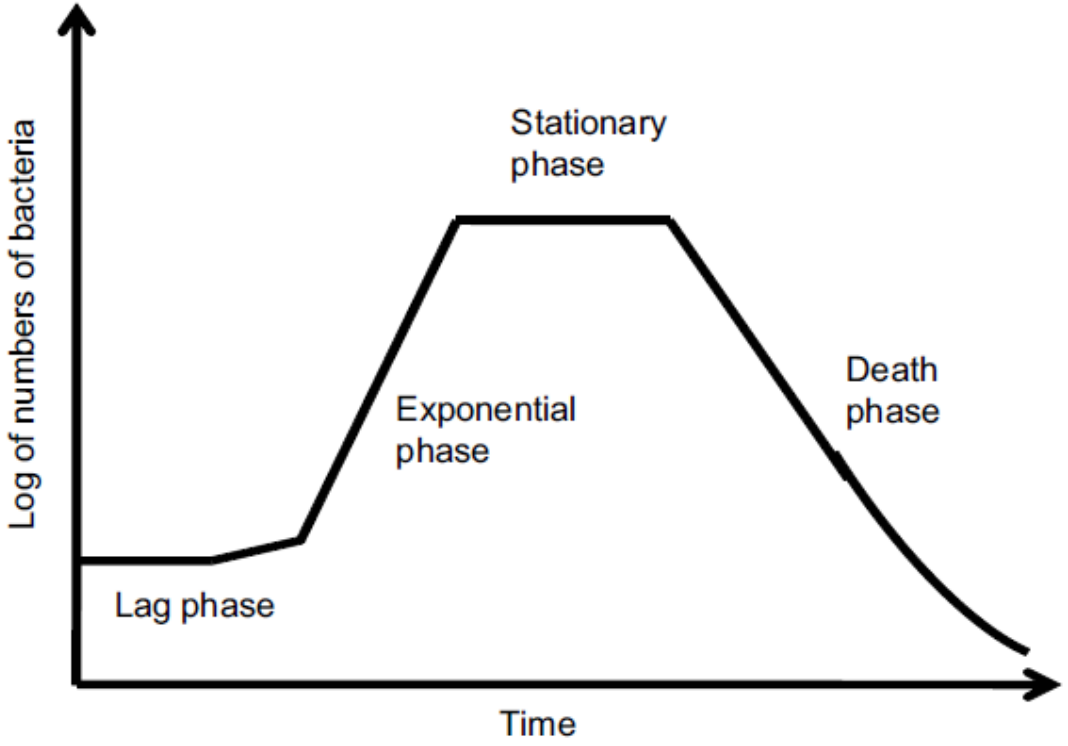
- where kd is a first-order rate constant for endogeneous metabolism, and
- Xso is the cell mass concentration at the beginning of the stationary phase.
- Because S is zero, μ_g is zero in the stationary phase.

- During the stationary phase, the cell catabolizes cellular reserves for new building blocks and for energy-producing monomers. This is called **endogenous metabolism**.

Microbial Cell Growth Cycle

- **The death phase** is also known as the **decline phase,** occurs at the end of the stationary phase, where the bacteria die **because of**

- either nutrient depletion
- or toxic product accumulation,
- environmental temperature above or below the tolerance limits for the species,
- other injurious conditions.



Growth cycle of the bacteria

Microbial Cell Growth Cycle

- **The death phase** is also known as the decline phase where the bacteria die.

- The rate of death usually follows first-order kinetics:

$$\frac{dN}{dt} = -k'_d N \qquad\qquad N = N_s e^{-k'_d t}$$

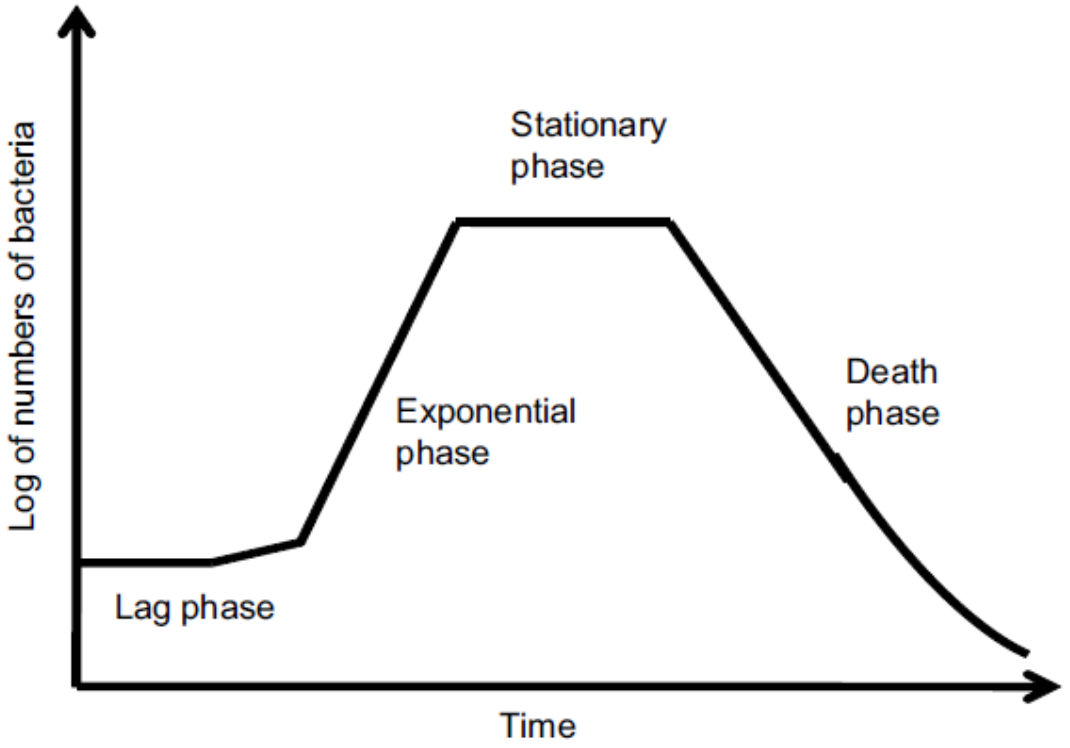
- where Ns is the concentration of cells at the end of the stationary phase and k'_d is the first order death-rate constant.

- A plot of ln N versus t yields a line of slope $-k'_d$.

Microbial Cell Growth Cycle

Functions of different phases of cell growth

Phase	Description	Specific growth rate
Lag	Cells acclimatize to the new environment, no growth	$\mu_{net} \sim 0$
Log	Cells are active and growth achieves its maximum rate	$\mu_{net} \sim \mu_{max}$
Stationary	Cells are under starvation and growth ceases due to starvation	$\mu_{net} \sim 0$
Death	Cell losses viability and lyse	$\mu_{net} < 0$



Growth cycle of the bacteria

Microbial Cell Growth: stoichiometrically related parameters

- To better describe growth kinetics, we define some stoichiometrically related parameters.

- **Yield coefficients** are defined based on the amount of consumption of another material.

  For example, the growth yield in a fermentation is

$$Y_{X/S} \equiv -\frac{\Delta X}{\Delta S}$$

- A **maintenance coefficient** is used to describe the specific rate of substrate uptake for cellular maintenance,

$$m \equiv -\frac{[dS/dt]_m}{X}$$

- However, during the stationary phase where little external substrate is available, endogeneous metabolism of biomass components is used for maintenance energy.

Microbial Cell Growth: Microbial products

- Microbial products can be classified in three major categories:

1) **Growth-associated products** are produced simultaneously with microbial growth.

- The specific rate of product formation is proportional to the specific rate of growth, μ_g

$$q_P = \frac{1}{X}\frac{dP}{dt} = Y_{P/X}\mu_g$$

- The production of a constitutive enzyme is an example of a growth-associated product.

2) **Nongrowth-associated product** formation takes place during the stationary phase when the growth rate is zero.

The specific rate of product formation is constant. $q_P = \beta = \text{constant}$

- Many secondary metabolites, such as antibiotics (for example, penicillin), are non-growth associated products.

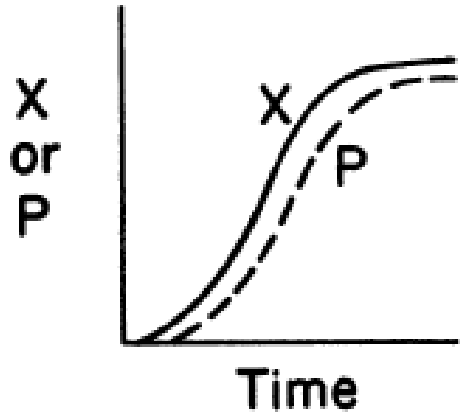
Microbial Cell Growth: Microbial products

3) **Mixed-growth-associated product** formation takes place during the slow growth and stationary phases.

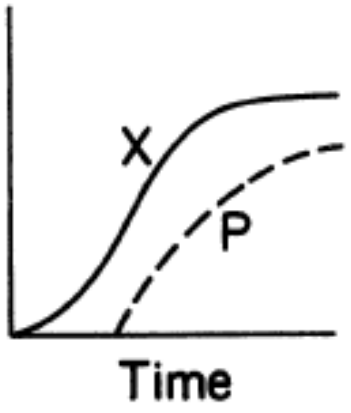
- In this case, the specific rate of product formation is given by the following equation:

$$q_P = \alpha\mu_g + \beta$$ Luedeking–Piret equation

- Lactic acid fermentation, xanthan gum, and some secondary metabolites from cell culture

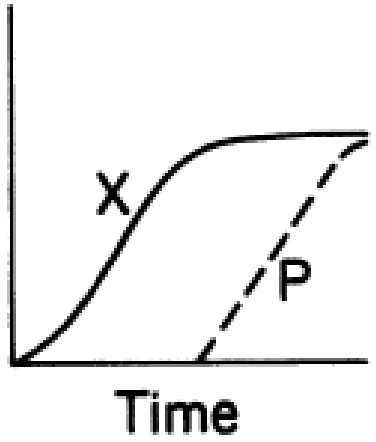


growth-associated product formation
If β = 0



mixed-growth-associated product formation

$$q_P = \alpha\mu_g + \beta$$



nongrowth-associated product formation
If α = 0

Problem statement: Growth rate and yield

A strain of mold was grown in a batch culture on glucose and the following data were obtained.

Time (h)	Cell concentration (g/l)	Glucose concentration (g/l)
0	1.25	100
9	2.45	97
16	5.1	90.4
23	10.5	76.9
30	22	48.1
34	33	20.6
36	37.5	9.38
40	41	0.63

a. Calculate the maximum net specific growth rate.

b. Calculate the apparent growth yield.

c. What maximum cell concentration could one expect if 150 g of glucose were used with the same size inoculum?

Problem statement: Growth rate and yield

a. Calculate the maximum net specific growth rate.

Solution: A plot of ln X versus t yields a slope of 0.1 h.

$$\mu_{net} = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} = \frac{\ln 37.5 - \ln 5.1}{36 - 16} \cong 0.1\ h^{-1}$$

Time (h)	Cell concentration (g/l)	Glucose concentration (g/l)
0	1.25	100
9	2.45	97
16	5.1	90.4
23	10.5	76.9
30	22	48.1
34	33	20.6
36	37.5	9.38
40	41	0.63

b) Calculate the apparent growth yield.

$$Y = -\frac{\Delta X}{\Delta S} = -\frac{41 - 1.25}{0.625 - 100} \cong 0.4\ \text{g cells/g substrate}$$

C) What maximum cell concentration could one expect if 150 g of glucose were used with the same size inoculum?

$$X_{max} = X_0 + YS_0 = 1.25 + 0.4(150) = 60.25\ \text{g cells/l}$$

How environmental conditions affect growth kinetics:

The patterns of microbial growth and product formation are influenced by environmental conditions such as

- temperature,
- pH, and
- dissolved-oxygen concentration.

How environmental conditions affect growth kinetics:

- **Temperature** is an important factor affecting the **performance of cells**
- According to their **temperature optima**, organisms can be classified in three groups:

  (1) Psychrophiles (T_{opt} < 20°C),

  (2) Mesophiles (T_{opt} = from 20° to 50°C), and

  (3) Thermophiles (T_{opt} > 50°C).

- As the temperature is increased toward optimal growth temperature, the **growth rate** approximately doubles for every 10°C increase in temperature.
- Above the optimal temperature range, the growth rate decreases and thermal death may occur.

How environmental conditions affect growth kinetics:

- The net specific replication rate can be expressed by the following equation for temperature above optimal level:

$$\frac{dN}{dt} = (\mu'_R - k'_d)N$$

- At high temperatures, the thermal death rate exceeds the growth rate, which causes a net decrease in the concentration of viable cells.

$$\mu'_R = Ae^{-E_a/RT} \qquad k'_d = A'e^{-E_d/RT}$$

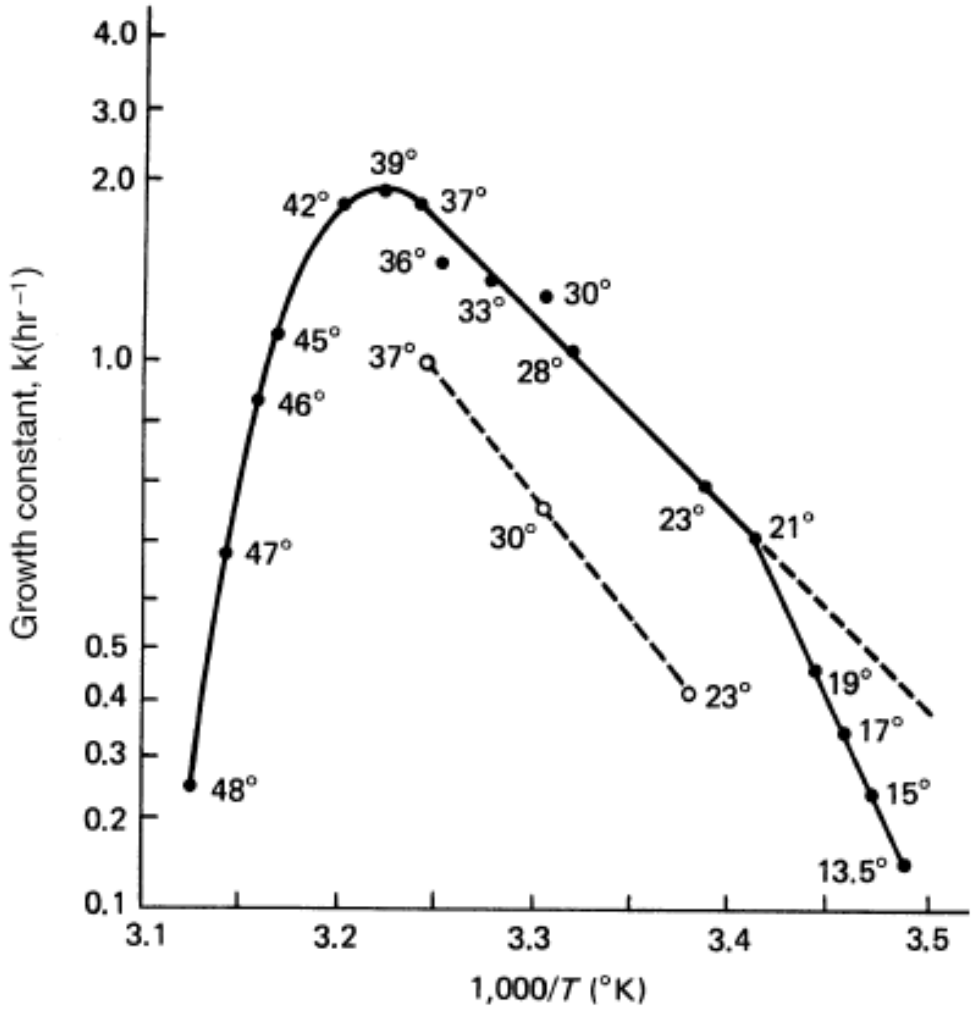
- Both μ'_R and k'_d vary with temperature according to the Arrhenius equation
- where Ea and Ed are activation energies for growth and thermal death.

- The activation energy for growth is typically 10 to 20 kcal/mol, and for thermal death 60 to 80 kcal/mol.
- That is, thermal death is more sensitive to temperature changes than microbial growth.

How environmental conditions affect growth kinetics:

- Temperature also affects **product formation**.
- The **yield coefficient** is also affected by temperature.
  - When temperature is increased above the optimum temperature, the maintenance requirements of cells increase.
  - That is, the maintenance coefficient increases with increasing temperature, resulting in a decrease in the yield coefficient.
- Temperature also may affect the **rate-limiting step in a fermentation process**
  - At high temperatures, the rate of bioreaction might become higher than the diffusion rate, and diffusion would then become the rate-limiting step (for example, in an immobilized cell system).
  - so diffusional limitations must be carefully considered at high temperatures.

How environmental conditions affect growth kinetics:



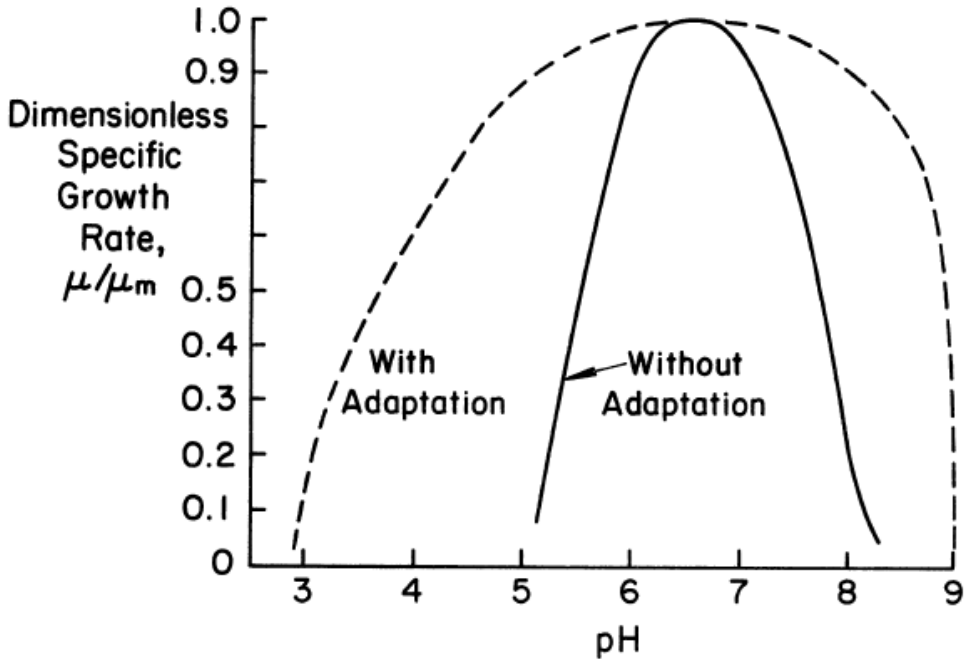
Arrhenius plot of growth rate

How environmental conditions affect growth kinetics:

- **Hydrogen-ion concentration (p^H)** affects the activity of enzymes and therefore the microbial growth rate.
- Different organisms have different pH optima:
  - for yeast, pH = 3 to 6;
  - for molds, pH = 3 to 7;
  - for plant cells, pH= 5 to 6; and
  - for animal cells, pH = 6.5 to 7.5.
- When pH differs from the optimal value, the maintenance-energy requirements increase.
- One consequence of different pH optima is that the pH of the medium can be used to select one organism over another.

