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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.TECH – BIOTECHNOLOGY

UNIT – I – Introduction to broad scope of Biochemical Engineering-SBT1303

1.1 DEFINITIONS

1. DEFINE BIO-REACTOR.

   An apparatus in which a biological reaction or process is carried out. It is a vessel for the growth of micro-organisms. (fermentation). A bioreactor can be defined as an apparatus , such as an large fermentation chamber, for growing organisms such as bacteria or yeast that are used in the biotechnological manufacture of substances such as pharmaceuticals, antibodies, or vaccines, or for the bioconversion of organic waste.

2. DEFINE FERMENTOR.

   AN APPARATUS FOR CARRYING OUT FERMENTATION.

   A fermenter is an enclosed and sterilised vessel that maintains optimal conditions for the growth of a microorganism. The microorganism undergoes fermentation to produce large quantities of a desired metabolite for commercial use.

3. DEFINE REACTOR.

   An apparatus or structure in which fissile material can be made to undergo a controlled, self- sustaining nuclear reaction with a consequent release of energy.

1.2 MEDIUM OPTIMISATION-PLACKETT BURMAN METHOD

Detailed investigation is needed to establish the most suitable medium for an individual fermentation process, but certain basic requirements must be met by any such medium. All micro-organisms require water, sources of energy, carbon, nitrogen, mineral elements and possibly vitamins plus oxygen if aerobic. On a small scale it is relatively simple to devise a medium

containing pure compounds, but the resulting medium, although supporting satisfactory growth may be unsuitable for use in a large-scale process.

On a large scale one must normally use sources of nutrients to create a medium which will meet as many as possible of the following criteria:

1. It will produce the maximum yield of product or biomass per gram of substrate used.
2. It will produce the maximum concentration of product or biomass.
3. It will permit the maximum rate of product formation.
4. There will be the minimum yield of undesired products.
5. It will be of a consistent quality and be readily available throughout the year.
6. It will cause minimal problems during media making and sterilization.
7. It will cause minimal problems in other aspects of the production process particularly aeration and agitation, extraction, purification and waste treatment.

Medium optimization by the classical method of changing *one* independent variable (nutrient, antifoam, pH, temperature, etc.) while fixing all the others at a certain level can be extremely time consuming and expensive for a large number of variables. To make a full factorial search which would examine each possible combination of independent variable at appropriate levels could require a large number of experiments x^n, where x is the number of levels and n is the number of variables. This may be quite appropriate for the three nutrients at two concentrations (2" trials) but not for six nutrients at three concentrations, in this instance 3' (729) trials would be needed. Industrially the aim is to perform the minimum number of experiments to determine optimal Conditions. Other alternative strategies must therefore be considered which allow more than one variable to be changed at a time.

When more than five independent variables are to be investigated, the **Plackett-Burman** design may be used to find the most important variables in systems, which are then optimized in further studies (Placket and Burman,1946). These authors give a series of designs for up to one hundred experiments using an experimental rationale known as balanced incomplete blocks. This technique allows for the evaluation of X - 1 variables by X experiments. X must be a multiple of 4, e.g. 8, 12, 16, 20, 24, etc. Normally one determines how many experimental variables needs to be included in an investigation and then selects the Plackett-Burman design which meets that

requirements most closely in multiples of 4. Any factors not assigned to variable can be designated as a dummy variable. Alternatively, factors known to not have any effect may be included and designated as dummy variables. As will be shown shortly in a worked example (Table 1), the incorporation of dummy variables into an experiment makes it possible to estimate the variance of an effect (experimental error).

Table (1) shows a Plackett-Burman design for seven variables (A-G) at high and low levels in which two factors, E and G, are designated as 'dummy' variables. These can then be used in the design to obtain an estimate of error. Normally three dummy variables with provide an adequate estimate of the error. However, more can be used if fewer real variables need to be studied in an investigation (Stowe and Mayer, 1966). Each horizontal row represents a trial, and each vertical column represents the H (high) and L (low) values of one variable in all the trials. This design (Table) requires that the frequency of each level of a variable in a given column should be equal and that in each test (horizontal row) the number of high and low variables should be equal. Consider the variable A; for the trials in which A is high, B is high in two of the trials and low in the other two. Similarly, C will be high in two trials and low in two. as will all the remaining variables. For those trails in which A is low B will be high two times and low two times. This will also apply to all the other variables.

Table (1) Plackett-Buman design for seven variables

Trial	Variables							Response (OD)
	A	B	C	D	E	F	G	
1	H	H	H	L	H	L	L	
2	L	H	H	H	L	H	L	
3	L	L	H	H	H	L	H	
4	H	L	L	H	H	H	L	
5	L	H	L	L	H	H	H	
6	H	L	H	L	L	H	H	
7	H	H	L	H	L	L	H	
8	L	L	L	L	L	L	L	

H. denotes a high-level value; L denotes a low-level value

Thus, the effects of changing the other variables cancel out when determining the effect of A. The same logic then applies to each variable. However, no changes are made to the high and low values for the E and G columns. Greasham and Inamine (1986) state that although the difference between the levels of each variable must be large enough to ensure that the optimum response will be included, caution must be taken when setting the level differential for sensitive variables, since a differential that is too large could mask the other variables. The trials are carried out in a randomized sequence.

The effects of the dummy variables are calculated in the same way as the effects of the experimental variables. If there are no interactions and no errors in measuring the response, the effect shown by a dummy variable should be 0. if the effect is not equal to 0. it is assumed to be a measure of the lack of experimental precision plus any analytical error in measuring the response (Stowe and Mayer, 1966)

This procedure will identify the important variables and allow them to be ranked in order of importance to decide which to investigate in a more detailed study to determine the optimum values to use.

The stages in analysing the data (Tables 1 and 2) using Nelson's (1982) example is as follows:

1. Determine the difference between the average of the H (high) and L (low) responses for each independent and dummy variable.

Therefore, the difference = $\sum A\ (H) - \sum A\ (L)$.

The effect of an independent variable on the response is the difference between the average response for the four experiments at the high level and the average value for four experiments at the low level.

Thus, the effect of

$$A = \frac{\sum A\ (H)}{4} - \frac{\sum A\ (L)}{4}$$

$$= \frac{2(\sum A\ (H) - \sum A\ (L))}{}$$

8

this value should be near zero for the dummy variables.

2. Estimate the mean square of each variable (the variance of effect).

$$\text{For A the mean square will be} = \frac{(\sum A(H) - \sum A(L))^2}{8}$$

3. The experimental error can be calculated by averaging the mean squares of the dummy effects of E and G.

$$\text{Thus, mean square for error} = \frac{\sum (\textit{mean square of dummy variables})}{\textit{Number of dummy variables}}$$

4. The final stage is to identify the factors which are showing large effects. This is done using an F-test which is given by:

F-Test= (Factor mean square) / (error mean square)

The factor which is having highest F-test value is identified as the most important factor.

1.3 INTRODUCTION TO BIOREACTOR CONFIGURATION

A Bioreactor, as the name suggests, is a reactor (or a vessel) which supports the growth of a biological substance (Organism).

A bioreactor can be defined as manufactured device or system that supports a biologically active environment. In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms.

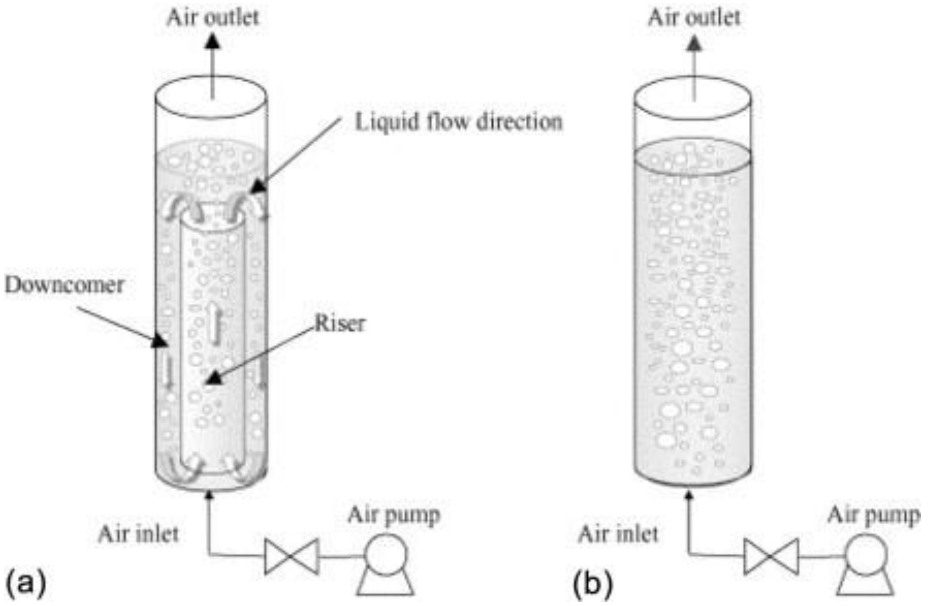
Bioreactors have been modified and enhanced according to functioning. That is, its design and configuration has been altered to perform a specialized and a specific kind of reaction.

A few of the several types of bioreactors and their configurations are discussed briefly below –

TYPES OF BIOREACTORS

1. Airlift bioreactors

   Airlift bioreactors are tower reactors for large-scale aerobic cultures where the mixing of the culture broth is done by the inserted gas via an airlift pump.



