

**BIO204 – MIDTERM MERGED PPT
(LECTURE 01 TO 45)**

Principles of Biochemical Engineering

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Note:

Students must follow the course announcement for midterm syllabus & any up-gradation in the course.

Advances in Fermentation Technology

Microbial Growth Kinetics: Introduction

Lesson No1

Microbial Growth Kinetics

❑ What is Microbial Growth Kinetics?

Growth → Change in state/number/size/volume or in other sense it is the ratio between birth (B) and death (D).

Microbial Growth Kinetics

**□ What is
Microbial Growth
Kinetics?**

Growth kinetics
refers to the
change in number
or size of
organisms with
respect to time.

END

Advances in Fermentation Technology

Microbial Growth Kinetics: Methods to Calculate/Measure Growth

Lesson No 2

Microbial Growth Kinetics

☐ Methods that can be used to measure the microbial growth:

1. Turbidity of the culture
2. Total count
3. Viable count
4. Biochemical analysis
5. Graduated capillary tube
6. Dry/wet cell mass

Microbial Growth Kinetics

□ Turbiditometric Method

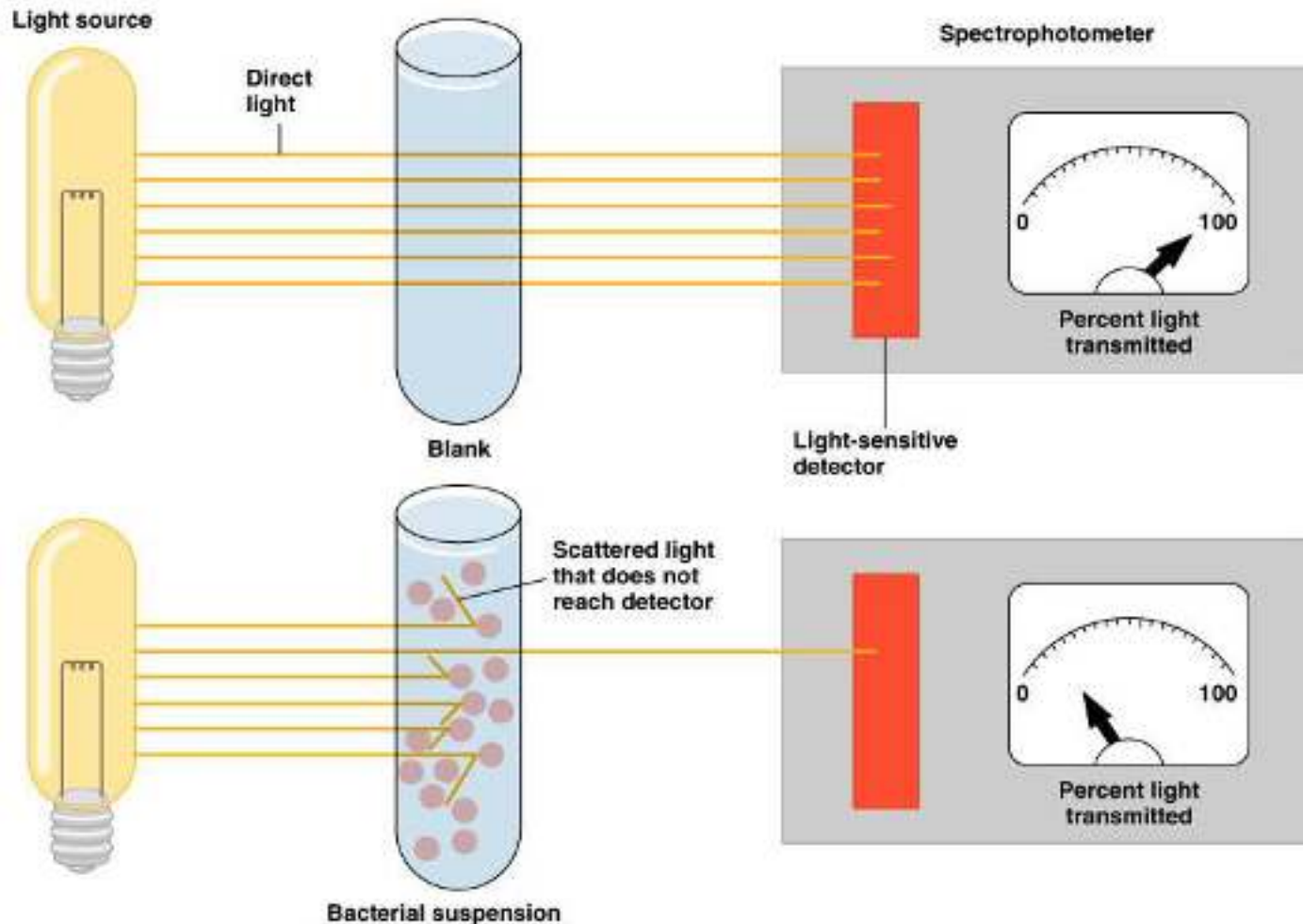
- A **spectrophotometer** is used to determine **turbidity** ("cloudiness") by measuring the amount of light that passed through a suspension of cells.
- More cells → more turbidity;
- more turbidity → less light passing through the suspension
- **%T** is **percent transmission** - fewer cells present (less turbidity) will allow more light to pass through, the %T is higher when the cell number is lower.
- *Absorbance is the opposite of %T.*

$$A = -\log_{10}(T)$$

$$A = abc \quad \text{or} \quad A = \epsilon lc \quad (\text{Lambert-Beer Law})$$

Microbial Growth Kinetics

Measure of Turbidity by using Spectrophotometer



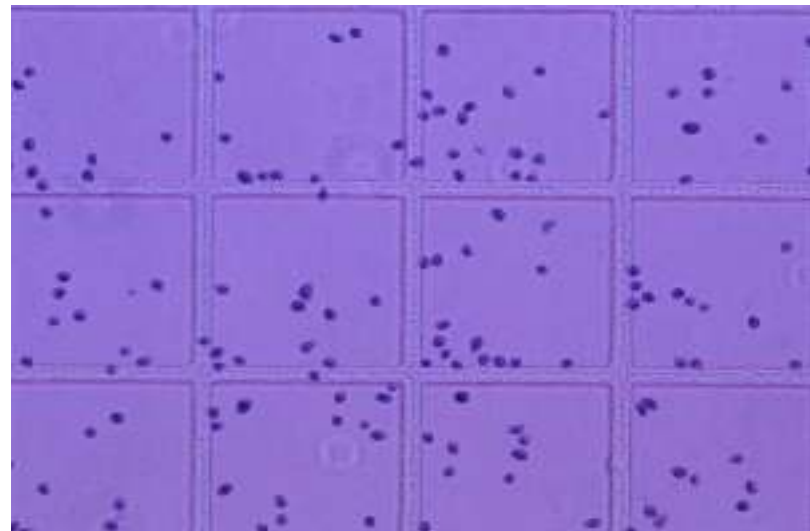
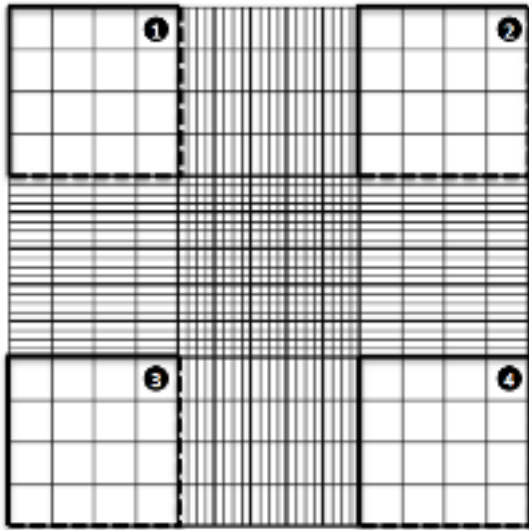
Microbial Growth Kinetics

□ Total Count

- Cell growth is also measured by counting total cell number of the microbes present in that sample. Total cells (both live and dead) of liquid sample are counted by using a **Haemocytometer/Neubauer Chamber**.
- If there is dilute culture, direct cell counting can be done. However, the cell culture of high density can be diluted. Otherwise clumps of cells would be formed which would create problem in exact counting of bacterial cells.

Microbial Growth Kinetics

Haemocytometer/Neubauer Chamber



Microbial Growth Kinetics

☐ Viable Count:

A **viable cell** is defined as a cell which is **able to divide and increase cell numbers**.

Microbial Growth Kinetics

☐ Viable Count:

The normal way to perform a viable count is to determine the number of cells in the sample which is capable of forming colonies on a suitable medium.

Microbial Growth Kinetics

☐ Viable Count:

Here it is assumed that each viable cell will form one colony. Therefore, viable count is often called **plate count** or **colony count**.

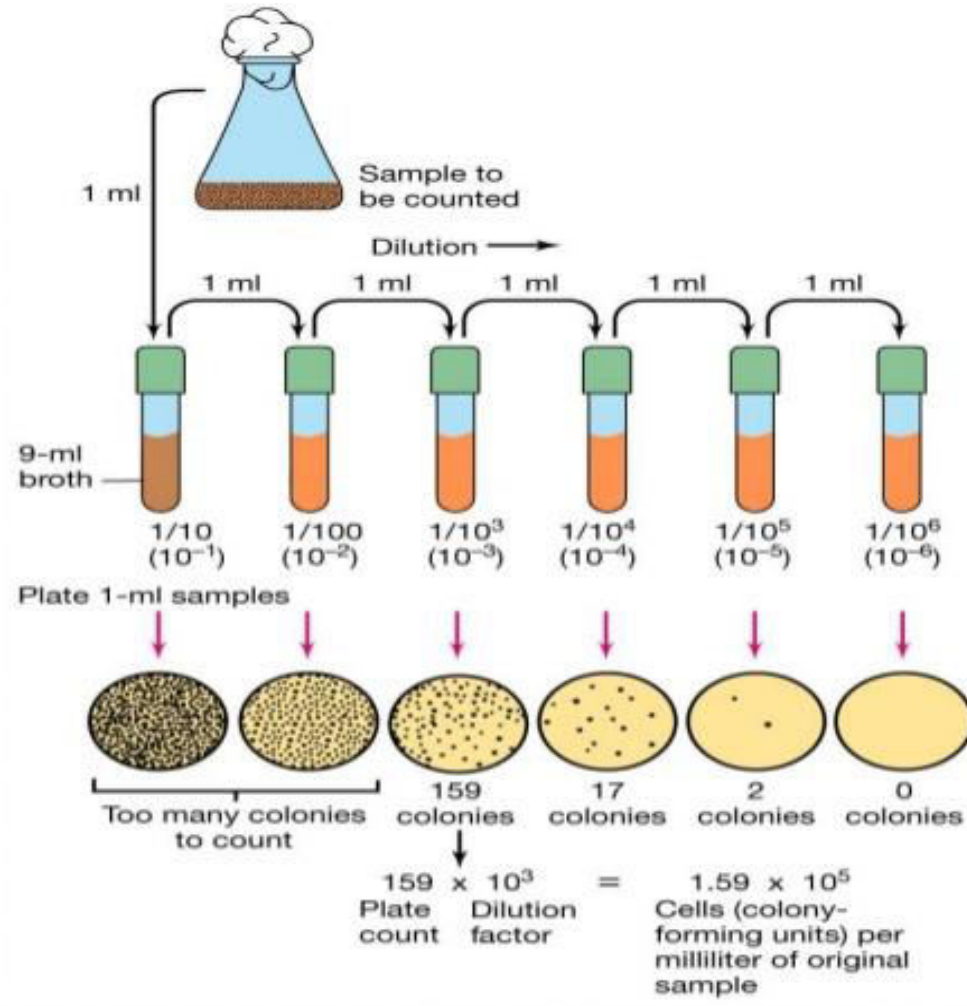
Microbial Growth Kinetics

☐ Viable Count:

A **colony-forming unit (CFU)** is a unit used to estimate the number of viable bacteria or fungal cells in a sample.

Microbial Growth Kinetics

Viable Cell Counting by CFU/ml Method



Microbial Growth Kinetics

☐ Biochemical Analysis:

Direct bio-**chemical measurement** of some cellular component such as Nitrogen content, total protein, or total DNA content or any special compound which is not the part of media constituent.

Microbial Growth Kinetics

☐ Biochemical Analysis:

Indirect **measurement of chemical activity** such as rate of O_2 consumption or production CO_2 production or vice versa, in short: rate of respiration.

END

Advances in Fermentation Technology

Microbial Growth Kinetics: Batch Culture-1

Lesson No 3

Microbial Growth Kinetics

❑ Batch Culture:

In batch processing, a batch of culture medium in a fermenter is inoculated with a microorganism (the 'starter culture').

Microbial Growth Kinetics

□ Batch Culture:

Fermentation proceeds for a certain duration (the 'fermentation time' or '**batch time**'), and the **product is harvested at the end.**

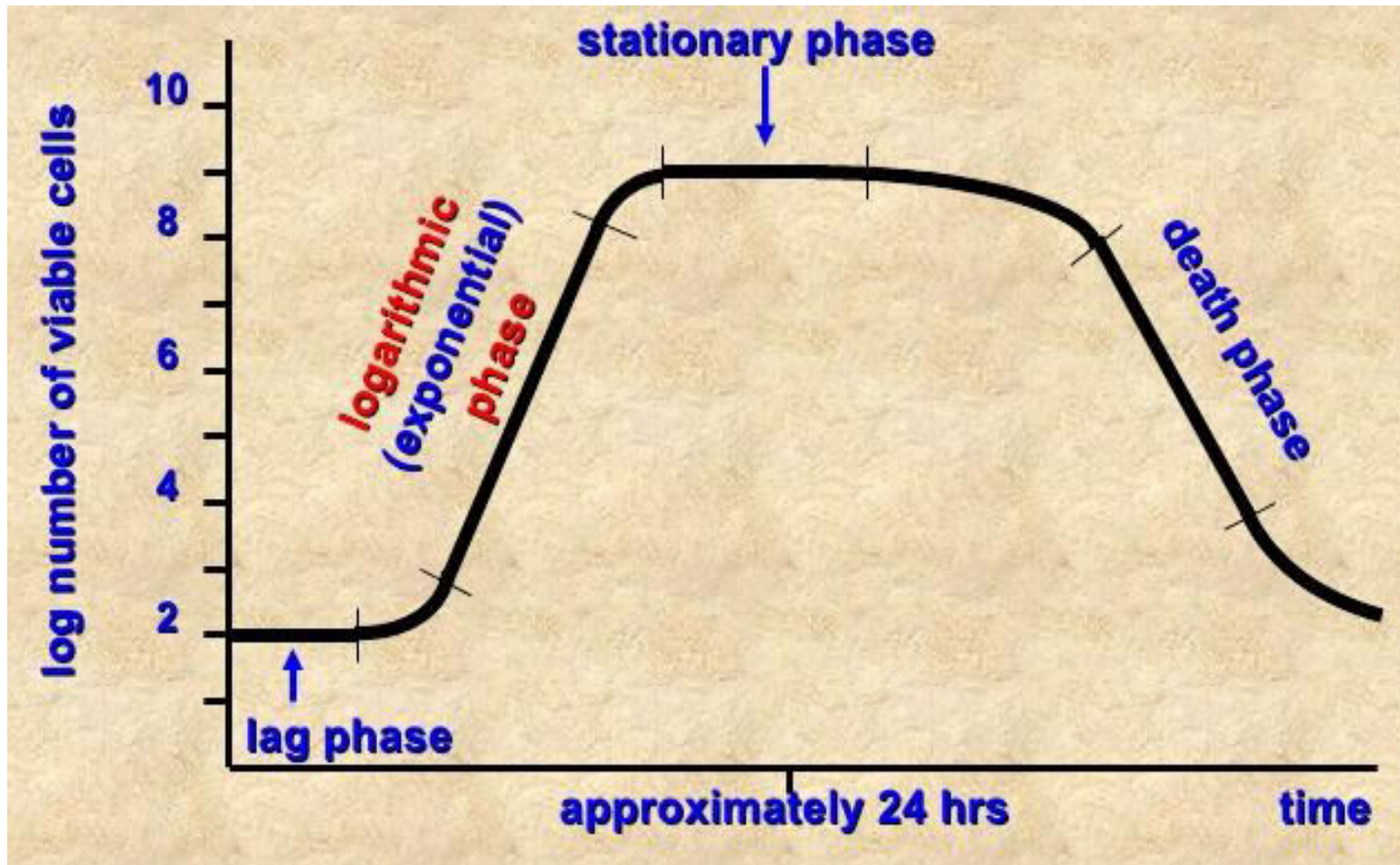
Microbial Growth Kinetics

❑ Batch Culture:

- It is a closed system which contains an **initial limited amount of nutrient**.
- We put all the things at one time here and '**s**' (concentration of substrate) **will remain same**.
- Minor change in volume.

Microbial Growth Kinetics

Microbial Growth Curve in Closed System



Microbial Growth Kinetics

□ Growth Kinetics in Batch Culture:

Biomass/Mass
Culture → 'x'

- Substrate → 's'
- Product → 'p'
(concentration of product)
- x, s, p changes with respect to time 't'.

Microbial Growth Kinetics

□ Growth Kinetics in Batch Culture:

In case of Product:
Sometimes 'x' is itself product (e.g. Baker's Yeast) and sometimes product is directly linked/associated with biomass (e.g. $\text{DNA} \propto \text{Biomass}$, Primary metabolites).

END

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Microbial Growth Kinetics: Batch Culture-2

Lesson No 4

Microbial Growth Kinetics

□ Relationship between 'x' & 't':

Following a period during which the growth rate of the cells gradually increases, the cells grow at a constant, maximum, rate and this period is known as **log** or **exponential, phase**.

Microbial Growth Kinetics

The exponential phase may be described as:
Microbial growth follows the pattern;

$$\begin{aligned} &2^0X_0 \rightarrow 2^1X_0 \rightarrow 2^2X_0 \\ &\rightarrow 2^3X_0 \rightarrow 2^4X_0 \dots\dots \\ &\dots 2^tX_0 \end{aligned}$$

Microbial Growth Kinetics

Doubling time
(t_d) is defined as the time required for cell mass to double. Hence number of generation 'n' after time t are given by:

$$N = t/t_d$$

END

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Microbial Growth Kinetics: Batch Culture-3

Lesson No 5

Microbial Growth Kinetics

Cell density (X_t)
after time 't' will
be:

$$X_t = X_o 2^n = X_o 2^{t/td}$$

$$x \propto t$$

$$dX \propto dt$$

change in biomass
 $\propto$ change in time

Microbial Growth Kinetics

$$dX = \mu \cdot X \cdot dt$$

$$dX/dt = \mu \cdot X$$

Where,

X = concentration
of microbial
biomass

t = time, (mostly
in hrs)

μ = is the Specific
Growth Rate per
unit cell mass

Microbial Growth Kinetics

On applying integration equation this equation ($dX/dt = \mu \cdot X$) becomes:

$$X_t = x_o e^{\mu t}$$

X_o = original biomass concentration

X_t = biomass concentration after the time interval t hours

e = base of the natural logarithm

Microbial Growth Kinetics

To convert equation $x_t = x_o e^{\mu t}$ into linear equation, take natural logarithm by which this equation becomes:

$$\begin{aligned} \ln x_t &= \ln x_o + \mu t (\ln e) \\ \ln x_t &= \ln x_o + \mu t, \text{ because } \ln e = 1 \end{aligned}$$

This equation fit to linear regression equation:

$$Y = a + bX$$

Where:

Y= Vertical axis (dependent variable)

X=Horizontal axis (independent variable)

a= intersect point on plot

b= slope of line (specific growth rate)

Microbial Growth Kinetics

If, $\ln X$ is taken along Y-axis & T is taken on X-axis, a plot of the natural logarithm of biomass concentration against time should yield a straight line, the slope of which would equal μ .

Microbial Growth Kinetics

By using this relationship:

$\ln X_t = \ln X_o + \mu t$, we can calculate **doubling time** by putting $X_t = 2X_o$

$$\ln 2X_o = \ln X_o + \mu t_d$$

$$\ln 2X_o - \ln X_o = \mu t_d$$

$$\ln (2X_o/X_o) = \mu t_d$$

$$\ln (2) = \mu t_d$$

$$\ln 2 / \mu = t_d$$

END

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**Microbial
Growth
Kinetics:
Batch Culture-4**

Lesson No 6

Microbial Growth Kinetics

There are two types of organisms:

- i. Unicellular
- ii. Multicellular

μ (specific growth)
is applied only when
cell is unicellular,
(bacteria & yeast)
e.g. independent of
other cells.

Microbial Growth Kinetics

In case of multicellular organisms, some cells are growing at one time while others are at rest at the same time. So, at one time different cells are at different phases of their growth e.g. some are at log phase while others are at stationary phase etc.). That's why we cannot calculate μ properly in this case.

$$Dx/dt = \mu x$$

$$dH/dt = \mu H$$

$$dA/dt = \mu A$$

Where,

H=total length, and A=number of growing tips or change in area of the colony

Microbial Growth Kinetics

In Submerged fermentation/culture a mycelial organism may grow as dispersed hyphal fragments or as pellets. The growth of pellets will be exponential until the density of the results in diffusion limitation. Under such limitation the central biomass of the pellet will not receive a supply of nutrients, nor will potentially toxic secretions/products diffuse out. Thus effect the growth of the pellet, only growth proceed from the outer shell of biomass which is the actively growing zone and was described by Pirt as:

$$M^{1/3} = kt + M_0^{1/3}$$

where M_0 and M are the mycelial mass at time 0 and t , respectively

Microbial Growth Kinetics

□ Effect of Substrate Concentration 'S':

The **Growth Yield** (Y) is the increase in cell mass (dX) which results due to consumption of a small amount of substrate (dS)

$$Y = dX/dS$$

END

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Microbial Growth Kinetics:

**Batch Culture-5
(Effect of Substrate
Concentration)**

Lesson No 7

Microbial Growth Kinetics

Monod proposed growth yield to constant reproducible quantity under identical conditions

$$X_t - X_o = Y(S_o - S_t)$$

Where, X and S are cell mass and substrate concentration respectively, at time t and X_o and S_o are initial cell mass and substrate concentration respectively. If the substrate in equation is growth limiting substrate and the X_{max} is the maximum cell mass level reached after total consumption of substrate, when there is zero residual substrate (s) then

$$X_{max} - X_o = YS_o$$

Microbial Growth Kinetics

Above than some extent if concentration of 's' is increased its become toxic.

$$X = Y (S_0 - s)$$

Where,

Y= Yield (concentration of biomass produced in fermentation)

S_0 = Initial substrate concentration

s= Residual substrate (which remains at end)

As, $s=0$ (residual substrate 's' remained at end is usually zero)

$$X = YS_0$$

Microbial Growth Kinetics

The specific growth rate is generally found to be function of three parameters:

1. The concentration of growth limiting substrate, S
2. The maximum specific growth rate, $\mu_{\max}$
3. A substrate-specific constant, K_s

$$\mu = \mu_{\max} / (K_s + S) \quad (\text{MONOD EQUATION})$$

Specific growth rate is independent of substrate concentration as long as excess substrate is present.