How environmental conditions affect growth kinetics:

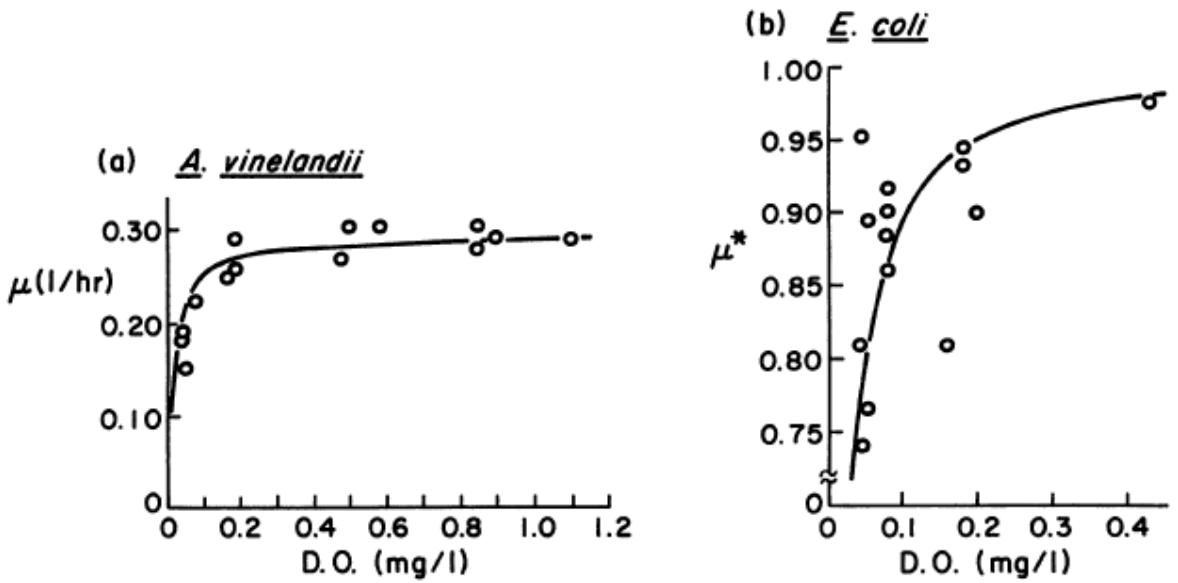
- **Hydrogen-ion concentration (p^H)** can **change because of**:
- production of organic acids,
- the utilization of acids (particularly amino acids),
- or the production of bases.
- The evolution or supply of CO_2 can alter pH greatly
- Thus, **pH control** by means of a
- buffer or an active pH control system is important.



Typical variation of specific growth rate with pH.

How environmental conditions affect growth kinetics:

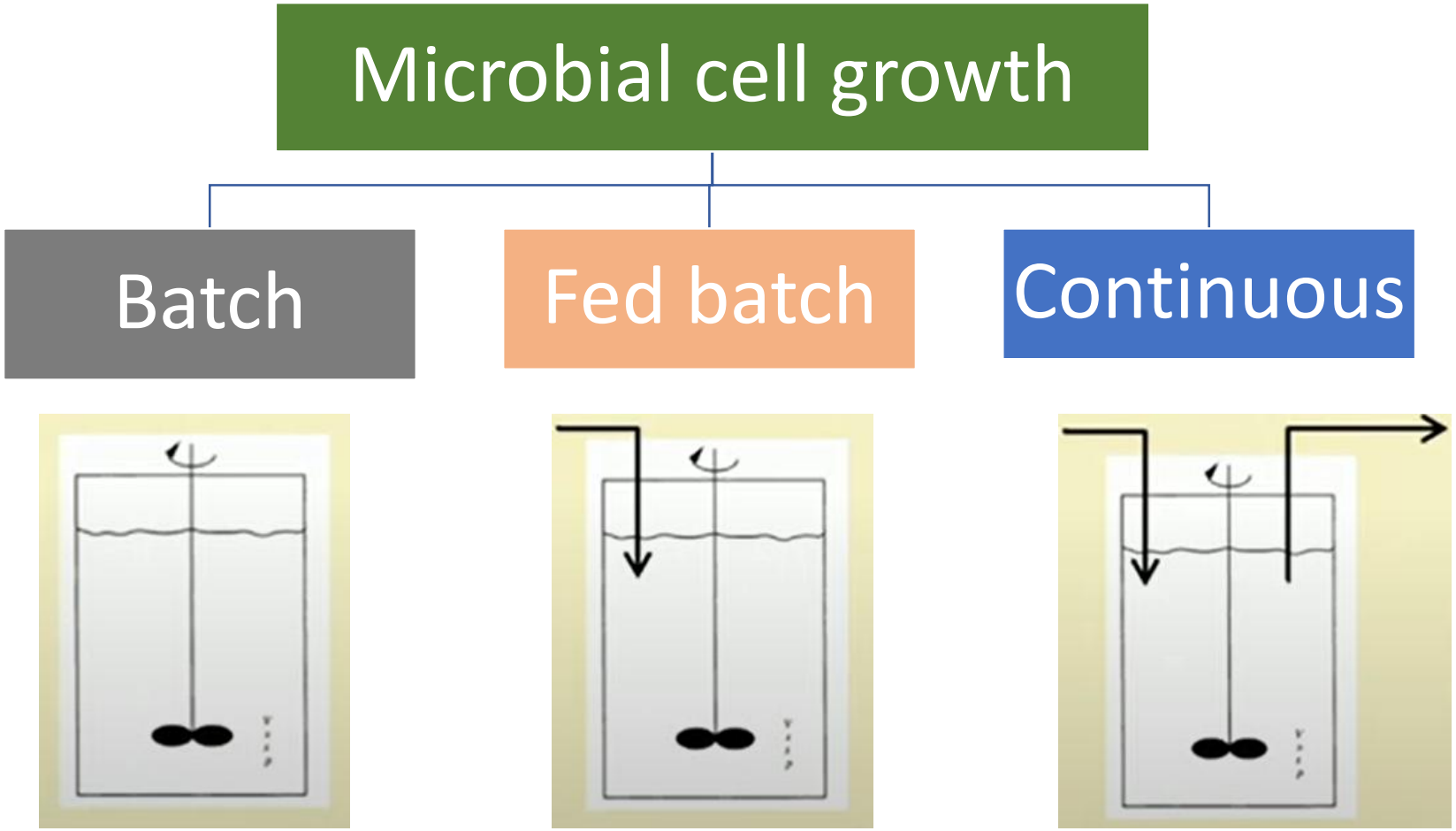
- **Dissolved oxygen (DO)** is an important substrate in aerobic fermentations
- May be a limiting substrate, since oxygen gas is sparingly soluble in water.
- At high cell concentrations, the rate of oxygen consumption may exceed the rate of oxygen supply, leading to oxygen limitations.
- Oxygen is a growth-rate-limiting factor when the DO level is below the critical DO concentration



variation of specific growth rate with dissolved-oxygen concentration

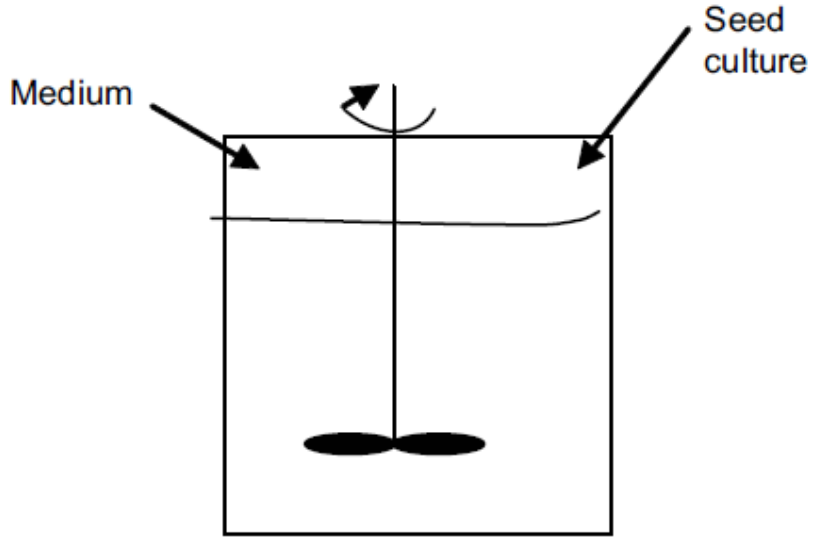
Microbial cell growth type:

- On the basis of mode of cultivation, microbial growth can be three different types:
- In this chapter, we will discuss how the specific growth rate changes with its environment.
- First, we will consider growth in batch culture, where growth conditions are constantly changing.



Batch Cell Growth Kinetics

- Several biochemical processes are carried out to study cell growth in batch process.
- In a batch reactor, the concentrations of nutrients, cells, and products vary with time as cell growth takes place.



- The microbial biomass and product formation can be represented as follows:

Substrate	+	Microbial Cells	→	Extracellular Products	+	More Microbial Cells
$\sum S$	+	X	→	$\sum P$	+	nX

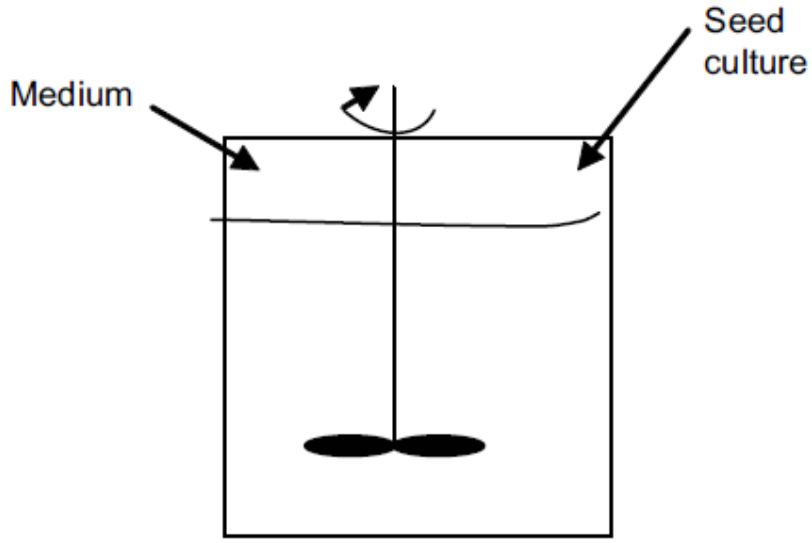
where S is the substrate concentration (mass/vol),

X is the cell mass concentration (mass/vol),

P is the product concentration (mass/vol), and

n is the number of cell divisions.

Batch Cell Growth Kinetics



- From the **material balance of any component "a,"** we g

$$\frac{d(S_a V_R)}{dt} = V_R \frac{dS_a}{dt} = V_R r_a$$

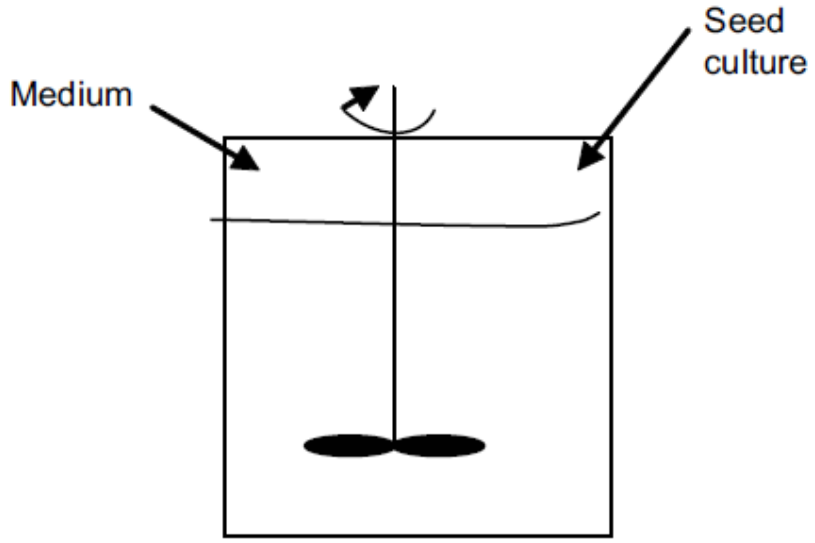
- where V_R is the working volume of the reactor,
  which is usually constant;
  S_a is the moles of "a" per volume;
  r_a is the rate of formation of "a" (in moles of "a" per volume per time).

Batch Cell Growth Kinetics

- If no liquid is added to or removed from the reactor,

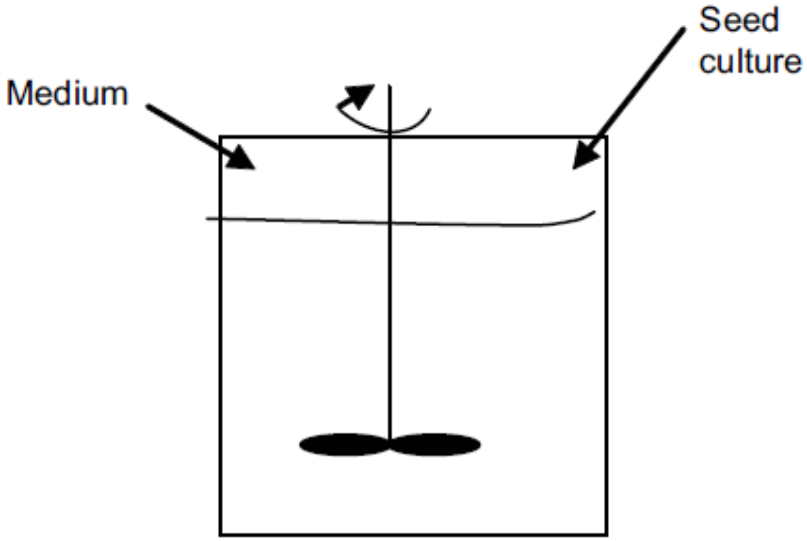
  and if the gas stripping of the culture liquid is negligible, then

$$\frac{dS_a}{dt} = r_a$$



- In general, the rate of formation, r_a, depends on the characteristics of the cell population (composition, morphology, and age distribution)

Batch Cell Growth Kinetics



- The batch process is an unsteady state operation where the the concentration of cell mass, substrate changes with time.

- The **cell mass (X) balance** can be written as:

  Input + Cell generation = Output + Accumulation + Cell death

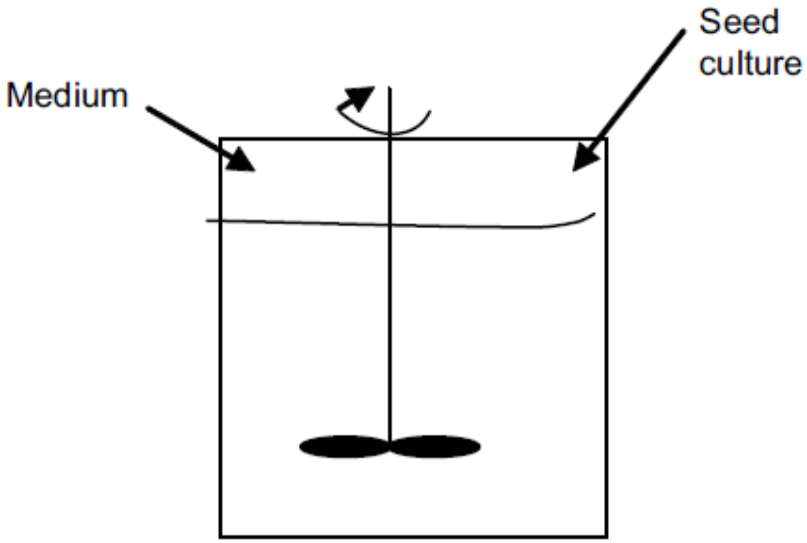
$$0 + \mu_g XV = 0 + \frac{d(XV)}{dt} + \mu_d XV$$

- where μ_g and μ_d are the rate of specific cell growth and specific cell death, respectively.

- For constant volume V, eq. becomes:

$$\frac{dX}{dt} = (\mu_g - \mu_d)X$$

Batch Cell Growth Kinetics



- If specific cell death rate (μ_d) is negligible as compared to cell growth ($\mu_g = \mu_{net} = \mu$),

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

- In batch growth in the log phase, μ_g remains approximately constant and approaches μ_{max}.

- Rearranging and integrating, we get

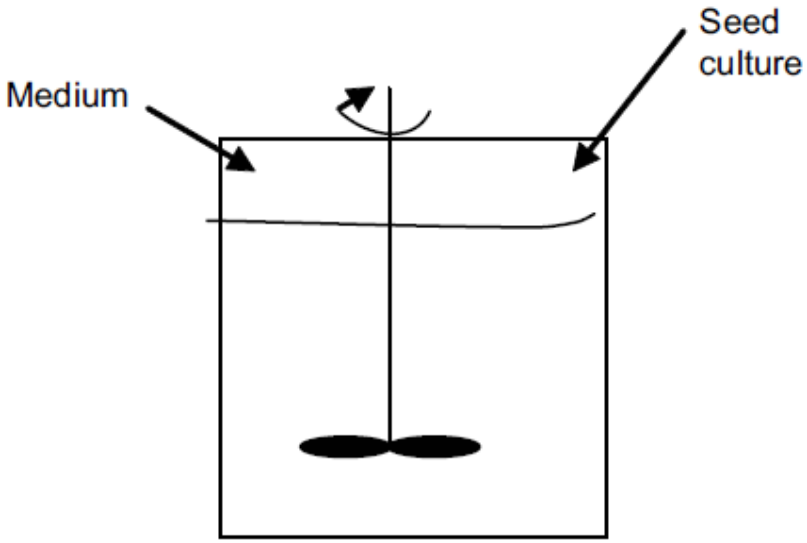
$$\int_{X_0}^{X} \frac{dX}{X} = \int_{0}^{t} \mu \, dt$$

$$\ln\left(\frac{X}{X_0}\right) = \mu t$$

- Time required for cell mass from X_0 to X

$$t = \frac{\ln\left(\frac{X}{X_0}\right)}{\mu}$$

Batch Cell Growth Kinetics



- The doubling time of the cell (t_d) is defined as the time required for doubling the microbial mass ($X=X_0$)

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

- The minimum doubling time is: $$t_{d\,min} = \frac{\ln 2}{\mu_{max}}$$

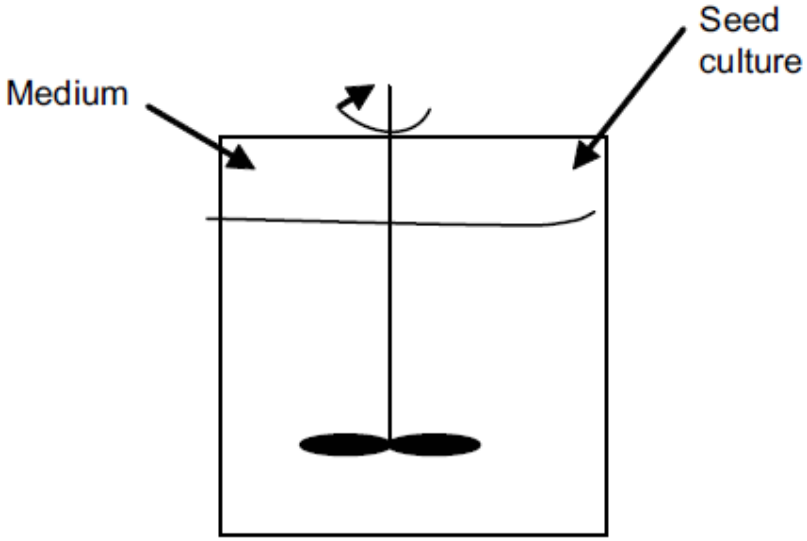
- The generation time of a cell is defined as the time required for cell division.
- If X_n is the number of cells produced during the cell division, the generation time (tgn) may be calculated as follows:

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

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

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

Batch Cell Growth Kinetics



- Like specific growth rate $\left(\mu = \frac{1}{X}\frac{dX}{dt}\right)$ specific substrate consumption rate and specific product formation rate may be expressed as follows:

- The **specific substrate consumption** rate is

$$q_S = \frac{1}{X}\frac{dS}{dt}$$

- The **specific product formation** rate is

$$q_P = \frac{1}{X}\frac{dP}{dt}$$

- Yield in cell culture

$$Y_{X/S} = \frac{\text{mass of biomass produced}}{\text{mass of substrate consumed}} = -\frac{dX}{dS} = \frac{X - X_0}{S_0 - S}$$

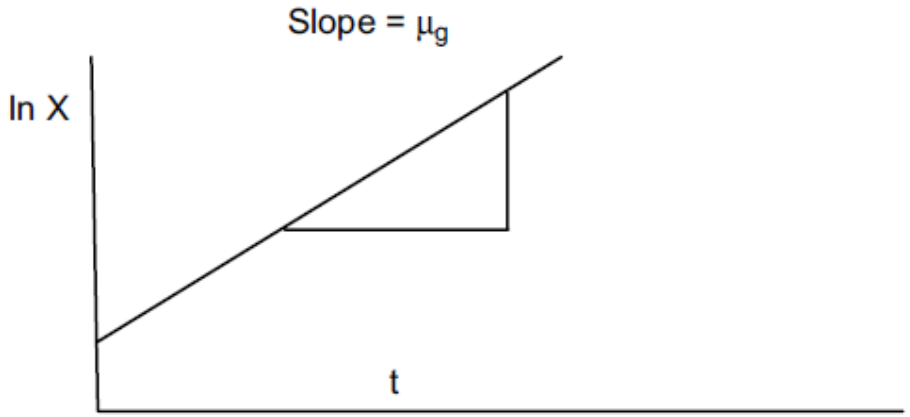
$$Y_{P/S} = \frac{\text{mass of product formed}}{\text{mass of substrate consumed}} = -\frac{dP}{dS} = \frac{P - P_0}{S_0 - S}$$

Kinetics of Microbial Cell Growth

- Microbial growth rate: In the case of no cell death equation can be modified as:

$$\mu_{net} = \mu_g = \frac{d \ln X}{dt}$$

- The slope of the straight line is equal to μ_g.