   Advantages –

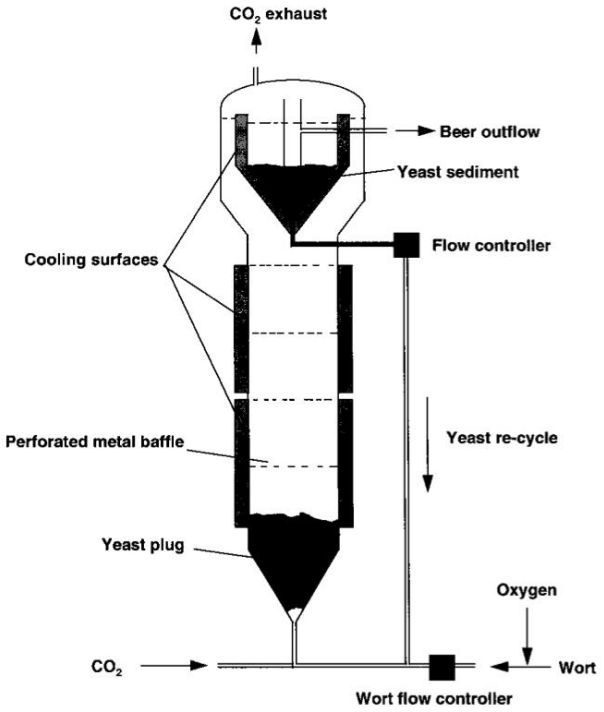
   - Simple design with no moving parts or agitator for less maintenance, less risk of defects.
   - Easier sterilization (No agitator shaft parts)
   - Low energy requirement and greater heat removal

   Disadvantages –

- Higher pressure needed
- Insufficient mixing
- Nonuniform nutrient supply

2. Tower fermenter

Tower fermenter is an elongated non- mechanically stirred fermenter with an aspect ratio of 6:1 through which there is a unidirectional flow of gases.



Advantages –

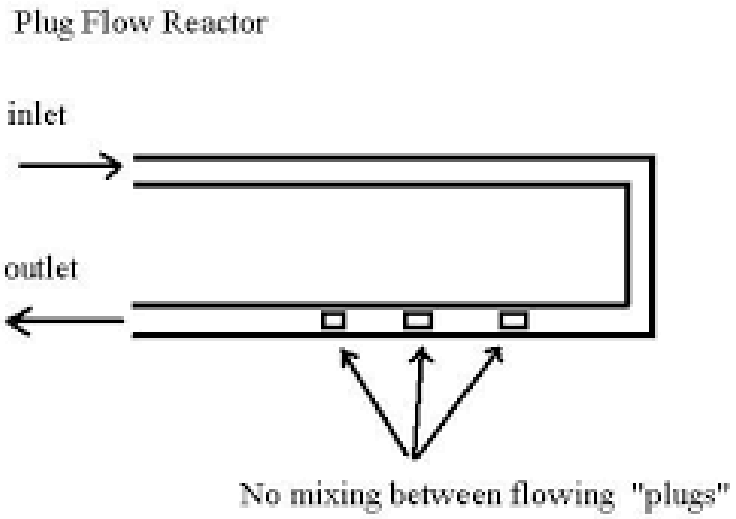
- Shorter fermentation time
- No yeast lag phase
- Very consistent product

Disadvantages –

- Less scope for differential product
- Has to operate continuously
- Difficult to maintain without contamination

3. Plug flow reactors

In the Plug flow bioreactors, the primary phase involved is the gas phase. It is used for the production of large scale homogenous, heterogenous and continuous reactions.



Advantages –

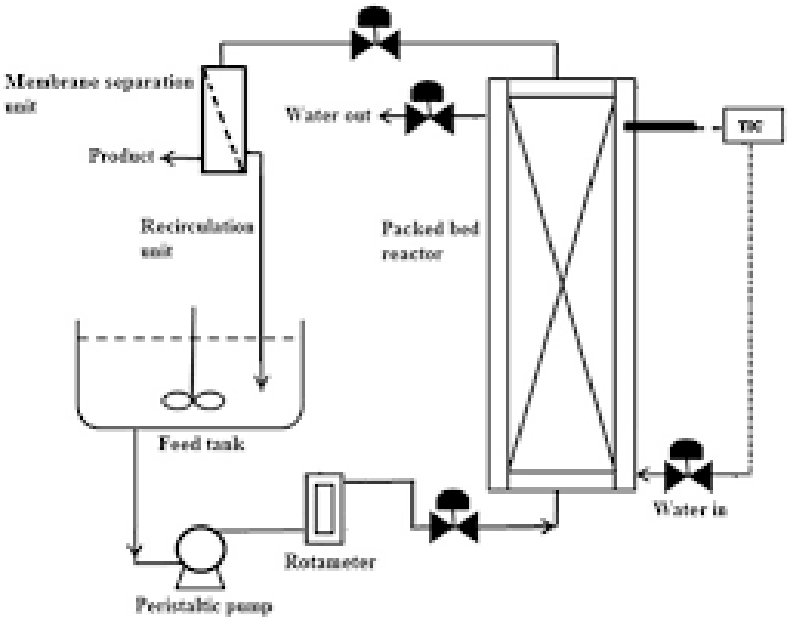
- High Conversion per unit volume
- Low operating cost
- Continuous operation
- Good heat transfer

Disadvantages –

- Undesired thermal gradients may exist
- Poor temperature control
- Shutdown and cleaning may be expensive

4. Packed bed bioreactors

Packed bed bioreactors or fixed bed bioreactors are commonly used with attached biofilms. They are widely used with immobilized cells and particulate biocatalysts. The medium can be fed either at the top or at the bottom and from a continuous liquid phase.



Advantages –

- High conversion per unit mass of catalyst
- Low operating cost
- Continuous operation

Disadvantages –

- Undesired thermal gradients may exist
- Poor temperature control
- Channeling may occur
- Unit may be difficult to service and clean

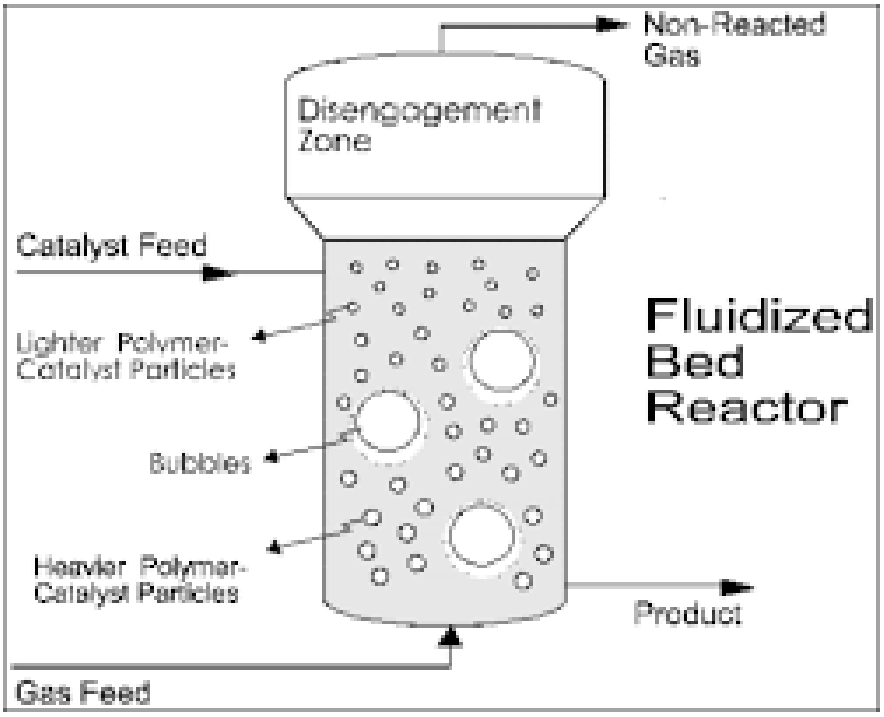
5. Fluidized bed Bioreactor

The fluidized bed reactors are designed in such a way that it can carry out a variety of multiphase (Solid, Liquid, Gaseous) chemical reactions.

When the packed beds are operated in an up-flow orientation, the bed expands at high liquid flow rates due to upward motion of the particles.

The particles used can be of the following types

- Inert core on which the biomass is created by cell attachment
- Porous particles in which the biocatalyst is entrapped
- Cell aggregates and self-immobilization



Advantages –

- The smaller particle size facilitates higher mass transfer rates and better mixing.
- The volumetric productivity attained in FBBs is usually higher than in stirred tank and packed bed bioreactors.
- Uniform particle mixing

Disadvantages –

- Additional separation steps needed limited by reaction equilibrium
- Lack of current understanding
- Particle entrainment
- Erosion of internal components

1.4 BATCH, FED-BATCH AND CONTINUOUS REACTOR

Batch Operation

In a batch operation, all necessary medium components and the inoculum are added at the beginning and not during period of fermentation. Therefore, their concentrations are not controlled but are allowed to vary as the living cells take them up. The products, be they intra- or extracellular, are harvested only at the end of the run. Basic controls for pH, temperature, dissolved oxygen, and foam are applied during the course of batch culture. The pH, dissolved oxygen, and temperature are normally held constant during the course of batch reactor operation. The only optimization parameters are the initial medium composition. However, profile optimizations of temperature and pH may lead to improved performance over the operations carried out at constant temperature and constant pH.