Microbial Growth Kinetics

$$\mu = \mu_{\max} / (K_s + S)$$

Taking the reciprocal values in the Monod equation and rearranging it:

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

or

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

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

Microbial Growth Kinetics

To convert Monod equation into Linear equation we can write it as:

$$\frac{1}{\mu} = \frac{1}{\mu_{\max}} + \frac{K_s}{\mu_{\max}} \frac{1}{S}$$
$$Y = a + bx$$

The plot of $\frac{1}{\mu}$ against $\frac{1}{S}$ produces a straight line with intercept on the y axis $\frac{1}{\mu_{\max}}$ and slope equals to:

$$\frac{K_s}{\mu_{\max}}$$

END

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Microbial Growth Kinetics: Batch Culture-6 Process Kinetics

Lesson No 8

Microbial Growth Kinetics

□ Process Kinetics:

Material balance for a process can be written as:

$$\text{Accumulation} = \text{Growth} - \text{Death}$$

Substrate accumulation = substrate feed – substrate used for growth – substrate used for product formation – substrate used for maintenance energy

$$-dS/dt = \mu \cdot X/Y_{x/s} - q_p \cdot X/Y_{p/s} - m \cdot X$$

Similarly product formation: **Product $\propto$ Time**

$$p \propto t$$

$$dp \propto dt$$

$$dp/dt = q_p$$

$$dp/dt = q_p X$$

$$dp/dt = q_p X - k_P$$

(k_P = kinetic parameters)

Microbial Growth Kinetics

In fermentation some products are directly proportional to biomass concentration, while some are partially proportional with biomass. **When product is partially proportional with biomass:**

$$dp/dt = q_p \cdot x$$

When product is directly proportional with biomass:

$$dp/dx = Y_{P/x}$$

Yield effected with substrate

$$dp/ds = Y_{P/s}$$

Microbial Growth Kinetics

As we have:

$$dp/dx = Y_{P/X}$$

$$dx/dt \cdot dp/dx = Y_{P/X} \cdot dx/dt$$

$$(\text{As: } dx/dt = \mu x)$$

$$dp/dt = Y_{P/X} \cdot \mu x$$

$$q_P x = Y_{P/X} \cdot \mu x$$

$$q_P = Y_{P/X} \cdot \mu$$

For Substrate:

$$q_S = Y_{P/S} \cdot \mu$$

END

Advances in Fermentation Technology

**Microbial
Growth
Kinetics:
Batch Culture-7**

Lesson No 9

Microbial Growth Kinetics

Borrow *et al.*, (1961)

divided the growth of organism into several phases:

- **The balanced phase:** equivalent to the early to middle exponential phase.
- **The storage phase:** equivalent to the late exponential phase where the increase in mass is due to the accumulation of lipid and carbohydrate.
- **The maintenance phase:** equivalent to the stationary phase.

Microbial Growth Kinetics

□ Growth Phases with respect to metabolites:

Following terms were coined by Bu'Lock *et al.*, (1965):

- i. Trophase
(Refers to exponential phase- stage where primary metabolites are produced).

Microbial Growth Kinetics

□ Growth Phases with respect to metabolites:

- ii. Idiophase
(Usually refers to stationary or death phase- where secondary, tertiary or other metabolites are produced).

END

Advances in Fermentation Technology

**Microbial
Growth
Kinetics:
Fed- Batch
Culture-1**

Lesson No 10

Microbial Growth Kinetics

❑ Fed-Batch Culture:

Yoshida *et al.*, (1973) introduced the term fed-batch culture to describe batch cultures which are fed continuously, or sequentially, **with medium, without the removal of culture fluid.**

Microbial Growth Kinetics

A fed-batch culture is established initially in batch mode and is then fed according to one of the following feed strategies:

- i. The same medium used to establish the batch culture is added, resulting in an increase in volume.
- ii. A solution of the limiting substrate at the same concentration as that in the initial medium is added, resulting in an increase in volume.
- iii. A concentrated solution of the limiting substrate is added at a rate less than in (i) and (ii), resulting in an increase in volume.
- iv. A very concentrated solution of the limiting substrate is added at a rate less than in (i), (ii) and (iii), resulting in an insignificant increase in volume.

Microbial Growth Kinetics

Fed-batch
systems
employing
strategies

(i) and (ii) are
described as
variable volume,
whereas a system
employing
strategy (iv) is
described as fixed
volume.

Microbial Growth Kinetics

The use of strategy (iii) gives a culture intermediate between the two extremes of variable and fixed volume.

END

Advances in Fermentation Technology

**Microbial
Growth
Kinetics:
Fed- Batch
Culture-2**

Lesson No 11

Microbial Growth Kinetics

Variable volume:

(The kinetics of variable volume in fed-batch culture have been developed by **Dunn and Mor** (1975) and **Pirt** (1974, 1975, 1979).

Microbial Growth Kinetics

Consider a batch culture in which growth is limited by the concentration of one substrate; the biomass at any point in time will be described by the equation:

$$x_t = x_o + Y (S_R - s)$$

where x_t is the biomass concentration after time, t hours,

and x_o is the inoculum concentration.

The final biomass concentration produced when $s = 0$ may be described as x_{max} and, provided that x_o is small compared with x_{max} :

$$x_{max} \simeq Y \cdot S_R$$

Microbial Growth Kinetics

If, at the time when $x = x_{max}$ ' a medium feed is started such that the dilution rate is less than μ_{max} , virtually all the substrate will be consumed as fast as it enters the culture, thus:

$$FS_R \simeq \mu (X/Y)$$

where F is the flow rate of the medium feed,
and X is the total biomass in the culture, described by $X = xV$, where V is the volume of the culture medium in the vessel at time t .

From this equation it may be concluded that input of substrate is equaled by consumption of substrate by the cells.

Microbial Growth Kinetics

Thus, $(ds/dt) \simeq 0$.
Although the total biomass in the culture (X) increases with time, cell concentration (x) remains virtually constant, that is $(dx/dt) \simeq 0$ and therefore $\mu \simeq D$.
This situation is termed a **quasi-steady state**.

END

Advances in Fermentation Technology

**Microbial
Growth
Kinetics:
Fed- Batch
Culture-3**

Lesson No 12

Microbial Growth Kinetics

As time progresses the dilution rate will decrease as the volume increases and D will be given the expression:

$$D = F / (V_o + F_t)$$

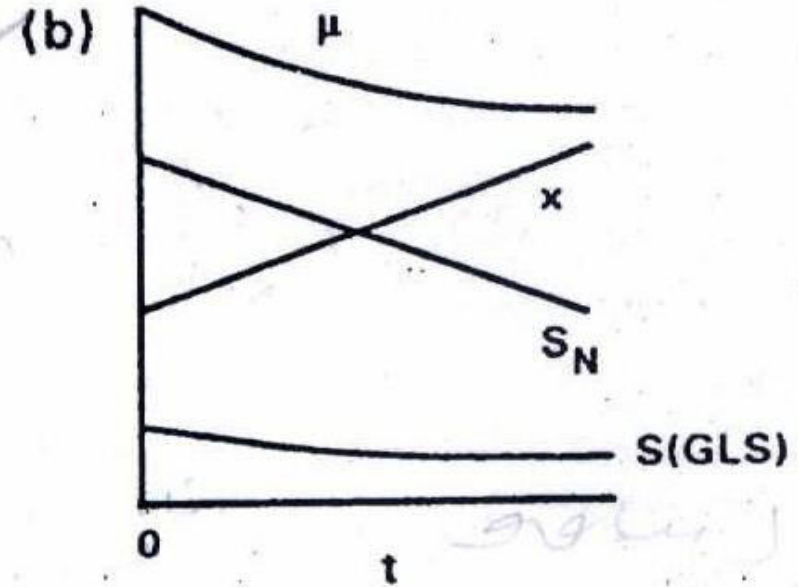
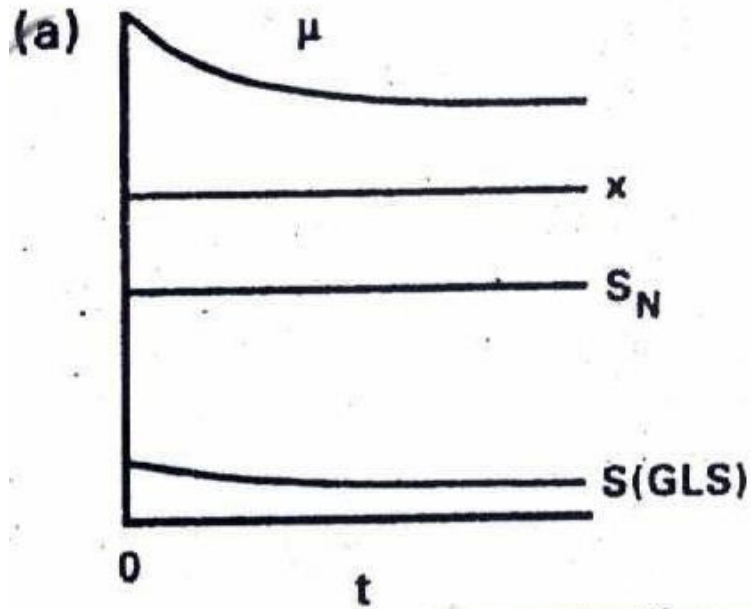
where V_o is the original volume.

Microbial Growth Kinetics

Thus, according to Monod kinetics, residual substrate should decrease as D decreases resulting in an increase in the cell concentration.

However, over most of the range of μ which will operate in fed-batch culture, S_R will be much larger than K_s so that, for all practical purposes, the change in residual substrate concentration would be extremely small and may be considered as zero. Thus, provided that D is less than $\mu_{\max}$ and K_s is much smaller than S_R , a quasi-steady state may be achieved..

Microbial Growth Kinetics



μ = specific growth rate

x = biomass concentration

$S(\text{GLS})$ = growth limiting substrate

S_N = any other substrate than $S(\text{GLS})$

(a) Variable volume fed-batch culture.

(b) Fixed volume fed-batch culture.

(Pirt, 1979).

Microbial Growth Kinetics

The major difference between the **steady state of a chemostat** and the **quasi steady state** of a fed-batch culture is that μ is constant in the chemostat but decreases in the fed-batch

END

Advances in Fermentation Technology

**Microbial
Growth
Kinetics:
Fed- Batch
Culture-4**

Lesson No 13

Microbial Growth Kinetics

Pirt (1979) has expressed the change in product concentration in variable volume fed-batch culture

$$dp/dt = q_p x - Dp$$

Microbial Growth Kinetics

Thus, product concentration changes according to the balance between production rate and dilution by the feed.

Fed-batch quasi steady state change over the time of the fermentation.

Product concentration in a fed-batch system over the time of the fermentation will be dependent on the relationship between **q_p and μ** (hence D).

If **q_p** is strictly growth related then it will change as **μ with D** and, thus, the product concentration remain constant.

However, if q_p is constant and independent of **μ** , then product concentration will the start of the cycle when Dp is greater than $q_p x$, *but will rise with time as D decreases and $q_p x$ become greater than Dp .*

Microbial Growth Kinetics

If q_p is related to μ in a complex manner then product concentration will vary according relationship. Thus, the feed strategy of a system would be optimized according to the ship between q_p and μ .

END

Advances in Fermentation Technology

**Microbial
Growth
Kinetics:
Fed- Batch
Culture-5**

Lesson No 14

Microbial Growth Kinetics

Fixed volume fed-batch Culture:

Pirt (1979) described the kinetics of fixed volume fed-batch culture as follows. Consider a batch culture in which the growth of the process organism depleted the limiting substrate to a limiting level.

Microbial Growth Kinetics

If the limiting substrate is then added in a concentrated feed such that the broth volume remains almost constant then:

$$dx/dt = GY$$

where G is the substrate feed rate ($\text{g dm}^{-3} \text{ h}^{-1}$)
and Y is the yield factor.

But $dx/dt = \mu x$, thus substituting for dx/dt in equation (2.34) gives:

$$\mu x = GY, \text{ and thus:}$$
$$\mu = GY/x$$

Microbial Growth Kinetics

Provided that GY/x does not exceed μ_{max} then the limiting substrate will be consumed as soon as it enters the fermenter and $ds/dt \simeq 0$. However, dx/dt may not be equated to zero, as in the case of variable volume fed- batch, because the biomass concentration, as well as the total amount of biomass in the fermenter, will increase with time. Biomass concentration is given by the equation:

$$x_t = x_a + GY_t$$

where x_t is the biomass after operating in fed- batch for t hours

and x_a is the biomass concentration at the onset of fed-batch culture

Microbial Growth Kinetics

As biomass increases then the specific growth rate will decline according to the equation.

END

Advances in Fermentation Technology

**Microbial
Growth
Kinetics:
Fed- Batch
Culture-6**

Lesson No 15

Microbial Growth Kinetics

Pirt (1979) described the product balance in a fixed volume fed-batch system as:

$$dp / dt = q_p x$$

but substituting for x from equation gives:

$$dp/dt = q_p(x_0 + GYt)$$

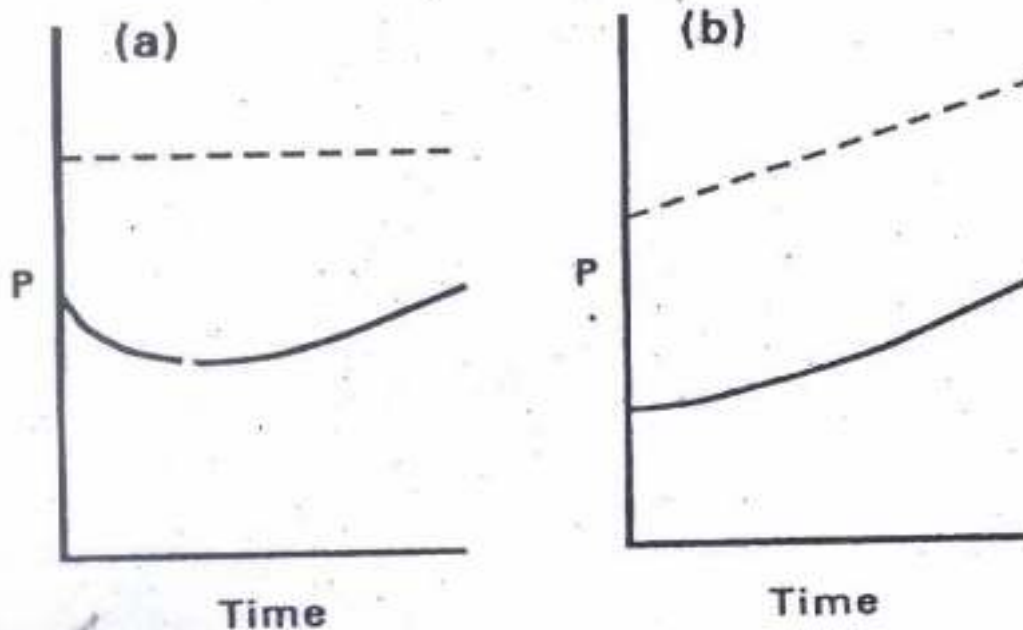
Microbial Growth Kinetics

If q_p is strictly growth-rate related then product concentration will rise linearly as for biomass.

However, if q_p is constant then the rate of increase in product concentration will rise as growth rate declines, i.e. as time progresses and x increases.

If q_p is related to μ in a complex manner then the product concentration will vary according to that relationship.

Microbial Growth Kinetics



Product concentration (p) in fed-batch culture when q_p is growth related (— — — — —) or non-growth related, i.e. q_p constant (———)

(a) Variable volume fed-batch culture.

(b) Fixed volume fed-batch culture.

(Modified from Pirt, 1979.)

Microbial Growth Kinetics

As in the case of variable volume fed-batch the feed profile would be optimized according to the relationship between q_p and μ .

END

Advances in Fermentation Technology

Microbial Growth Kinetics: Continuous Culture-1

Lesson No 16

Microbial Growth Kinetics

- Exponential growth in batch culture may be prolonged by the addition of fresh medium to the vessel. Provided that the medium has been designed such that growth is substrate limited (i.e. by some component of the medium), and not toxin limited, exponential growth will proceed until the additional substrate is exhausted. This exercise may be repeated until the vessel is full.
- However, if an overflow device were fitted to the fermenter such that the added medium displaced an equal volume of culture from the vessel then continuous production of cells could be achieved.

Microbial Growth Kinetics

If medium is fed continuously to such a culture at a suitable rate, steady state is achieved eventually, that is, formation new biomass by the culture is balanced by the loss cells from the vessel.

Microbial Growth Kinetics

The flow of medium into the vessel is related to the volume of the vessel by the term dilution rate, D , defined as:

$$D = F/V$$

Where, F is the flow rate ($\text{dm}^3 \text{ h}^{-1}$)
and V is the volume (dm^3)

Thus, D is expressed in the units h^{-1} . The net change in cell concentration over a time period may be expressed as:

$$dx/dt = \text{growth} - \text{output}$$

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

Microbial Growth Kinetics

OR

Under steady state conditions the cell concentration remains constant, thus $dx/dt = 0$ and:

$$\mu x = Dx$$

$$\mu = D$$

Microbial Growth Kinetics

Thus, under steady-state conditions the specific growth rate is controlled by the dilution rate, which is an experimental variable. It will be recalled that under batch culture conditions an organism will grow at its maximum specific growth rate and, therefore, it is obvious that a continuous culture may be operated only at dilution rates below the maximum specific growth rate. Thus, within certain limits, the dilution rate may be used to control the growth rate of the culture.

Microbial Growth Kinetics

The growth of the cells in a continuous culture of this type is controlled by the availability of the growth limiting chemical component of the medium and, thus, the system is described as a **chemostat**.

END

Advances in Fermentation Technology

Microbial Growth Kinetics: Continuous Culture-2

Lesson No 17

Microbial Growth Kinetics

The mechanism underlying the controlling effect of the dilution rate is essentially the relationship expressed in following equation as demonstrated by Monod in 1942:

$$\mu = \mu_{max} s / (K_s + s)$$

At steady state, $\mu = D$, and, therefore,

$$D = \mu_{max} \bar{s} / (K_s + \bar{s})$$

Where $\bar{s}$ is the steady state concentration of substrate in the chemostat, and

$$\bar{s} = K_s D / (\mu_{max} - D) \text{ (Eq. 1)}$$

Microbial Growth Kinetics

Equation: $\bar{s} = K_s D / (\mu_{max} - D)$ predicts that the substrate concentration is determined by the dilution rate.

In effect, this occurs by growth of the cells depleting the substrate to a concentration that supports the growth rate equal to the dilution rate. If substrate is depleted below the level that supports the growth rate dictated by the dilution rate, the following sequence of events takes place:

(mentioned in next slide)

Microbial Growth Kinetics

- i. The growth rate of the cells will be less than the dilution rate and they will be washed out of the vessel at a rate greater than they are being produced, resulting in a decrease in biomass concentration.
- ii. The substrate concentration in the vessel will rise because fewer cells are left in the vessel to consume it.
- iii. The increased substrate concentration in the vessel will result in the cells growing at a rate greater than the dilution rate and biomass concentration will increase.
- iv. The steady state will be re-established.

Microbial Growth Kinetics

Thus, a chemostat is a nutrient-limited self-balancing culture system which may be maintained in a steady state over a wide range of sub-maximum specific growth rates. The concentration of cells in the chemostat at steady state is described by the equation:

$$\bar{x} = Y (S_R - \bar{s}) \dots\dots\dots \text{Eq. 2}$$

Microbial Growth Kinetics

Where, $\bar{x}$ in **Eq. 2** is the steady-state cell concentration in the chemostat. By combining equations (1) and (2), then:

$$\bar{x} = Y [S_R - \{K_s D / (\mu_{max} - D)\}]$$

Thus, the biomass concentration at steady state is determined by the operational variables, S_R and D . If S_R is increased, $\bar{x}$ will increase but $\bar{s}$, the residual substrate concentration in the chemostat, will remain the same.

Microbial Growth Kinetics

If D is increased, μ will increase ($\mu = D$) and the residual substrate at the new steady state would have increased to support the elevated growth rate; thus, less substrate will be available to be converted into biomass, resulting in a lower steady state value.

END

Advances in Fermentation Technology

**Microbial
Growth
Kinetics:
Continuous
Culture-3**

Lesson No 18

Microbial Growth Kinetics

An alternative type of continuous culture to the **chemostat** is the **turbidostat**, where the concentration of cells in the culture is kept constant by controlling the flow of medium such that the turbidity of the culture is kept within certain, narrow limits. This may be achieved by monitoring the biomass with a photoelectric cell and feeding the signal to a pump supplying medium to the culture such that the pump is switched on if the biomass exceeds the set point and is switched off if the biomass falls below the set point. Systems other than turbidity may be used to monitor the biomass concentration, such as CO₂ concentration or pH in which case it would be more correct to term the culture a **biostat**.