Plot ln X versus t

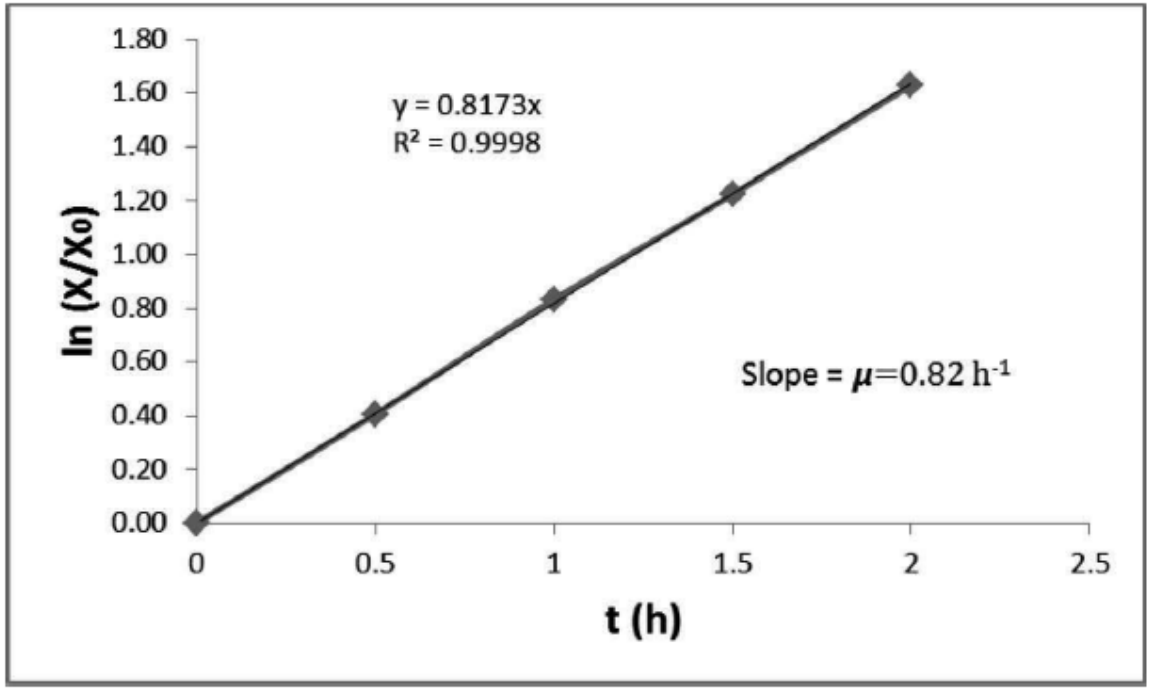
- **Problem statement:** At each cell division, a new microorganism produced three daughters.
  The cell growth rate data is given in Table
  Estimate the generation time of the microorganism.

Time (h)	Dry cell weight (g/L)
0	0.10
0.5	0.15
1.0	0.23
1.5	0.34
2.0	0.51

Kinetics of Microbial Cell Growth

- **Solution:** From the given data, the following table can be obtained.

t	X	$\ln (X/X_0)$
0	0.1	0.00
0.5	0.15	0.41
1	0.23	0.83
1.5	0.34	1.22
2	0.51	1.63



From the graph, $\mu = 0.82\ h^{-1}$

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

Given $n = 3$ Therefore, $t_{gn} = \frac{\ln(3)}{0.82} = 1.34\ h$

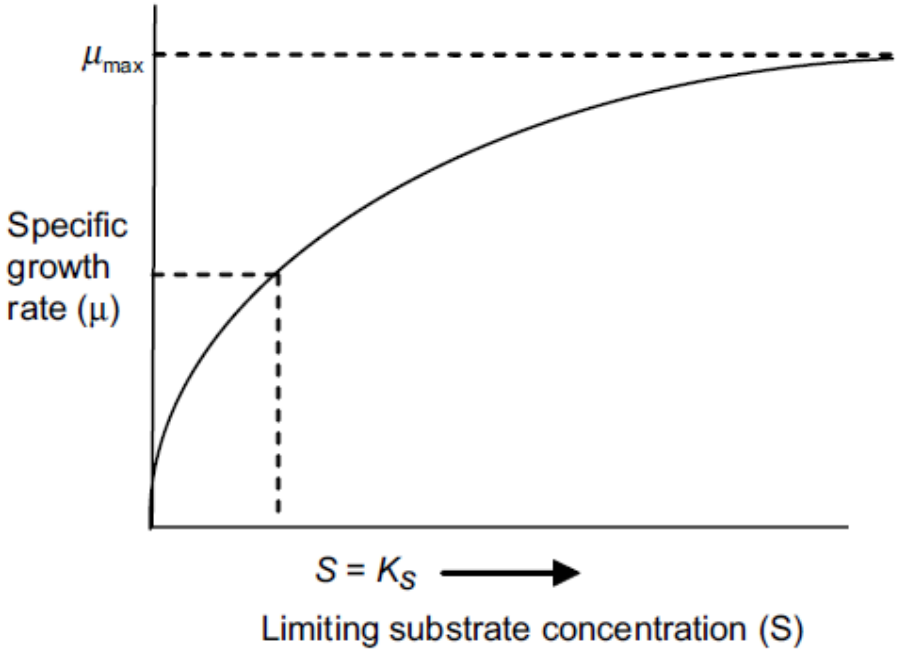
Kinetic Models for microbial cell Growth

- Different mathematical models have been proposed to describe the growth kinetics of microbial cells.

- As shown in fig., the relationship of specific growth rate to substrate concentration often assumes the form of saturation kinetics.

- Here, we assume that a single chemical species, *S*, is growth-rate limiting (i.e., an increase in *S* influences growth rate, while changes in other nutrient concentrations have no effect).

- These kinetics are similar to the Langmuir–Hinshelwood (or Hougen–Watson) kinetics in traditional chemical kinetics or Michaelis–Menten kinetics for enzyme reactions.



Correlation between specific growth rate (μ) and limiting substrate (S).

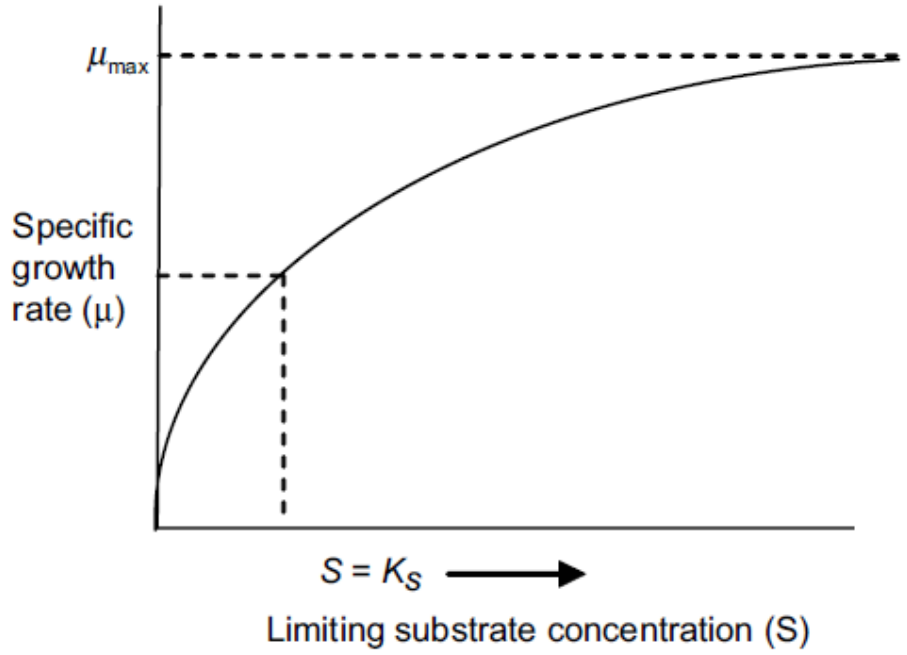
Kinetic Models for microbial cell Growth

- When applied to cellular systems, these kinetics can be described by the *Monod equation:*

- This is considered an ideal equation,
  i.e., an unstructured, unsegregated model

$$\mu_g = \frac{\mu_m S}{K_s + S} \qquad \mu = \frac{\mu_{max} S}{K_S + S}$$

- where μ, μ_g is the specific growth rate (h^{-1}),
  μ_{max}, μ_m is the maximum specific growth rate (h^{-1}),
  K_S is the saturation constant (g/L),
  and S is the growth-limiting substrate concentration (g/L).



Correlation between specific growth rate (μ) and limiting substrate (S).

Kinetic Models for microbial cell Growth

- where μ_m is the maximum specific growth rate when S >> Ks.

- If endogeneous metabolism is unimportant, then $\mu_{net} = \mu_g$.

- The constant Ks is known as the saturation constant or half velocity constant and is equal to the concentration of the rate-limiting substrate when the specific rate of growth is equal to one-half of the maximum.

  That is, Ks = S when μg = ½ μmax.

  In general, $\mu_g = \mu_m$ for S >> Ks and $\mu_g = (\mu_m/Ks)S$ for S < < Ks.

- The Monod equation empirically fits a wide range of data satisfactorily and is the most commonly applied unstructured, non-segregated model of microbial growth.

The limitations of the Monod model:

- When $S \rightarrow \infty$, $\mu \rightarrow \mu_{max}$.
- μ is finite when S is finite.
- Does not explain what will happen when $S \rightarrow 0$.
- Does not take care of cell death.
- Not applicable in the case of substrate and product inhibitions.

The limitations of the Monod model:

- The Monod equation describes substrate-limited growth only when growth is slow and population density is low.

- At high population levels, the buildup of toxic metabolic by-products becomes more important.

- The following rate expressions have been proposed for rapidly growing dense cultures:

- Or

$$\mu_g = \frac{\mu_m S}{K_{s0} S_0 + S}$$

$$\mu_g = \frac{\mu_m S}{K_{s1} + K_{s0} S_0 + S}$$

- where S_0 is the initial concentration of the substrate and K_{s0} is dimensionless.

Other substrate limited growth models:

- Other equations have been proposed to describe the substrate-limited growth phase. as an alternative to the Monod equation are as follows:

- Blackman equation:

$$\mu_g = \mu_{max} \quad \text{if } S \geq 2K_s$$

$$\mu_g = \frac{\mu_{max}}{2K_S} S \quad \text{if } S < K_S$$

- Tessier equation:

$$\mu_g = \mu_{max}(1 - e^{-K_S})$$

- Moser equation:

$$\mu_g = \frac{\mu_{max} S^n}{K_S + S^n} = \mu_{max}(1 + K_S S^{-n})^{-1}$$

- Contois equation:

$$\mu_g = \frac{\mu_{max} S}{K_{SX} X + S}$$

The Contois model very much resembles with the Monod equation, where the saturation constant depends on the cell mass concentration.

Other substrate limited growth models:

- Although the **Blackman equation** often fits the data better than the Monod equation, the discontinuity in the Blackman equation is troublesome in many applications.

- The **Tessier equation** has two constants (μ_m, K), and the **Moser equation** has three constants (μ_m, Ks, n).

- The **Moser equation** is the most general form of these equations, and it is equivalent to the Monod equation when n = 1.

- The **Contois** equation has a saturation constant proportional to cell concentration that describes substrate-limited growth at high cell densities.

- According to this equation, the specific growth rate decreases with decreasing substrate concentrations and eventually becomes inversely proportional to the cell concentration in the medium (i.e., mg μ X^{-1}).

Generalized growth model:

- The different growth equations can be described by a single differential equation as follows:

$$\frac{dv}{dS} = K v^a (1 - v)^b$$

- where u = µg/µmax ; S is the rate-limiting substrate concentration; and K, a, and b are constants.

Values of a, b, and K for different growth equations

a		***b***	***K***
Monod	0	2	$1/K_s$
Moser	$1 - (1/n)$	$1 + (1/n)$	$n/K_s^{1/n}$
Contois	0	2	$1/K_{SX}$
Tessier	0	1	$1/K$

Advantages and disadvantages of batch culture

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

Generalized growth model:

- Three types of inhibitions may take place in microbial growth:
    - Substrate inhibition
    - Product inhibition
    - Inhibition by toxic compounds
- The inhibition pattern of microbial growth is **analogous** to the inhibition of enzymatic reaction.
- Often the **underlying mechanisms** are **complicated**.
- The **kinetic constants** are obtained from the experimental data by **curve fitting** and do not have any biological meaning.

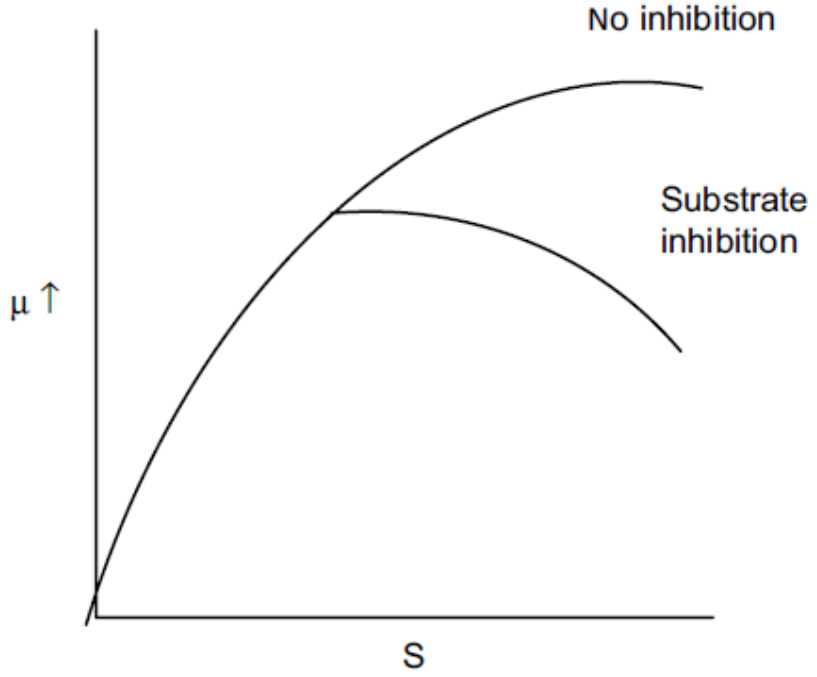
Substrate inhibition :

- At high substrate concentrations, the microbial growth rate is inhibited by the higher substrate concentration, as shown in Fig.

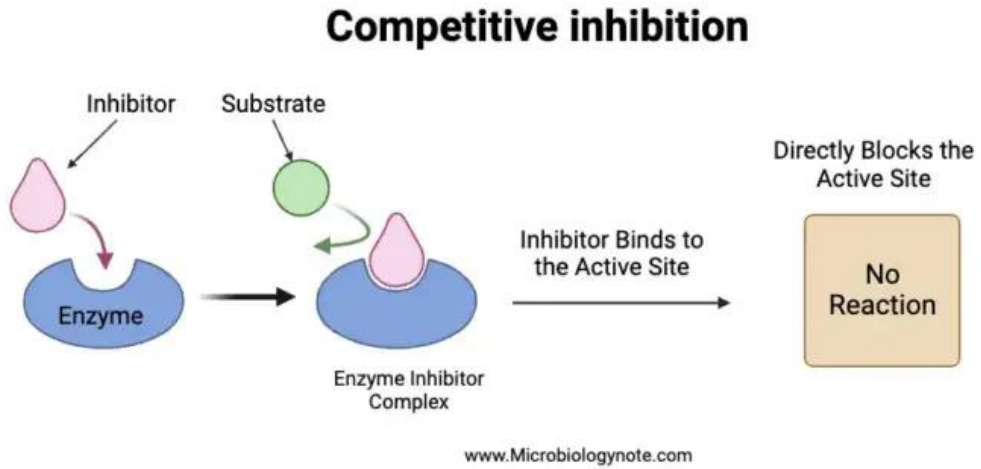
- The substrate inhibition for cell growth may be either competitive or non-competitive.

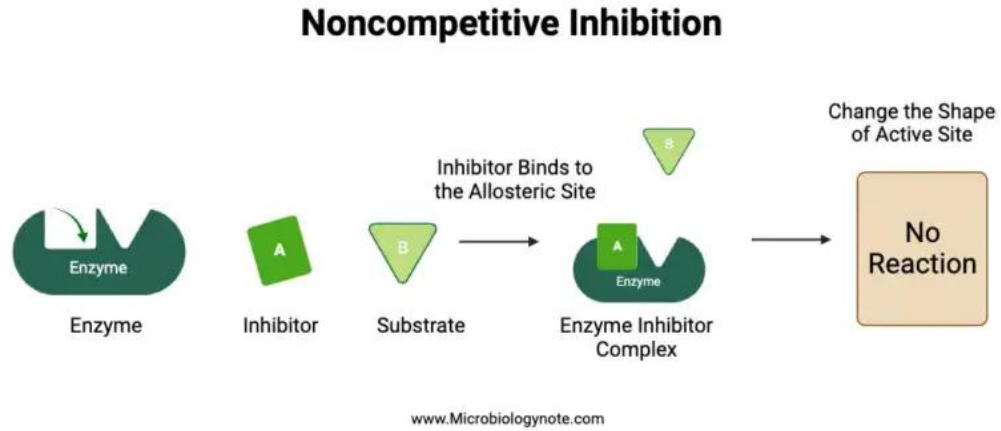
- In non-competitive substrate inhibition, the specific growth rate of the cell may be expressed as:

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



Correlation between μ and S in the case of substrate inhibition.