Batch Reactor

- A batch reactor has no input or output when the reaction is occurring ($F_{A0} = F_A = 0$), so

$$\frac{dN_A}{dt} = \int r_A \, dV$$

and if the reaction mixture is perfectly mixed so that r_A is independent of position,

$$\boxed{\frac{dN_A}{dt} = r_A V}$$

- The time, t, needed to reduce the number of moles from N_{A0} to N_{A1} is given as

$$t = \int_{N_{A1}}^{N_{A0}} \frac{dN_A}{-r_A V}$$

- Since

$$\boxed{N_A = C_A V}$$

it can also be stated that (for a constant-volume batch reactor)

$$\boxed{\frac{dC_A}{dt} = r_A}$$

Batch Reactor Design Equations

Conversion (of substance A) is defined as

$$\boxed{X = \frac{\text{moles of A reacted}}{\text{moles of A fed}}}$$

– This can be rephrased mathematically as

$$X_i = \frac{N_{i0} - N_i}{N_{i0}} = 1 - \frac{C_i V}{C_{A0} V_0}$$

The number of moles of A in the reactor after a conversion X has been achieved is

$$\boxed{N_A = N_{A0}(1 - X)}$$

By differentiating the above expression with respect to t and plugging it into the expression for the batch reactor, $\frac{dN_A}{dt} = r_A V$, we get

$$\boxed{N_{A0}\frac{dX}{dt} = -r_A V}$$

and

$$t = N_{A0}\int_0^X \frac{dX}{-r_A V}$$

Fed-Batch Cultures

A fed-batch culture is a semi-batch operation in which the nutrients necessary for cell growth and product formation are fed either intermittently or continuously via one or more feed streams during the course of an otherwise batch operation. The culture broth is harvested usually only at the end of the operational period, either fully or partially (the remainder serving as the inoculum for the next repeated run). This process may be repeated (repeated fed-batch) a number of times if the cells are fully viable and productive. Thus, there are one or more feed streams but no effluent during the course of operation. Sources of carbon, nitrogen, phosphates, nutrients, precursors, or inducers are fed either intermittently or continuously into the culture by manipulating the feed rates during the run. The products are harvested only at the end of the run. Therefore, the culture volume increases during the course of operation until the volume is full. Thereafter, a batch mode of operation is used to attain the final results. Thus, the fed-batch culture is a dynamic operation. By manipulating the feed rates, the concentrations of limiting nutrients in the culture can be manipulated either to remain at a constant level or to follow a predetermined optimal profile until the culture volume reaches the maximum, and then a batch mode is used to provide a final touch. In so doing, the concentration of the desired product or the yield of product at the end of the run is maximized. This type of operation was first called a fed-batch culture or fed-batch fermentation. 1,2 It is also known as Zulaufverfahren in German or ryukaho2 (a flow addition method) in Japanese. Obviously, this type of operation is a

semi-batch reactor operation that is used for chemical and biochemical reactions. In environmental engineering dealing with toxic waste, this type of operation is known as a fill and draw operation or as a sequencing batch reactor. In biomedical engineering, the breathing process in and out of the lung is known as stick and balloon, as the volume of the lung increases as we inhale and decreases as we exhale, which is a form of fed-batch process.

Mathematical model of a fed-batch bioreactor can be written as;

- for the reactor volume:

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

assuming that $\rho_{feed} = \rho_{liquid\ in\ fermenter}$

- for biomass:

$$\begin{aligned} \frac{d(xV_R)}{dt} &= F_i x_i + rV_R \\ V_R\frac{dx}{dt} + x\frac{dV_R}{dt} &= F_i x_i + (\mu x)V_R \\ V_R\frac{dx}{dt} &= F_i x_i - x\frac{dV_R}{dt} + \mu x V_R \end{aligned}$$

from equation (6.1) above, and also

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

with $F_i = F$;

$$\begin{aligned} \frac{dx}{dt} &= \frac{F}{V_R}x_i - \frac{F}{V_R}x + \left(\frac{\mu_{max} s}{K_s + s}\right)x \\ \frac{dx}{dt} &= Dx_i - Dx + \left(\frac{\mu_{max} s}{K_s + s}\right)x \\ \frac{dx}{dt} &= (x_i - x)D + \left(\frac{\mu_{max} s}{K_s + s}\right)x \end{aligned}$$

for a *sterile feed*,

$$x_i = 0$$

hence;

$$\frac{dx}{dt} = -xD + \left(\frac{\mu_{max} s}{K_s + s}\right)x$$

- for substrate/nutrient:

$$\begin{aligned} \frac{d(sV_R)}{dt} &= F_i s_i - \frac{1}{Y_{X/S}} rV_R \\ s\frac{dV_R}{dt} + V_R\frac{ds}{dt} &= F_i s_i - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)xV_R \\ V_R\frac{ds}{dt} &= F_i s_i - s\frac{dV_R}{dt} - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)xV_R \end{aligned}$$

let $F_i = F$ and according to equation (6.1) above;

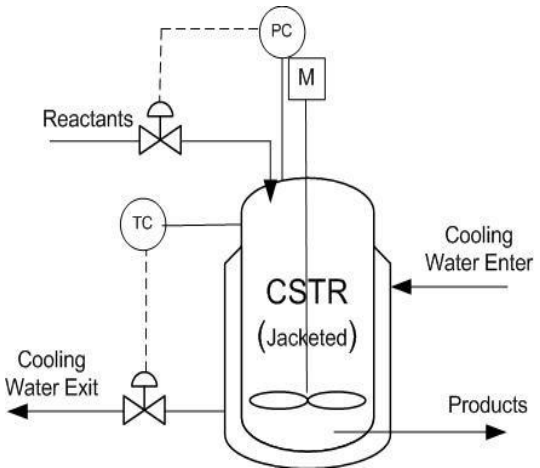
$$\begin{aligned} \frac{ds}{dt} &= \frac{F}{V_R}(s_i - s) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)x \\ \frac{ds}{dt} &= D(s_i - s) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)x \end{aligned}$$

- for product:

$$\begin{aligned} \frac{d(pV_R}{dt} &= \frac{Y_{P/S}}{Y_{X/S}} rV_R \\ p\frac{dV_R}{dt} + V_R\frac{dp}{dt} &= \frac{Y_{P/S}}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)xV_R \\ V_R\frac{dp}{dt} &= \frac{Y_{P/S}}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)xV_R - p\frac{dV_R}{dt} \end{aligned}$$

DESIGN OF CONTINUOUS STIRRED TANK REACTOR

Continuous Stirred Tank Reactor (CSTR), also known as vat- or Back mix reactor, is a common type of ideal reactor in chemical industry. CSTR is a complex nonlinear system.



The Schematic of a CSTR is shown below.

Mathematical Model for CSTR Process

A simple exothermic reaction A→B takes place in the reactor, which is in turn cooled by a coolant that flows through a jacket around the reactor.

The fundamental dependent quantities for the reactor are:

(a) Total mass of the reacting mixture in tank

(b) Mass of chemical A in the reacting mixture

(c) Total energy of the reacting mixture in the tank

In this process the heat produced due to the reaction is removed by a coolant medium that flows through a jacket around the reactor.

As known from the analysis of a CSTR system, the amount of heat released by the exothermic reaction is a nonlinear function of the temperature T inside the reactor.

On the other hand, the heat removed by the coolant is a linear function of the temperature T. When the CSTR is at steady state, heat produced by the reaction should be equal to the heat removed by the coolant. Let us apply the conservation principle on the three fundamental quantities:

Total Mass Balance:

$$\frac{d(\rho V)}{dt} = \rho_i F_i - \rho F \pm 0 \qquad (1)$$

Mass Balance on Component A:

$$\frac{d(n_A)}{dt} = \frac{d(c_A V)}{dt} = C_{A_i} F_i - C_A F - rV \qquad (2)$$

Total Energy Balance:

E + U+ K= P (3)

Assume the reactor does not move (i.e., dK/dt = dP/dt = 0), the left-hand side of the total energy balance yields:

$$\frac{dE}{dt} = \frac{d(U+K+P)}{dt} = \frac{dU}{dt} \quad (4)$$

Since the system is a liquid system, we can make the following approximation:

$$\frac{dU}{dt} \approx \frac{dH}{dt} \quad (5)$$

Characterize Total Mass:

$$\frac{d(\rho V)}{dt} = \rho \frac{dV}{dt} \qquad \rho_i = \rho \quad (6)$$

Characterize the Mass of A:

$$\frac{dC_A}{dt} = \frac{F_i}{V}(C_{Ai} - C_A) - k_o e^{-E/RT} C_A$$

State variables:

$$V, C_A, T \quad (8)$$

State Equations:

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

$$\frac{dT}{dt} = \frac{F_i}{V}(T_i - T) + Jk_o e^{-E/RT} C_A - \frac{Q}{\rho C_p V}$$

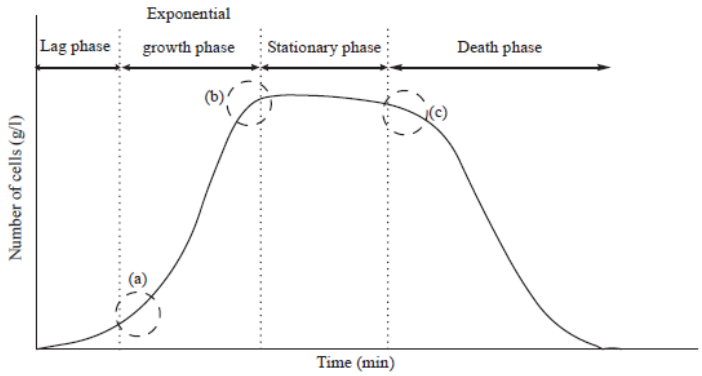
$$\frac{dC_A}{dt} = \frac{F_i}{V}(C_{Ai} - C_A) - k_o e^{-E/RT} C_A$$

The Transfer Function Model of the CSTR is given as:

$$G(s) = \frac{-1.1170s + 3.1472}{s^2 + 4.6429s + 5.3821}$$

1.5 BIOREACTOR LAY OUT KINETICS OF BATCH AND CONTINUOUS CULTURE

Batch culture there are a number of biochemical processes that involve batch culture/growth of cell. This type of culture requires enough nutrients to maintain the growth. A typical growth profile is given in the figure below.