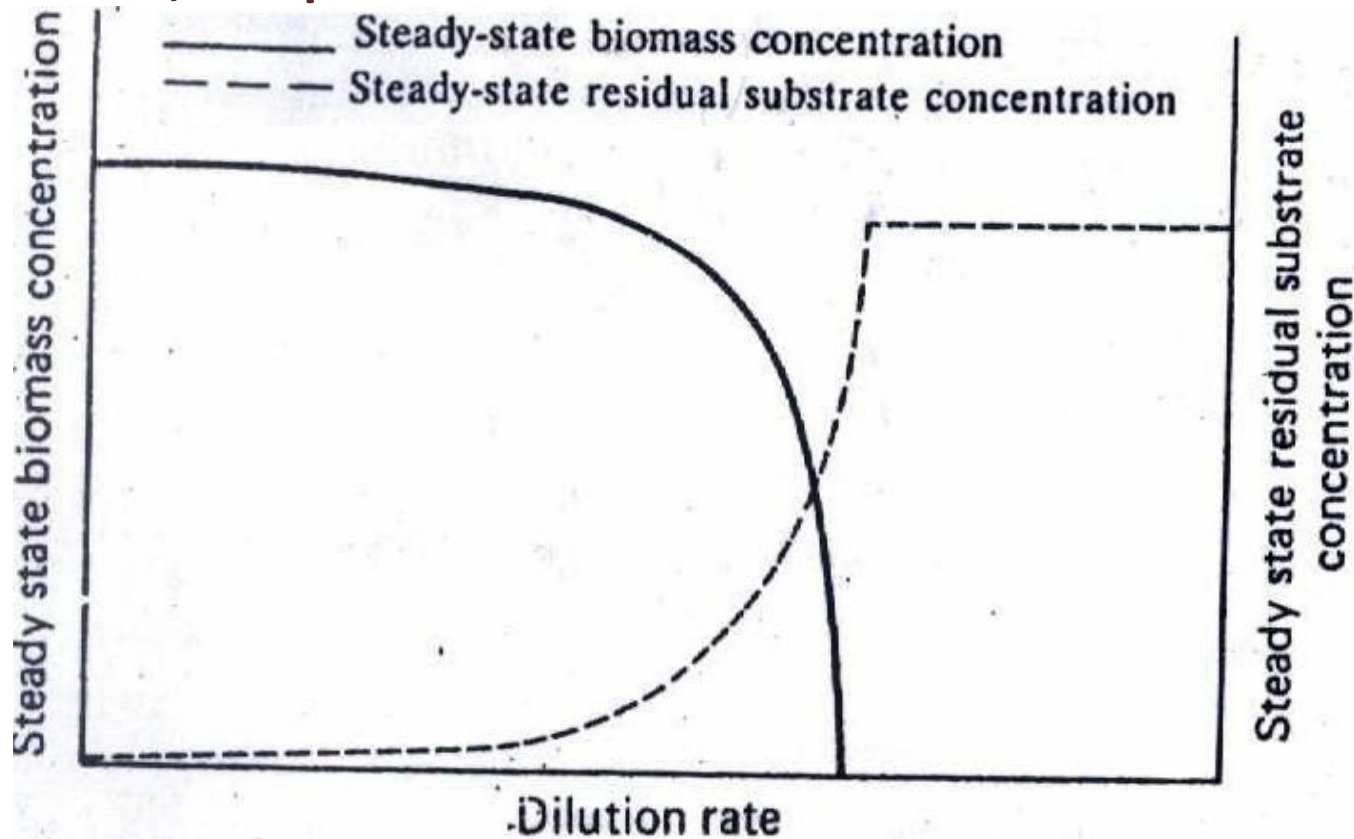
Microbial Growth Kinetics

Efforts by: AARISH ALI

The chemostat is the more commonly used system because it has the advantage over the biostat of not requiring complex control systems to maintain a steady state. However, the biostat may be advantageous in continuous enrichment culture in avoiding the total washout of the culture in its early stages.

Microbial Growth Kinetics

The effect of dilution rate on the steady-state biomass and residual substrate concentrations in a chemostat of a microorganism with a low K_s value for the limiting substrate, compared with the initial substrate concentration



Microbial Growth Kinetics

Figure in previous slide illustrates the continuous culture behavior of a hypothetical bacterium with a low K_s value for the limiting substrate, compared with the initial limiting substrate concentration. With increasing dilution rate, the residual substrate concentration increases only slightly until D approaches μ_{max} when s increases significantly. The dilution rate at which x equals zero (that is, the cells have been washed out of the system) is termed the critical dilution rate (D_{crit}) and is given by the equation:

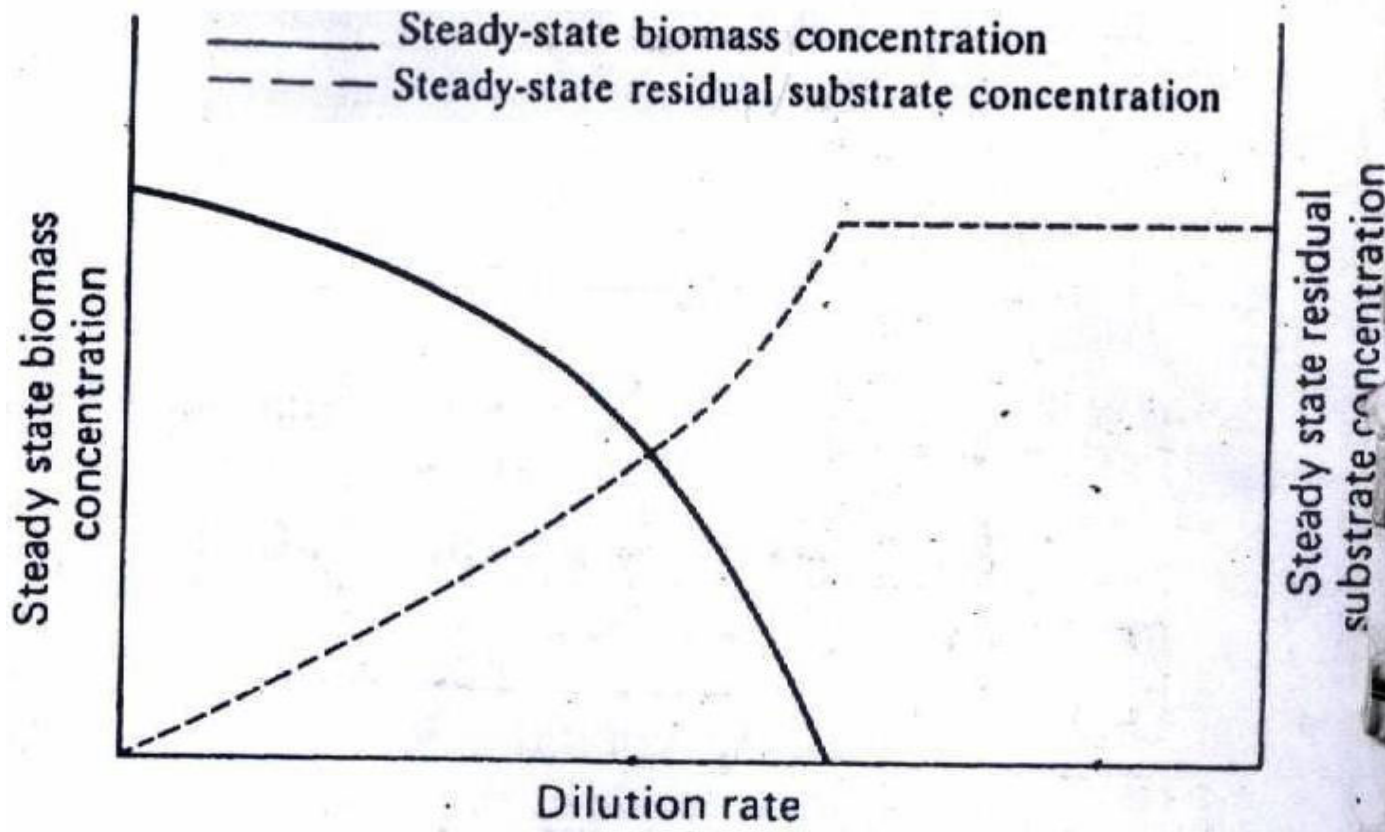
$$D_{crit} = \mu_{max} S_R / (K_s + S_R)$$

Microbial Growth Kinetics

The kinetic characteristics of an organism (and, therefore, its behavior in a chemostat) are described by the numerical values of the constants Y , μ_{max} and K_s . The value of Y affects the steady-state biomass concentration; the value of μ_{max} affects the maximum dilution rate that may be employed and the value of K_s affects the residual substrate concentration (and, hence, the biomass concentration) and also the maximum dilution rate that may be used.

Microbial Growth Kinetics

The effect of dilution rate on the steady-state biomass and residual substrate concentrations in a chemostat of a microorganism with a high K_s value for the limiting substrate, compared with the initial substrate concentration



Microbial Growth Kinetics

Figure in previous slide illustrates the continuous culture behavior of a hypothetical bacterium with high K_s for the limiting substrate compared with the initial limiting substrate concentration. With increasing dilution rate, the residual substrate concentration increases significantly to support the increased growth rate. Thus, there is a gradual increase in s and a decrease in x as D approaches D_{crit} .

Microbial Growth Kinetics

Thus, D_{crit} is affected by the constants, μ_{max} and K_s , and the variable, S_R ; the larger S_R the closer is D_{crit} to μ_{max} .

However, μ_{max} cannot be achieved in a simple steady state chemostat because substrate limited conditions must always prevail.

END

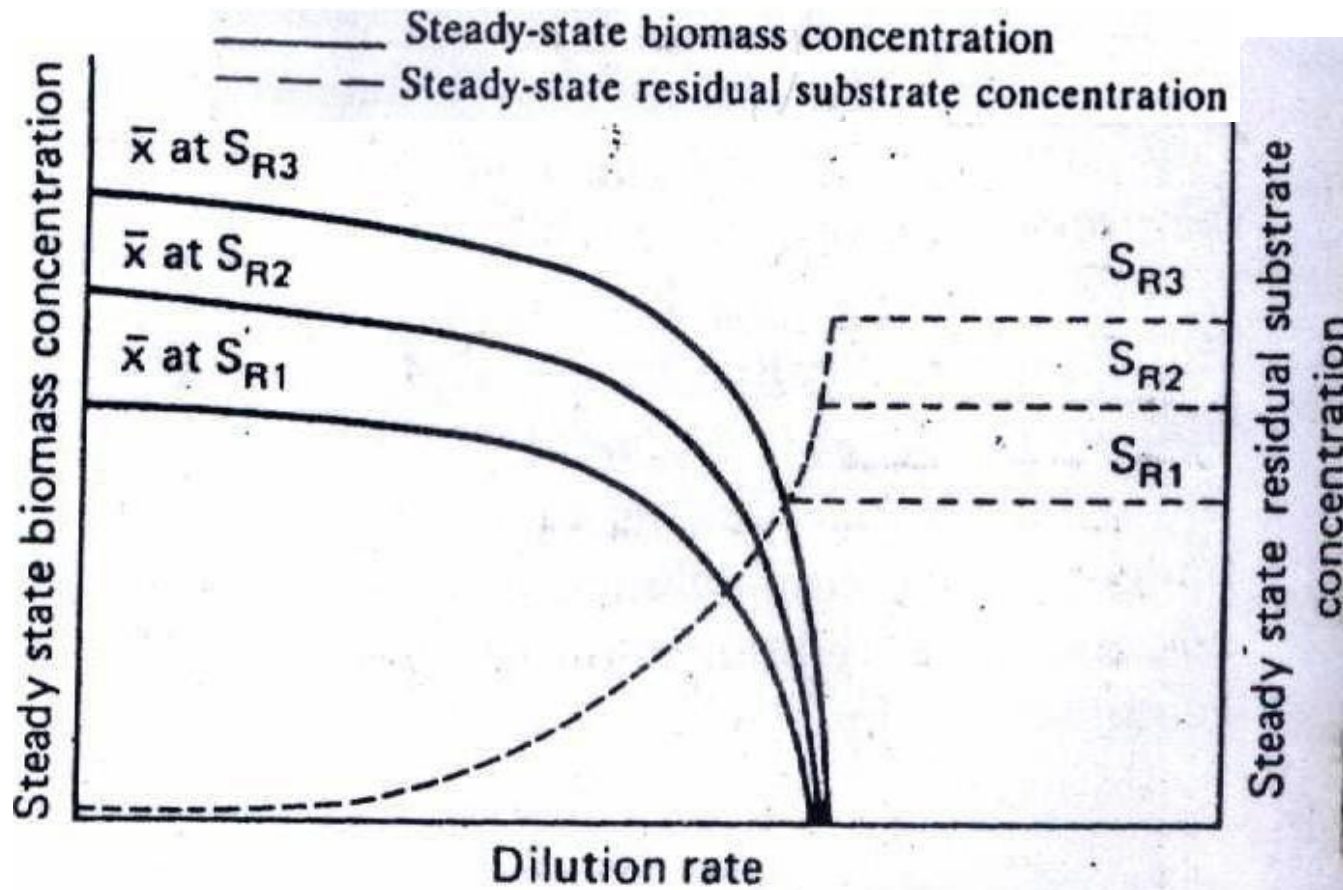
Advances in Fermentation Technology

**Microbial
Growth
Kinetics:
Continuous
Culture-4**

Lesson No 19

Microbial Growth Kinetics

The effect of increased initial substrate concentration on the steady-state biomass and residual substrate concentrations in a chemostat



Microbial Growth Kinetics

Figure in previous slide illustrates the effect of increasing the initial limiting substrate concentration on $\bar{x}$ and $\bar{s}$. As S_R is increased, $\bar{x}$ so increases, but the residual substrate concentration is unaffected. Also D_{crit} increases slightly with an increase in S_R .

Whereas, S_{R1} , S_{R2} and S_{R3} in the figure represent increasing concentrations of the limiting substrate in the feed medium.

Microbial Growth Kinetics

The results of chemostat experiments may differ from those predicted by the foregoing theory. The reasons for these deviations may be anomalies associated with the equipment or the theory not predicting the behavior of the organism under certain circumstances. Practical anomalies include imperfect mixing and wall growth. Imperfect mixing would cause an increase in the degree of heterogeneity in the fermenter when some organisms being subject to nutrient excess whilst others are under severe limitation. This phenomenon is particularly relevant to very low dilution rate system when the flow of medium is likely to be very intermittent. This problem may be overcome by the use of feedback system. Wall growth is another commonly encountered practical difficulty in which the organism adheres to the inner surface of the reactor resulting, again, in an increase in heterogeneity.

Microbial Growth Kinetics

The immobilized cells are not subject to removal from the vessel but will consume substrate resulting in the suspended biomass concentration being lower than predicted. Wall growth may be limited by coating the inner surface of the vessel with Teflon.

Microbial Growth Kinetics

- A frequent observation in carbon and energy limited chemostats is that the biomass concentration at lower dilution rates is lower than predicted. This is attributed to the phenomenon of microorganisms utilizing a greater proportion of substrate for maintenance at lower dilution rates. Effectively the yield factor decreases at lower dilution rates.
- Bull (1974) has reviewed the major cause of deviation from basic chemostat theory.

Microbial Growth Kinetics

The basic chemostat may be modified in a number of ways, but the most common modifications are the addition of extra stages (vessels) and the feedback of biomass into the vessel.

END

Advances in Fermentation Technology

Microbial Growth Kinetics: Principles of Growth in Continuous Systems

Lesson No 20

Microbial Growth Kinetics

Continuous cultivation at steady rate is possible only when all factors contributing to the accumulation of biomass are exactly balanced by all factors contributing to the loss of biomass from the system:

$$\left(\begin{array}{c} \text{Cells added} \\ \text{to the system} \end{array} \right) - \left(\begin{array}{c} \text{Cells removed} \\ \text{from the system} \end{array} \right) = \left(\begin{array}{c} \text{Cells produced} \\ \text{through growth} \end{array} \right) - \left(\begin{array}{c} \text{Cells consumed} \\ \text{through death} \end{array} \right)$$

or

$$(FX_0/V) - (FX/V) + \mu X - aX = dX/dt \dots\dots\dots (\text{Eq.3})$$

F=Flow rate (liter/hour) in and outflow from fermenter;

V = Liquid volume (liters) in fermenter;

X₀ and X = cell masses (gl⁻¹) in the feed and fermenter;

μ=specific growth rate;

a=specific death rate (reciprocal hours);

dX/dt = rate of change in cell mass (gl⁻¹h⁻¹)

Microbial Growth Kinetics

At steady state

$$dX/dt = 0,$$

V and F are fixed,

$a \ll \mu$, so aX is ignored,

And $X_0 = 0$, (because
Feed is sterile)

Hence at steady state
Eq. 3 is simplified as:

$$\mu = F/V = D \dots\dots (Eq.4)$$

Microbial Growth Kinetics

Specific growth rate of the population within the continuous fermenter is determined by the dilution rate, D .

Microbial Growth Kinetics

Chemostat is operated with sterile feed and not recycled hence $X_0=0$ and specific growth rate is equal to dilution rate ($\mu=D$). This is maintained by controlling the availability of a limiting nutrient through the addition of fresh medium.

Microbial Growth Kinetics

□ Limiting Nutrient Balance for a Chemostat

{Input-output-consumed} = Accumulation

or

$$DS_0 - DS - \mu X / Y_{x/s} = ds/dt \text{ (Eq.5)}$$

D = F/V; S_0 and S are substrate concentration in the feed and fermenter, respectively;

X = dry cell mass in fermenter, $Y_{x/s}$ = yield coefficient;

ds/dt = rate of change of substrate concentration in the fermenter

If product formation other than cells occurs in chemostat at steady state equation 5 will be:

$$D(S_0 - S) = \mu X / Y_{x/s} \text{ (Eq.6)}$$

substitution of equation 4 into this equation give:

$$X = Y_{x/s}(S_0 - S) \text{ (Eq.7)}$$

Microbial Growth Kinetics

The overall growth yield is assumed to be dependent on the limiting nutrient concentration and independent of specific growth rate. Substituting and rearranging the previous equations:

$$D = d_c S / K_s + S \text{ (Eq.8)}$$

d_c = critical dilution rate corresponding to the maximum specific growth rate (μ_{max})

Operation of the chemostat at dilution rates above D_c results in complete washout of the culture.

Microbial Growth Kinetics

Eq. 8 can be rearranged to

$$S = DK_s / D_c - D \dots (\text{Eq.8})$$

Substitution into eq. 7 gives:

$$X = Y_{x/s} \{ S_0 - (DK_s / D_c - D) \}$$

END

Advances in Fermentation Technology

Microbial Growth Kinetics: Calculation of Growth Yield in Chemostat

Lesson No 21

Microbial Growth Kinetics

The yield substrate by $Y_{x/s}$, a ratio of the respective concentrations. Yields can be easily calculated by determining the production of dry cell mass over a given period of the cultivation and dividing by mass of carbon substrate consumed.

$Y_{x/s}$ = g dry cells produced / g substrate consumed

$Y_{x/ATP}$ = g dry cells produced / moles of ATP formed

Y_{x/O_2} = g dry cells produced / moles of O_2 consumed

Yield with respect to ATP synthesis or oxygen consumption is relatively constant for many organisms, e.g. the yield of cells per mole of ATP synthesized under conditions of energy substrate limitation and high growth rate = approximately $10 + 2$ g cells. Yields of cells per mole of ATP is not constant for all bacteria and is variable if the energy substrate is not growth limiting or the growth rate is lower than the maximum rate.

Microbial Growth Kinetics

❑ Establishment of Steady-State Condition in a Chemostat

In terms of cell and substrate concentrations, steady-state condition can be ensured by allowing at least 4 changes of fermenter liquid volume.

Example: In a 2-litre chemostat with flow rate of (inlet and outlet) $F = 0.5 \text{ L/h}$, and with dilution rate of $F/V = D = 0.25 \text{ h}^{-1}$

The time necessary for 4 changes of medium volume = $4/D$ or $4/0.25 \text{ h}^{-1} = 16 \text{ h}$

Microbial Growth Kinetics

Steady-state will be established after 16 h of changing the growth conditions of a chemostat. Only then the overall growth yield can be determined as below:

$$Y_{x/s} = (X - X_0) / (S_0 - S)$$

X=g cells dry weight per liter of effluent medium.

X₀=g cells dry weight per liter of influent medium i.e. zero in sterile medium,

S=g substrate per liter of effluent medium,

S₀=g substrate per liter of influent medium

Microbial Growth Kinetics

Overall growth Yield is related maintenance and growth requirement for limiting substrates that act as energy sources:

$$1/Y_{x/s} = (m/m) + (1/Y_G)$$

m=specific rate of substrate uptake for cellular maintenance,

$Y_{x/s}$ =overall yield,

Y_G =growth specific yield,

Microbial Growth Kinetics

m and Y_G can be estimated by plotting $(1/Y_{x/s})$ vs. $(1/\mu)$ on rectangular paper. If this gives a straight-line relationship, The intercept = $1/Y_G$; Slope of the line = m ($\mu = D$, in simple chemostat without cell recycle).