Competitive inhibition
Inhibitor
Substrate
Enzyme
Enzyme Inhibitor Complex
Inhibitor Binds to the Active Site
Directly Blocks the Active Site
No Reaction
www.Microbiologynote.com


Noncompetitive Inhibition
Enzyme
A
B
Inhibitor Binds to the Allosteric Site
Change the Shape of Active Site
No Reaction
Enzyme
Inhibitor
Substrate
Enzyme Inhibitor Complex
www.Microbiologynote.com

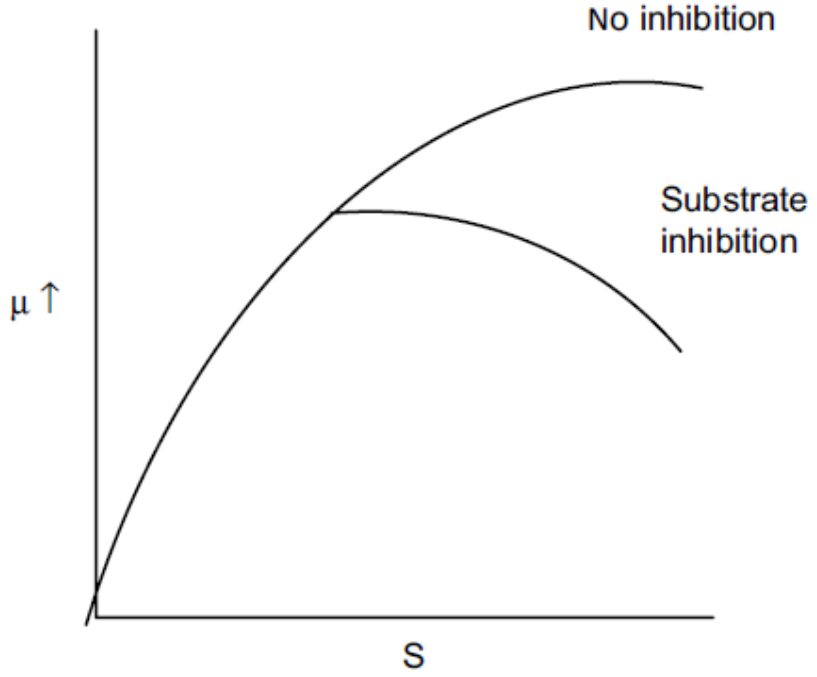
Substrate inhibition :

- $K_I >> K_S$, then

$$\mu = \frac{\mu_{max} S}{K_S + S + \left(\frac{S^2}{K_I}\right)}$$

- In the case of competitive substrate inhibition, the cell growth equation may be written as

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



Correlation between μ and S in the case of substrate inhibition.

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

Product inhibition :

- High product concentration can be inhibitory for microbial growth.
- The product inhibition of growth may also be competitive or noncompetitive.
- The non-competitive product inhibition may be expressed as

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

- where K_P is the product inhibition constant.
- The competitive product inhibition is given as follows:

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

- Other cell growth rate expressions for the product inhibition are shown below

$$\mu = \frac{\mu_{max} S}{\left(1+\frac{K_S}{S}\right)}\left(1-\frac{P}{P_m}\right)^n$$

$$\mu = \frac{\mu_{max}}{K_S\left(1+\frac{K_S}{S}\right)} e^{-P/K_P}$$

- where Pm is the product concentration at which cell growth stops. Kp is product inhibition constant.

Toxic compound inhibition :

- Inhibition by toxic compounds is analogous to enzyme inhibition as follows:

- **Non-competitive inhibition:**

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

- **Competitive inhibition:**

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

- **Uncompetitive inhibition:**

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

Logistic Equation:

- The logistic equation characterizes cell growth in terms of carrying capacity, i.e., the maximum cell mass that can be obtained (X_m). It does not consist of substrate concentration.

- The expression for cell growth rate can be represented as:

$$\frac{dX}{dt} = \mu_{max} X\left(1 - \frac{X}{X_{max}}\right)$$

- where μ_{max} is the maximum specific growth rate constant (h^{-1}), X_{max} is the maximum biomass concentration at the end (g/L), and X is the biomass concentration at any time t (g/L).

- The integral form of the above equation can be written as:

Boundary conditions $X=X_0$ at t=0

$$X = \frac{X_0 e^{\mu_{max} t}}{\left\{1 - \frac{X_0}{X_{max}}\left(1 - e^{\mu_{max} t}\right)\right\}}$$

Comparison of logistic equation and Monod equation:

Logistic equation	Monod equation
Independent of substrate concentration and is only related to biomass concentration.	Microbial growth is related to biomass concentration and limiting substrate concentration.
Cell growth rate is directly proportional to biomass concentration and the carrying capacity $(X_m - X)$.	It does not consider more than one growth-limiting substrate concentration.

Monod equation is related to limiting substrate concentration and
The logistic equation is related to maximum biomass concentration

Cell Growth Characteristics of Multicellular Cells Like Mold:

- Filamentous organisms such as **mold** 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 the pellet in the submerged culture increases linearly with time, such as:

$$\frac{dR}{dt} = k_P$$

- where R is the pellet radius.
- The biomass M can be expressed as follows:

$$M = \rho \frac{4}{3} \pi R^3$$

$$\frac{dM}{dt} = \rho 4\pi R^2 \frac{dR}{dt} = k_p \rho 4\pi R^2$$

Cell Growth Characteristics of Multicellular Cells Like Mold:

$$\frac{d\mathrm{M}}{dt} = \gamma \mathrm{M}^{2/3}$$

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

Integrating with an initial biomass of M_0, we get

$$M = \left(M_0^{1/3} + \frac{\gamma t}{3} \right)^3$$

Since Mass of the pellet ($M_{0)}$ << Final mass (M),

$$M = \left(\frac{\gamma t}{3} \right)^3$$

- Thus, the above equation gives the cubic dependence of M on t.

Product Formation Kinetics in Cell Culture:

- The **Luedeking–Piret model** is considered to correlate the rate of product formation (other than cell mass) with the cell growth rate.
- It combines growth-associated and non-growth-associated product formation as follows:

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

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

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

- where dP/dt is the rate of product formation,

  dX/dt is the biomass growth rate,

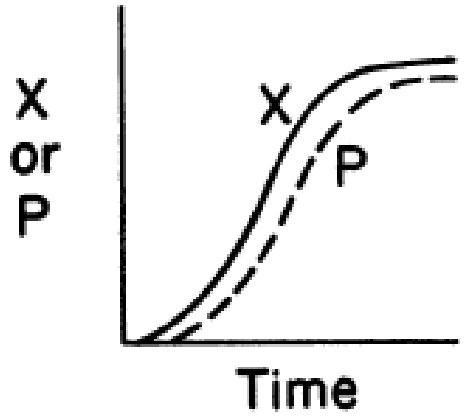
  α is the growth-associated coefficient, and

  β is the nongrowth- associated coefficient.

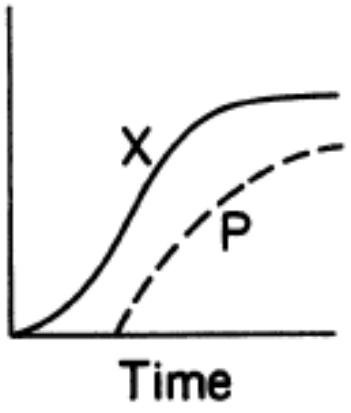
Microbial Cell Growth: Microbial products

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

$$q_P = \alpha \mu_g + \beta$$ Luedeking–Piret equation

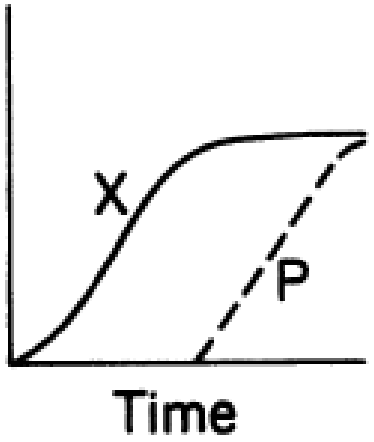


growth-associated product formation
If β = 0



mixed-growth-associated product formation

$$q_P = \alpha \mu_g + \beta$$



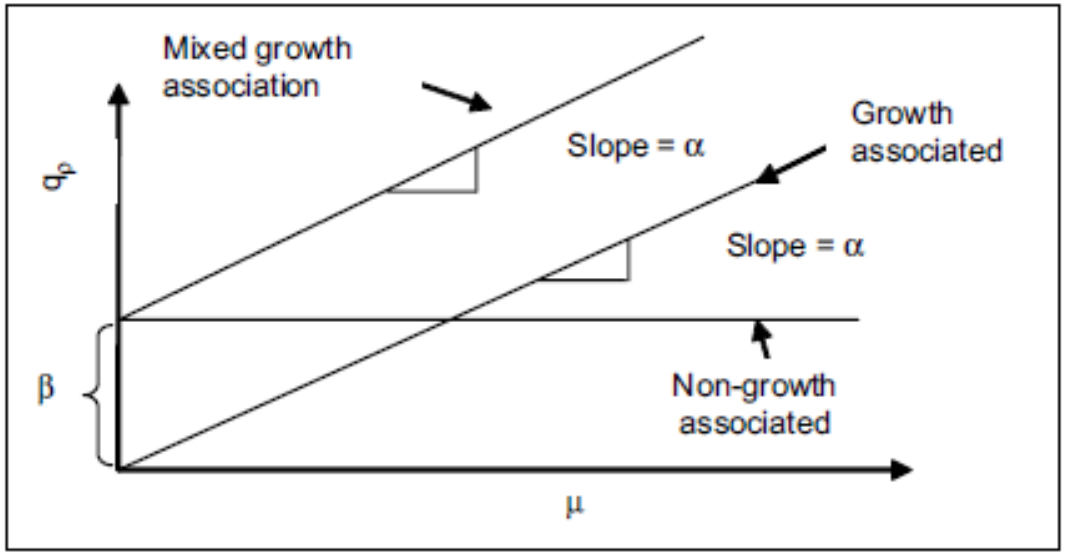
nongrowth-associated product formation
If α = 0

Product Formation Kinetics in Cell Culture:

- The values of α and β determine the product formation kinetics of the cell.

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

  - If α = 0, the product is non-growth associated.
  - If β = 0, the product is growth associated.
  - If α ≠ 0 and β ≠ 0, the product is mixed growth associated.



- Examples of growth-associated products are ethanol, enzymes, etc.,
- Examples of non-growth-associated products are all antibiotics such as penicillin, streptomycin, etc.
- Examples of mixed growth associated are lactic acid fermentation, etc.

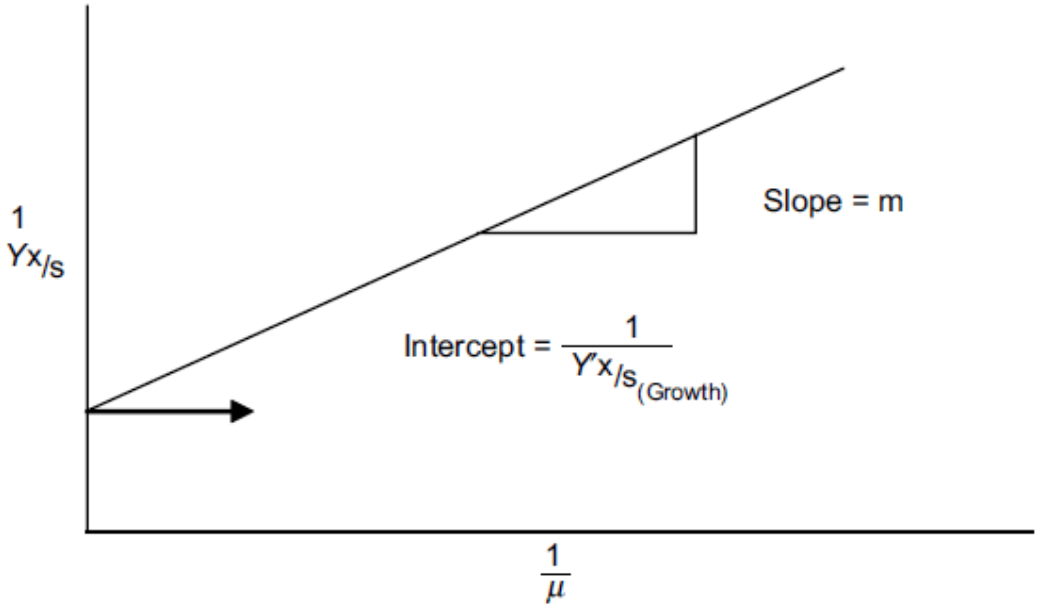
Determination of Maintenance Coefficient of Cells:

- In cell growth, a part of the substrate contributes to cell maintenance.
- The **Pirt model** deals with the maintenance of cells.
- The model is developed as follows:

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

$$\frac{\mu X}{Y_{X/S(\text{overall})}} = \frac{\mu X}{Y'_{X/S(\text{growth})}} + mX$$

$$\frac{1}{Y_{X/S(\text{overall})}} = \frac{1}{Y'_{X/S(\text{growth})}} + \frac{m}{\mu}$$



Plot of $1/Y_{X/S}$ versus $1/\mu$.

where

- m is the maintenance coefficient (time^{-1}),
- μ is the specific growth rate of cell (time^{-1}), and
- $Y'_{X/S}$ (growth) is the true cell yield coefficient.
- The slope of the line of the plot $1/Y_{X/S}$ (overall) versus 1/m gives the value of m,
- The intercept gives the value of $Y'_{X/S}$ (growth)

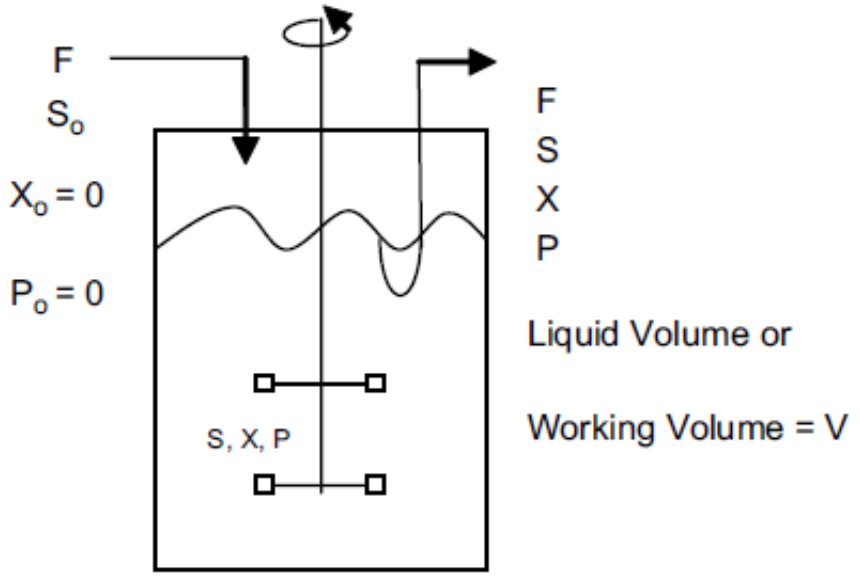
Continuous culture:

- 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 the culture at the same time.

Ideal Continuous Flow Stirred Tank Reactor

- A schematic diagram of a continuous stirred tank reactor (CSTR) / chemostat is shown in Fig.
- 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.

- A substrate is continuously added and the feed is continuously removed.
- A quasi-steady state is developed.



Continuous flow stirred-tank reactor or chemostat.

Ideal Continuous Flow Stirred Tank Reactor

- The **substrate balance** at steady-state conditions can be written as:

Input + Generation = Output + Consumption + Accumulation

$$FS_0 + 0 = FS_{SS} + \frac{dS}{dt}V + 0$$

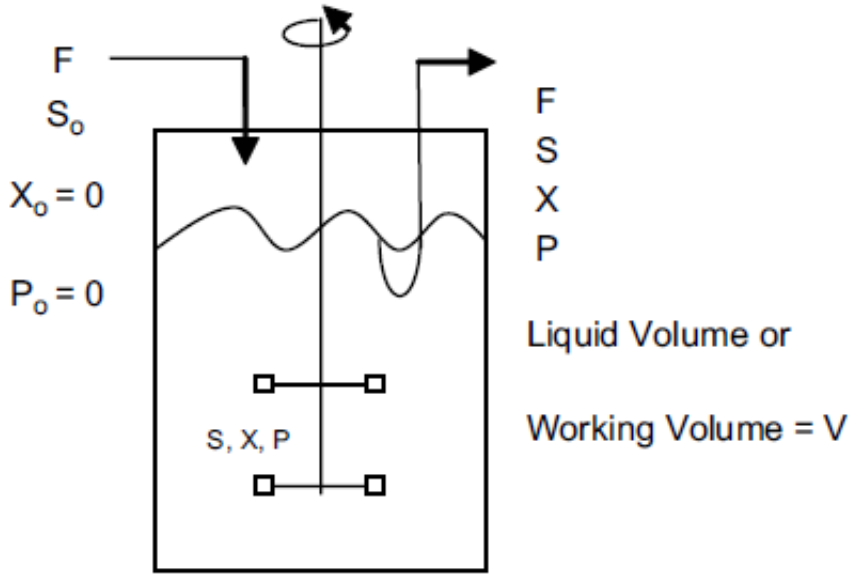
$$FS_0 = FS_{SS} + V\frac{dS}{dt}$$

where F is the volumetric flow rate (L/h),

S_0 is the substrate concentration in the feed (g/L),

S_{SS} is the steady-state substrate concentration (g/L),

and V is the working or liquid volume of the reactor (L).



Continuous flow stirred-tank reactor or chemostat.

The dilution rate (that is, the rate of addition of fresh medium determines the specific growth rate of the culture $D = \frac{F}{v}$

Ideal Continuous Flow Stirred Tank Reactor

- Dividing the above equation by V and rearranging, we get

$$\frac{F}{V}S_0 = \frac{F}{V}S_{SS} + \left(\frac{dS}{dX}\frac{dX}{dt}\right)$$

$$DS_0 = DS_{SS} + \left(\frac{1}{Y_{X/S}}\frac{dX}{dt}\right)$$

Ideal chemostat model

where D is the dilution rate (h^{-1}).

- Hydraulic retention time is

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

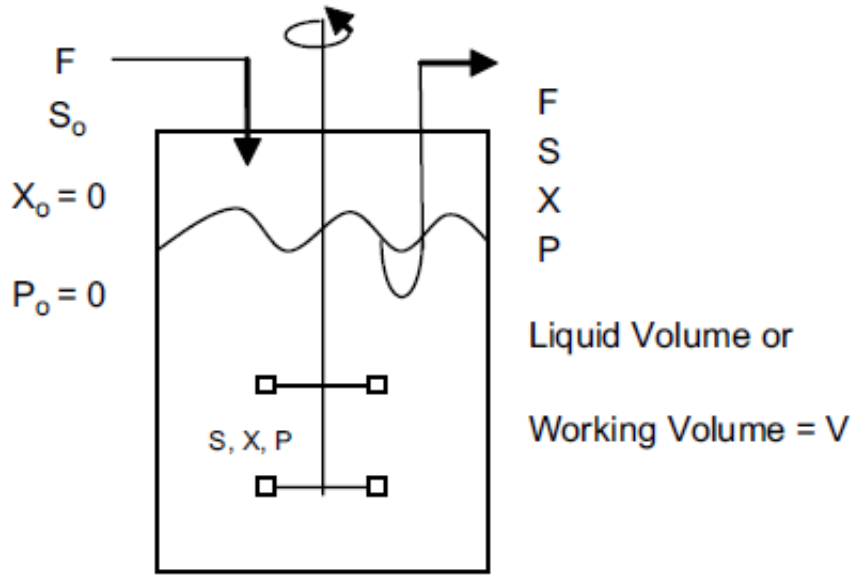
- We know,

$$\frac{dx}{dt} = \mu X_{SS}$$

$$D(S_0 - S_{SS}) = \left(\frac{1}{Y_{X/S}}\right)\mu X_{SS}$$

Ideal chemostat model

where X_{SS} is the biomass concentration at steady-state conditions.



Continuous flow stirred-tank reactor or chemostat.