The graph shows an increase of cell at the start of the cultivation (fermentation) process. This is due to the presence of enough nutrient for the cell to grow. At the same time the amount of nutrient decreases as it being consumed by the cell. Other side products such as carbon dioxide or ethanol is also formed simultaneously. In batch cultures, the cell properties such as.

• size of cells

• internal nutrient

• metabolic function varies considerably during the above growth phases. No apparent increase of the amount of cell at the start of cultivation, this is termed as the lag phase. After this period (can be between 10 to 15 mins) the number of cells increases exponentially thus, this stage is called the exponential growth phase; • the cell properties tend to be constant

• last for a short period of time

The next stage is the stationary phase where the population of cell achieves it maximum number. This is because:

• all nutrient in the closed system has been used up by the cell.

• lack of nutrient will eventually stop the cell from multiplying. The final stage of cell cultivation is the death phase. The decrease of the number of cells occurs exponentially which happens when the cell breaks open (lysed).

$$\frac{dN}{dt} = -k_d' N$$

The rate of death normally follows the first-order kinetics given by which upon integration leads to

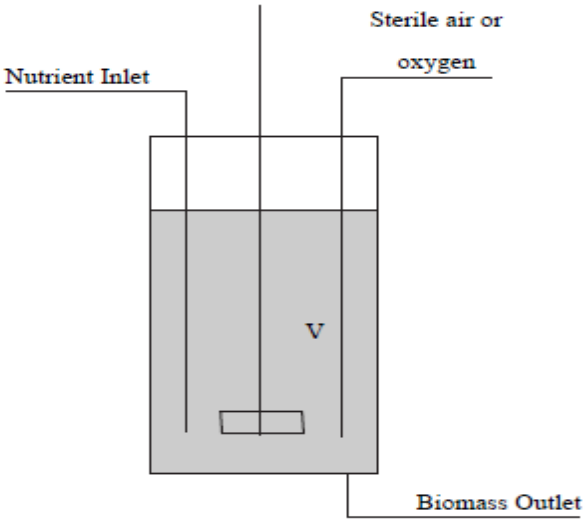
$$N = N_s e^{-k_d' t}$$

where Ns is the concentration of cells at the end of the stationary phase and at the beginning of the death phase and k 0 d is the first order death rate constant. In both stationary and death phase, it is important to recognise that there is a distribution of properties among the cells in a population. A summary of the different phases of cell growth is given in Table.

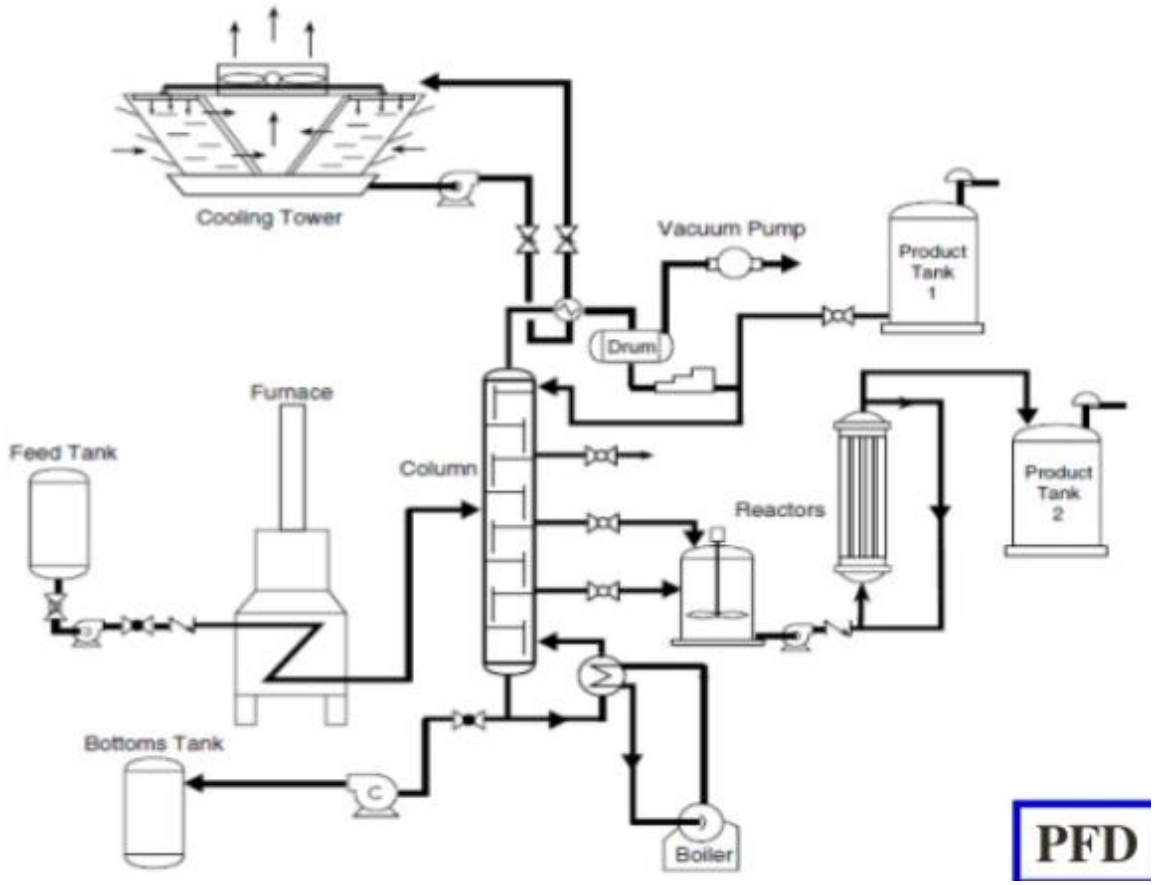
Growth phase	Rate of growth	Comments
Lag	Zero	Innoculum adapting with the changing condition (temperature, pH)
Acceleration	Increasing	Trivial
Exponential	Constant	Population growth changes the environment of the cells
Retardation	Decreasing	The effect of changing conditions appear
Stationary	Zero	One or more nutrients are exhausted to the threshold level of the cell
Decline	Negative	The duration of stationary phase and the rate of decline are strongly dependent on the kind of organism
Death phase	Negative	Cells lyse due to lack of nutrient

Continuous culture

Batch and continuous culture systems differ in that, in a continuous culture system, nutrients are supplied to the cell at a constant rate and in order to maintain a constant volume of biomass in the reactor, an equal volume of cell culture is removed. This will allow the cell population to reach a steady-state condition. The reactor configuration of a continuous process.



1.6 UNDERSTANDING A PIPING AND INSTRUMENTATION DIAGRAM OF A BIOREACTOR


Cooling Tower
Vacuum Pump
Product Tank 1
Drum
Furnace
Feed Tank
Column
Reactors
Product Tank 2
Bottoms Tank
Boiler
PFD

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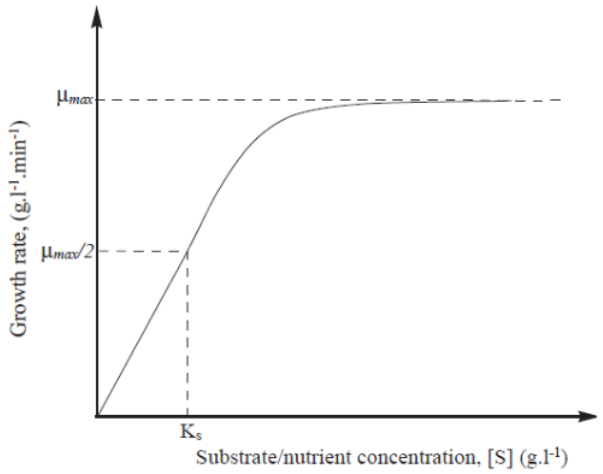
UNIT – III- DESIGN AND OPERATION OF BIOREACTORS-SBT1303

3.1 MONOD EQUATION

Monod growth kinetics

The growth of most of the bacterial cells is in the form of hyperbolic curve. A simple growth model describing such a curve was first proposed by Monod in 1942 by linking the specific growth rate and the concentration of the nutrient used by the cells. The model is similar to that of the Langmuir isotherm and the famous Michaelis-Menten model of enzyme-catalysed reactions. It is given by.

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



where μmax is the maximum growth rate when there is enough substrate supplied to the cell and the value exceeds the limiting substrate concentration, Ks.

$$[S] \gg K_s.$$

The corresponding curve resulted from equation (2.4) is given in Figure (2.1). The model described by Monod requires the yield factor, YX/S which is based on the stoichiometric parameters. Thus, the substrate balance is now can be written as:

$$\frac{F}{V}([S]_i - [S]) - \frac{1}{Y_{X/S}}\mu x = 0 \quad (2)$$

and YX/S is defined as.

$$Y_{X/S} = \frac{mass\ \ of\ \ biomass/cells\ \ produced}{mass\ \ of\ \ substrate\ \ used}$$

substituting equation (1) into (2) gives:

$$D([S]_i - [S]) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max}[S]}{K_s + [S]}\right)x = 0 \quad (3)$$

while the biomass (cell) balance at steady state is given as:

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$$\begin{aligned} \frac{d[S]}{dt} &= D([S]_i - [S]) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max}[S]}{K_s + [S]}\right)x \\ \frac{dx}{dt} &= \left(\frac{\mu_{max}[S]}{K_s + [S]}\right)x - Dx \end{aligned} \quad (5)$$

Type of operation	Advantages	Disadvantages
Batch	Versatile: can be used for different reaction everyday Safe: can be properly sterilised. Little risk of infection or strain mutation. Complete conversion of substrate is possible	High labour cost: skilled labour is required Much idle time: Sterilisation, growth of inoculum, cleaning after fermentation Safety problem: when filling, emptying, cleaning
Continuous, steady-state (chemostat)	Works all the time: low labour cost, good utilisation of reactor Often efficient: due to the autocatalytic nature of microbial reactions, the productivity can be high. Automation may be very appealing. Constant product quality.	Often disappointing: promised continuous production for months fails due to (a) infection, e.g. a short interruption of the continuous feed sterilisation, (b) spontaneous mutation of microorganism to non producing strain. Very inflexible: can rarely be used for other productions without substantial retrofitting. Downstream: all the downstream process equipment must be designed for low volumetric rate, continuous operation.
Semi-batch (fed-batch)	Combines the advantages of batch and continuous operation. Excellent for control and optimisation of a given production criterion.	Some of the advantages of both batch and continuous operation but the advantages far outweigh the disadvantages, and fed-batch is used to produce high biomass (baker's yeast) and important secondary metabolites (e.g. penicillin).