END

Advances in Fermentation Technology

Fermenter Design-1

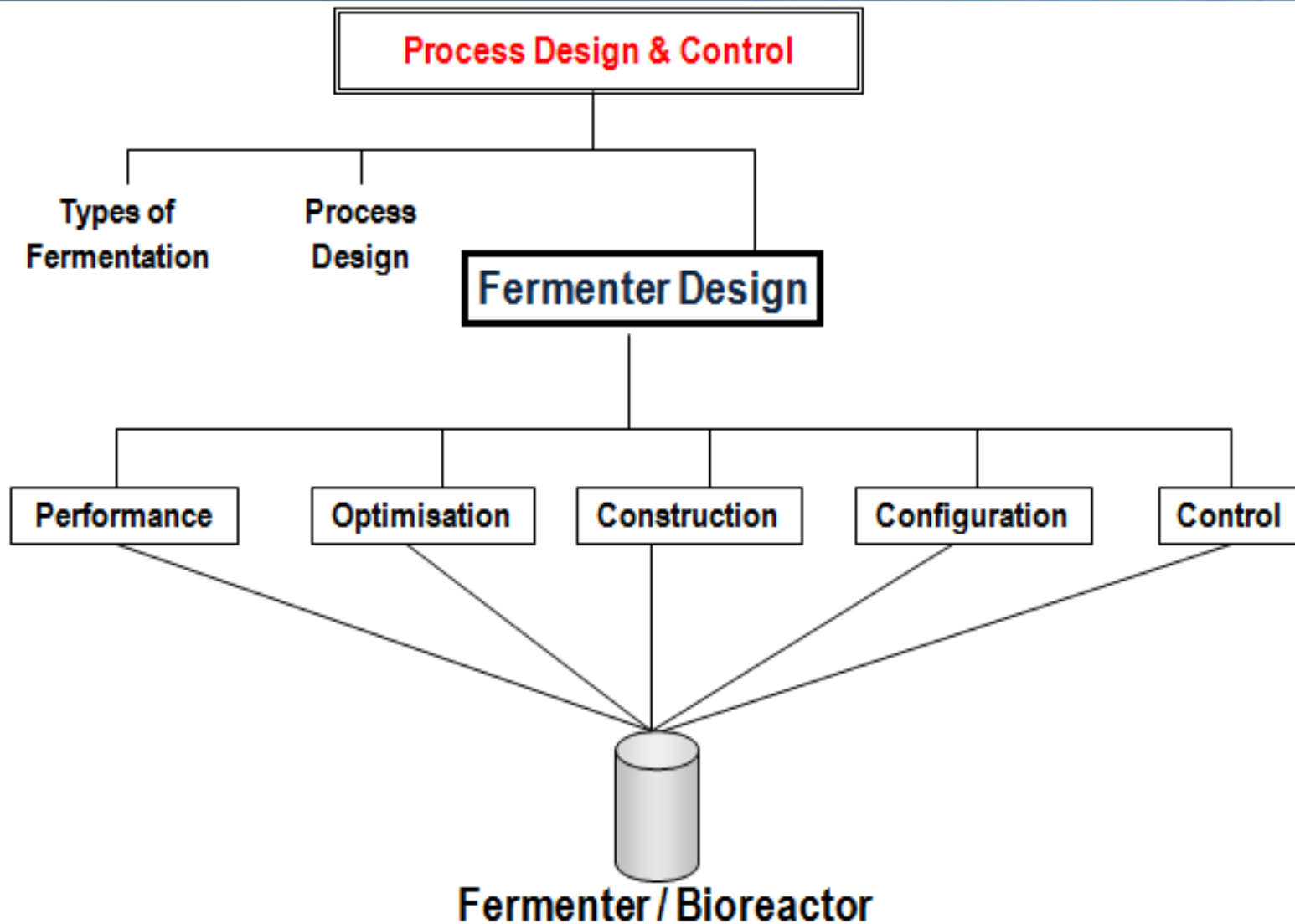
Lesson No 22

Fermenter Design

□ Key points for Industrial Production of Bio-products

- As soon as possible (initial start)
- As fast as possible (rate)
- As much as possible (concentration, yield)
- As long as possible (duration)
- As cheap as possible (cost)
- As simple as possible (ease of work)
- As desirable as possible (regulatory issues)

Fermenter Design



Fermenter Design

**Better productivity
of Biological
products depends
on:**

- ✓ **Strain
improvement**
- ✓ **Media
development**
- ✓ **Process Design &
Control**

END

Advances in Fermentation Technology

**Fermenter
Design-2**

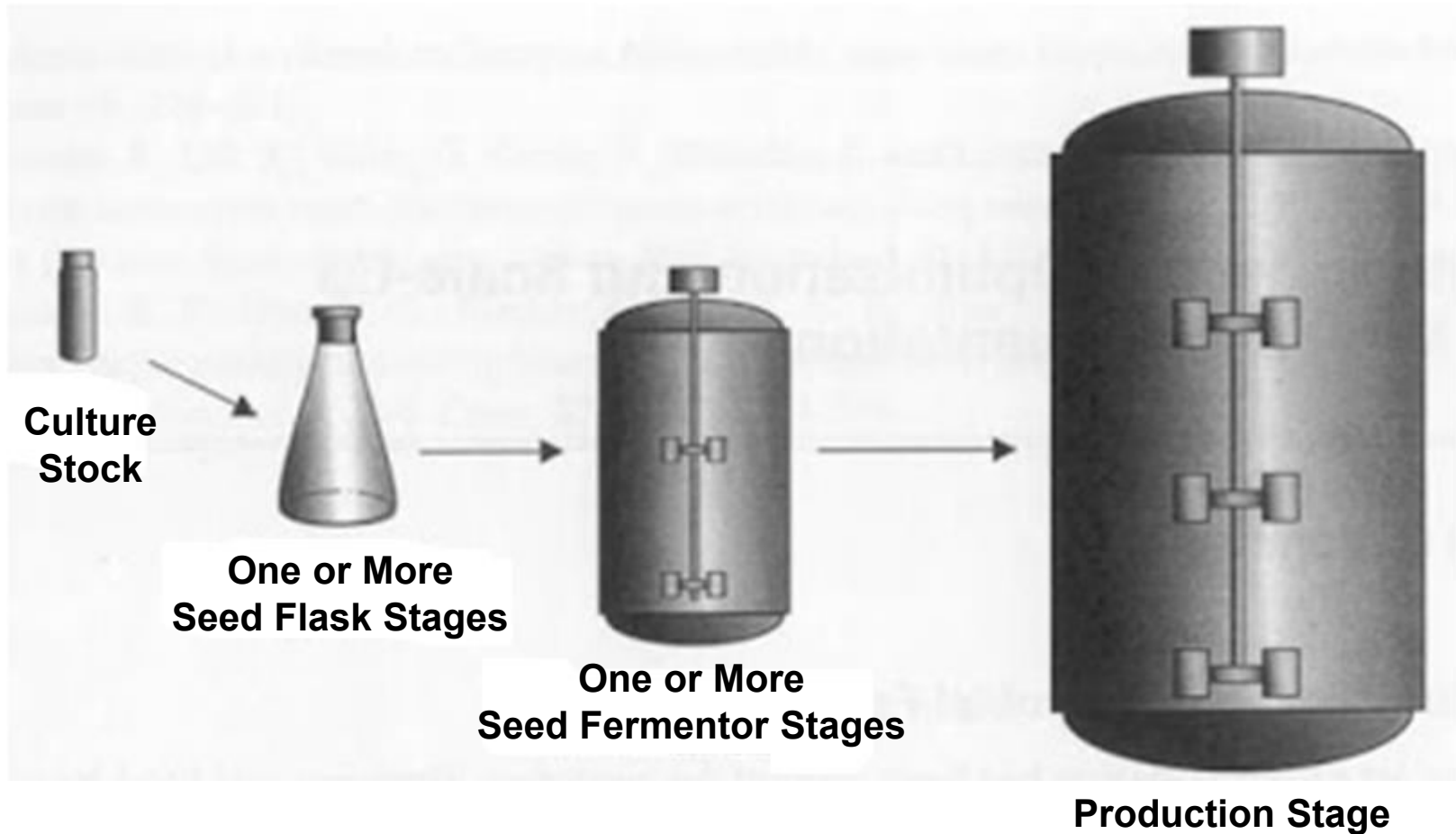
Lesson No 23

Fermenter Design

The **heart** of the Industrial fermentation process is the fermenter/bioreactor. The function of the fermenter or bioreactor is to provide a suitable environment in which an organism can efficiently produce a target product-the target product might be:

- **Cell biomass**
- **Metabolite**
- **Bioconversion Product**

Fermenter Design



Fermenter Design

□ Range/Scale of Fermenter/Bioreactor

- **Lab-scale Fermenters**

Ex-situ sterilization but seed fermenter is not required for inoculum development, slants or flask cultures can be utilized.

- **Pilot-scale Fermenters**

In-situ sterilization but seed fermenter is not required for inoculum development, slants or flask cultures can be utilized.

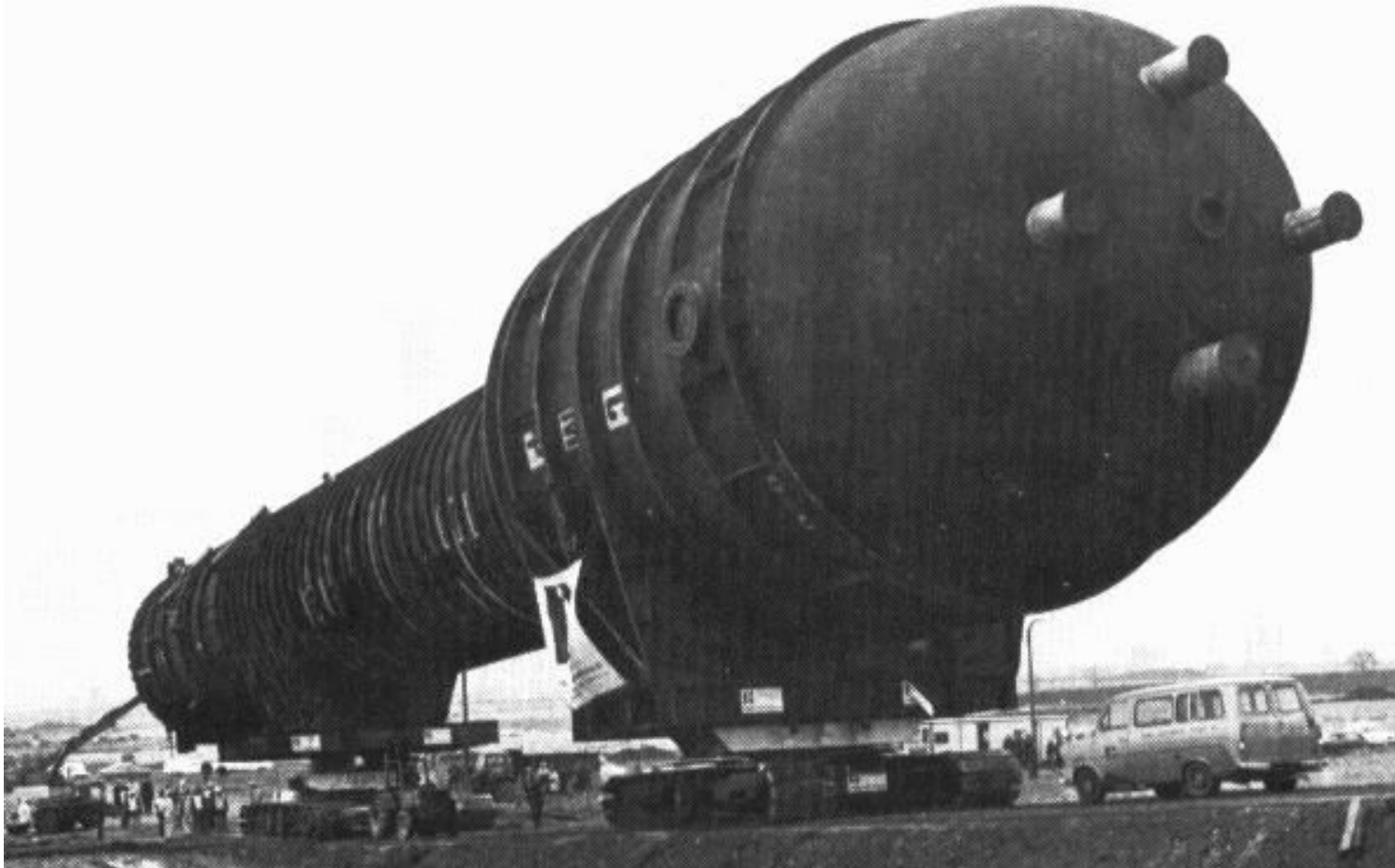
- **Industrial-scale Fermenters**

In-situ sterilization and seed fermenter is required for inoculum development.

Fermenter Design



Fermenter Design



Industrial Fermenter:
The fermenter is 200' high and 25 ft dia

Fermenter Design

□ Commonly used Types of Fermenter

- Stirred tank reactor (STR)
- Air lift fermenter
- Fermenter with Venturi eductor
- Waldhof type fermenter
- Self priming fermenter
- Tower fermenter
- Koji fermenter

Fermenter Design

Some other types of Fermenter include:

- **Packed bed**
- **Acetators and Cavitators**
- **Cyclone Column**
- **Cylindero-Conical Vessel**
- **Deep Jet Fermenter**
- **Rotating Disk Fermenters**
- **Paddle wheel reactor**
- **Basket reactor**
- **Spinfilter perfusion reactor**
- **Tray reactor**
- **Membrane Reactors**
- **Photo bioreactor**

END

Advances in Fermentation Technology

**Fermenter
Design-3**

Lesson No 24

Fermenter Design

☐ Accessories for a Fermenter:

- Baffle
- Sterile compressed air (at 1.5 to 3.0 atmospheres)
- Chilled water (12 to 15°C)
- Cold water (4°C)
- Hot water
- Steam (high pressure)

Fermenter Design

- **Steam condensate**
- **Electricity**
- **Stand-by generator**
- **Drainage of effluents**
- **Motors**
- **Storage facilities for media components**

END

Advances in Fermentation Technology

Fermenter Design: General Requirements of Fermenters/ Bioreactors

Lesson No 25

Fermenter Design

There is no universal bioreactor. The general requirements of the bioreactor are as follows:

1. The vessel should be capable of being operated aseptically for a number of days and should be reliable in long-term operation and meet the requirements of containment regulations.
2. Adequate aeration and agitation should be provided to meet the metabolic requirements of the micro-organism. However, the mixing should not cause damage to the organism.
3. Power consumption should be as low as possible.
4. A system of temperature control should be provided.
5. A system of pH control should be provided.
6. Sampling facilities should be provided.

Fermenter Design

7. Evaporation losses from the fermenter should not be excessive.
8. The vessel should be designed to require the minimal use of labour in operation, harvesting, cleaning and maintenance.
9. Ideally the vessel should be suitable for a range of processes, but this may be restricted because of containment regulations.
10. The vessel should be constructed to ensure smooth internal surfaces, using welds instead of flange joints whenever possible.
11. The vessel should be of similar geometry to both smaller and larger vessels in the pilot plant or plant to facilitate scale-up.

Fermenter Design

12. The cheapest materials which enable satisfactory results to be achieved should be used.

13. There should be adequate service provisions for individual plants.

END

Advances in Fermentation Technology

Fermenter Design: Aseptic Operation & Containment

Lesson No 26

Fermenter Design

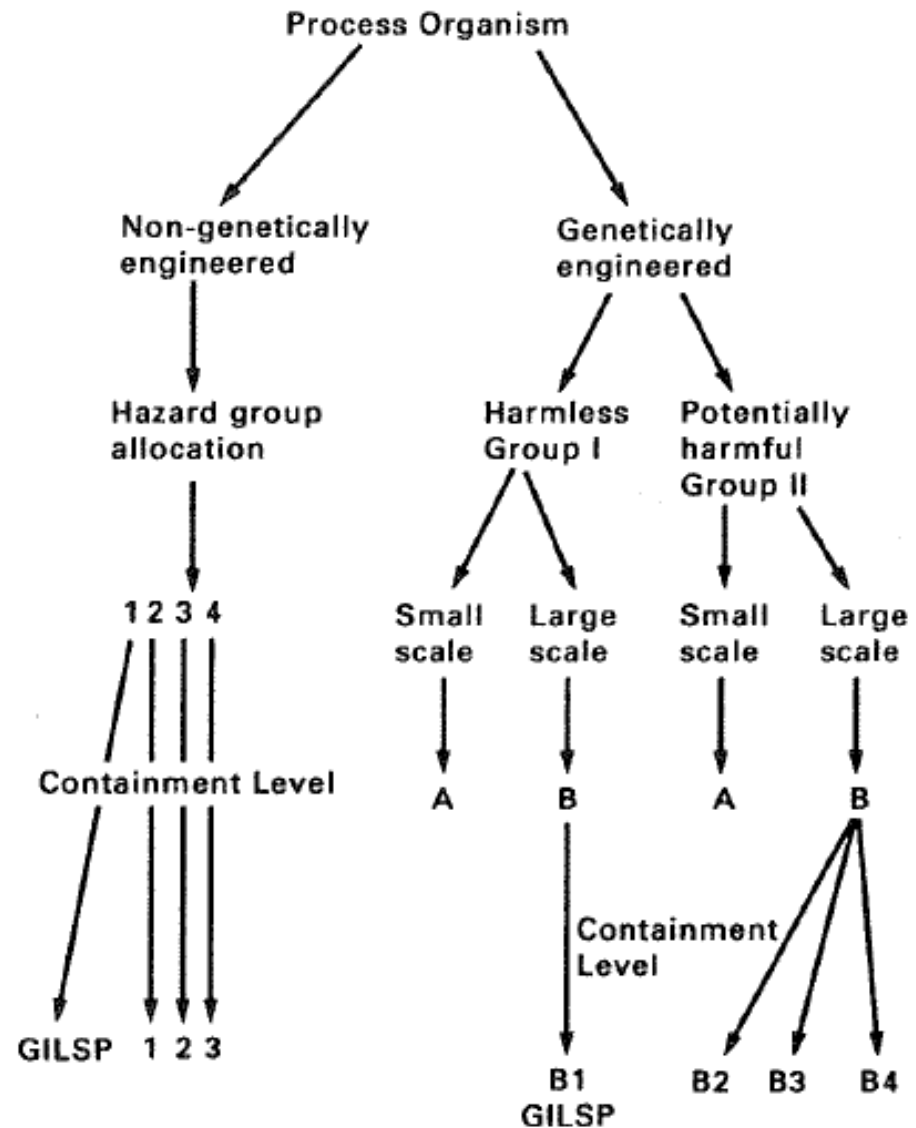
➤ Aseptic operation involves protection against contamination and containment involves prevention of escape of viable cells from a fermenter or downstream equipments

➤ Containment guidelines were initiated during the 1970s. To establish the appropriate degree of containment which will be necessary to grow a micro-organism, it, and in fact the entire process, must be carefully assessed for potential hazards that could occur should there be accidental release. Different assessment procedures are used depending on whether or not the organism contains foreign DNA (genetically engineered). Once the hazards are assessed, an organism can be classified into a hazard group for which there is an appropriate level of containment.

Fermenter Design

Categorization of a process micro-organism and designation of its appropriate level of containment at research or industrial sites within the European Federation of Biotechnology

GILSP = Good Industrial Large Scale Practice



Fermenter Design

Risk such as those given by Collins (1992):

- 1. The known pathogenicity of the micro-organism.**
- 2. The virulence or level of pathogenicity of the microorganism are the diseases it causes mild or serious?**
- 3. The number of organisms required to initiate an infection.**

Fermenter Design

- 4. The routes of infection.**
- 5. The known incidence of infection in the community and the existence locally of vectors and potential reserves.**
- 6. The amounts or volumes of organisms used in the fermentation process.**

Fermenter Design

- 7. The techniques or processes used.**
- 8. Ease of prophylaxis and treatment.**

END

Advances in Fermentation Technology

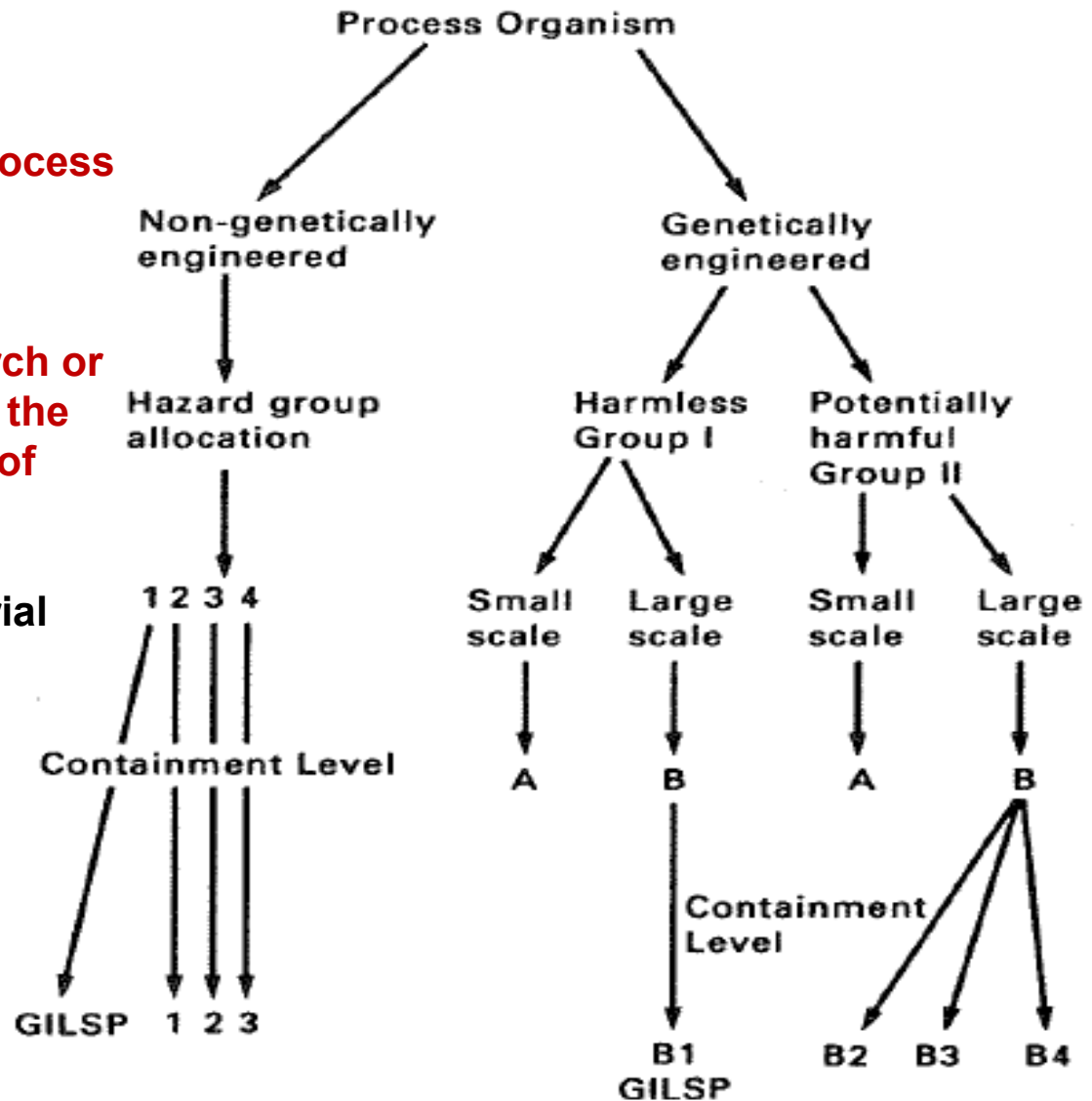
Fermenter Design: Aseptic Operation & Containment-2

Lesson No 27

Fermenter Design

Categorization of a process micro-organism and designation of its appropriate level of containment at research or industrial sites within the European Federation of Biotechnology

GILSP = Good Industrial Large Scale Practice



Fermenter Design

□ *Summary of safety precautions for biotechnological operations in the European Federation for Biotechnology (EFB)* (Frommer *et al.*, 1989)

Procedures	GILSP*	Containment Category		
		1	2	3
Written instructions and code of practice	+	+	+	+
Biosafety manual	—	+	+	+
Good occupational hygiene	+	+	+	+
Good Microbiological Techniques (GMT)	—	+	+	+
Biohazard sign	—	+	+	+
Restricted access	—	+	+	+
Accident reporting	+	+	+	+
Medical surveillance	—	+	+	+

* Unless required for product quality, — , not required; +, required.