- Applying the Monod equation to Eq.

$$D(S_0 - S_{SS}) = \left(\frac{1}{Y_{X/S}}\right)\frac{\mu_{max} S_{SS}}{K_s + S_{SS}} X_{SS}$$

Monod chemostat model

Ideal Continuous Flow Stirred Tank Reactor

- Similarly, the **cell mass balance** at steady-state conditions can be written as

  Input + Generation = Output + Consumption + Accumulation

$$FX_0 + \frac{dX}{dt}V = FX_{SS} + 0 + 0$$

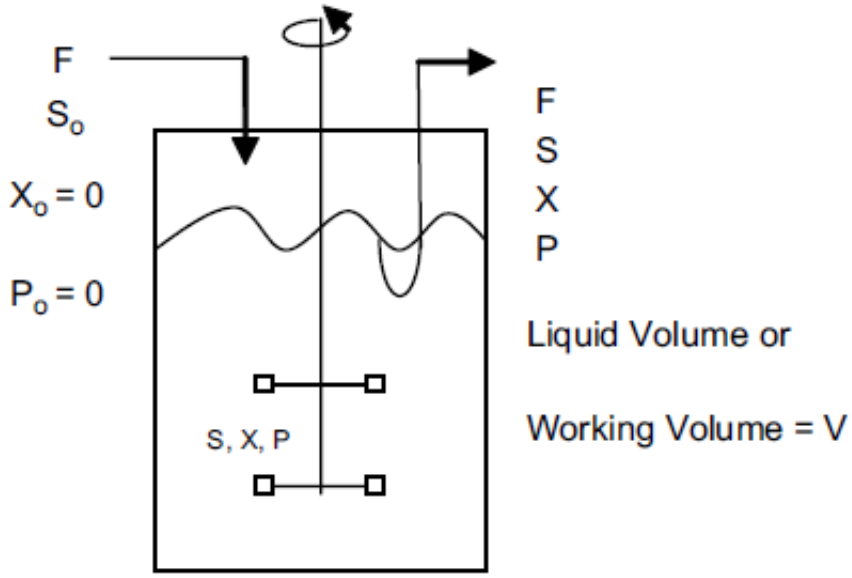
At steady state, accumulation = 0,

$$D(X_{SS} - X_0) = \mu X_{SS}$$

Applying the Monod equation to Eqs. gives rise to the Monod chemostat models as follows:

$$D(X_{SS} - X_0) = \frac{\mu_{max} S_{SS}}{K_s + S_{SS}} X_{SS}$$

Monod chemostat model



Continuous flow stirred-tank reactor or chemostat.

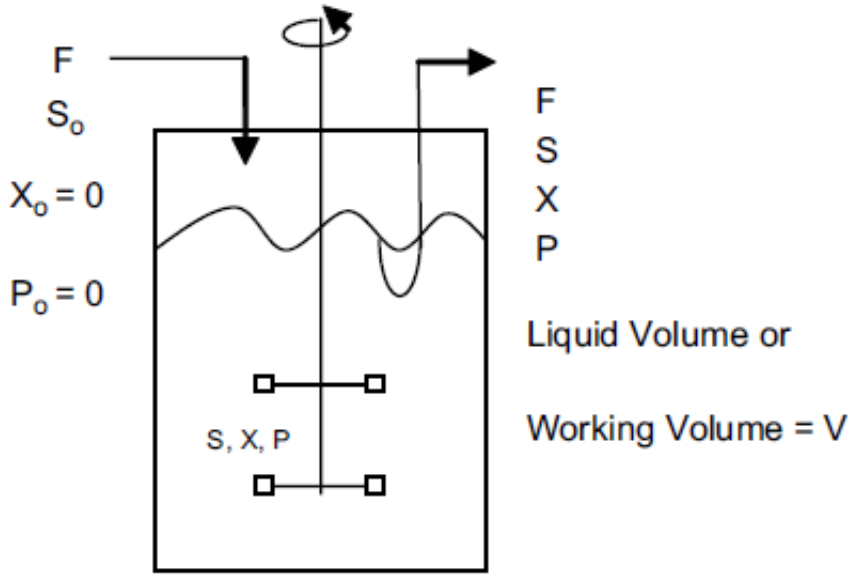
Ideal Continuous Flow Stirred Tank Reactor

- Applying the Monod equation to Eqs. gives rise to the Monod chemostat models as follows:

From Substrate balance $$D(S_0 - S_{SS}) = \left(\frac{1}{Y_{X/S}}\right)\frac{\mu_{max} S_{SS}}{K_s + S_{SS}} X_{SS}$$

From Cell mass balance $$D(X_{SS} - X_0) = \frac{\mu_{max} S_{SS}}{K_s + S_{SS}} X_{SS}$$

These equations are known as the **Monod chemostat models**.



Continuous flow stirred-tank reactor or chemostat.

Ideal Continuous Flow Stirred Tank Reactor

- Similarly, the **cell mass balance** at steady-state conditions can be written as

  Input + Generation = Output + Consumption + Accumulation

$$FX_0 + \frac{dX}{dt}V = FX_{SS} + 0 + 0$$

At steady state, accumulation = 0,

In the case of **sterile feed**, $X_0 = 0$,

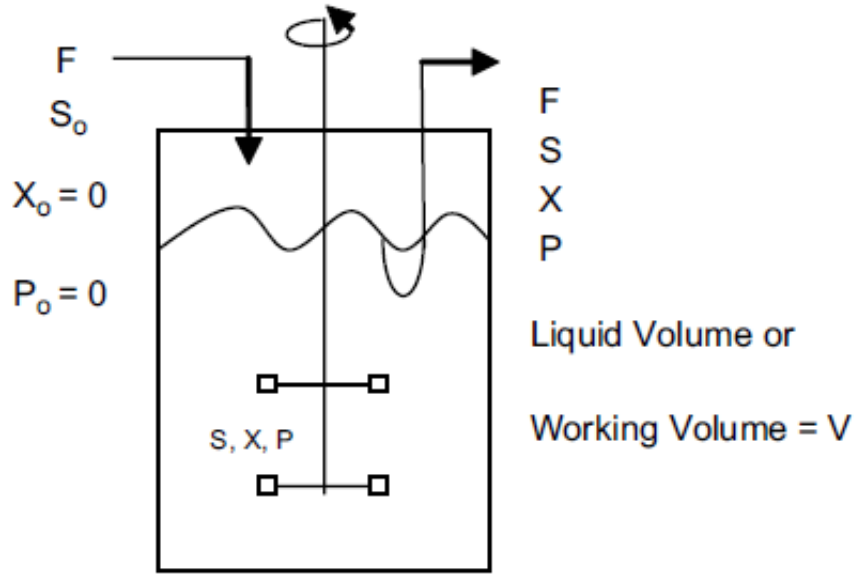
$$\frac{F}{V} = \frac{1}{X_{SS}}\frac{dX}{dt} = \mu$$

Applying Monod eq.

$$D = \mu = \frac{\mu_{max} S_{SS}}{K_s + S_{SS}}$$

Rearranging Eq., for sterile feed, we get

$$S_{SS} = \frac{K_s D}{\mu_{max} - D}$$



Continuous flow stirred-tank reactor or chemostat.

- We know that $Y_{X/S} = \frac{X_{SS} - X_0}{S_0 - S_{SS}}$

Ideal Continuous Flow Stirred Tank Reactor

- From substrate balance

$$D(S_0 - S_{SS}) = \left(\frac{1}{Y_{X/S}}\right)\mu X_{SS}$$

- Since for sterile feed $X_0 = 0$, $D = \mu$

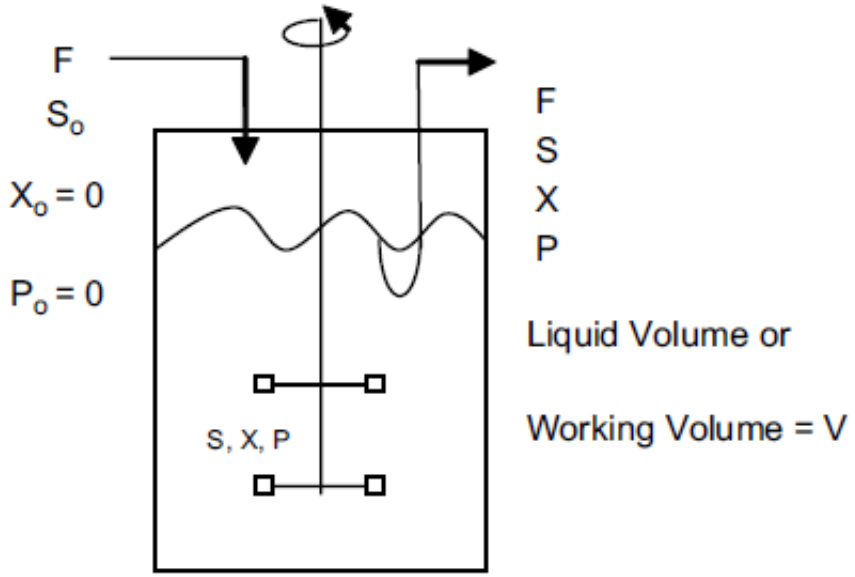
$$X_{SS} = Y_{X/S}\,(S_0 - S_{SS})$$

- Therefore,

$$X_{SS} = Y_{X/S}\left(S_0 - \frac{K_s D}{\mu_{max} - D}\right)$$

- **Cell mass productivity** is
- Multiply by D

$$DX_{SS} = Y_{X/S}\left(DS_0 - \frac{K_s D^2}{\mu_{max} - D}\right)$$



Continuous flow stirred-tank reactor or chemostat.

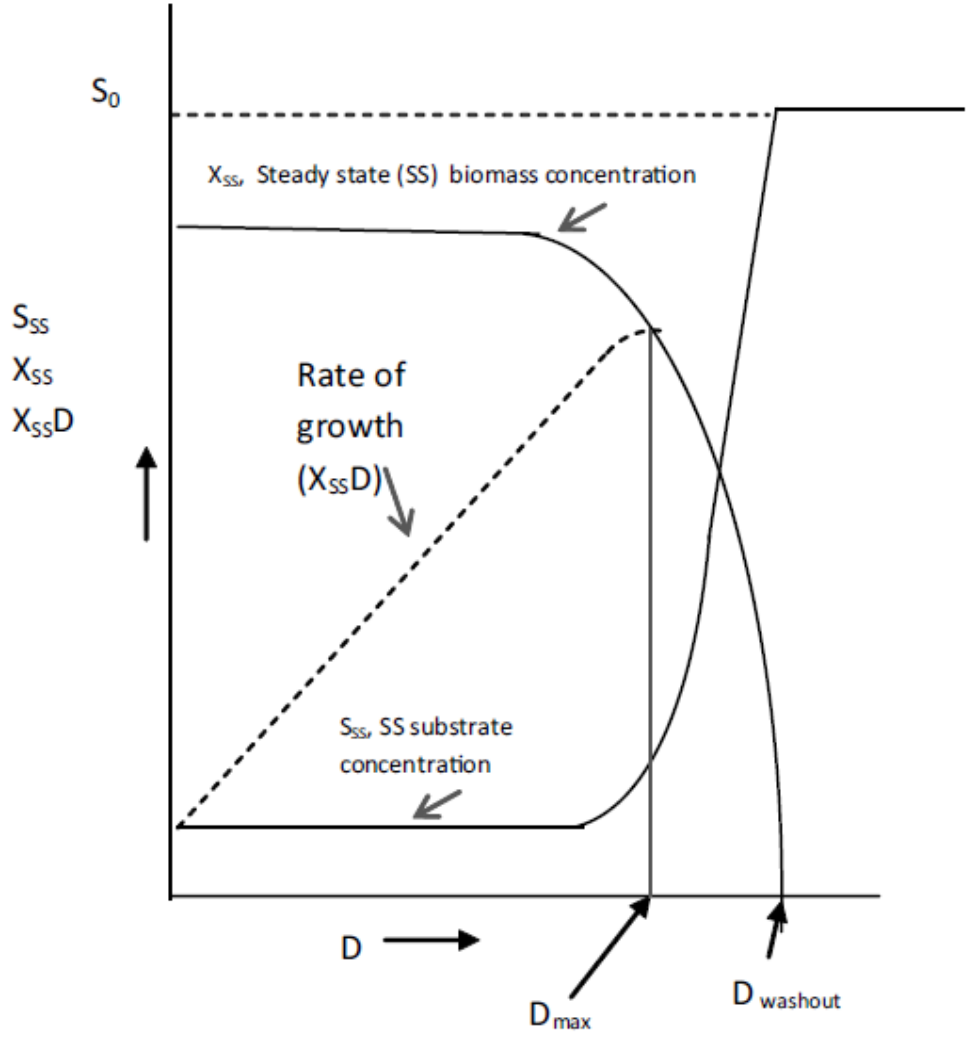
Ideal Continuous Flow Stirred Tank Reactor

- Figure depicts the plot of X_{SS}, S_{SS}, DX_{SS} versus the dilution rate D.
- The maximum productivity of cell (D_{max}) can be obtained when $dDX_{SS}/dD = 0$.
- D_{max} is obtained by differentiating Eq. with respect to D:

$$\frac{d}{dD}(DX_{SS}) = \frac{d}{dD}\left(Y_{X/S}\left(DS_0 - \frac{K_s D^2}{\mu_{max} - D}\right)\right)$$

$$= Y_{X/S}\left(S_0 - \frac{2K_s D}{\mu_{max} - D} - \frac{K_s D^2}{(\mu_{max} - D)^2}\right)$$

Now, when $D \rightarrow D_{max}$, $\frac{d}{dD}(DX_{SS}) = 0$



Plot of X_{SS}, S_{SS}, DX_{SS} versus the dilution rate D.

Ideal Continuous Flow Stirred Tank Reactor

- Therefore,

$$S_0 - \frac{2K_sD}{\mu_{max} - D} - \frac{K_sD^2}{(\mu_{max} - D)^2} = 0$$

- Because $Y_{X/S} \neq 0$,

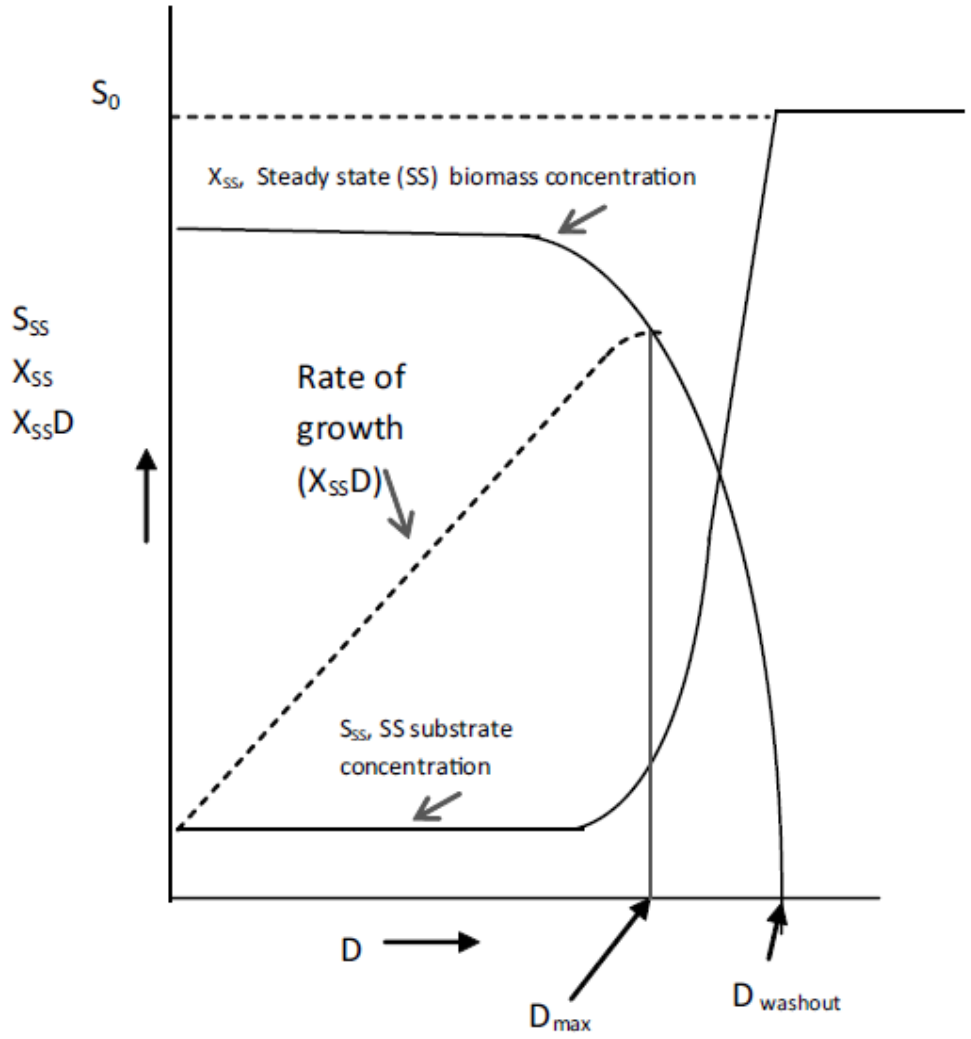
$$S_0 = \frac{2K_sD}{\mu_{max} - D} + \frac{K_sD^2}{(\mu_{max} - D)^2}$$

$$\frac{S_0}{K_S} + 1 = \frac{2D}{\mu_{max} - D} + \frac{D^2}{(\mu_{max} - D)^2} + 1$$

$$\frac{S_0}{K_S} + 1 = \left(1 + \frac{D}{\mu_{max} - D}\right)^2$$

- Continuous growth using chemostat:

$$\frac{S_0 + K_S}{K_S} = \left(\frac{\mu_{max} - D + D}{\mu_{max} - D}\right)^2$$



Plot of X_{SS}, S_{SS}, DX_{SS} versus the dilution rate D.

Ideal Continuous Flow Stirred Tank Reactor

- Taking reciprocal on both sides, we get

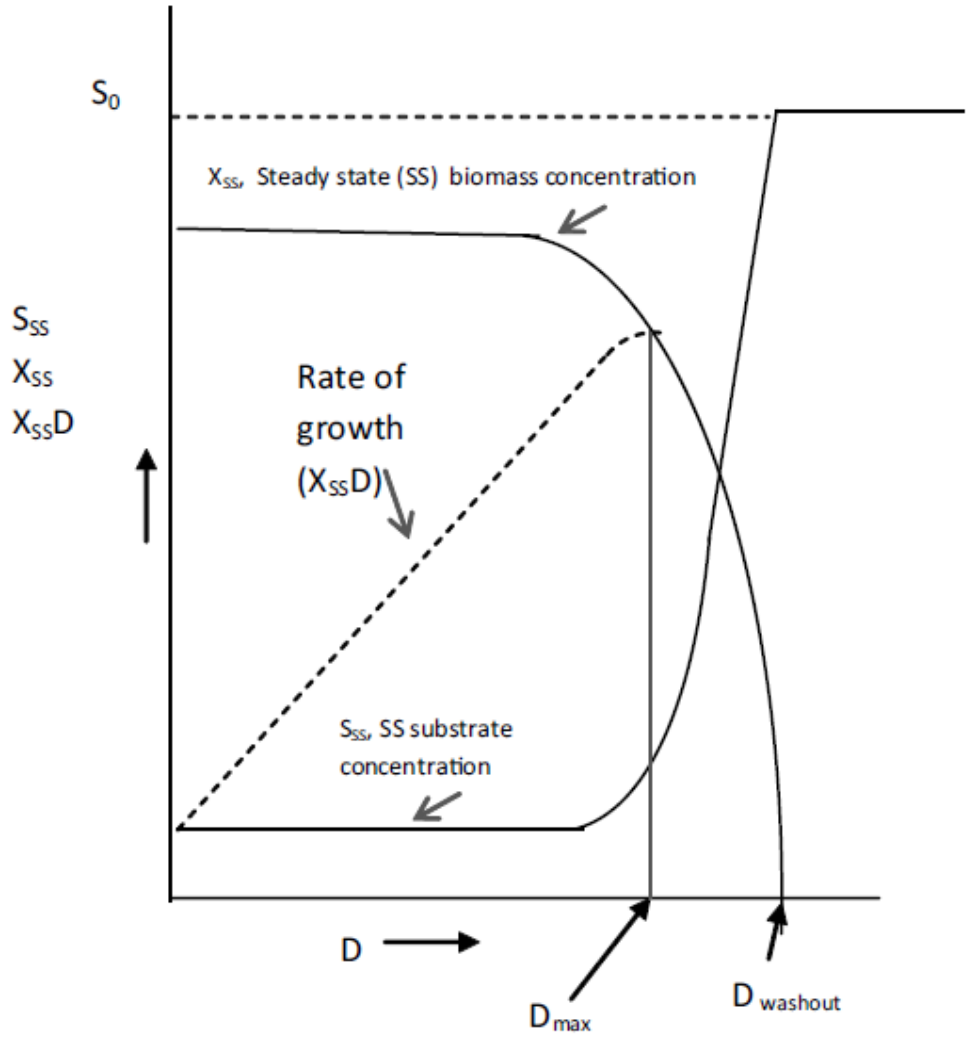
$$\frac{K_S}{S_0 + K_S} = \left(\frac{\mu_{max} - D}{\mu_{max}}\right)^2$$

- Taking square root on both sides, we get

$$\pm\sqrt{\frac{K_S}{S_0 + K_S}} = 1 - \frac{D}{\mu_{max}}$$

- (Since $D_{max} < \mu_{max}$)
- Therefore,

$$D_{max} = \mu_{max}\left(1 - \sqrt{\frac{K_S}{S_0 + K_S}}\right)$$



Plot of X_{SS}, S_{SS}, DX_{SS} versus the dilution rate D.