3.2 ALTERNATIVES TO MONOD EQUATION

Unstructured, non segregated models:

Monod model:

$$\mu = \frac{\mu_m C_S}{K_S + C_S}$$

μ : specific (cell) growth rate

μ_m : maximum specific growth rate at saturating substrate concentrations

C_S : substrate concentration

K_S : saturation constant ($C_S = K_S$ when $\mu = \mu_m / 2$)

Blackman equation:

$$\mu = \mu_m \quad \text{if } C_S \geq 2K_S$$

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Tessier equation:

$$\mu = \mu_m [1 - \exp(-KC_S)]$$

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ALTERNATIVE TO MONOD MODEL EQUATION BLACKMAN, TESSIER, MOSER, CONTOIS EQUATION

BLACKMAN EQUATION:

$\mu = \mu m$ if $CS \geq 2KS$ μm CS

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if $CS < 2KS$

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μm CSn

MOSER EQUATION:

$\mu = KS + CSn$

μm CS

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3.3 UNSTRUCTURED AND STRUCTURED MODELS

UNSTRUCTURED MODEL

WHAT IS A MODEL:

STRUCTURED (multi component system) MODEL UNSTRUCTURED (single component system) SEGREGATED (heterogeneity) MODEL NON-SEGREGATED (homogeneity)

CHOICE OF THE MODEL

- The choice among these properties depends on the objective of the model
- structured models are used to describe in more details the intrinsic complexity of the system (Most realistic, but are computationally complex)
- unstructured models consider living cells regardless their intracellular sub processes. While they focus on the process behaviour, they usually involve only the most significant signals known as macroscopic species (e.g. substrates, biomass, and products of interest)

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Monod model: $\mu = \mu m \, CS / KS + CS$

μ: specific (cell) growth rate

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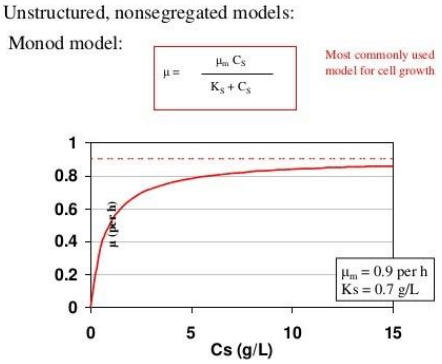

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Monod model modified for substrate inhibition:

Monod model does not model substrate inhibition. Substrate inhibition means increasing substrate concentration beyond certain value reduces the cell growth rate.

For non-competitive substrate inhibition: $\mu = \mu m (1 + KS/CS)/ (1 + CS/KI)$

For competitive substrate inhibition: $\mu = \mu m/ (1+Ks/Cs) (1+Cs/Kl)$ where KI is the substrate inhibition constant.

Monod model modified for cell growth with toxic compound inhibition: Monod model does not model product inhibition (where increasing product concentration beyond certain value reduces the cell growth rate)

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Assumptions behind Monod model:

- One limiting substrate Semi-empirical relationship Single enzyme system with M-M kinetics being responsible for the uptake of substrate Amount of enzyme is sufficiently low to be growth limiting Cell growth is slow Cell population density is low

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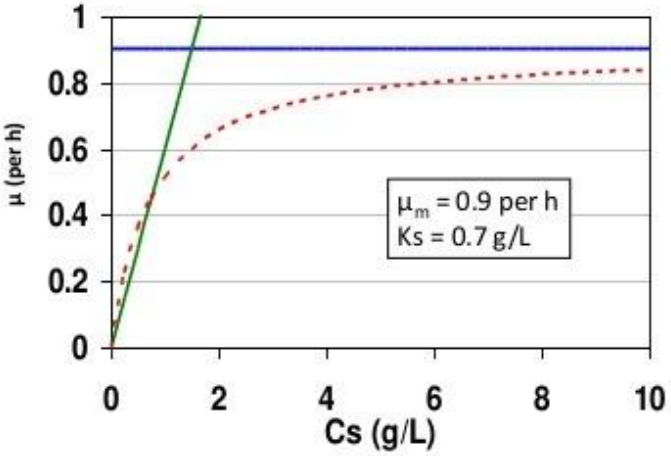

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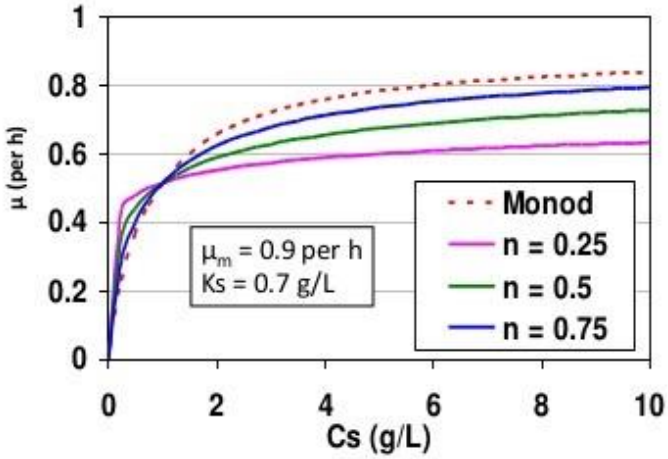

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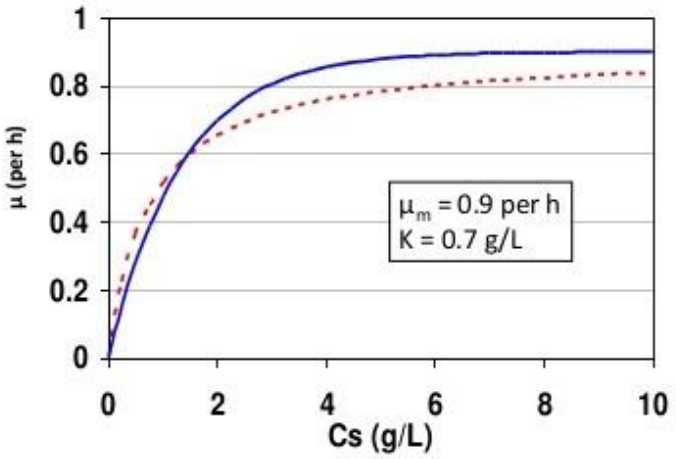
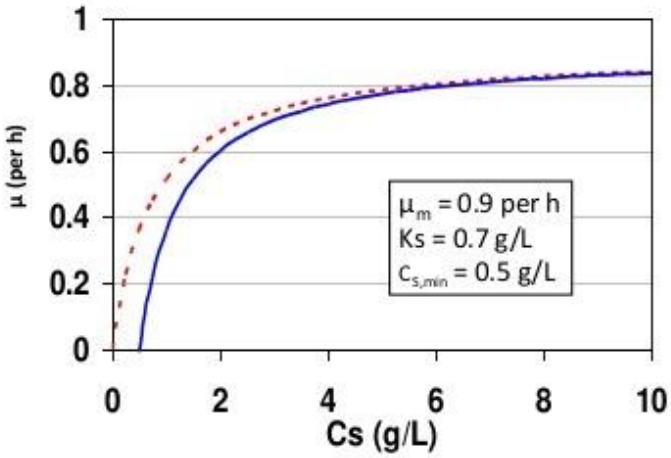


Contois equation: Saturation constant (KSX CX) is proportional to cell concentration = µm

Extended Monod model:

$$\mu = \frac{\mu_m (C_s - C_{s,min})}{K_s + C_s - C_{s,min}}$$

Extended Monod model includes a $C_{s,min}$ term, which denotes the minimal substrate concentration needed for cell growth.



CS KSX CX + CS

Other Unstructured, Nonsegregated Models (Assuming One Limiting Substrate): Luedeking-Piret model: rP = □ rX + β CX Used for lactic acid formation by Lactobacillus debruickii where production of lactic acid was found to occur semi- independently of cell growth.

Limitations of Unstructured Non-segregated Models:

- No attempt to utilize or recognize knowledge about cellular metabolism and regulation
- Show no lag phase
- Give no insight to the variables that influence growth
- Assume a black box
- Assume dynamic response of a cell is dominated by an internal process with a time delay on the order of the response time
- Most processes are assumed to be too fast or too slow to influence the observed response.