Fermenter Design

Procedures	GILSP*	Containment Category		
		1	2	3
<i>Primary containment: Operation and equipment</i>				
Work with viable micro-organisms should take place in closed systems (CS) , which minimize (m) or prevent (p) the release of cultivated micro-organisms	—	m	p	p
Treatment of exhaust air or gas from CS	—	m	p	p
Sampling from CS	—	m	p	p
Addition of materials to CS, transfer of cultivated cells	—	m	p	p
Removal of material, products and effluents from CS	—	m	p	p
Penetration of CS by agitator shaft and measuring devices	—	m	p	p
Foam-out control	—	m	p	p

m, minimize release. The level of contamination of air, working surface and personnel shall not exceed the level found during microbiological work applying Good Microbiological Techniques.

p, prevent release. No detectable contamination during work should be found in the air, working surfaces and personnel.

Fermenter Design

Procedures	GILSP*	Containment Category		
		1	2	3
<i>Secondary containment: Facilities</i>				
Protective clothing appropriate to the risk category	+	+	+	+
Changing/washing facility	+	+	+	+
Disinfection facility	—	+	+	+
Emergency shower facility	—	—	+	+
Airlock and compulsory shower facilities	—	—	—	+
Effluents decontaminated	—	—	+	+
Controlled negative pressure	—	—	—	+
HEPA filters in air ducts	—	—	+	+
Tank for spilled fluids	—	—	+	+
Area hermetically sealable	—	—	—	+

* Unless required for product quality, —, not required; +, required.

Fermenter Design

Once the organism has been allocated to a hazard group, the appropriate containment requirements can be applied (*as mentioned in the Table in previous slides*).

END

Advances in Fermentation Technology

Fermenter Design: General Introduction of a Fermenter

Lesson No 28

Fermenter Design



• Detachable stirrer motor

Fermenter Design



• Detachable stirrer motor

• Vessel

Fermenter Design



- Detachable stirrer motor
- Vessel
- pH/O₂ Electrodes

Fermenter Design



- Detachable stirrer motor
- Vessel
- pH/O₂ Electrodes
- Exhaust Gas Condenser

Fermenter Design



- Detachable stirrer motor
- Vessel
- pH/O₂ Electrodes
- Exhaust Gas Condenser
- Dialysis Unit (Not Usual)

Fermenter Design



- ❖ Control Console Note:
 - Microprocessor logging & Control

Fermenter Design



❖ Control Console Note:

- Microprocessor logging & Control
- Gas supply rotameters

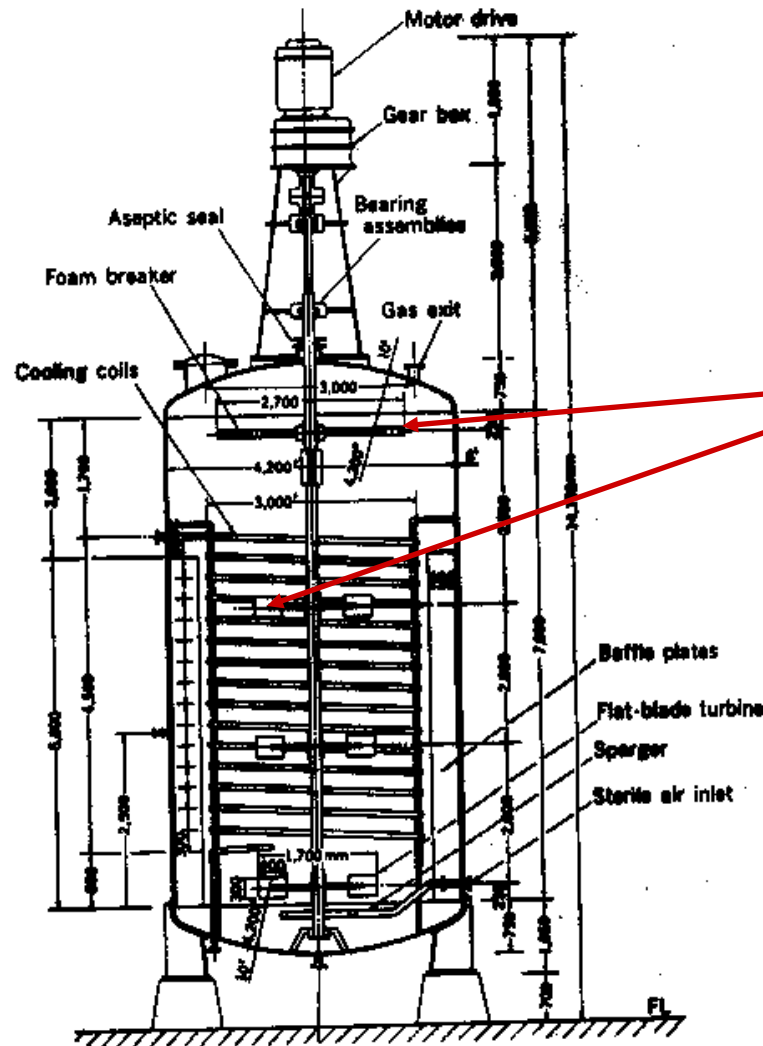
Fermenter Design



❖ Control Console Note:

- Microprocessor logging & Control
- Gas supply rotameters
- Pumps for pH control, antifoam, nutrient feed, etc.

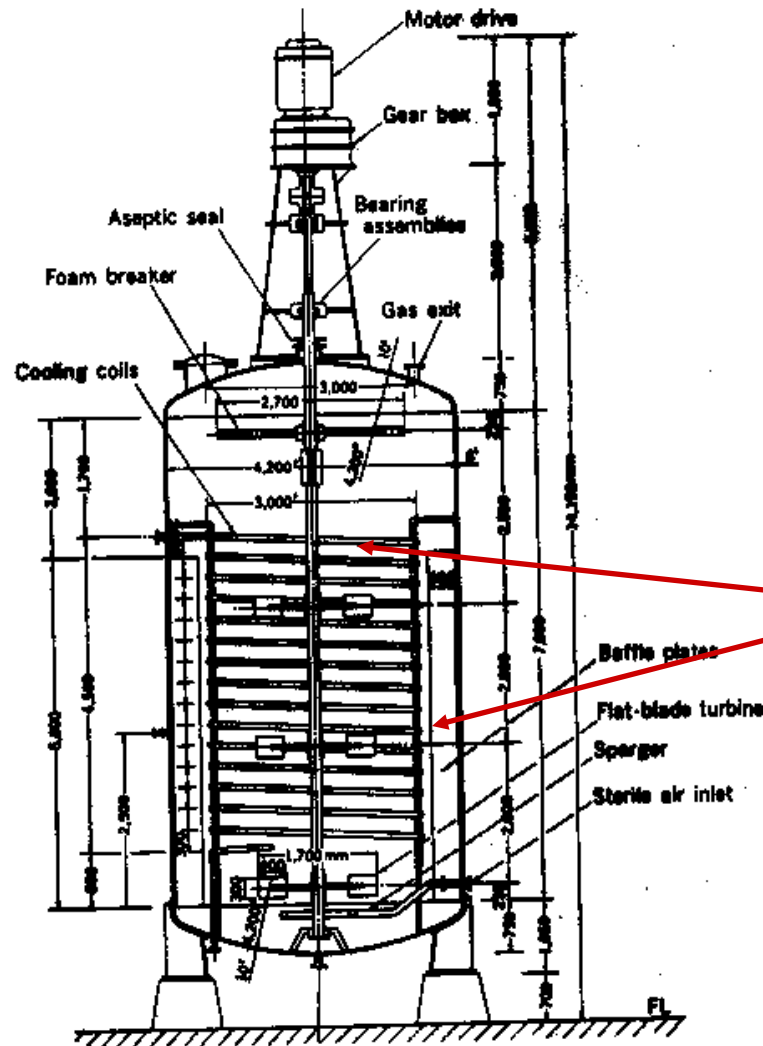
Fermenter Design



❖ Schematic representation of 100,000 L Production Factor with:

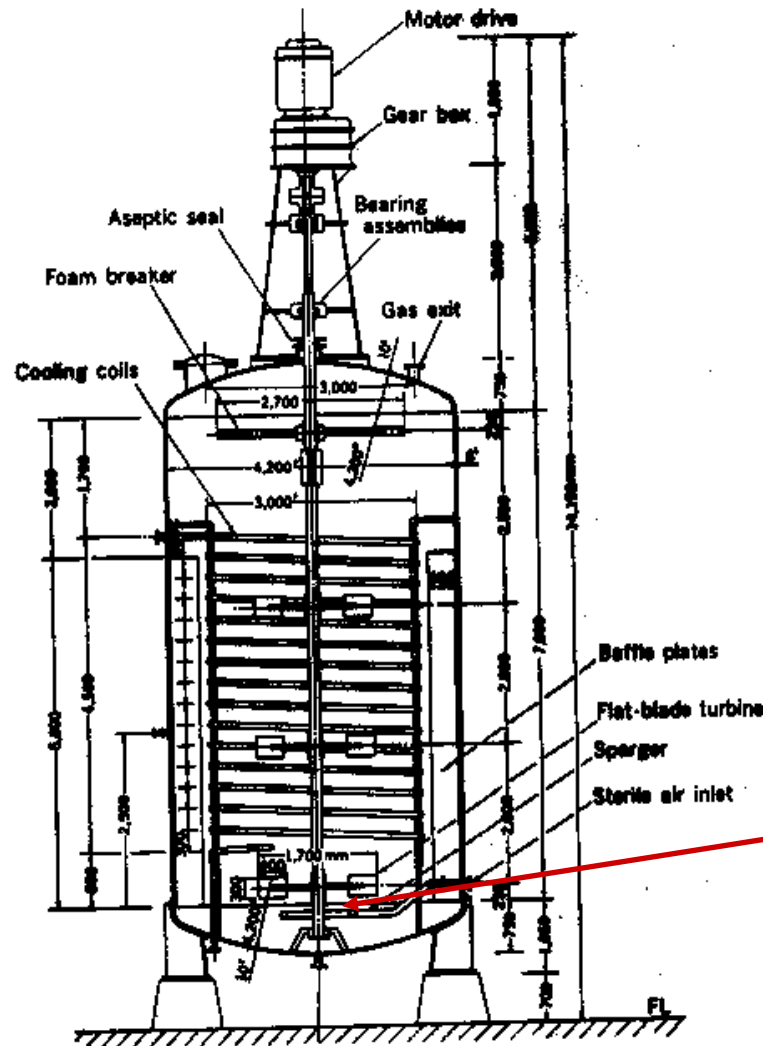
Top drive agitators foam breakers

Fermenter Design



- ❖ Schematic representation of 100,000 L Production Factor with:
 - **Top drive agitators foam breakers**
 - **Internal cooling coils and baffles**

Fermenter Design



❖ Schematic representation of 100,000 L Production Factor with:

- Top drive agitators foam breakers
- Internal cooling coils and baffles
- Sparger (air input)

Fermenter Design

Therefore, basic features/accessories of a bioreactor associated for monitoring, control & record:

- An agitator system
- An oxygen delivery system
- A foam control system
- A temperature control system
- A pH control system
- A cleaning and sterilization system
- A sump and dump line system (only on pilot and Industrial Scale)

END

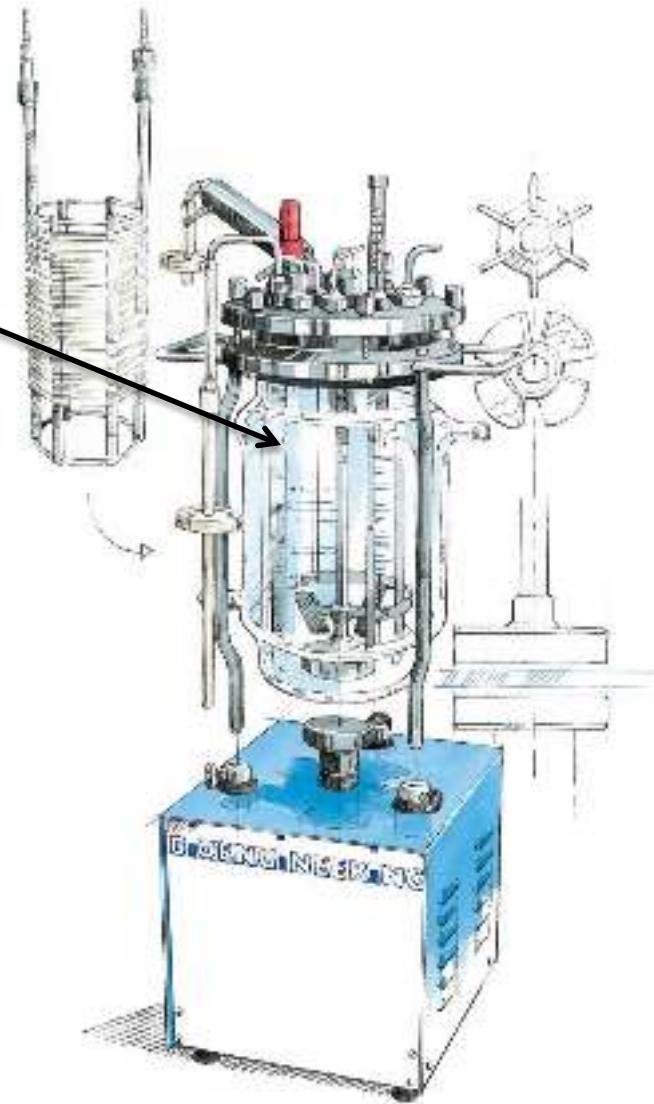
Advances in Fermentation Technology

Fermenter Design: Basic Parts of a Stirred Fermenter

Lesson No 29

Fermenter Design

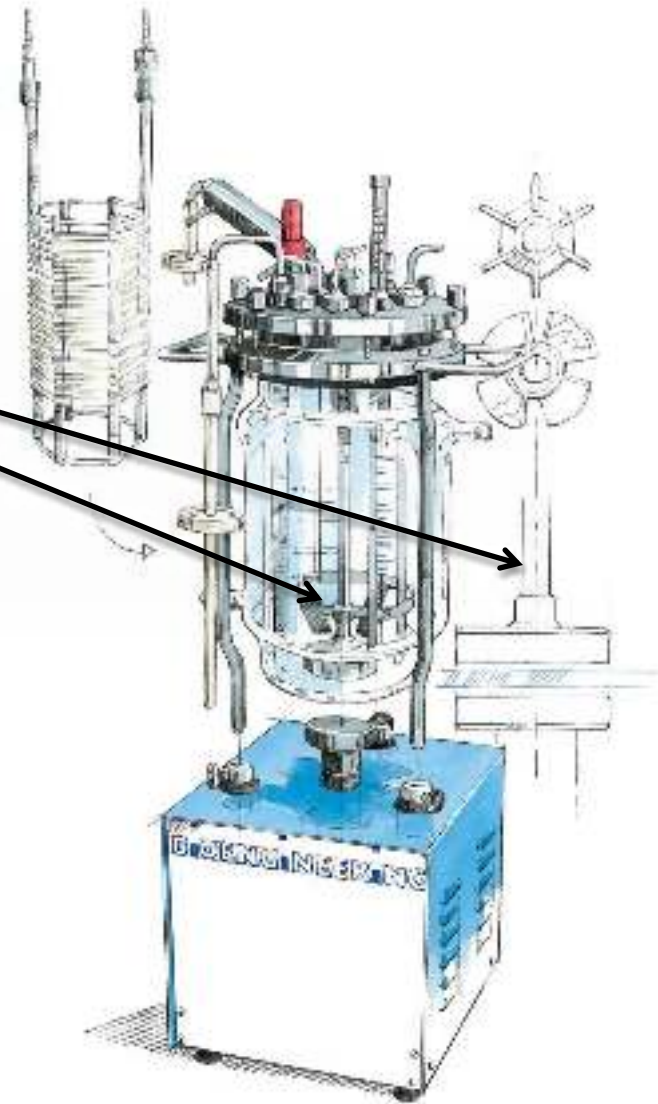
1. *Body vessel*



Fermenter Design

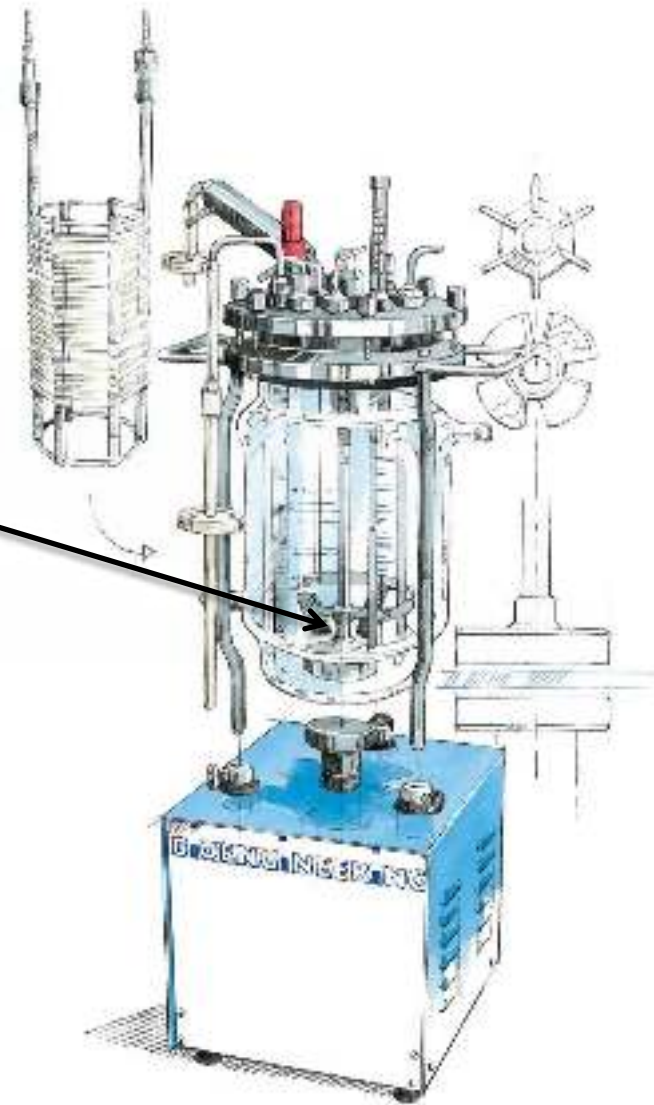
1. *Body vessel*

2. *Agitator*

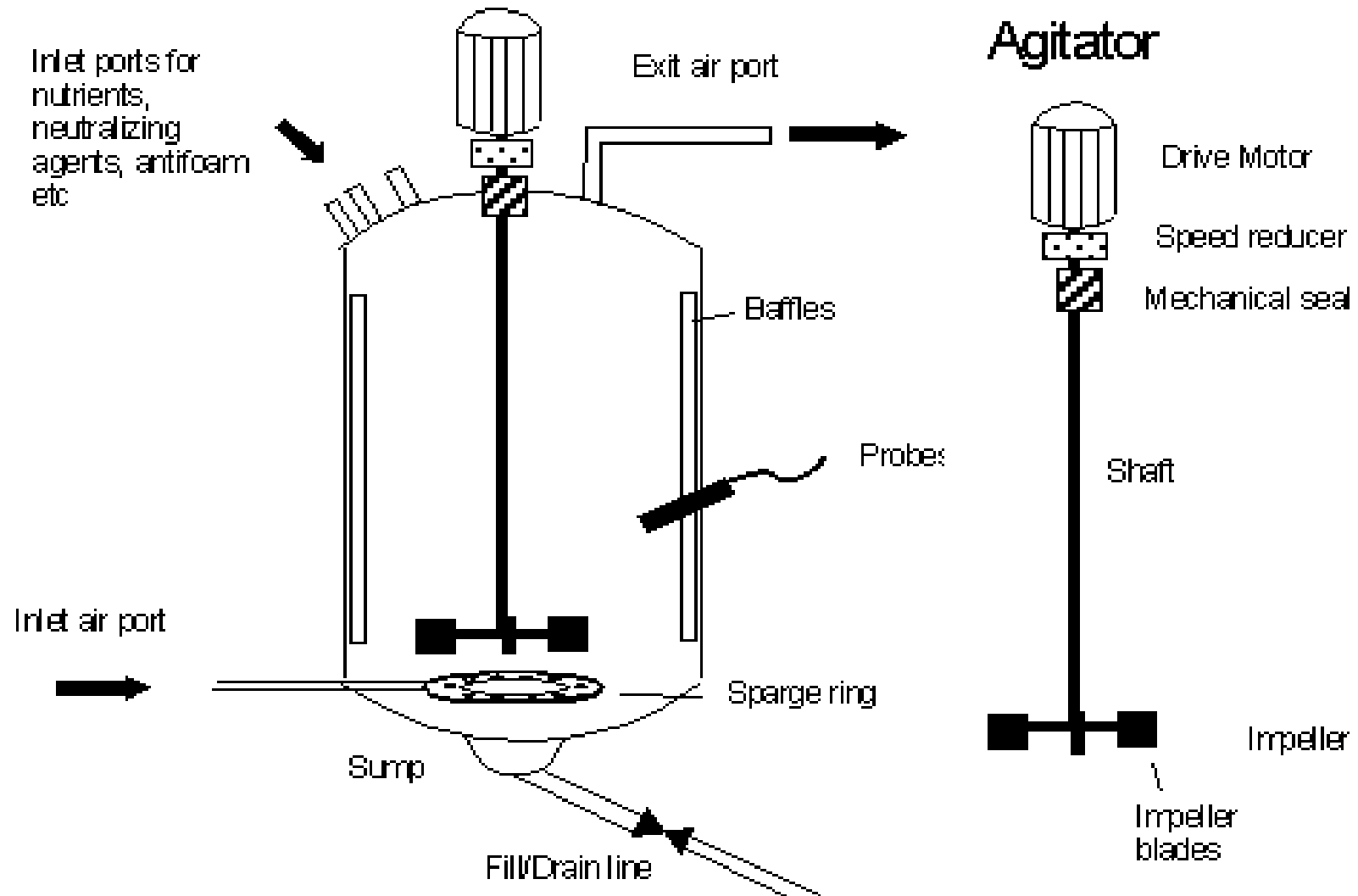


Fermenter Design

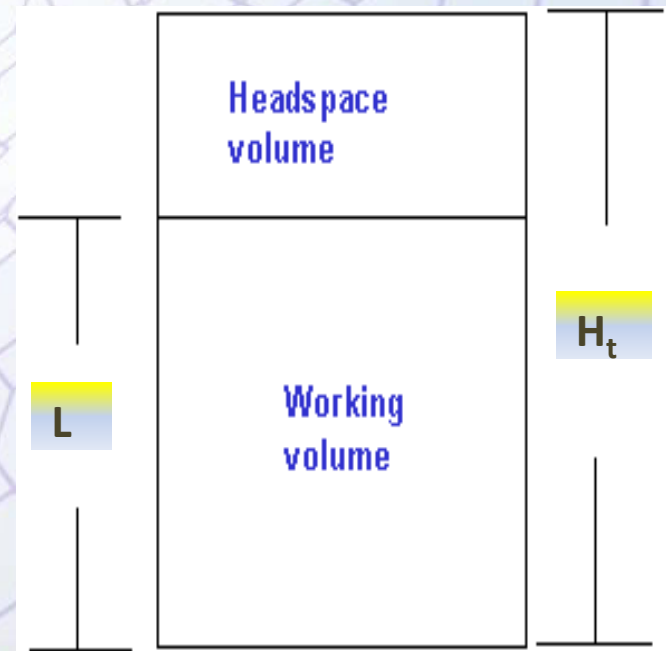
1. *Body vessel*
2. *Agitator*
3. *Sparger*