Ideal Continuous Flow Stirred Tank Reactor

Cell mass concentration at D_{max} can be given as

- We know that,

$$X_{SS} = Y_{X/S}\left(S_0 - \frac{K_s D}{\mu_{max} - D}\right)$$

$$X_{max} = Y_{X/S}\left(S_0 - \frac{K_s}{\frac{\mu_{max}}{D_{max}} - 1}\right)$$

$$X_{max} = Y_{X/S}\left(S_0 - \frac{K_S}{\frac{\sqrt{K_S + S_0}}{(\sqrt{K_S + S_0} - \sqrt{K_S})} - 1}\right)$$

$$X_{max} = Y_{X/S}\left(S_0 - \left(\sqrt{K_S + S_0} - \sqrt{K_S}\right)\sqrt{K_S}\right)$$

$$X_{max} = Y_{X/S}\left(S_0 - \sqrt{K_S}\left(K_S + S_0\right) + K_S\right)$$

$$X_{max} = Y_{X/S}\left(S_0 - \frac{K_s D_{max}}{\mu_{max} - D_{max}}\right)$$

$$X_{max} = Y_{X/S}\left(S_0 - \frac{K_S}{\frac{\mu_{max}}{\mu_{max}\left(1 - \sqrt{\frac{K_S}{K_S + S_0}}\right)} - 1}\right)$$

$$X_{max} = Y_{X/S}\left(S_0 - \frac{\left(\sqrt{K_S + S_0} - \sqrt{K_S}\right)K_S}{\sqrt{K_S + S_0} - \sqrt{K_S + S_0} + \sqrt{K_S}}\right)$$

Ideal Continuous Flow Stirred Tank Reactor

- The maximum cell productivity Q_{max} can be given as

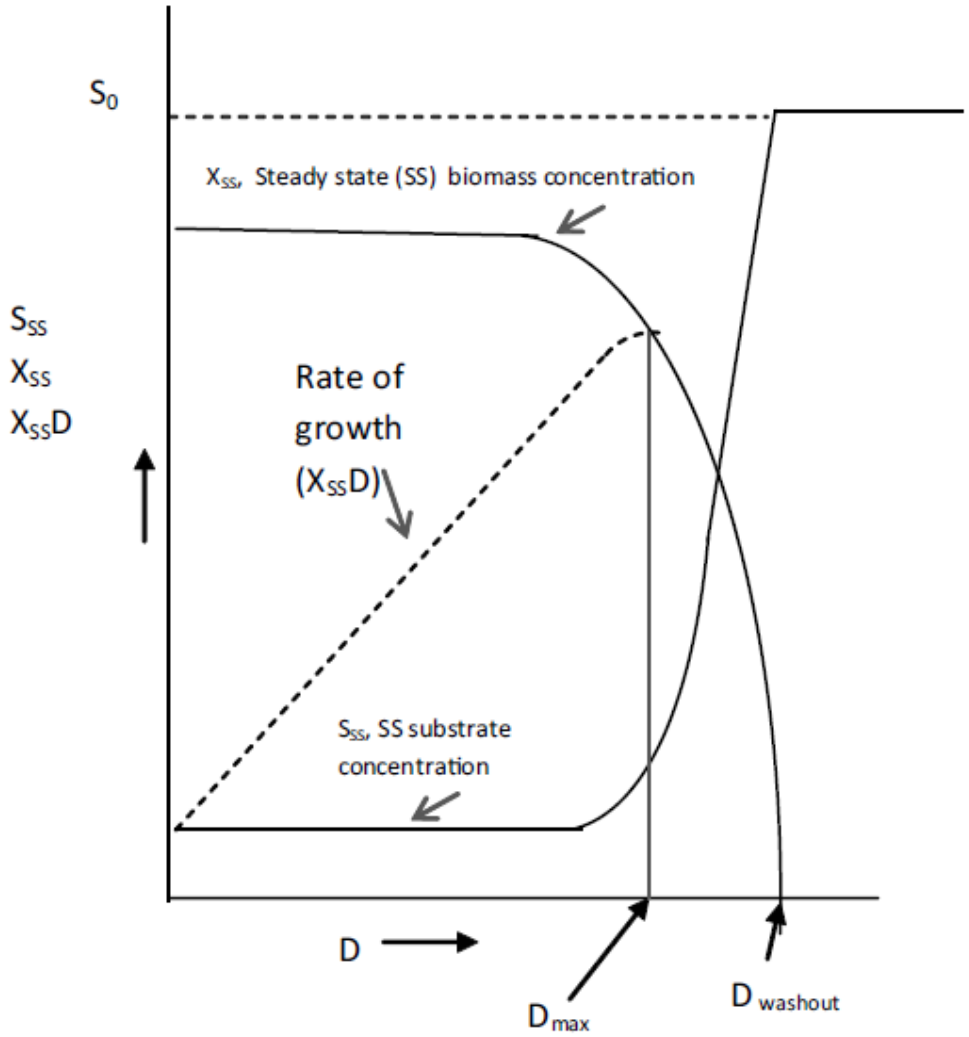
$$Q_{\max} = DX_{\max} = Y_{X/S}\left(D_{\max}S_0 - \frac{K_s D_{\max}^2}{\mu_{\max} - D_{\max}}\right)$$

- At $D_{washout}$, Xss → 0, μ → μ_{max}
- i.e., washout of cells will take place.
- At $D_{washout}$, no cell will remain present in the reactor.
- This occurs mainly because the cell will not get sufficient time for cell division.

- $D_{washout}$ can be expressed as

$$D_{\text{washout}} = \frac{\mu_{\max} S_0}{K_s + S_0}$$

- where S_0 is the initial substrate concentration.



Plot of X_{SS}, S_{SS}, DX_{SS} versus the dilution rate D.

Ideal Continuous Flow Stirred Tank Reactor

- It should be noted that $D_{max} < D_{washout} \leq \mu_{max}$.

- If a mixed microbial culture contains both slow-growing and fast-growing microorganisms, then by adjusting the dilution rate higher than the $D_{washout}$ of slow-growing but less than that of fast-growing, it is possible to separate fast-growing organisms from the slow-growing organisms.

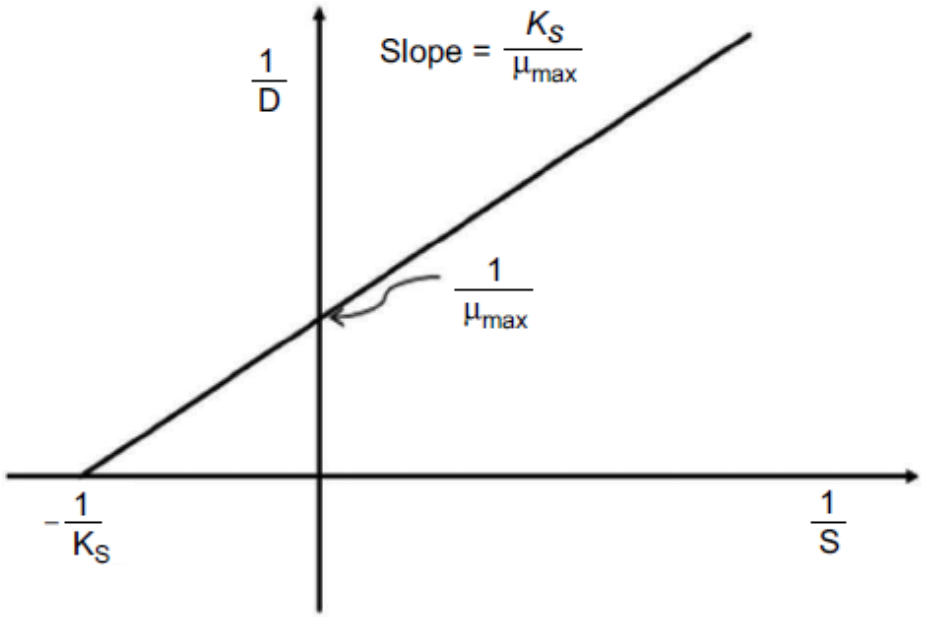
- This is applicable in the anaerobic digestion process where acidogens are fast-growing compared to methanogens.

- So acidogens can be easily separated from a mixed culture by adjusting the dilution rate.

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.

Advantages and disadvantages of chemostat compared to batch process:

- **Advantages:**

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

- The effect **of growth-limiting substrate can be easily monitored.**

- Results obtained are **reliable** and **reproducible**.

Advantages and disadvantages of chemostat compared to batch process:

- **Disadvantages:**

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

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

Chemostat

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

- Chemostat with cell recycling

- Whole-cell immobilization (Home work)

Chemostat

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

- Cell recycling is carried out **to keep the cell concentration higher than the normal steady-state level in a chemostat**.

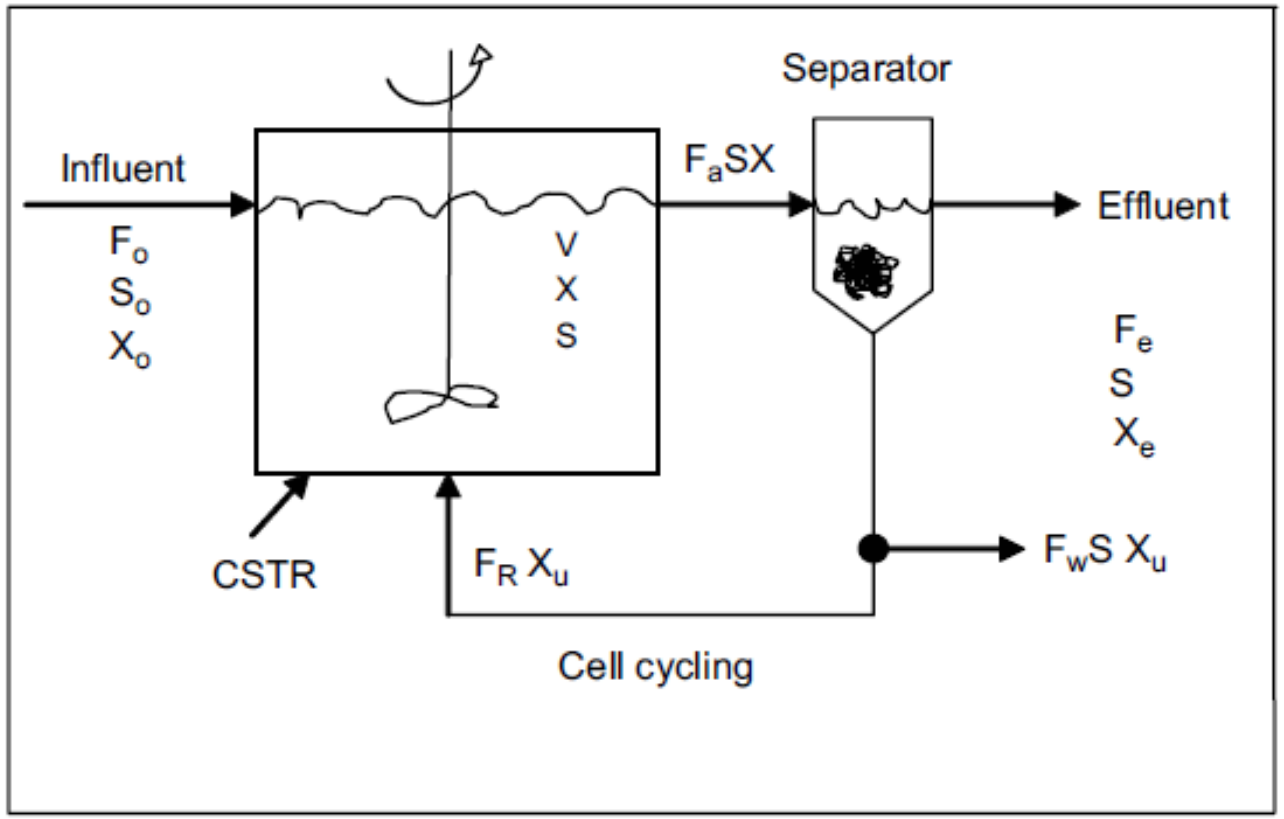
- Cell recycle **increases the rate of conversion** (or productivity).

- **Increases critical dilution rate for washout**, thereby increasing operating flexibility.

- Can be operated by using a centrifuge or **settling tank to concentrate biomass and recycle a portion of the settled biomass** to the reactor for maintaining cell mass concentration constant.

Analysis of Chemostat with Cell Recycle

- The Fig. shows a 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: Steady-state 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 recycle.

Analysis of Chemostat with Cell Recycle

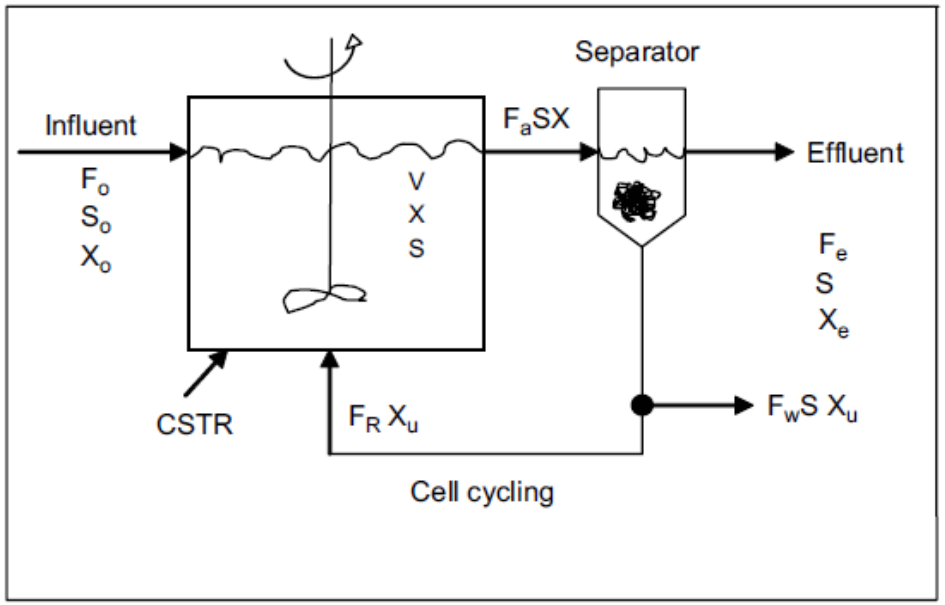
- At a steady state, the **cell mass balance** across the chemostat can be given as

Input + Generation = Output + Accumulation+ Cell death

$$(F_0X_0 + F_RX_u) + V\frac{dX}{dt} = F_aX + 0 + 0$$

- Now, $\alpha = \frac{F_R}{F_0}$ where α is the recycle ratio. So, $F_R = \alpha F_0$

- Also, $F_a = F_0 + F_R$
- Therefore, $F_a = F_0 + \alpha F_0$
- So, $F_a = F_0(1 + \alpha)$
- Now mass balance equation can be written as



Chemostat with cell recycle.

$$(F_0X_0 + \alpha F_0X_u) + V\frac{dX}{dt} = F_0(1+\alpha)X + 0 + 0$$

Analysis of Chemostat with Cell Recycle

- **Chemostat with recycle**
- For sterile feed, $X_0 = 0$; so mass balance Eq. becomes

$$\alpha F_0 X_u + V\mu X = F_0(1+\alpha)X$$

$$\left(\text{Since } \frac{dX}{dt} = \mu X\right)$$

- Dividing Eq. by V, we get

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

$$\alpha D X_u + \mu X = D(1+\alpha)X$$

$$\left(\text{Since } \frac{F_0}{V} = D\right)$$

Analysis of Chemostat with Cell Recycle

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

- So Xu = CX, putting in Eq., we get

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

- By rearranging, we get

$$\mu = D\,[1 + \alpha(1 - C)]$$

- In eq. $\alpha < 1$ and $C \gg 1, \frac{\mu}{D} < 0$

- **That is D > μ**

- So in the case of a chemostat with cell recycling, the process can be easily operated at D_{max} because the cell washout problem will not arise.

Analysis of Chemostat with Cell Recycle

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

Input + Generation = Output + Consumption + Accumulation

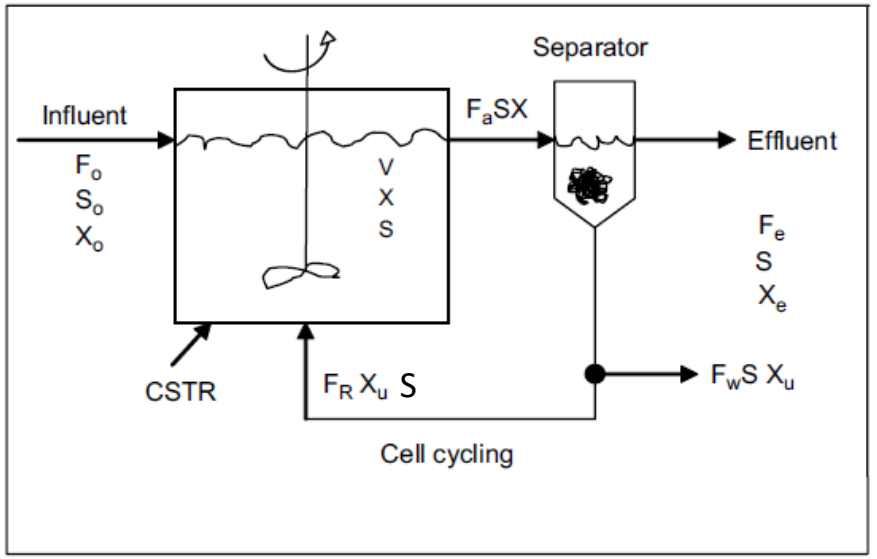
$$(F_0 S_0 + F_R S) + 0 = F_a S + V\frac{dS}{dt} + 0$$

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

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

$$\frac{dX}{dS} = Y_{X/S};\ \frac{dX}{dt} = \mu X$$

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



Chemostat with cell recycle.