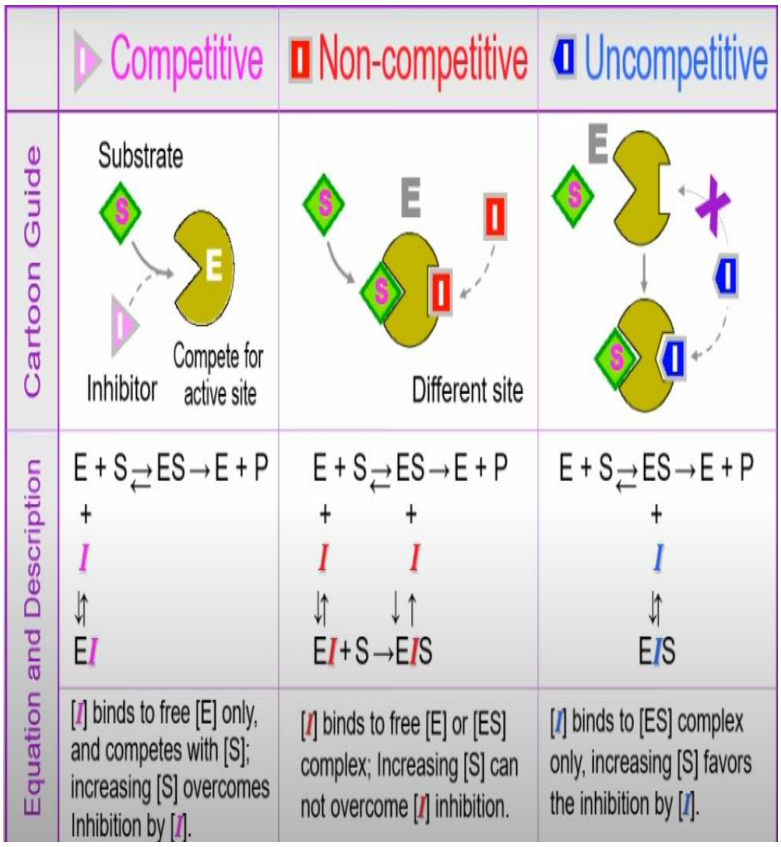
3.4 MODELS WITH GROWTH INHIBITORS

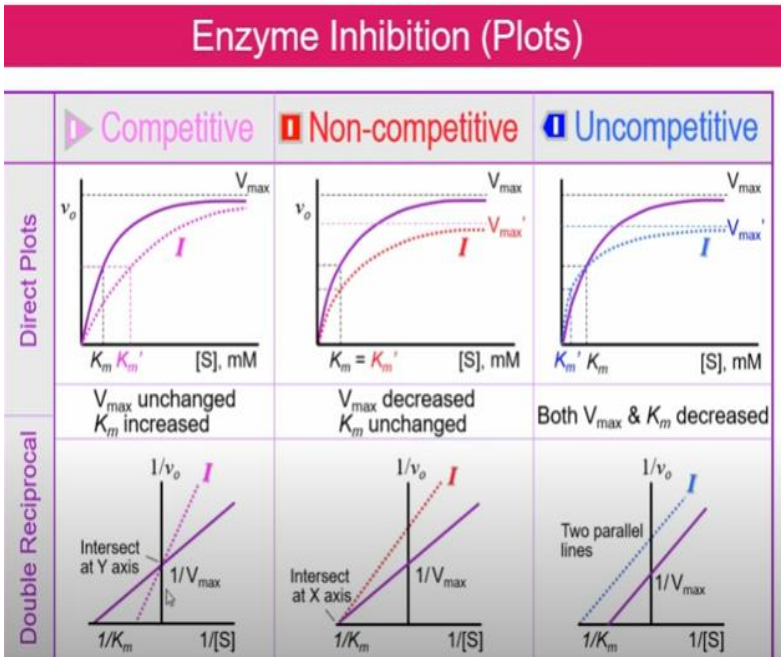
- At high concentrations of substrate or product and in the presence of inhibitory substances in the medium, growth becomes inhibited, and growth rate depends on inhibitor concentration
- The inhibition pattern of microbial growth is analogous to enzyme inhibition. If a single-substrate enzyme-catalysed reaction is the rate-limiting step in microbial growth, then kinetic constants in the rate expression are biologically meaningful.
- Often, the underlying mechanism is complicated, and kinetic constants do not have biological meanings and are obtained from experimental data by curve fitting.

3.5 SUBSTRATE INHIBITION, PRODUCT INHIBITION

SUBSTRATE INHIBITION

- At high substrate concentrations, microbial growth rate is inhibited by the substrate. As in enzyme kinetics, substrate inhibition of growth may be co. If a single-substrate enzyme-catalysed reaction is the rate limiting step in microbial growth, then inhibition of enzyme activity results in inhibition of microbial growth by the same pattern.
- The major substrate-inhibition patterns and expressions are as follows: competitive or non-competitive.





COMPRTITIVE AND NON-COMPETITIVE

For competitive substrate inhibition: $$\mu_g = \frac{\mu_m S}{K_s\left(1+\frac{S}{K_I}\right)+S}$$

Noncompetitive substrate inhibition: $$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)\left(1+\frac{S}{K_I}\right)}$$

Or if $K_I \gg K_s$, then: $$\mu_g = \frac{\mu_m S}{K_s + S + S^2/K_I}$$

PRODUCT INHIBITION

- High concentrations of product can be inhibitory for microbial growth. Product inhibition may be competitive or non-competitive, and in some cases when the

underlying mechanism is not known, the inhibited growth rate is approximated to exponential or linear decay expressions. Important examples of the product inhibition rate expression are as follows:

Competitive product inhibition: $$\mu_g = \frac{\mu_m S}{K_s\left(1+\frac{P}{K_p}\right)+S}$$

Noncompetitive product inhibition: $$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)\left(1+\frac{P}{K_p}\right)}$$

- Ethanol fermentation from glucose by yeasts is a good example of non-competitive product inhibition, and ethanol is the inhibitor at concentrations above about 5%. Other rate expressions used for ethanol inhibition are

$$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)}\left(1-\frac{P}{P_m}\right)^n$$

where P_m is the product concentration at which growth stops, or

$$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)}e^{-P/K_p}$$

where K_p is the product inhibition constant.

3.6 COMPETITIVE AND NON-COMPETITIVE PRODUCT INHIBITION

INHIBITION BY TOXIC COMPOUNDS

The following rate expressions are used for competitive, non-competitive, and uncompetitive inhibition of growth in analogy to enzyme inhibition.

Uncompetitive inhibition: $$\mu_g = \frac{\mu_m S}{\left(\frac{K_s}{\left(1+\frac{I}{K_I}\right)}+S\right)\left(1+\frac{I}{K_I}\right)}$$

Competitive inhibition: $$\mu_g = \frac{\mu_m S}{K_s\left(1+\frac{I}{K_I}\right)+S}$$

Noncompetitive inhibition: $$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)\left(1+\frac{I}{K_I}\right)}$$

In some cases, the presence of toxic compounds in the medium results in the inactivation of cells or death.

The net specific rate expression in the presence of death has the following form:

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.TECH - BIOTECHNOLOGY

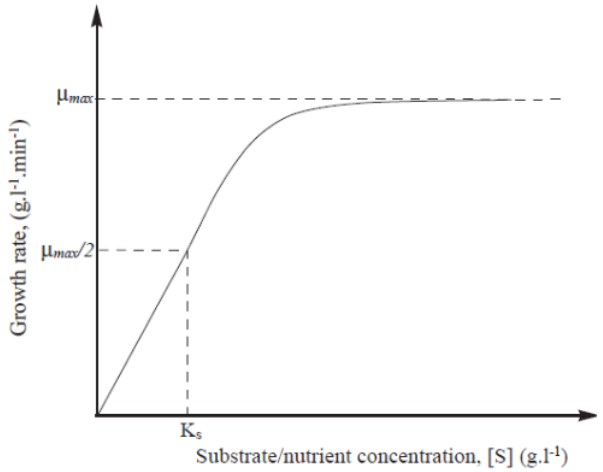
UNIT –III- DESIGN AND OPERATION OF BIOREACTORS SBT1303

3.1 MONOD EQUATION

Monod growth kinetics

The growth of most of the bacterial cells is in the form of hyperbolic curve. A simple growth model describing such a curve was first proposed by Monod in 1942 by linking the specific growth rate and the concentration of the nutrient used by the cells. The model is similar to that of the Langmuir isotherm and the famous Michaelis-Menten model of enzyme-catalysed reactions. It is given by.

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



where μmax is the maximum growth rate when there is enough substrate supplied to the cell and the value exceeds the limiting substrate concentration, Ks.

$$[S] \gg K_s.$$

The corresponding curve resulted from equation (2.4) is given in Figure (2.1). The model described by Monod requires the yield factor, YX/S which is based on the stoichiometric parameters. Thus, the substrate balance is now can be written as:

$$\frac{F}{V}([S]_i - [S]) - \frac{1}{Y_{X/S}}\mu x = 0 \tag{2}$$

and YX/S is defined as.

$$Y_{X/S} = \frac{mass\ \ of\ \ biomass/cells\ \ produced}{mass\ \ of\ \ substrate\ \ used}$$

substituting equation (1) into (2) gives:

$$D([S]_i - [S]) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max}[S]}{K_s + [S]}\right)x = 0 \tag{3}$$

while the biomass (cell) balance at steady state is given as:

$$\left(\frac{\mu_{max}[S]}{K_s + [S]}\right)x - Dx = 0 \tag{4}$$

The transient state of the system is obviously given by

$$\begin{aligned} \frac{d[S]}{dt} &= D([S]_i - [S]) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max}[S]}{K_s + [S]}\right)x \\ \frac{dx}{dt} &= \left(\frac{\mu_{max}[S]}{K_s + [S]}\right)x - Dx \end{aligned} \tag{5}$$