Fermenter Design



Fermenter Design



END

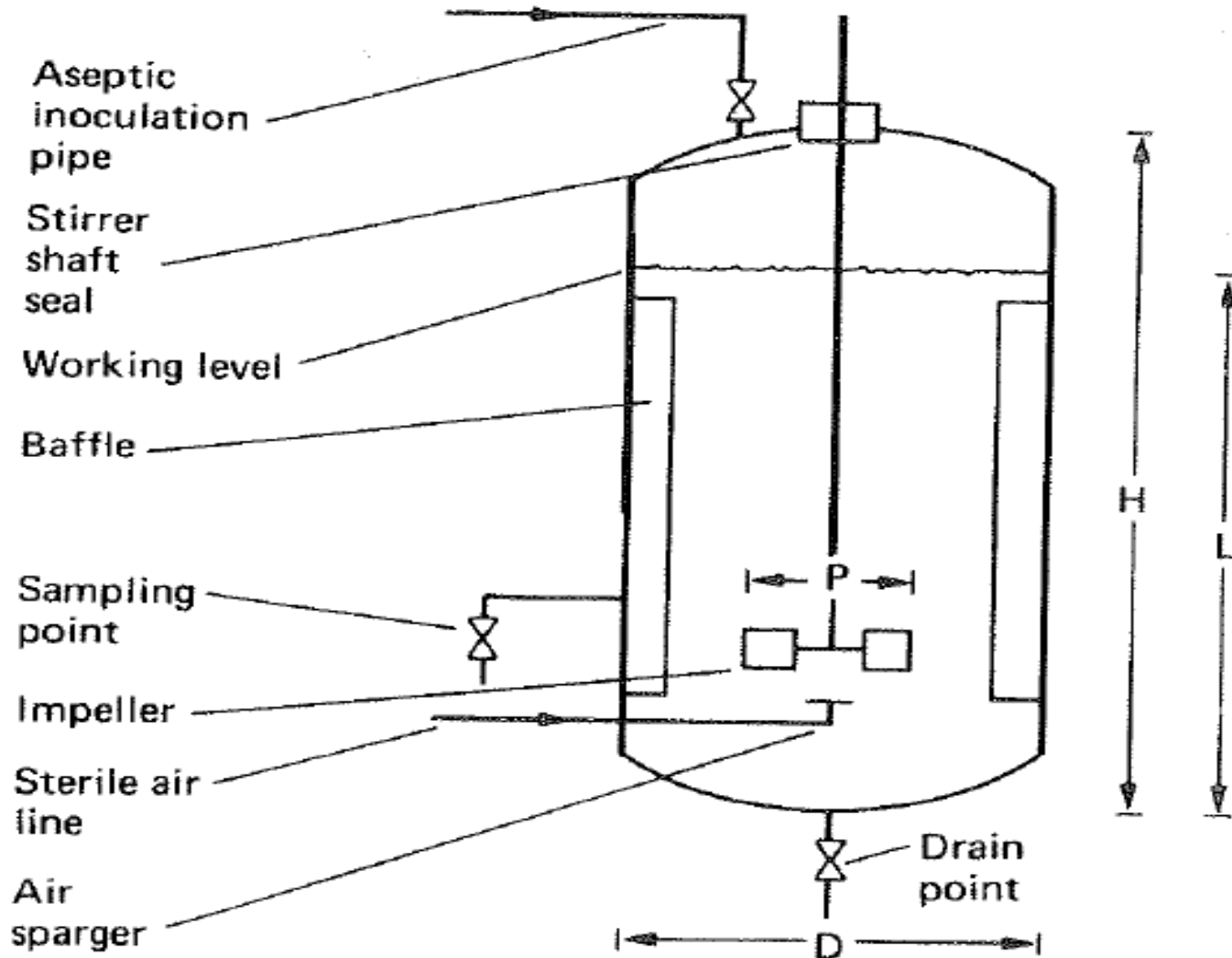
Advances in Fermentation Technology

**Fermenter
Design:
Body
Construction**

Lesson No 30

Fermenter Design

❑ Schematic representation of a fermenter with one multi-blade impeller

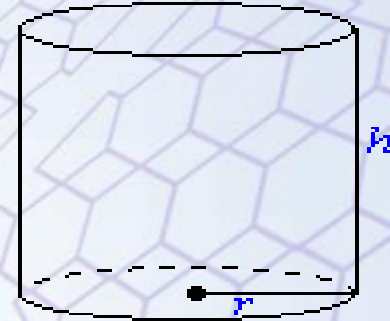


Fermenter Design

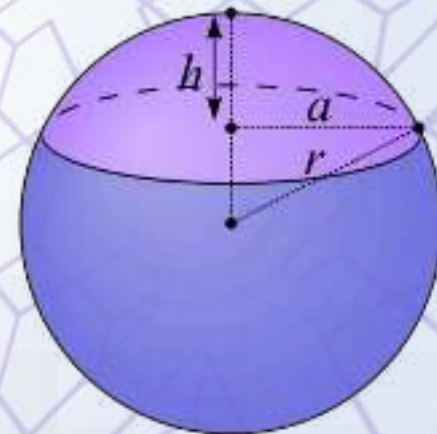
□ Details of geometrical ratios of Fermenters with single multi-blade impellers

Dimension	Steel and Maxon (1961)	Wegrich and Shurter (1963)	Blakeborough (1967)
Operating volume	250L	12L	--
Liquid height (L)	55cm	27cm	--
L/D (tank diameter)	0.72	1.1	1.0 – 1.5
Impeller diameter (P/D)	0.4	0.5	0.33
Baffle width/D	0.10	0.08	0.08 – 0.10
Impeller height/D	--	--	0.33

Fermenter Design



Volume of a cylinder: $V = \pi r^2 h$



END

$$V = \frac{\pi h}{6}(3a^2 + h^2)$$

Advances in Fermentation Technology

**Fermenter
Design:
with
Multi-impeller**

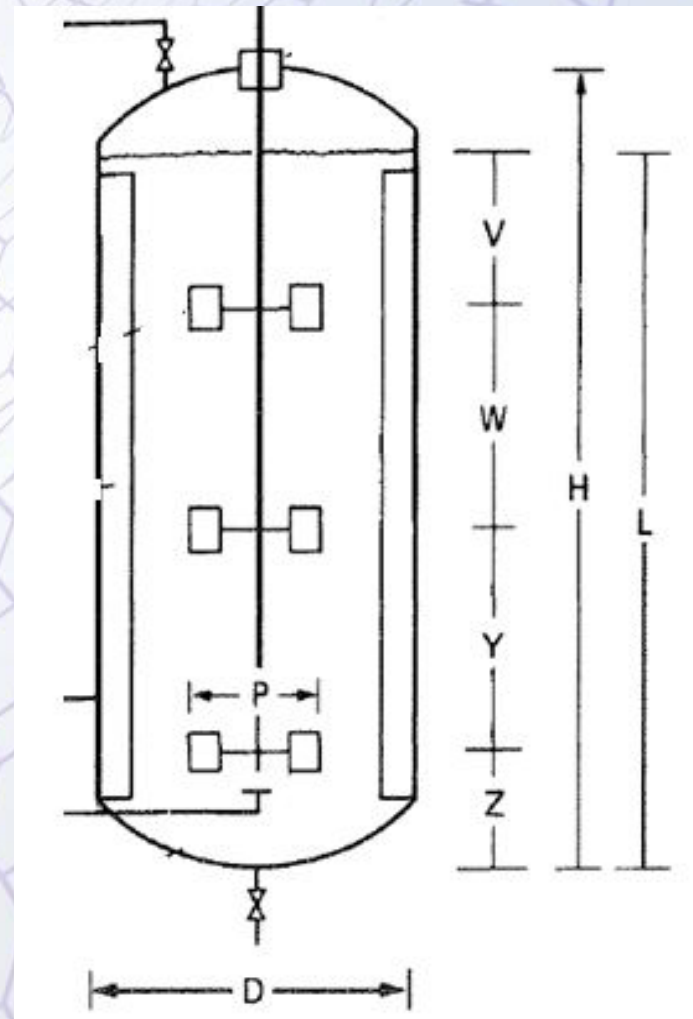
Lesson No 31

Fermenter Design

Details of geometrical ratios of Fermenters with multi-blade impellers

Dimension	Jackson (1958)	Aiba <i>et al.</i> , (1973)	Paca <i>et al.</i> , (1976)
Operating volume	--	100,000 L	170 L
Liquid height (L)	--	--	150cm
L/D (tank diameter)	--	--	1.7
Impeller diameter (P/D)	0.34-0.5	0.4	0.33
Baffle width/D	0.08-0.1	0.095	0.098
Impeller height/D	0.5	0.25	0.37
P/V	0.5-1.0	--	0.74
P/W	0.5-1.0	0.85	0.77
P/Y	0.5-1.0	0.85	0.77
P/Z	--	2.1	0.91
H/D	1.0 -1.6	2.2	2.95

Fermenter Design



END

Advances in Fermentation Technology

**Fermenter
Design:
Construction
Materials**

Lesson No 32

Fermenter Design

❑ Construction Materials

In fermentations with strict aseptic requirements it is important to select materials that can **withstand repeated steam sterilization cycles**. On a small scale (1 to 30 L) it is possible to use **glass and/or stainless steel**. Glass is useful because it gives smooth surfaces, is non-toxic, corrosion proof and it is usually easy to examine the interior of the vessel. Two basic types of fermenter are used at **lab scale**:

1. A glass vessel with a round or flat bottom and a top flanged carrying plate. The large glass containers originally used were borosilicate battery jars (Brown and Peterson, 1950). All vessels of this type have to be sterilized by autoclaving. Cowan and Thomas (1988) state that the **largest practical diameter** for glass fermenters is **60 cm**.

Fermenter Design

2. A glass cylinder with stainless-steel top and bottom plates . These fermenters may be sterilized *in situ*, but **30 cm** diameter is the upper size limit to safely withstand working pressures (**Solomons, 1969**). Vessels with two stainless steel plates cost approximately 50% more than those with just a top plate.

END

Advances in Fermentation Technology

Fermenter Design: Pilot Scale & Industrial Scale Fermenters

Lesson No 33

Fermenter Design

- Pilot-scale and industrial scale vessels are normally constructed of stainless steel or at least have a stainless-steel cladding to limit corrosion.
- The American Iron and Steel Institute (AISI) states that steels containing less than 4% chromium are classified as **steel alloys** and those containing more than 4% are classified as **stainless steels**. Mild steel coated with glass or phenolic epoxy materials has occasionally been used (Gordon *et al.*, 1947; Fortune *et al.*, 1950; Buelow and Johnson, 1952; Irving, 1968).
- Wood, Plastic and concrete have been used when contamination was not a problem in a process (Steel and Miller, 1970).

Fermenter Design



Fermenter Design

- ❖ The corrosion resistance of stainless steel is thought to depend on the existence of a thin **hydrous oxide film** on the surface of the metal. The composition of this film varies with different steel alloys and different manufacturing process treatments such as **rolling, pickling or heat treatment**. The film is stabilized by chromium and is considered to be continuous, non-porous, insoluble and self healing. If damaged, the film will repair itself when exposed to air or an oxidizing agent (Cubberly *et al.*, 1980).
- ❖ The minimum amount of chromium needed to resist corrosion will depend on the corroding agent in a particular environment, such as acids, alkalis, gases, soil, salt or fresh water. Increasing the chromium content enhances resistance to corrosion, but only grades of steel containing at least **10 to 13% chromium** develop an effective film.

Fermenter Design

The inclusion of **nickel in high percent chromium steels** enhances their resistance and improves their engineering properties. The presence of **molybdenum improves the resistance of stainless steels to solutions of halogen salts** and pitting by chloride ions in brine or sea water. Corrosion resistance can also be improved by **tungsten, silicone** and other elements.

END

Advances in Fermentation Technology

Fermenter Design:

**Grading quality of
Construction Material**

Lesson No 34

Fermenter Design

AISI Grade 316 steels which contain 18% chromium, 10% nickel and 2-2.5% molybdenum are now commonly used in fermenter construction.

In a citric acid fermentation where the pH may be 1 to 2 it will be necessary to use a stainless steel with 3-4% molybdenum (AISI grade 317) to prevent leaching of heavy metals from the steel which would interfere with the fermentation.

AISI Grade 304, which contains 18.5% chromium and 10% nickel, is now used extensively for brewing equipment.

Fermenter Design

AISI	UNS	C	S	Si	Cr	Ni	Cu	Mo	Nb
302B	S30215	0.03 Max.	0.03 Max.	2.0 3.0	17.0 19.0	8.0 10.0	.50 Max.	.50 Max.	-----
303L	S30300	0.03 Max.	.150 .300	1.0 Max.	17.0 19.0	8.0 10.0	.50 Max.	.50 Max.	-----
304L	S30403	0.03 Max.	.030 Max.	1.0 Max.	17.0 19.0	8.0 10.0	.50 Max.	.50 Max.	-----
304Cu	S30430	0.12 Max.	.030 Max.	1.0 Max.	17.0 19.0	8.0 10.0	3.0 4.0	.50 Max.	-----
316L	S31603	0.03 Max.	.030 Max.	1.0 Max.	16.0 18.0	10.0 14.0	.50 Max.	2.00 3.00	-----
316Cb	S31640	0.03 Max.	0.03 Max.	1.0 Max.	16.0 18.0	10.0 14.0	.50 Max.	2.00 3.00	.40 .60
317L	S31703	0.03 Max.	.030 Max.	1.0 Max.	18.0 20.0	11.0 15.0	.50 Max.	3.00 4.00	-----
321L	S32100	0.03 Max.	.030 Max.	1.0 Max.	17.0 19.0	9.0 12.0	.50 Max.	.50 Max.	.40 .60

Fermenter Design

- The thickness of the construction material will increase with scale.
- At **300,000-400,000 L** capacity, **7-mm** plate may be used for the side of a vessel and **10 mm plate** for the top and bottom, which should be hemispherical to withstand pressures.

END

Advances in Fermentation Technology

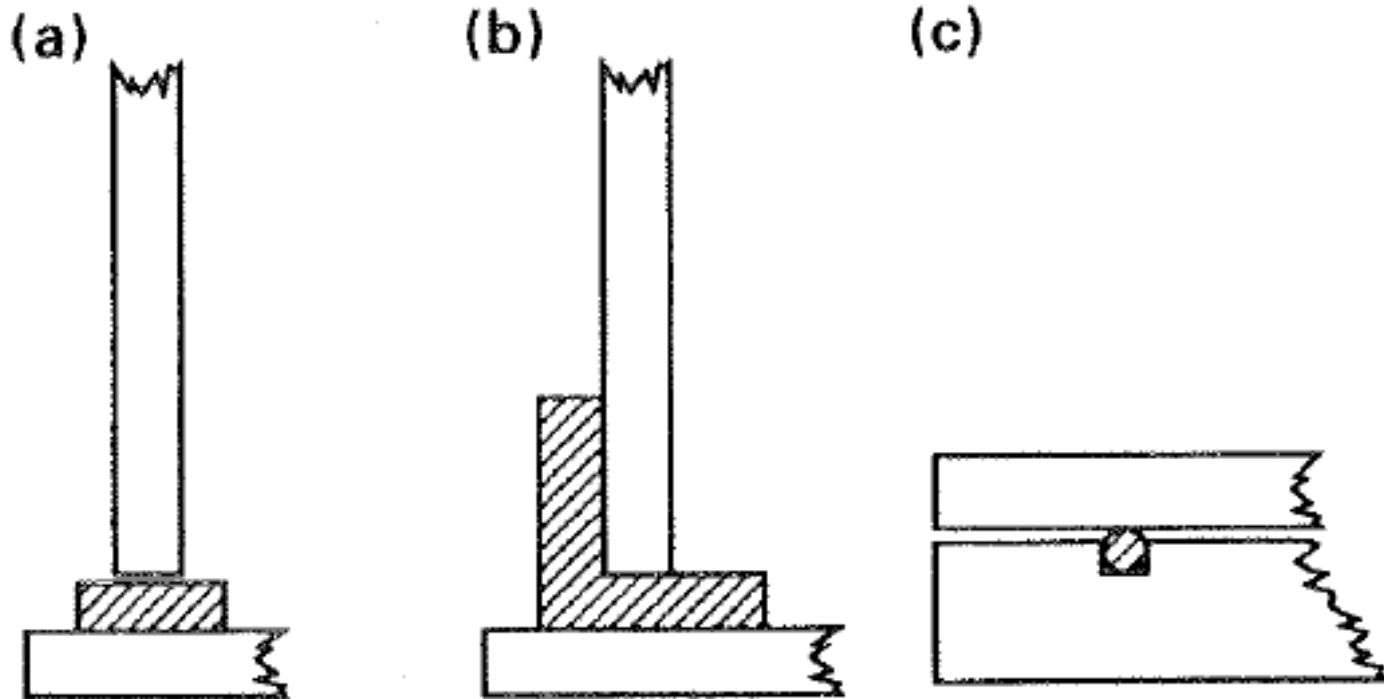
**Fermenter
Design:
Seals & O Rings**

Lesson No 35

Fermenter Design

- ✓ Reliable aseptic seal is made between glass and glass, glass and metal or metal and metal joints such as between a fermenter vessel and a detachable top or base plate.
- ✓ With glass and metal, a seal can be made with a compressible gasket, a lip seal or an 'O' ring. With metal to metal joints only an 'O' ring is suitable. This is placed in a groove, machined in either the end plate, the fermenter body or both.
- ✓ This seal ensures that a good liquid-and/or gas-tight joint is maintained in spite of the glass or metal expanding or contracting at different rates with changes in temperature during a sterilization cycle or an incubation cycle.
- ✓ **Nitryl or butyl rubbers** are normally used for these seals as they will withstand fermentation process conditions. These rubber seals have a finite life and should be checked regularly for damage or perishing.