Analysis of Chemostat with Cell Recycle

- Since, $D = F_0 / V$

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

- We know that,

$$\mu = D\,[1 + \alpha(1 - C)]$$

- Putting the value of μ, we get

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

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

Analysis of Chemostat with Cell Recycle

- The substrate concentration S can be obtained by applying the Monod kinetics

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

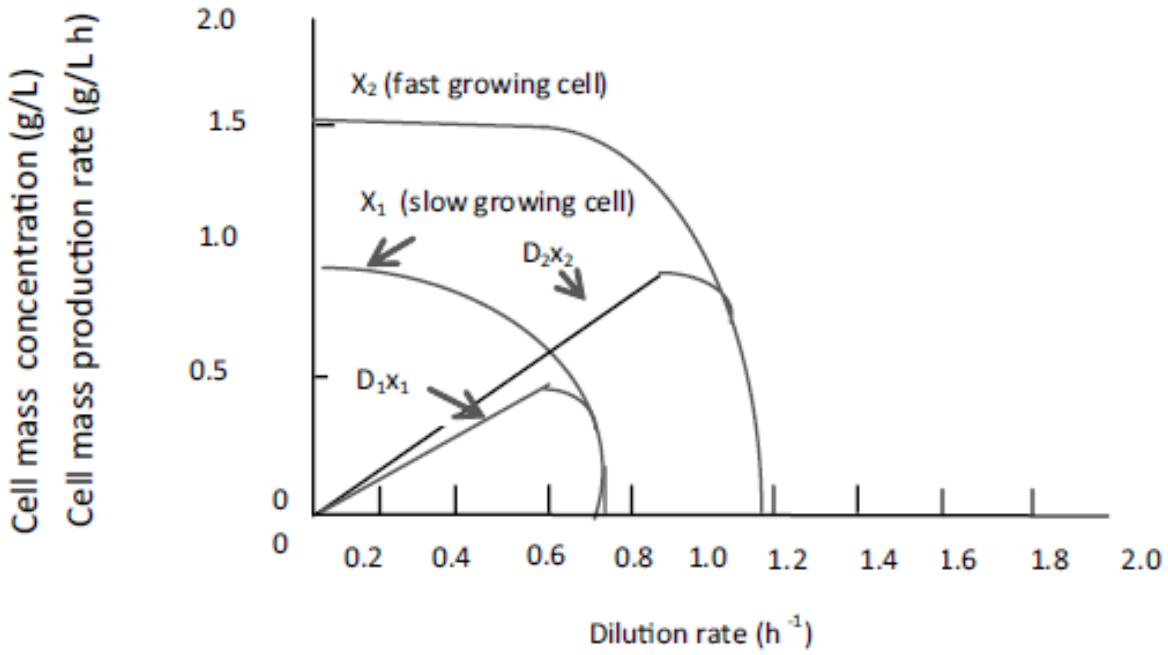
- Rearranging

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

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

- Using S value,

$$X = \frac{Y_{X/S}}{\left[1 + \alpha(1 - C)\right]}\left[S_0 - \frac{K_S\left[1 + \alpha(1 - C)\right]D}{\mu_{max} - \left[1 + \alpha(1 - C)\right]D}\right]$$



Effect of dilution rate in case of cell mass without recycling (D_1X_1) and with recycling (D_2X_2) on the cell mass productivity.

The productivity of cell mass increases with cell recycling as compared to that of without recycling.

Continuous Operation Using Plug Flow Reactor

- The analysis of a plug flow reactor for cell culture follows the same procedure as that for an enzymatic reaction.
- The material balance for **cell mass** in a small section (ΔZ) can be represented as follows:

Input + Generation = Output + Consumption + Accumulation

$$FX_z + \mu XA\Delta z = FX_{z+\Delta z} + 0 + 0$$

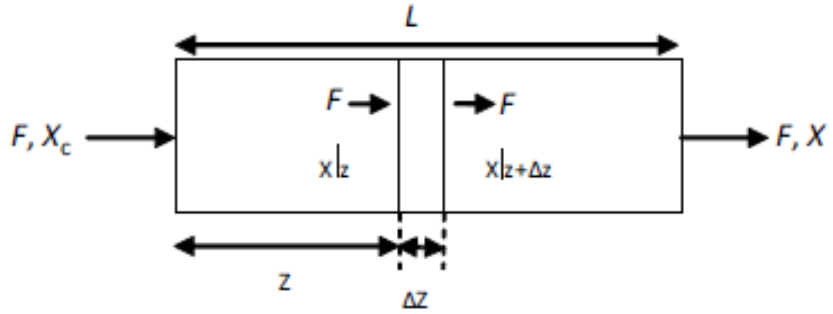
- where A is the cross-sectional area of the reactor,

  F is the volumetric flow rate, and dV = A Δz.

$$F(X_{z+\Delta z} - X_z) = \mu XA\Delta z$$

$$u\frac{(X_{z+\Delta z} - X_z)}{\Delta z} = \mu X$$

where u is the velocity of the liquid.



Cell mass balance across a plug flow reactor.

Continuous Operation Using Plug Flow Reactor

- Applying the limit z → 0 to the above equation, we get

$$u\left[\frac{(X_{z+\Delta z}-X_z)}{\Delta z}\right]=\mu X$$

$$u\left(\frac{dX}{dz}\right)=\mu X$$

- After separating the variables and solving integration, we get

$$u\int_{x_0}^{x}\frac{\mathrm{d}x}{x}=\mu\int_{0}^{L}\mathrm{d}z$$

$$\ln\frac{x}{x_0}=\mu\frac{L}{u}\ =\mu\frac{V/A}{F/A}$$

$$\ln\frac{x}{x_0}=\mu\tau \qquad\qquad \tau=\frac{1}{\mu}\ln\frac{x}{x_0}$$

- The above equation suggests that $\tau_{\text{PFR}} = \tau_{\text{CSTR}}$

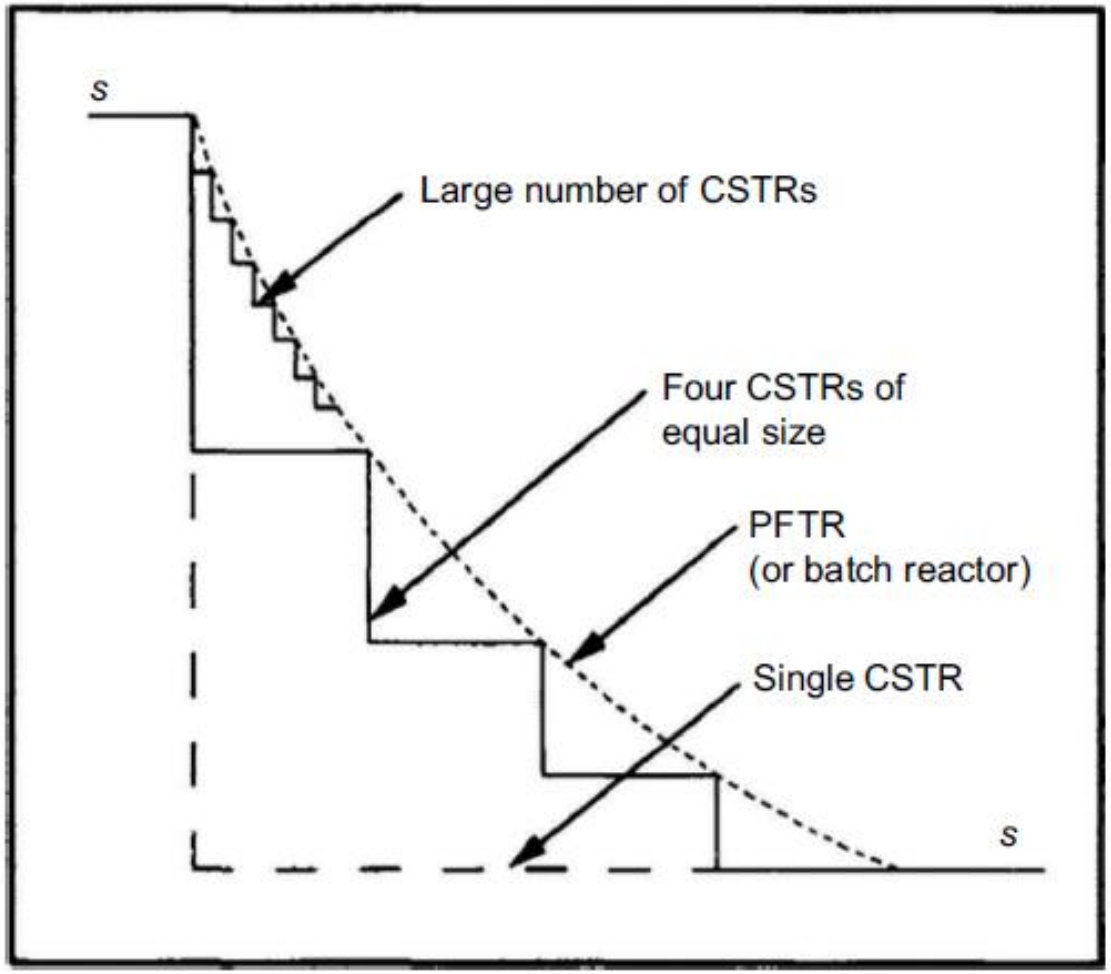
The drawbacks of a plug flow reactor:

- **Not suitable** for **suspended cell growth**.
- Can be used for **cell recycling** or **immobilized cell reactions**, but difficult to operate.
- Difficult to control due to **temperature and composition** variations.
- **Maintenance** is more **expensive** in PFR.
- **Rarely** employed in **industrial fermentations**.

Comparison between major modes of cultivation:

- **Kinetic characteristics** of **PFRs** are the same as those of the **batch** reactors.
- When **many CSTRs** are connected in series, the **conversion characteristics approach** those of batch and PFRs.
- The rate of conversion in the chemostat operated at D_{max} is **greater** than PFR or Batch.
- For most fermentations, **CSTRs offer significant** theoretical advantages over other modes of reactor operations.
- Despite the benefits of CSTR the **majority of commercial fermentations** are conducted in **Batch.**

Comparison between major modes of cultivation:



Comparison between major modes of cultivation:

- Batch fermentations have a lower risk of contamination compared to CSTR.
- Equipment and control failures during long-term operation are the associated problems with CSTR.
- A decrease in substrate concentration in PFR or batch is observed.
- In a single CSTR, the outlet substrate concentration is the same under steady-state conditions at a particular flow rate and feed substrate concentration.
- In the case of cascade (CSTRs are connected in series), there is a step-wise drop in substrate concentration between each stage, mimicking PFR or batch.

Fed-Batch culture:

- A fed-batch culture is a semi-batch operation
- Nutrients are fed either intermittently or continuously
- The culture broth is harvested only at the end of the operational period, either fully or partially
- Repeated fed-batch till the cells remain fully viable and productive
- One or more feed streams but no effluent
- Manipulating the feed rates during the run
- The culture volume increases continuously

Fed-Batch culture:

- A dynamic operation
- Concentrations of limiting nutrients in the culture can be manipulated
- Nutrient concentration profile: can remain at a constant level or to follow a predetermined profile
- A batch mode is used to end the fermentation (maximum product or yield)

- Examples are Baker's yeast production, penicillin fermentation, etc.

Objectives of Fed-Batch culture:

- Maximizing the cell formation rate for constant cell mass yield
- The substrate concentration is maintained at a value that maximizes the specific growth rate
- Maximum cell concentration achieved at the end of the run
- Feed rate regulated to hold the substrate concentration constant at the desired value until the reactor is full
- Once the fermenter is full, it is running in batch mode

Advantages of Fed-Batch culture:

- A low level of limiting substrate concentration helps in **avoiding substrate inhibition**.
- **High cell density**
- Relieves **catabolite repression** when this is an issue in a production process.
- **Higher yield** resulting from a well-defined cultivation period.
- Avoids the toxic effects of some medium components.

Constant Volume Fed Batch:

- **Disadvantages:**

- **Lower productivity** levels due to **high downtime** for filling, heating, sterilization, cooling, emptying, and cleaning the reactor.

- **Higher costs** in labor and/or dynamic process control for the process.

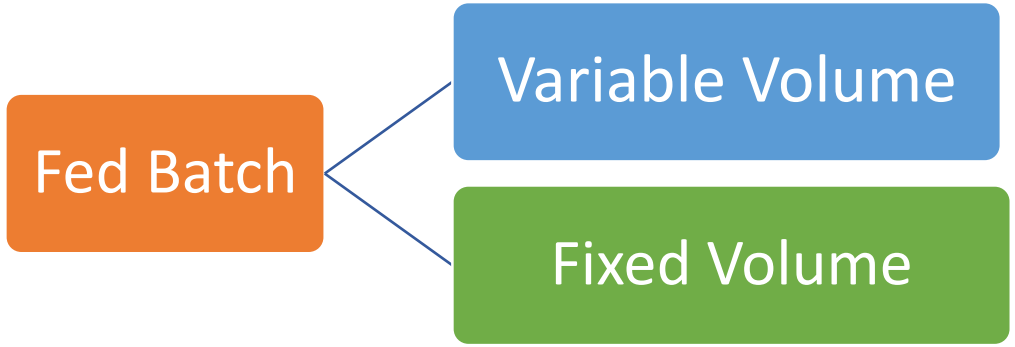
Feeding strategy in Fed-Batch culture:

- Manipulation of one or more feed rates,
- A means of regulating the nutrient concentrations
- Controlling the key reaction rates

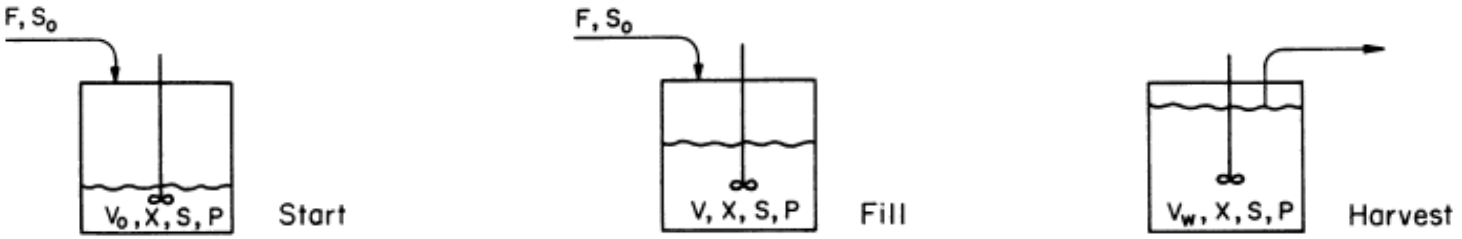
- What compound(s) should be fed and
- How they should be added.

Kinetics of Fed Batch Cell Growth:

- The Fed batch process can be operated in two ways:



- **Variable Volume Fed Batch:**
- In this system a feed at the same concentration as that in the initial medium is added resulting in an increase in volume



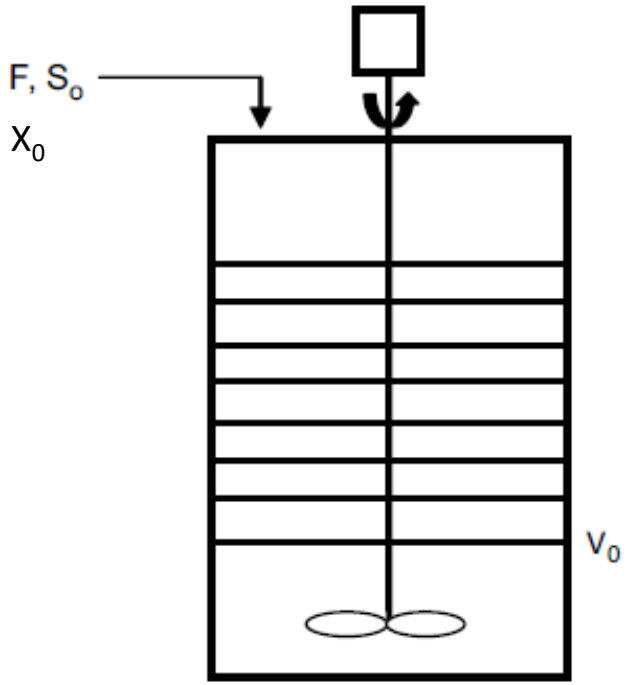
Kinetics of Fed Batch Cell Growth:

- **Variable Volume Fed Batch:**
- If the feed is added continuously at a constant flow rate F, the rate of change in volume (V) can be given as

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

- Rearranging and integrating the above equation, we get

$$\int_{V_0}^{V} dV = \int_{0}^{t} F dt \qquad V = V_0 + Ft$$



Fed-batch process with
The variable volume of feed

- where V is the volume of the reactor at time t and
- V_0 is the initial volume of the reactor (at t = 0)
- **At a quasi-steady state, Sadded → Sconsumed and X is constant**.

Variable Volume Fed Batch:

- The **cell mass balance** can be given as

Input + Cell generation = Output + Accumulation + Cell death

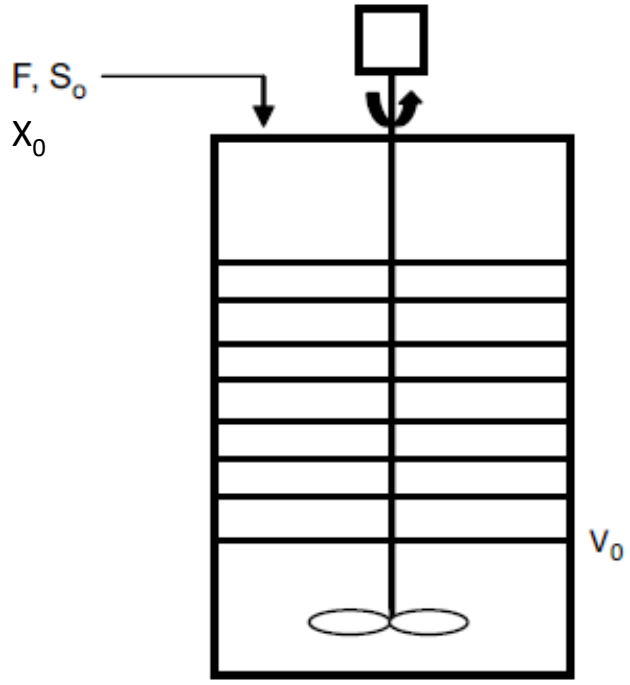
$$FX_0 + \mu XV = 0 + \frac{dXV}{dt} + 0$$

assuming cell death is negligible

$$FX_0 + \mu XV = X\frac{dV}{dt} + V\frac{dX}{dt}$$

At quasi-steady state and using sterile feed, $X_0 = 0;\ \frac{dX}{dt} = 0$

Therefore, $\mu XV = X\frac{dV}{dt}$ $\qquad \mu = \frac{1}{V}\frac{dV}{dt} = \frac{F}{V}$



Fed-batch process with
The variable volume of feed

Variable Volume Fed Batch:

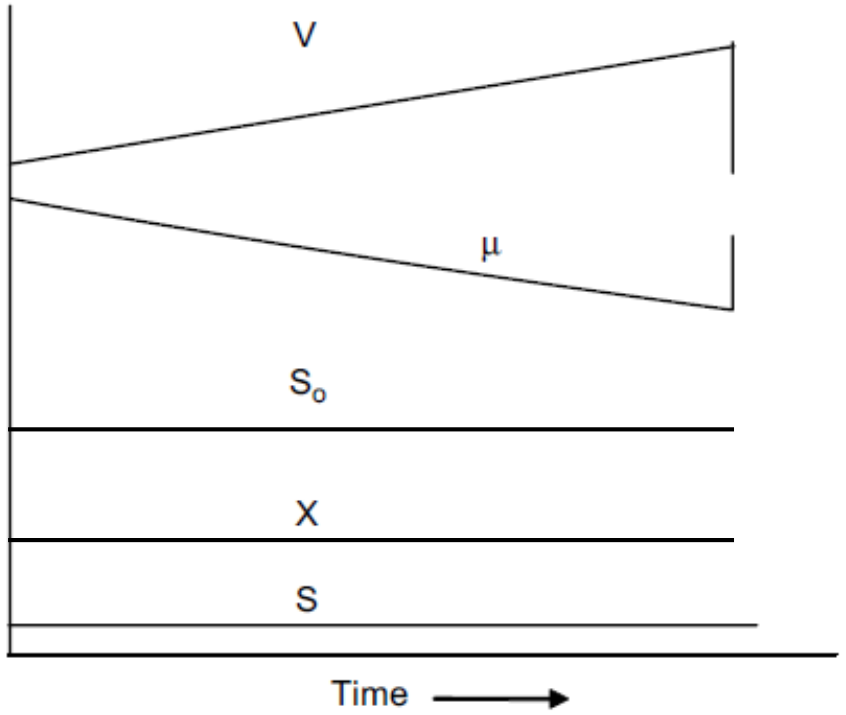
- The ratio of flow rate (F) and volume (V) is called
- dilution rate (D), which can be represented as

$$D = \frac{F}{V}$$

- Thus, $$\mu = D = \frac{F}{V_0 + Ft}$$

- Applying the Monod kinetics gives
- $$\mu = D = \frac{\mu_{max} S}{K_s + S} = \frac{F}{V_0 + Ft} = \frac{D_0}{1 + D_0 t}$$

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



Profiles of cell mass concentration, substrate concentration, specific cell growth rate, and volume of a fed batch reactor.