Type of operation	Advantages	Disadvantages
Batch	Versatile: can be used for different reaction everyday Safe: can be properly sterilised. Little risk of infection or strain mutation. Complete conversion of substrate is possible	High labour cost: skilled labour is required Much idle time: Sterilisation, growth of inoculum, cleaning after fermentation Safety problem: when filling, emptying, cleaning
Continuous, steady-state (chemostat)	Works all the time: low labour cos, good utilisation of reactor Often efficient: due to the autocatalytic nature of microbial reactions, the productivity can be high. Automation may be very appealing. Constant product quality.	Often disappointing: promised continuous production for months fails due to (a) infection, e.g. a short interruption of the continuous feed sterilisation, (b) spontaneous mutation of microorganism to non producing strain. Very inflexible: can rarely be used for other productions without substantial retrofitting. Downstream: all the downstream process equipment must be designed for low volumetric rate, continuous operation.
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$$\mu = \frac{\mu_m C_S}{K_S + C_S}$$

μ : specific (cell) growth rate

μ_m : maximum specific growth rate at saturating substrate concentrations

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K_S : saturation constant ($C_S = K_S$ when $\mu = \mu_m / 2$)

ALTERNATIVE TO MONOD MODEL EQUATION BLACKMAN, TESSIER, MOSER, CONTOIS EQUATION

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$\mu = \mu m$ if $CS \geq 2KS$ μm CS

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$$\mu = \frac{\mu_m C_S}{2 K_S} \quad \text{if } C_S < 2K_S$$

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Contois equation:
$$\mu = \frac{\mu_m C_S}{K_{SX} C_X + C_S}$$

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MOSER EQUATION:

μ = KS + CSn

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3.3 UNSTRUCTURED AND STRUCTURED MODELS

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STRUCTURED (multi component system) MODEL UNSTRUCTURED (single component system) SEGREGATED (heterogeneity) MODEL NON-SEGREGATED (homogeneity)

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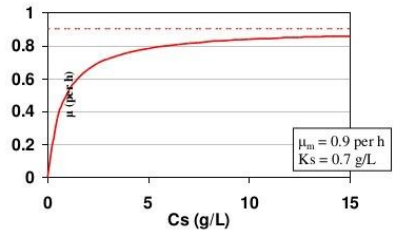
Most commonly used model for cell growth



KS: saturation constant (CS = KS when μ = μm / 2)

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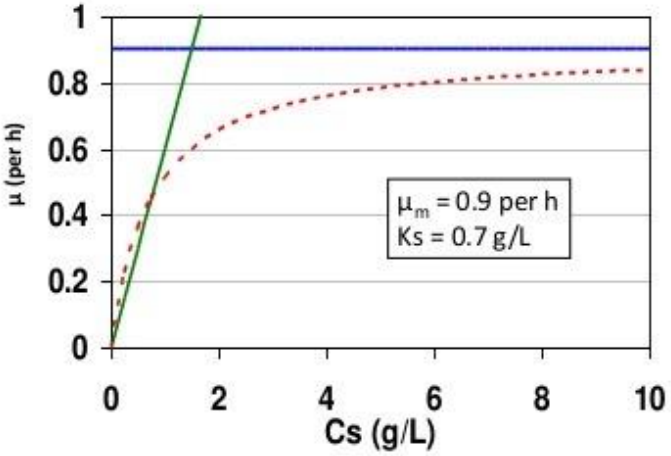

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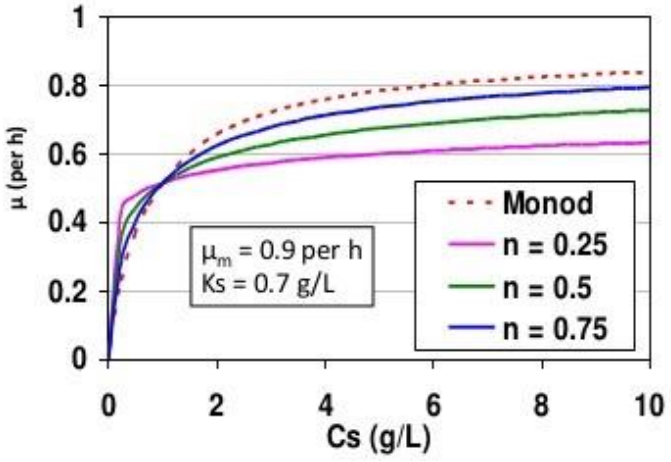

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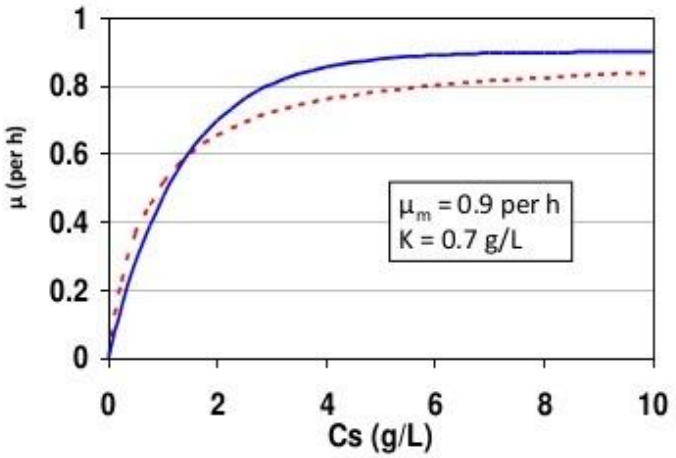

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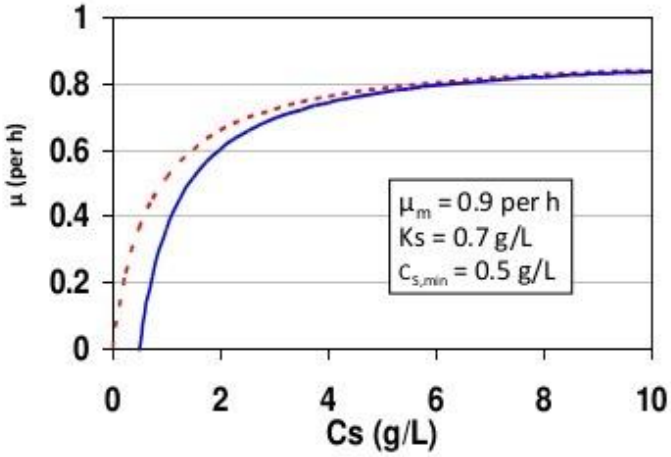

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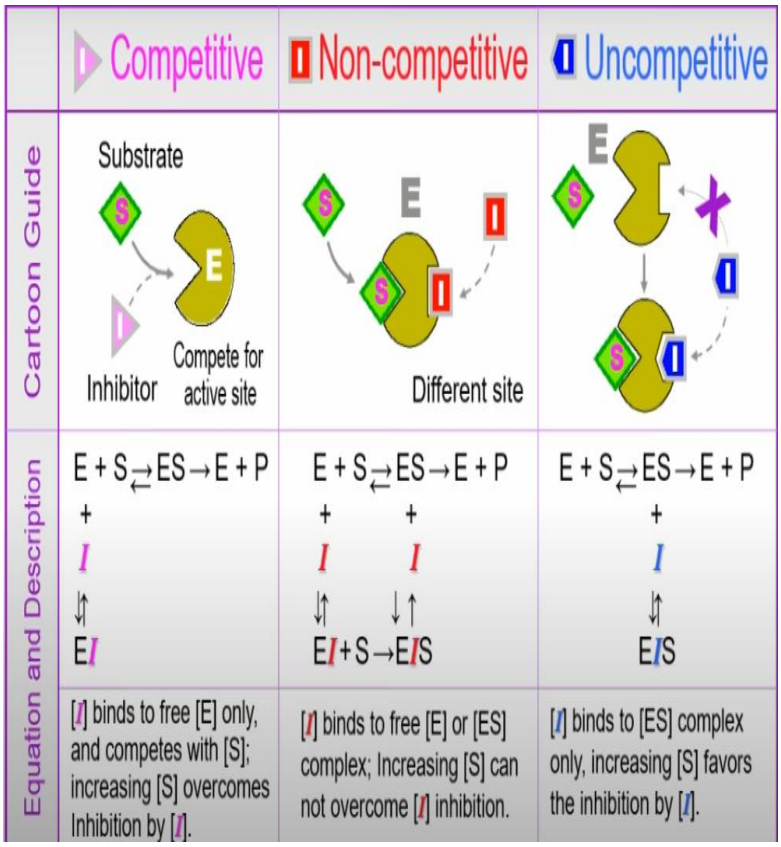

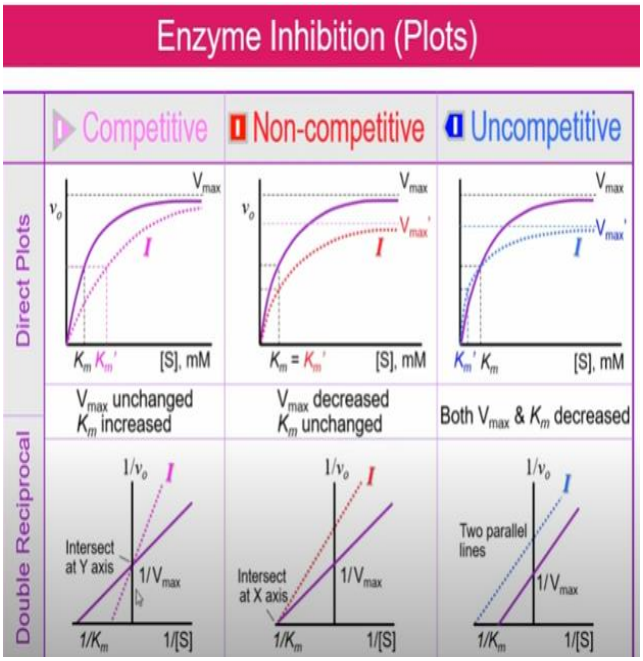

COMPRTITIVE AND NON-COMPETITIVE

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Noncompetitive substrate inhibition: $$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)\left(1+\frac{S}{K_I}\right)}$$

Or if $K_I \gg K_s$, then: $$\mu_g = \frac{\mu_m S}{K_s + S + S^2/K_I}$$

PRODUCT INHIBITION

- High concentrations of product can be inhibitory for microbial growth. Product inhibition may be competitive or non-competitive, and in some cases when the underlying mechanism is not known, the inhibited growth rate is approximated to exponential or linear decay expressions. Important examples of the product inhibition rate expression are as follows:

Competitive product inhibition: $$\mu_g = \frac{\mu_m S}{K_s\left(1+\frac{P}{K_p}\right)+S}$$

Noncompetitive product inhibition: $$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)\left(1+\frac{P}{K_p}\right)}$$

- Ethanol fermentation from glucose by yeasts is a good example of non-competitive product inhibition, and ethanol is the inhibitor at concentrations above about 5%. Other rate expressions used for ethanol inhibition are

$$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)}\left(1-\frac{P}{P_m}\right)^n$$

where P_m is the product concentration at which growth stops, or

$$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)} e^{-P/K_p}$$

where K_p is the product inhibition constant.