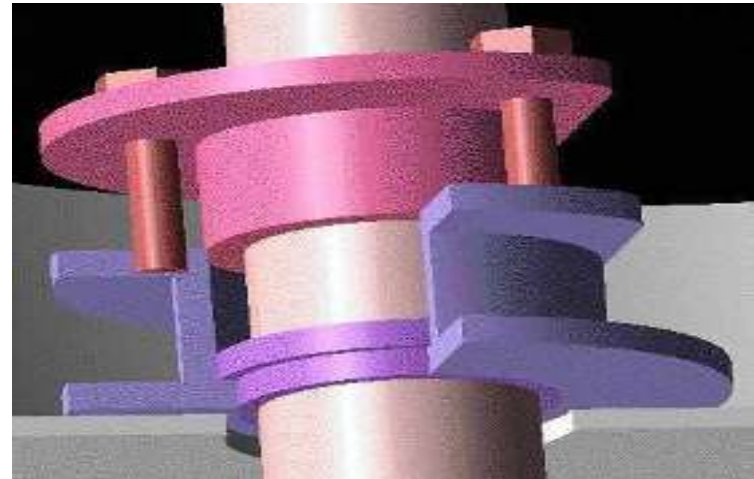
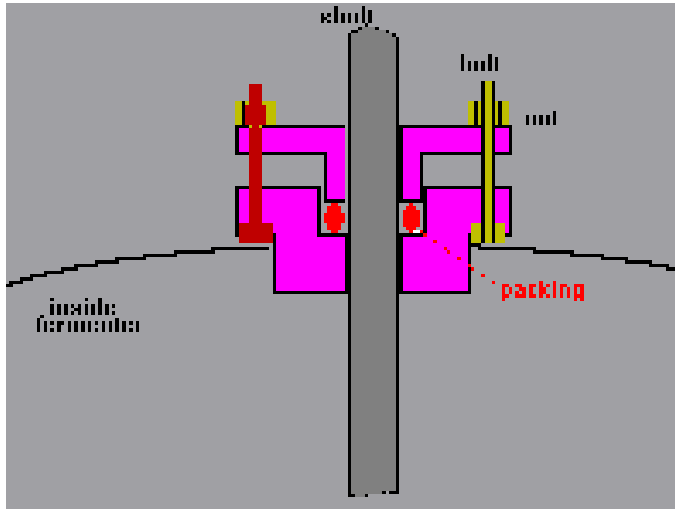
Fermenter Design



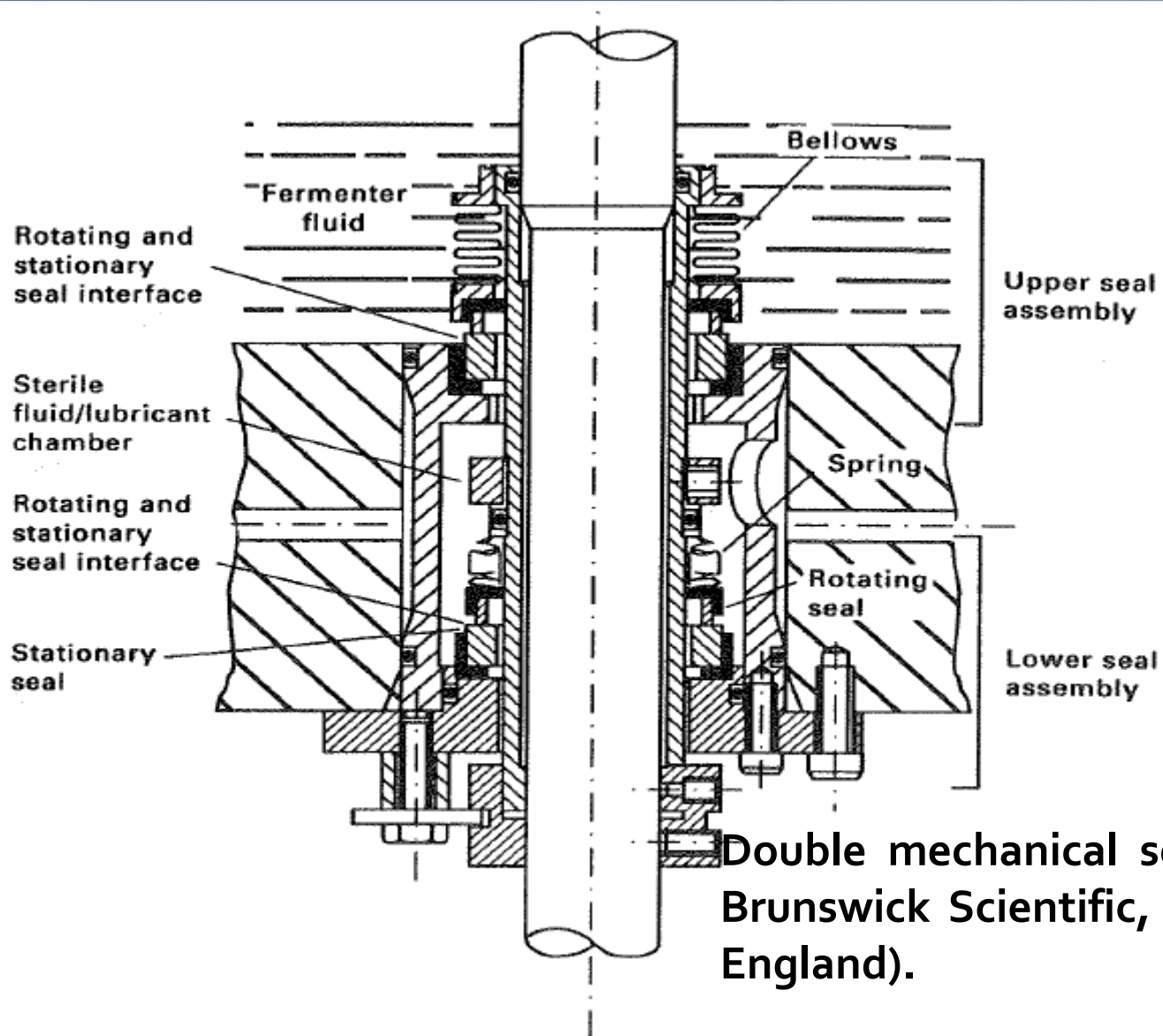
Joint seals for glass-glass, glass-metal and metal-metal; (a) **gasket**; (b) **lip seal**; (c) **'O' ring in groove**.

Fermenter Design

Main Seals



Fermenter Design



Fermenter Design

Without Main Seal



END

Advances in Fermentation Technology

Efforts by: AARISH ALI

Fermenter Design: Basic Features of a Stirred Tank Bioreactor: Agitation System-1

Lesson No 36

Fermenter Design

The function of the agitation system is to

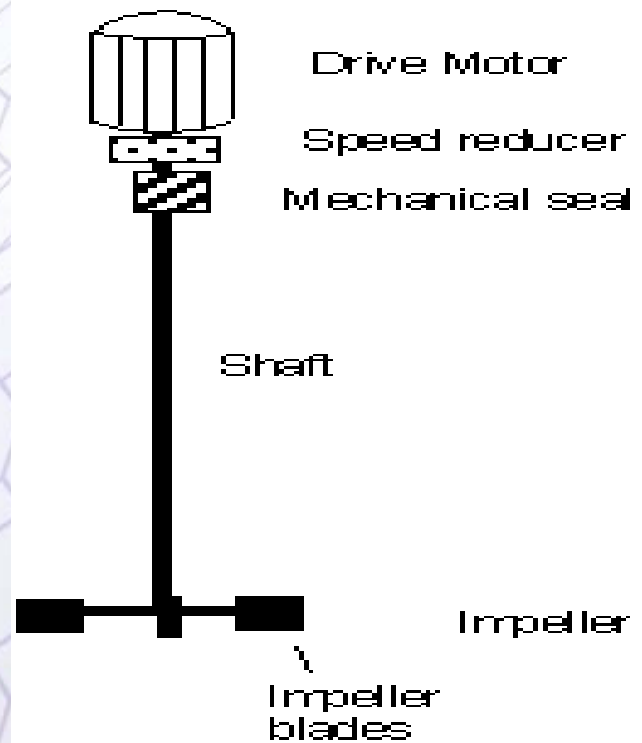
- provide good mixing and thus increase mass transfer rates through the bulk liquid and bubble boundary layers.
- provide the appropriate shear conditions required for the breaking up of bubbles.

The agitation system consists of the agitator and the baffles.

The baffles are used to break the liquid flow to increase turbulence and mixing efficiency.

Fermenter Design

Agitator



END

Advances in Fermentation Technology

Fermenter Design:

**Basic Features of a
Stirred Tank Bioreactor:
Agitation System-2**

Lesson No 37

Fermenter Design

Number of impellers will depend on the height of the liquid in the reactor. Each impeller will have between 2 and 6 blades. Most microbial fermentations use a Rushton turbine impeller.

A single phase agitator drive motor can be used with small reactors. However for large reactors, a 3 phase motor should be used. The latter will tend to require less current and therefore generate less heat.

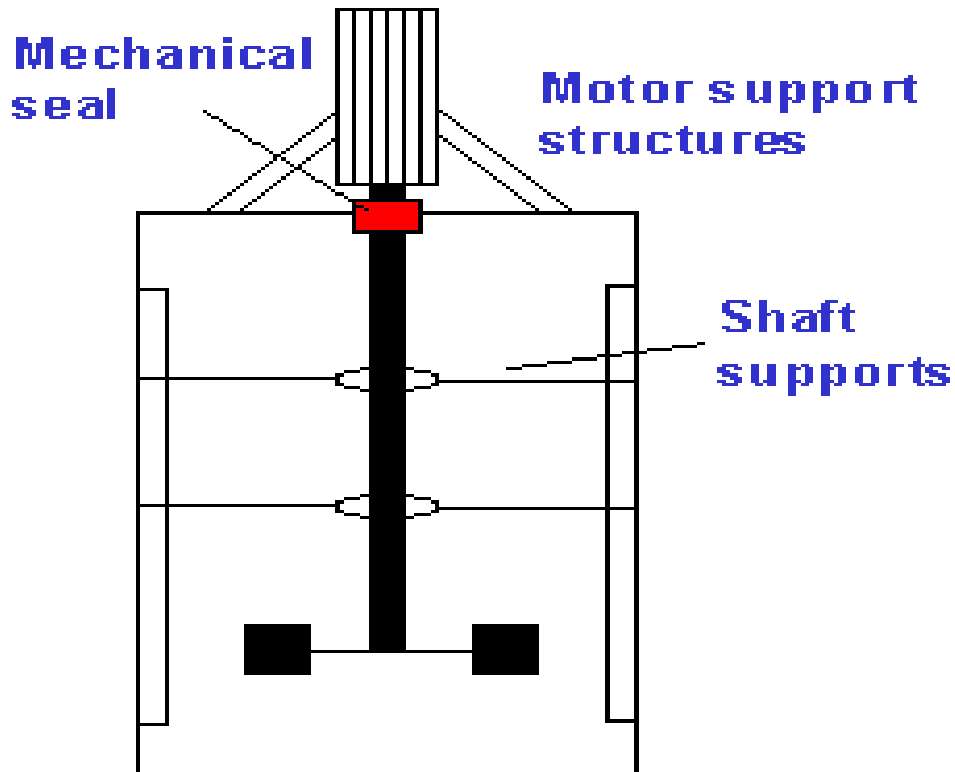
Speed control or speed reduction devices are used to control the agitation speed.

Fermenter Design



Fermenter Design

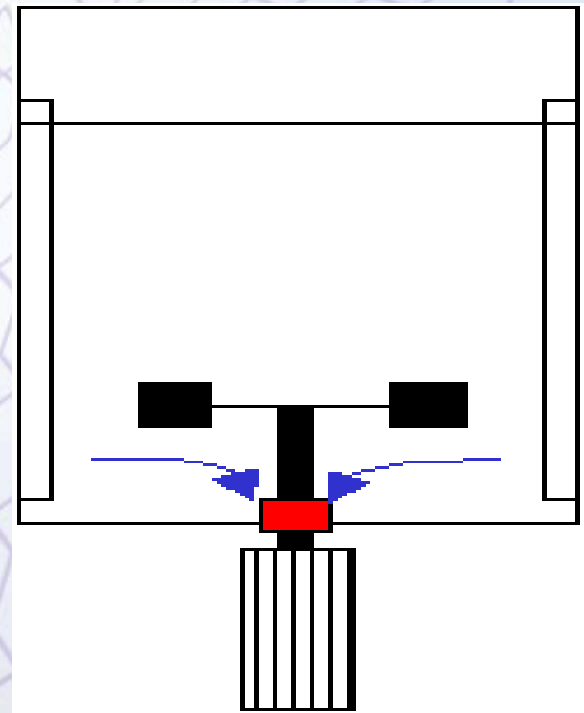
The impeller shaft can enter from the **bottom** of the tank or from the **top**. A top entry impeller ("overhung shaft") is more expensive to install as the motor and the shaft will need to be structurally supported:



Fermenter Design

A reactor with bottom entry impeller however will need higher maintenance due to damage of the seal by particulates in the medium and by medium components that crystallize in the seal when reactor is not in use.

Fermenter Design



Fermenter Design

Bottom entry agitators tend to require more maintenance than top entry impellers due to the formation of crystals and other solids in the seals.

END

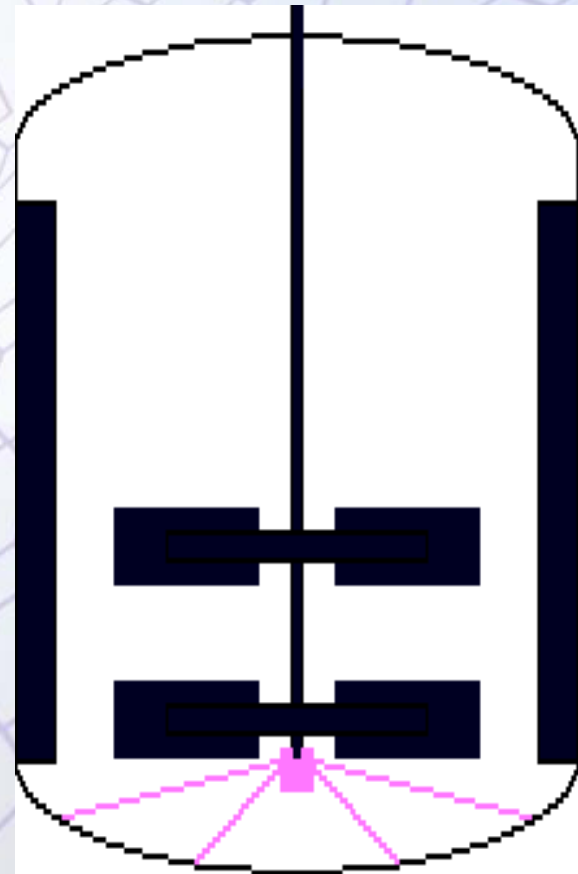
Advances in Fermentation Technology

**Fermenter
Design:
Agitator design
and
Operation-1**

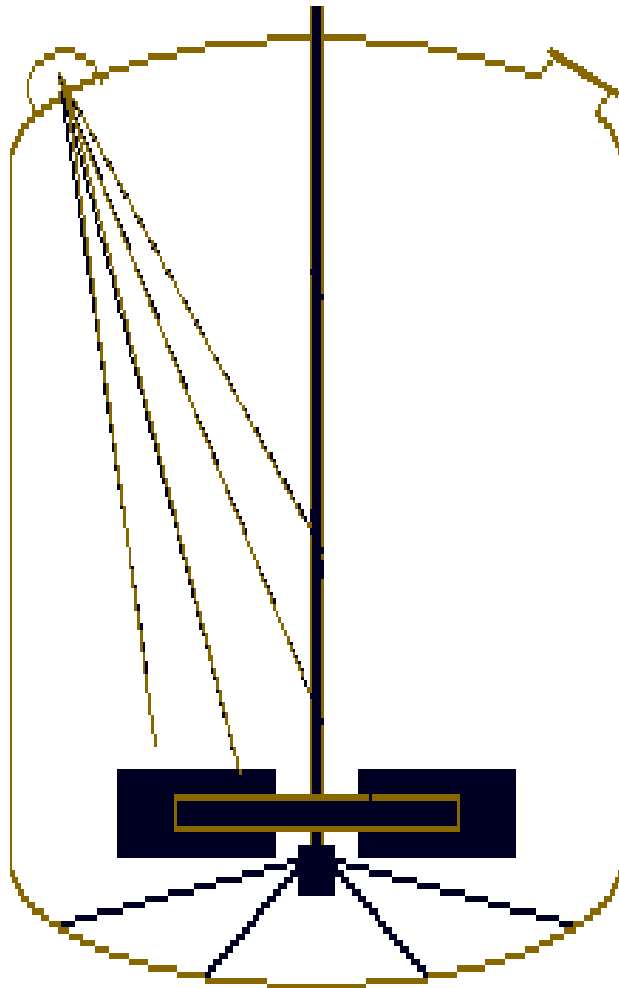
Lesson No 38

Fermenter Design

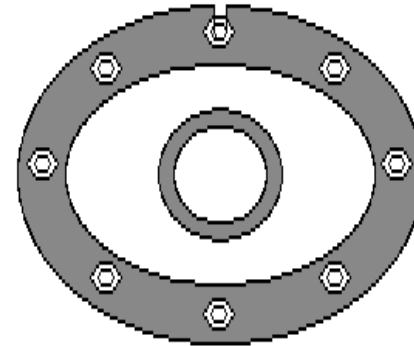
Baffles



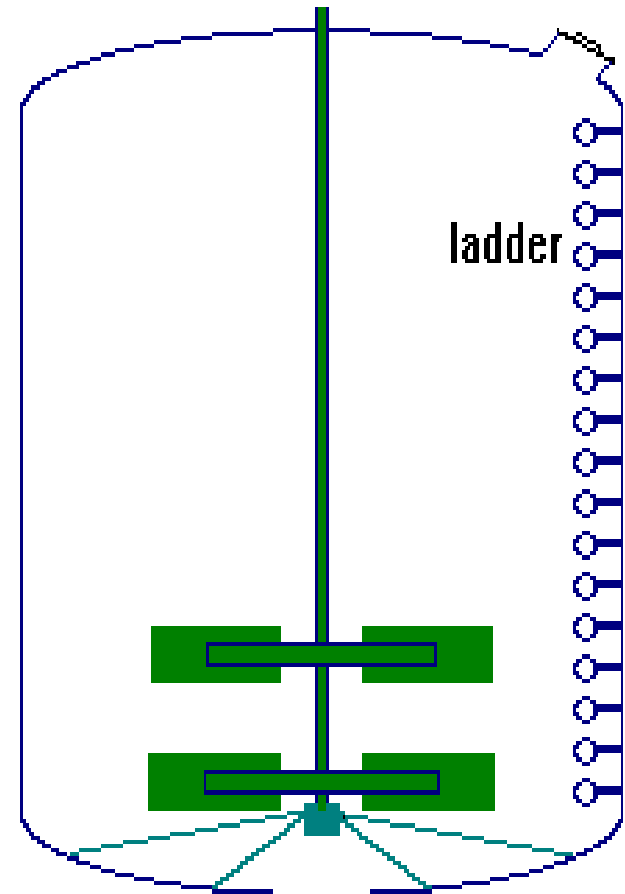
Fermenter Design



Only top bolt slot is shown



Fermenter Design



END

Advances in Fermentation Technology

**Fermenter
Design:
Agitator design
and
Operation-2**

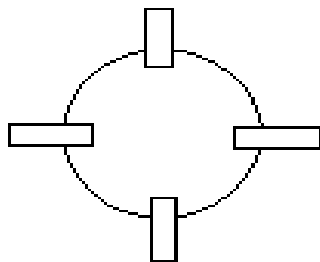
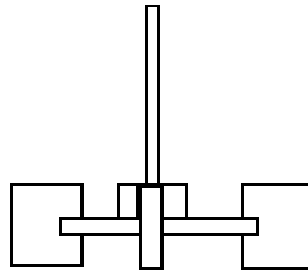
Lesson No 39

Fermenter Design

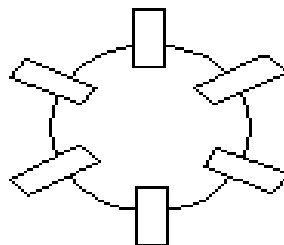
- ❑ Agitators are classified as having radial flow or axial flow characteristics.
- ❑ With radial flow mixing, the liquid flow from the impeller is initially directed towards the wall of the reactor; i.e. along the radius of the tank.
- ❑ With axial flow mixing, the liquid flow from the impeller is directed downwards towards the base of the reactor, i.e. in the direction of the axis of the tank.
- ❑ Radial flow impellers are primarily used for gas-liquid contacting (such as in the mixing of sparged bioreactors) and blending processes.
- ❑ Axial flow impellers provide more gentle but efficient mixing and are used for reactions involving shear sensitive cells and particles.

Fermenter Design

Rushton turbine



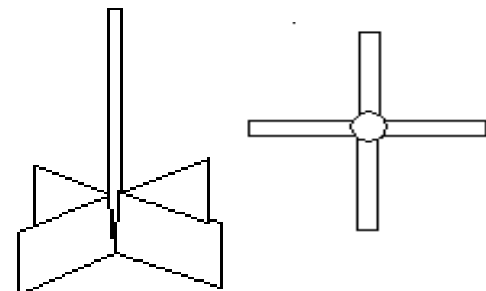
Four bladed



Six bladed

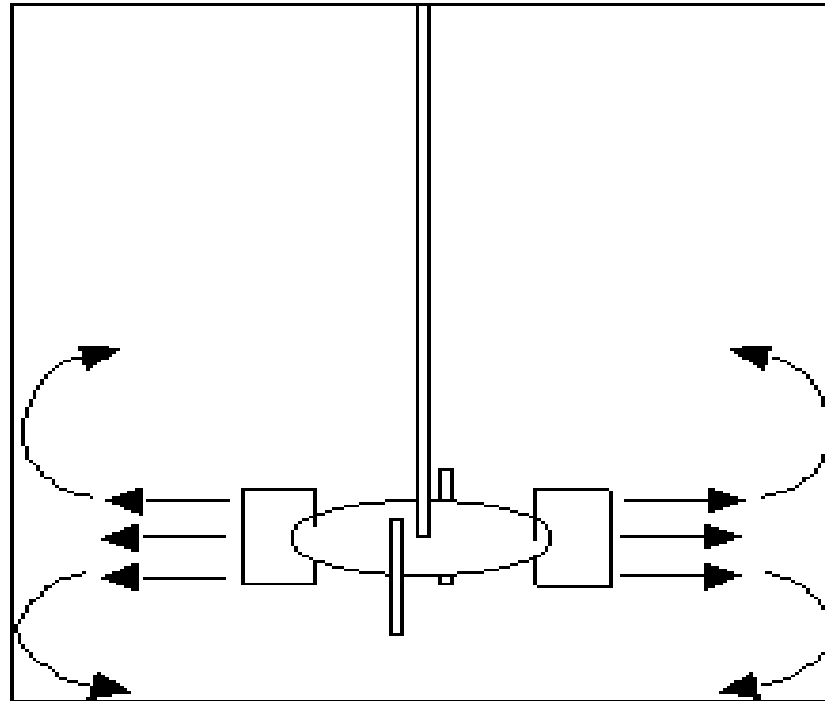
Radial flow impellers contain two or more impeller blades which are set at a vertical pitch:

Flat bladed Impeller



Fermenter Design

The liquid flow from the blades is directed towards the walls of the reactor; i.e. along the radius of the tank.