Variable Volume Fed Batch:

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

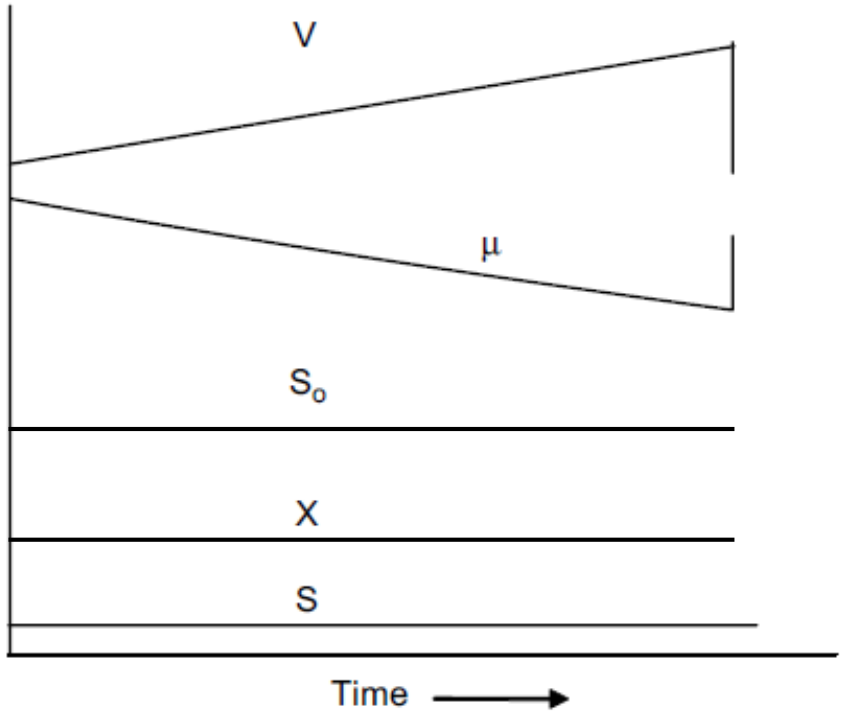
$$X = \frac{X_t}{V}$$

- where X_t is the total biomass concentration.
- At quasi-steady state,

$$\frac{dX}{dt} = 0 \text{ i.e., } \frac{d\left(\frac{X_t}{V}\right)}{dt} = 0 \qquad \left(\text{Since } \frac{d}{dx}(a/b) = \frac{b\frac{da}{dx} - a\frac{db}{dx}}{b^2}\right)$$

$$\frac{V\left(\frac{dX_t}{dt}\right) - X_t\left(\frac{dV}{dt}\right)}{V^2} = 0 \qquad \frac{dX_t}{dt} = \frac{X_t}{V}\frac{dV}{dt} = FX$$

- Xt / V is some concentration and $\frac{dV}{dt} = F$



Profiles of cell mass concentration, substrate concentration, specific cell growth rate, and volume of a fed batch reactor.

Variable Volume Fed Batch:

- The purpose of the fed-batch is to maximize the cell mass concentration
- So let's assume Xt / V to be X_m, At a constant yield of the biomass,
- Maximum biomass concentration that can be achieved

  (which means S ~ 0 at quasi steady state, i.e. as the substrate is coming in, it is getting consumed so there is no substrate and then only we assume maximum biomass production)
- Therefore S ~ 0 for the maximum biomass possible. $X_m = y_{x/s} s_0$

- And the total biomass concentration (X_t) can be expressed as

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

- Now, when S = 0 and X_0 <<X_t, the above equation can be written as $X_t = Y_{X/S} S_0$

- The total biomass concentration is nothing but the maximum biomass concentration at steady state

Variable Volume Fed Batch:

- Therefore,

$$\frac{\mathrm{d}X_t}{\mathrm{d}t} = \frac{X_t}{V}\frac{\mathrm{d}V}{\mathrm{d}t} = X_m F = Y_{X/S} S_0$$

$$x_m = x_t = y_{x/s} s_0$$

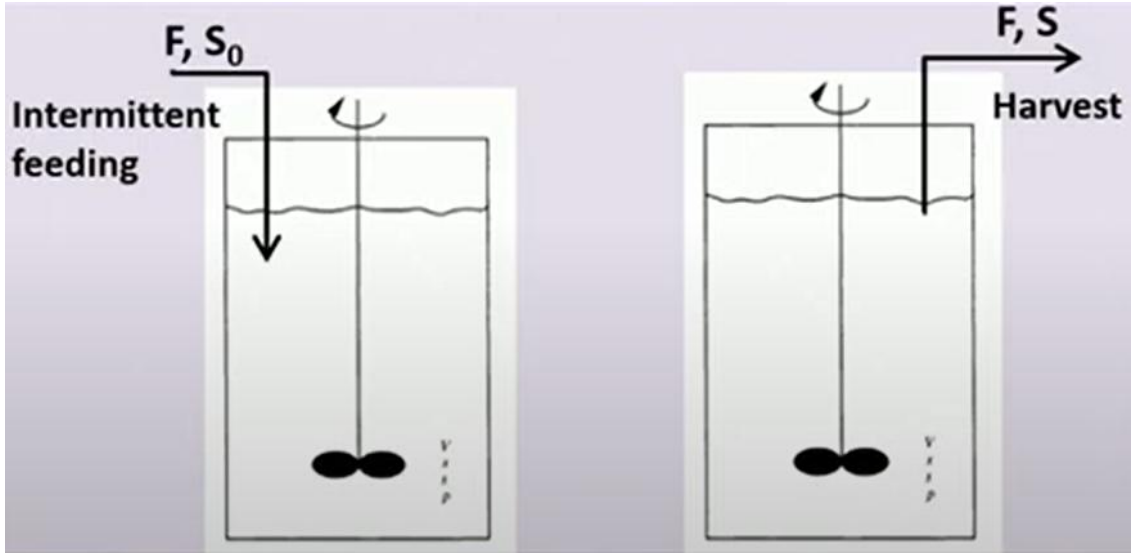
$$\frac{dX_t}{dt} = FY_{X/S} S_0$$

$$\int_{X_0}^{X_t} dX = FY_{X/S}\, S_0 \int_0^t dt$$

$$\boxed{X_t = X_0 + FY_{X/S}\, S_0 t}$$

Constant Volume Fed Batch:

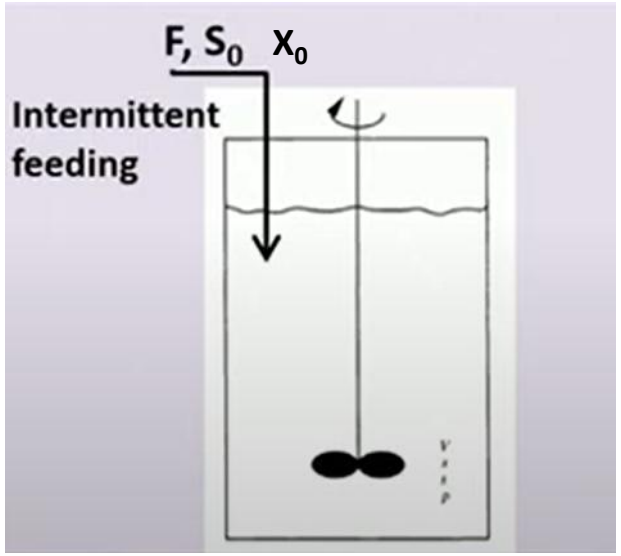
- A **very concentrated solution** of the **limiting substrate** is added **intermittently** at a very low flow rate (lower than that of variable fed-batch) resulting in **an insignificant increase in volume**.



Constant Volume Fed Batch:

- Since the feed is added intermittently, the rate of change in cell mass is dependent on the flow rate such that

$$\frac{dX}{dt} = G\frac{dX}{dS} = GY_{X/S}$$



- where G is the limiting substrate feed rate in g/L.h.

- The **cell mass balance** can be given as

Input + Cell generation = Output + Accumulation + Cell death

$$FX_0 + \mu XV = 0 + \frac{dXV}{dt} + 0$$

Constant Volume Fed Batch:

- In case volume is constant and $X_0 = 0$, the above equation can be written as

$$\mu X = \frac{dX}{dt} = GY_{X/S}$$

- Therefore,

$$\mu = \frac{1}{X} GY_{X/S}$$

- From the above equation, if $\frac{1}{X} GY_{X/S}$ is less than μ_{max}, the limiting substrate is consumed as soon as it enters the fermenter and thus $\frac{dS}{dt} = 0$
- The biomass concentration changes with time and can be found by rearranging and integrating Eq. as

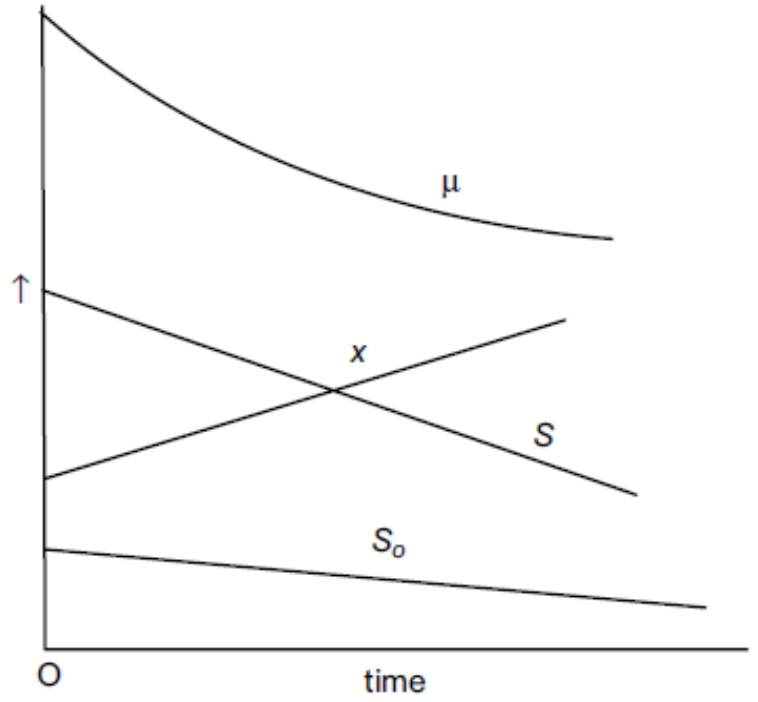
$$\int_{X_0}^{X_t} dX = GY_{X/S} \int_{0}^{t} dt \qquad X_t = X_0 + GY_{X/S}t$$

- where X_t is the total biomass concentration after the time t and X_0 is the initial biomass concentration.

Constant Volume Fed Batch:

- The kinetic parameters (μ_{max} and Ks) can be estimated by applying the Monod kinetics in Eq. and plotting 1/μ versus 1/S using the Lineweaver–Burk plot like in the batch system.

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



Profiles of different parameters in the case of constant volume fed batch.

Problem statement and solution:

Steady-state cell biomass and substrate concentrations in a chemostat are 0.04 g/L and 0.02 g/L of phenol, respectively. The feed (sterile) contains 0.1 g/L of phenol. Find out the biomass yield?

- **Solution:** Given X = 0.04 g/L, S = 0.02 g/L, and S_0 = 0.1 g/L

For a sterile feed, $X_0 = 0$.

Now,

$$Y_{X/S} = \frac{(X - X_0)}{(S_0 - S)}$$

$$= \frac{(0.04 - 0)}{(0.1 - 0.02)}$$

$$= 0.5 \text{ g/g}$$

Problem statement and solution:

Lactococcus lactis has a maximum specific growth rate (µm) of 1.23 h^{-1} in a glucose–yeast extract medium. Find out the specific growth rate of this organism at a steady state in a 4 L reactor at a flow rate of 2 L/h?

Solution: Given µmax = 1.23 h^{-1}, V = 4 L, and F = 2 L/h

$$D = \frac{F}{V} = \frac{2}{4} = 0.5$$

At steady state, µ = D. Therefore, µ = 0.5 h^{-1}

Problem statement and solution:

The initial volume of a repeated fed-batch reactor is 2 L. The feeding rate is 1 L/h. Find out the volume of the reactor after 10 h?

Solution: Given V_0 = 2 L, F = 1 L/h, and t = 10 h.

For a variable volume fed batch reactor, $V = V_0 + Ft$

Therefore,

V = 2 L + (1 L/h)(10 h) = 12 L

Problem statement and solution:

A chemostat (100 m^3 bioreactor) is used for the cultivation of Rhizobium sp. The sucrose concentration in the feed is 12 g/L. K_S and µmax for the organism are 0.2 g/L and 0.3 h^{-1}, respectively.

(a) Find out the flow rate required to obtain a steady-state concentration of sucrose as 1.5 g/L in the bioreactor?

(b) The steady-state cell concentration and yield coefficient of the above culture in the bioreactor are 4 g/L and 0.4 g/g, respectively. Find out the steady-state substrate concentration.

Solution:

Problem statement and solution:

(a) Find out the flow rate required to obtain a steady-state concentration of sucrose as 1.5 g/L in the bioreactor?

Solution: Given: K_S = 0.2 g/L, µmax = 0.3 h^{-1}

Volume of the reactor is V = 100 m^3

Initial substrate concentration is S_0 = 12 g/L

Steady-state concentration is S = 1.5 g/L

Under steady-state conditions and sterile feed,

Dilution rate (D) = Specific cell growth rate (µ)

Problem statement and solution:

Solution: The Monod model for the cell growth kinetics is

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

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

$$D = \frac{0.3 \times 1.5}{0.2 + 1.5} = 0.26\ \mathrm{h}^{-1}$$

Now,

$$F = D \times V \qquad = 0.26 \times 100 \qquad = 26\ \mathrm{m}^3/\mathrm{h}$$

Problem statement and solution:

(b) The steady-state cell concentration and yield coefficient of the above culture in the bioreactor are 4 g/L and 0.4 g/g, respectively. Find out the steady-state substrate concentration.

Solution: Given data:

Yield coefficient = $Y_{X/S}$ = 0.4 g/g

Steady-state cell concentration = X = 4 g/L

For sterile condition = X_0 = 0

Initial substrate concentration, S_0 = 12 g/L

$$Y_{X/S} = \frac{X - X_0}{S_0 - S} \qquad Y_{X/S} = \frac{X}{S_0 - S} \qquad S = S_0 - \frac{X}{Y_{X/S}}$$

Putting all the known values, we get S = 2 g/L.

Problem statement:

Nicotiana tabacum cells are cultured to high density to produce polysaccharide gum. A stirred-tank reactor was used containing initially 100 L medium. The maximum specific growth rate of the culture is 0.18 d^{-1}, and the yield of biomass from the substrate is 0.5 g/g. The concentration of the growth-limiting substrate in the medium is 3%(w/v). The reactor is inoculated with 1.5 g/L cells and operated in batch until the substrate is totally exhausted. Medium flow is then started at a rate of 4 L/d. Fed-batch operation occurs under quasi-steady-state conditions.

(a) Estimate the batch culture time and final biomass concentration.

Problem statement solution:

(a) Estimate the batch culture time and final biomass concentration.

Solution: Given μmax = 0.18 d^{-1}, S_0 = 3% (w/v) = 30 g/L, X_0 = 1.5 g/L, $Y_{X/S}$ = 0.5 g/g

- For a batch culture in the log phase, we know that

$$t_b = \frac{\ln(X/X_0)}{\mu_{max}} \qquad Y_{X/S} = \frac{X - X_0}{S_0 - S}$$

$$X = X_0 + Y_{X/S}(S_0 - S) \qquad t_b = \frac{1}{\mu_{max}} \ln\left[\frac{(Y_{X/S}(S_0 - S) + X_0)}{X_0}\right]$$

- We know that for a batch reactor, tb is the time required to achieve S = 0, which can be expressed as (assuming μ = μmax)

$$t_b = \frac{1}{0.18} \ln\left[1 + \frac{(0.5(30 - 0))}{1.5}\right] = 13.32 \text{ d}$$

Problem statement solution:

Now, cell density at this time can be given as

$$X = X_0 e^{\mu t_b}$$

$$= 1.5\ e^{0.18(13.32)}$$

$$= 16.5\ \text{g/L}$$

Problem statement:

The growth of a microorganism follows the Monod model for cell growth kinetics where μ_{max} = 0.5 h^{-1} and Ks = 2 g/L.

(a) In a chemostat reactor at a steady state with no cell death, if S_0 = 50 g/L and $Y_{X/S}$ = 1, what dilution rate D will give the maximum total rate of cell production?

(b) Determine the number of reactors required to reduce the substrate concentration to 1 g/L if the same value of D is used in the operation of subsequent reactors in series.

Problem statement and solution:

The growth of a microorganism follows the Monod model for cell growth kinetics where μ_{max} = 0.5 h^{-1} and Ks = 2 g/L.

(a) In a chemostat reactor at a steady state with no cell death, if S_0 = 50 g/L and $Y_{X/S}$ = 1, what dilution rate D will give the maximum total rate of cell production?

Solution:

$$D_{max} = \mu_{max}\left(1 - \sqrt{\frac{K_s}{S_0 + K_s}}\right)$$

$$= 0.5\left(1 - \sqrt{\frac{2}{50+2}}\right)$$

$$= 0.402\ h^{-1}$$

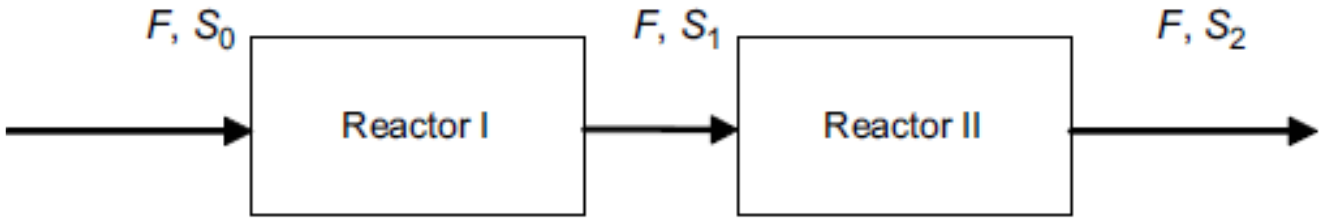
Problem statement and solution:

(b) Determine the number of reactors required to reduce the substrate concentration to 1 g/L if the same value of D is used in the operation of subsequent reactors in series.

- **Solution:** Steady state outlet concentration at reactor 1

$$S_{SS} = \frac{K_s D}{\mu_{max} - D}$$

$$S_1 = \frac{K_s D}{\mu_{max} - D} = 8.2 \text{ g/L}$$



$$X_{SS} = Y_{X/S}\left(S_0 - \frac{K_s D}{\mu_{max} - D}\right)$$

$$X_1 = X_{SS} = Y_{X/S}\left(S_0 - \frac{K_s D}{\mu_{max} - D}\right) = 41.8 \text{ g/L}$$

Problem statement and solution:

(b) Determine the number of reactors required to reduce the substrate concentration to 1 g/L if the same value of D is used in the operation of subsequent reactors in series.

- **Solution:** Steady state outlet concentration at reactor 2

$$D(S_0 - S_{SS}) = \left(\frac{1}{Y_{X/S}}\right)\frac{\mu_{max} S_{SS}}{K_s + S_{SS}} X_{SS}$$

$$D(S_1 - S_2) = \left(\frac{1}{Y_{X/S}}\right)\frac{\mu_{max} S_2}{K_s + S_2} X_2$$

$$Y_{X/S} = \frac{(X_2 - X_1)}{(S_1 - S_2)}$$

$$X_2 = X_1 + Y_{X/S}(S_1 - S_2)$$

$$D(S_1 - S_2) = \left(\frac{1}{Y_{X/S}}\right)\frac{\mu_{max} S_2}{K_s + S_2} \{X_1 + Y_{X/S}(S_1 - S_2)\}$$

Putting the values of D, S_1, μ_{max}, $Y_{X/S}$, K_s, we get

$S_2 = 0.293$ g/L < 1 g/L

Therefore, two reactors will be enough for the above conversion.