With radial flow impellers, the liquid is pushed towards the wall of the tank; that is, along the radius of the reactor

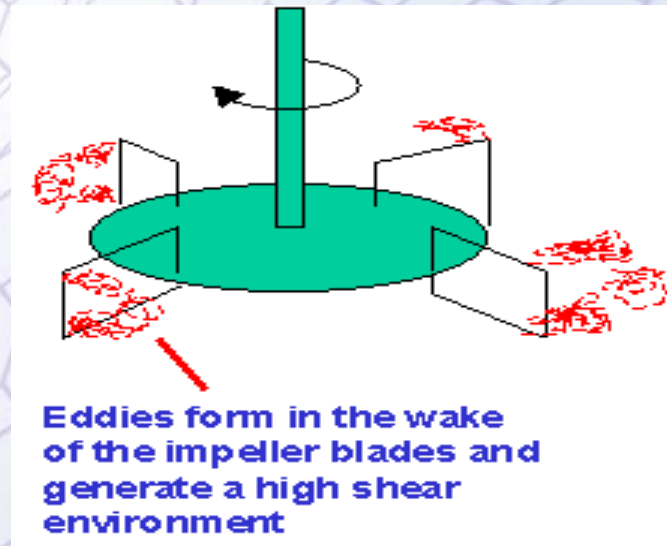
Fermenter Design

Radial flow mixing is not as efficient as axial flow mixing.

For radial flow impellers, a much higher input of energy is required to generate a given level of flow.

Radial flow impellers do and are designed to, generate high shear conditions. This is achieved by the formation of vortices in the wake of the impeller.

Fermenter Design



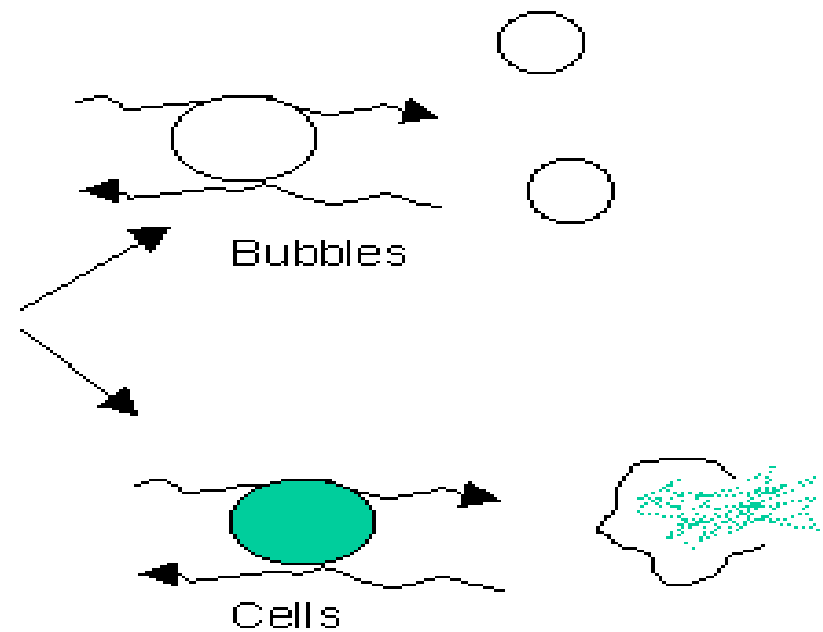
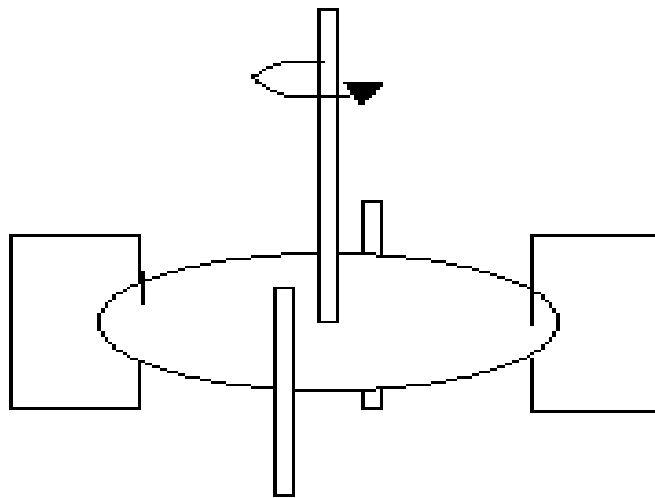
END

Advances in Fermentation Technology

**Fermenter
Design:
Agitator design
and
Operation-3**

Lesson No 40

Fermenter Design



Fermenter Design

The high shear is effective at breaking up bubbles. For this reason, radial flow impellers are used for the culture of aerobic bacteria.

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High shear can also damage shear sensitive materials such as crystals and precipitates and shear sensitive cells such as filamentous fungi and animal cells.

END

Advances in Fermentation Technology

**Fermenter
Design:
Agitator design
and
Operation-4**

Lesson No 41

Fermenter Design

The most commonly used agitator in microbial fermentations is the **Rushton turbine**.

Like all radial flow impellers, the Rushton turbine is designed to provide the high shear conditions required for breaking bubbles and thus increasing the oxygen transfer rate.

The Rushton turbine has 4 or 6 blades which are fixed onto a disk.

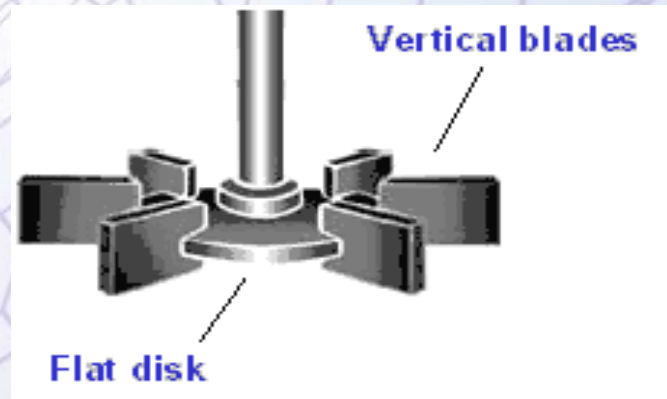
The diameter of the Rushton turbine should be $\frac{1}{3}$ of the tank diameter.

Fermenter Design

A Rushton turbine is often referred to as a disk turbine.

The disk design ensures that most of the motor power is consumed at the tips of the agitator and thus maximizing the energy used for bubble shearing.

Fermenter Design



END

Advances in Fermentation Technology

Fermenter Design:

**Agitator design
and**

Operation:

Axial Flow Impellers-1

Lesson No 42

Fermenter Design

Axial flow mixing is considerably more energy efficient than radial flow mixing.

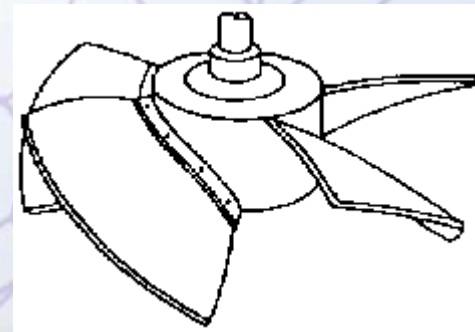
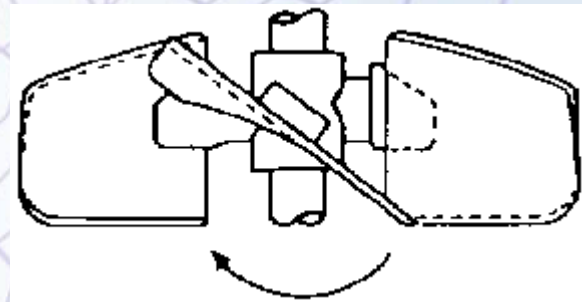
They are also more effective at lifting solids from the base of the tank.

Axial flow impellers have low shear properties. The angled pitch of the agitators coupled with the thin trailing edges of the impeller blades reduces formation of eddies in the wake of the moving blades.

Fermenter Design

Axial flow impeller blades are pitched at an angle and thus direct the liquid flow towards the base of the tank. Examples of axial flow impellers are marine impellers and hydrofoil impellers.

Fermenter Design



END

Advances in Fermentation Technology

Fermenter Design:

**Agitator design
and**

Operation:

Axial Flow Impellers-2

Lesson No 43

Fermenter Design

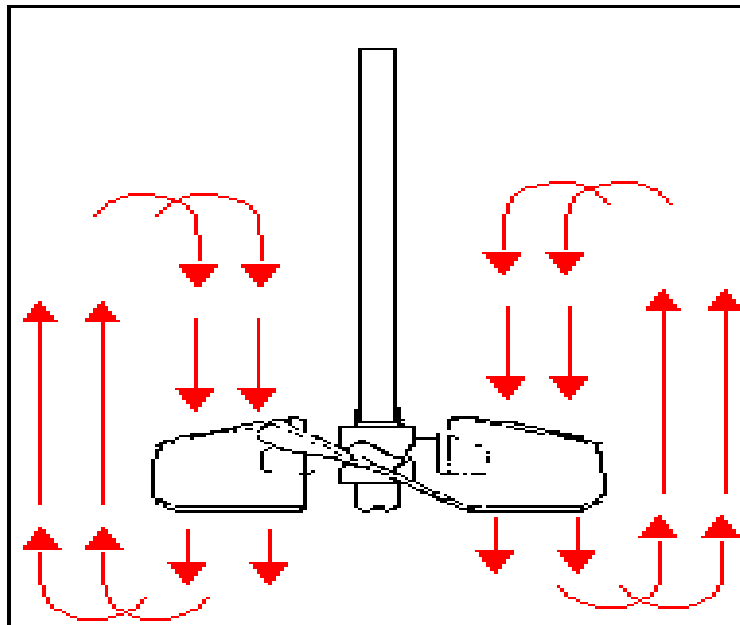
Axial flow impellers are used for mixing shear sensitive processes such as crystallization and precipitation reactions.

They are also used widely in the culture of animal cells.

Their low shear characteristics generally makes them ineffective at breaking up bubbles and thus unsuitable for use in aeration of bacterial fermentations.

Fermenter Design

With the use of Axial Flow Impellers, the radial resultant flow pattern is thus predominantly vertical; i.e. along the tank axis.



With axial impellers, the liquid is pushed in a downward direction; that is, along the axis of the reactor.

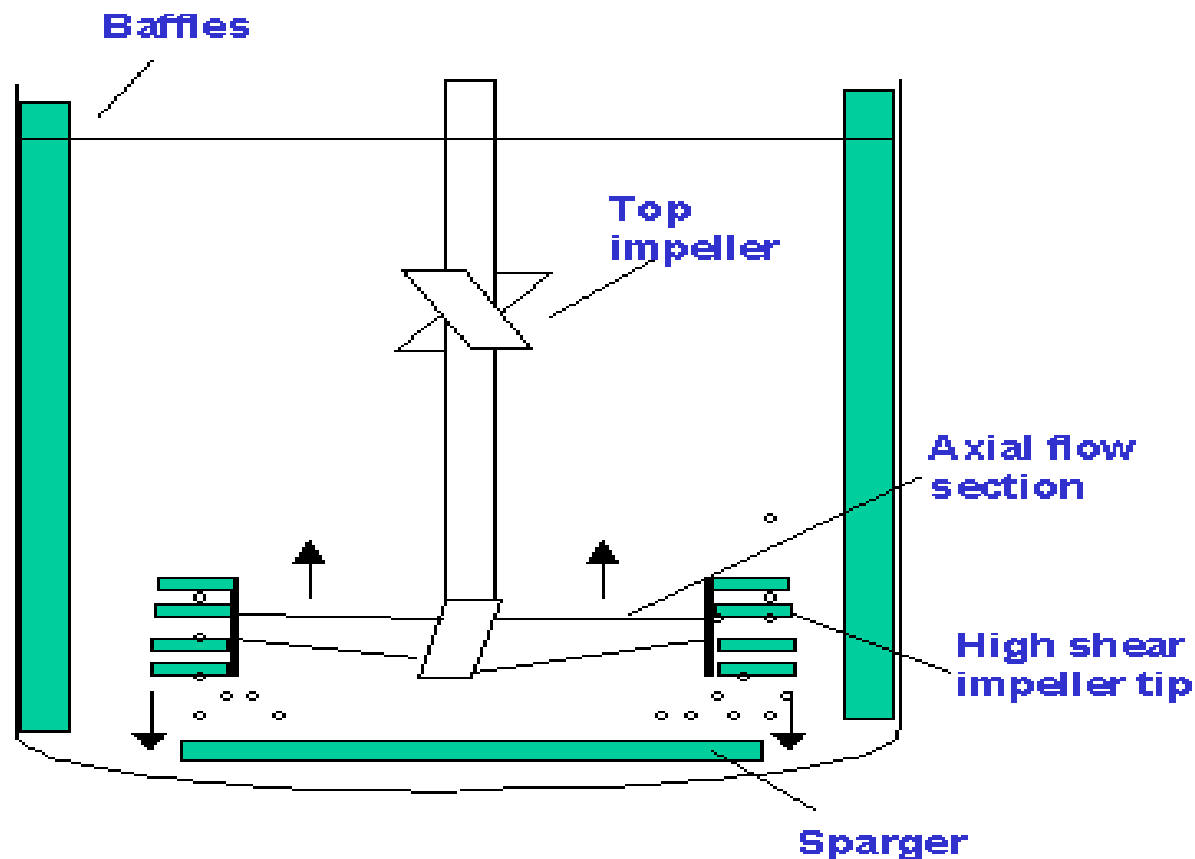
Fermenter Design

Low shear conditions are achieved by pitching the impeller blades at an angle and by making the edges of the impeller blades thin and smooth.



Fermenter Design

Intermig impeller is a axial flow impeller which is used for microbial fermentations. Following is the figure of axial flow impeller:



Fermenter Design

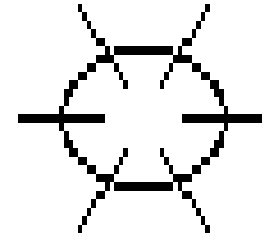
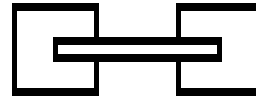
The agitation system has two impellers. The bottom impeller has a large axial flow section. The tips of the impeller contain finger like extensions which create a turbulent wake for breaking bubbles.

As the high shear region exists only at the tip, the overall shear conditions in the reactor are lower than would be generated by a radial flow impeller such as a Rushton Turbine.

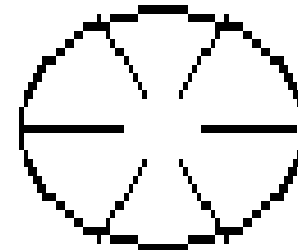
Intermig impellers are used widely for agitation and aeration in fungal fermentations.

Fermenter Design

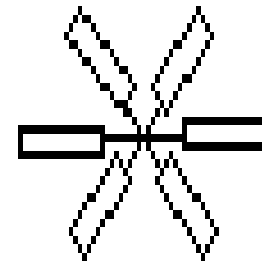
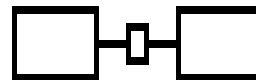
Disc Turbine



Vaned Disc



Open Turbine
(Variable Pitch)



Fermenter Design



6 blade disk turbine



6 curved blade turbine

Fermenter Design



Screw Propeller



**High efficiency hydrofoil
propeller**

END

Advances in Fermentation Technology

Fermenter Design: Temperature Control System-1

Lesson No 44

Fermenter Design

The temperature control system consists of

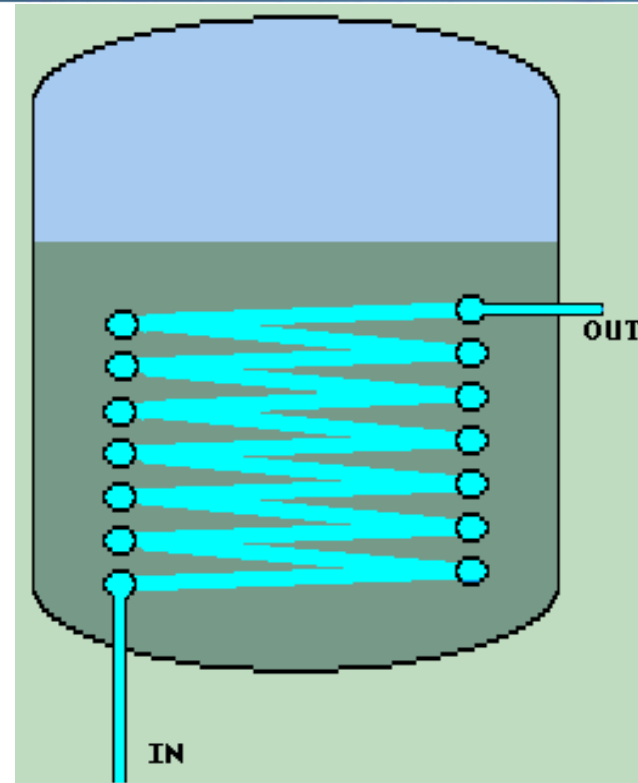
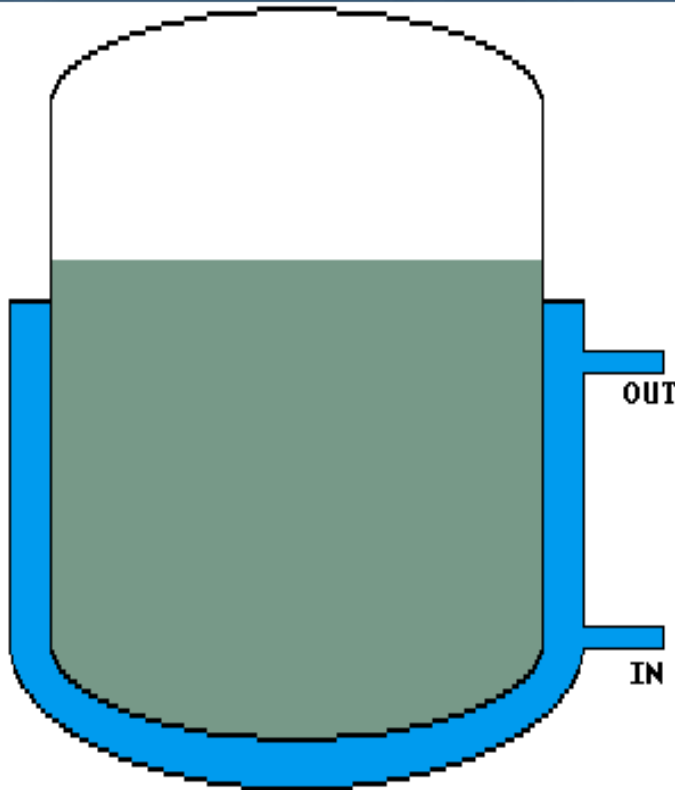
i. Temperature probes

ii. Heat transfer system

Fermenter Design

Typically the heat transfer system will use a "jacket" to transfer heat in or out of the reactor. The jacket is a shell which surrounds part of the reactor. The liquid in the jacket does not come in direct contact with the fermentation fluid.

Fermenter Design



The jacket will typically be "dimpled" to encourage turbulence in the jacket and thus increase the heat transfer efficiency.

Fermenter Design

An alternative to using jackets are coils. Coils have a much higher heat transfer efficiency than jackets.

However coils take up valuable reactor volume and can be difficult to clean and sterilize.

As probe; that is mostly pt100

END

Advances in Fermentation Technology

Fermenter Design: Temperature Control System-2

Lesson No 45

Fermenter Design

The heating/cooling requirements are provided by the following methods:

	Laboratory-scale Reactors	Pilot and Production scale reactors
Heating Requirements	Electric Heaters	Steam generated in boilers
Cooling Requirements	Tap waters or refrigerated water baths	Cooling water produced by cooling towers or refrigerants such as ammonia

Fermenter Design

To make an accurate estimate of heating/cooling requirements for a specific process it is important to consider the **contributing factors**. An overall energy balance for a fermenter during normal operation can be written as:

$$Q_{\text{met}} + Q_{\text{ag}} + Q_{\text{gas}} = Q_{\text{acc}} + Q_{\text{exch}} + Q_{\text{evap}} + Q_{\text{sen}}$$

where Q_{met} = heat generation rate due to microbial metabolism,

Q_{ag} = heat generation rate due to mechanical agitation,

Q_{gas} = heat generation rate due to aeration power input,

Q_{acc} = heat accumulation rate by the system,

Q_{exch} = heat transfer rate to the surroundings and/or heat exchanger,

Q_{evap} = heat loss rate by evaporation,

Q_{sen} = rate of sensible enthalpy gain by the flow streams (exit – inlet).

Fermenter Design

Equation in the previous slide can be re-arranged as:

$$Q_{\text{exch}} = Q_{\text{met}} + Q_{\text{ag}} + Q_{\text{gas}} - Q_{\text{acc}} - Q_{\text{sen}} - Q_{\text{evap}}$$

Q_{exch} is the heat which will have to be removed by a cooling system.

The cooling requirements (jacket and/or pipes) to remove the excess heat from a fermenter may be determined by the following formula:

$$Q_{\text{exch}} = U \cdot A \cdot \Delta T$$

where A = the heat transfer surface available, m^2 ,

Q = the heat transferred, W,

U = the overall heat transfer coefficient,
 $\text{W}/\text{m}^2\text{K}$,

ΔT = the temperature difference between the heating or cooling agent and the mass itself, K.

Fermenter Design

It is impossible to specify accurately the necessary cooling surface of a fermenter since the temperature of the cooling water, the sterilization process, the cultivation temperature, the type of microorganism and the energy supplied by stirring can vary considerably in different process.

Fermenter Design

A cooling area of 50 to 70m² may be taken as average for 55,000L fermenter and with a coolant temperature of 14°C the fermenter may be cooled from 120 to 30°C in 2.5 to 4.0 hrs without stirring. The consumption of cooling water in this size of vessel during bacterial fermentation ranges from 500 to 2000 L/hr while fungi needs 2000 to 10,000 L/hr.

END

Final Term Syllabus

Lecture 01 to 45

Efforts By

AARISH ALI (+96569002641)

Contact us to join our esteemed group

“All About Biotech”

Note:

Students must follow the course announcement for midterm syllabus & any up-gradation in the course.

REMEMBER IN PRAYERS
KEEP SMILING
SAY NO TO CURRUPTION
HELP TO OTHERS

Regards,
AARISH ALI & TEAM

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