3.6 COMPETITIVE AND NON-COMPETITIVE PRODUCT INHIBITION

INHIBITION BY TOXIC COMPOUNDS

The following rate expressions are used for competitive, non-competitive, and uncompetitive inhibition of growth in analogy to enzyme inhibition.

Uncompetitive inhibition: $$\mu_g = \frac{\mu_m S}{\left(\frac{K_s}{\left(1+\frac{I}{K_I}\right)}+S\right)\left(1+\frac{I}{K_I}\right)}$$

Competitive inhibition: $$\mu_g = \frac{\mu_m S}{K_s\left(1+\frac{I}{K_I}\right)+S}$$

Noncompetitive inhibition: $$\mu_g = \frac{\mu_m}{\left(1+\frac{K_s}{S}\right)\left(1+\frac{I}{K_I}\right)}$$

In some cases, the presence of toxic compounds in the medium results in the inactivation of cells or death.

The net specific rate expression in the presence of death has the following form:

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B.TECH - BIOTECHNOLOGY

UNIT – IV DESIGN AND OPERATION OF BIOREACTORS SBT1303

4.1HENRY'S LAW

Henry's law states that at a constant temperature, the amount of a given gas that dissolves in a given type and volume of liquid is directly proportional to the partial pressure of gas in equilibrium with that liquid.

$$S_g = K.P_g$$

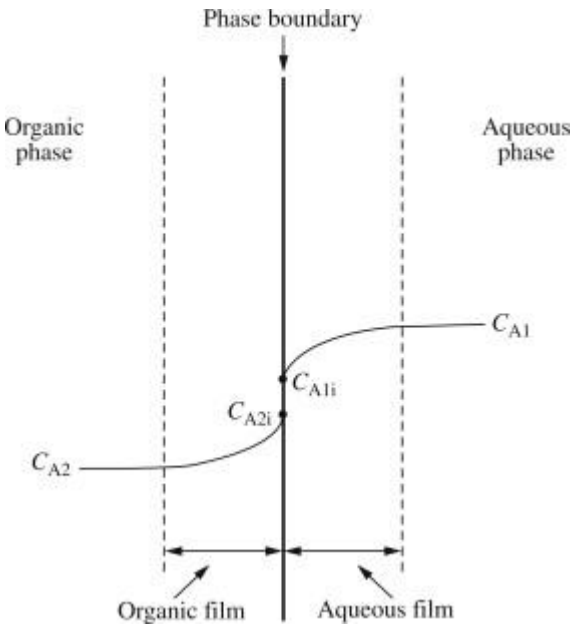
S_g - Solubility of gas

K - Henry's law constant

P_g - Pressure of gas

4.2 MASS TRANSFER, TWO FILM THEORY, DEFINITIONS OF OXYGEN TRANSFER RATE, OXYGEN UPTAKE RATE

TWO FILM THEORY:



The two-film theory is useful for mass transfer between phases- mass transfer of solute from one phase to another involves transport from the bulk of one phase to the phase boundary or interface into the bulk of second phase.

OXYGEN TRANSFER RATE (OTR):

Amount of oxygen transfer from gas to bulk liquid.

$$n = K_L A(C_S - C_L)$$

n – mass of oxygen transfer per unit

K_L – liquid film coefficient

A – interfacial area in transfer

C_S – saturation concentration of liquid

C_L – oxygen concentration in bulk of liquid phase

OXYGEN UPTAKE RATE (OUR):

The amount of oxygen uptake by microbes.

$$OUR = q_{o2}X = \mu_g X/Y_{g/o2}$$

X – cell concentration

q_{o2} – specific rate of O_2 consumption

$Y_{g/o2}$ – yield coefficient of O_2

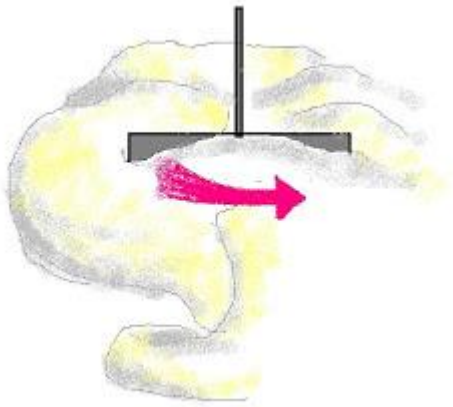
SPECIFIC OXYGEN UPTAKE RATE (SOUR)

O_2 requirement per gram of cells per second and related to the total O_2 uptake.

4.2MASS TRANSFER AND RHEOLOGY: RHEOLOGY OF BROTHS

Fermentation is largely affected by the rheological properties of the medium. These properties are primarily controlled by the biomass concentration and the morphology of the

organisms. The accumulation of biomass leads to an increase in viscosity of the fermentation broth. Phenomena such as yield stress may also appear. The fermentation broth of microorganisms, especially filamentous fungi, is a complex rheological system where the accumulation of biomass or biosynthesized product leads to the continuous modification of the rheological properties of the medium produced in a bioreactor.



One of the most important singular properties of the fermentation broth will be its rheological or viscosity characteristics. We always 'picture' fermentation broth as a thick gooey sticky mixture that is thick and viscous compounded by rising bubbles of gas exploding at the broth surface. Maybe this picture is too dramatic but, in a way, it is true!

The viscous nature or the rheological properties will affect the mixing regimes of the fermenter.

Viscosity is not a simple but a complex phenomenon that is always changing and responding to various parameters. Very rarely can we describe a fermentation broth as following a Newtonian behaviour. In most cases it is a complex combination of various Non-Newtonian behaviours.

The viscosity of the fermentation broth is caused by the interactions of the various components in the fermentation broth. The interactions may occur between the components of the broth and the water or it could result from the interactions between the components themselves. Such interactions in the viscosity of the broth could occur at the level of the ions and molecules which involve the various ionic forces or it could involve at the macrolevel

such as between the various bio-polymers tangling and sliding with each other. The overall result will be that the fermentation broth will be viscous.

Contribution of carbohydrates to the viscosity:

Sugar or the carbohydrates are the main carbon source in any fermentation media and supply the carbon needed for energy and skeleton structures of the cells and organic compounds. The stickiness or viscosity of the sugar in solution is caused by hydrogen bonding which develop between the sugar molecules and water. During the interactions of sugar and water the hydrogens in the water molecules and the hydrogen in the sugar molecules have an attraction for each other. Thus, it is the hydrogen bonding that make the sugar sticky!

Thus, we see that most of the viscosity in the fermentation broth is caused by the various hydrogen and other ionic bonding

Extracellular proteins produced an increase in the viscosity:

Cell cultures of Beta vulgaris were developed in an air lift bioreactor of 10 dm3. Culture broth rheology exhibited non-Newtonian, shear thinning characteristics. The pseudoplasticity of the broth was governed by the presence of the cells as well as by the proteins secreted by the cells in the medium. The accumulation of extracellular proteins produced an increase in the viscosity and a change in the rheological properties of the cell-free medium. This phenomenon may be a response of the cells to hydrodynamic stress. The accumulation of extracellular proteins and the change in the rheology of cell-free medium were discussed with respect to those data reported in literature obtained in shake flasks and stirred tank bioreactor.

Rheological properties determined by:

Many authors have proposed correlations between cell concentration (X) and consistency index (K), calculated from the power law model (t = K.g n). Rheological properties were determined offline, employing a digital Brookfield Rheometer (LVDV III) with a small sample adapter, connected to a microcomputer running Rheocalc software. The spindle speed varied between 1 and 150 rpm. The spindles used were: ULAdapter (shear rate in the range between 0 and 322 s -1 , which corresponds to an apparent viscosity between 1.0 and 5000 cP), SSA-18 (shear rate in the range between 0 and 330 s -1 , which corresponds to an apparent viscosity between 1.3 and 30000 cP) and SSA-31 (shear rate in the range between 0 and 85 s-1 , which corresponds to an apparent viscosity between 12 and 30000 cP).

For the flow behaviour index, the value obtained was near 1.0, in the beginning of cultivations, showing Newtonian behaviour at the beginning of fermentation.

In the course of fermentation, the flow behaviour index decreased to a minimum value about 0.25, showing pseudoplastic behaviour (Non-Newtonian fluid)

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UNIT – V DESIGN AND OPERATION OF BIOREACTORS SBT1303

5.1 ANIMAL AND PLANT CELL BIOREACTORS

5.1.1 ANIMAL CELL BIOREACTORS

- Animal cell culture is the process of culturing animal cells outside the tissue (in vitro)
- It will continue to grow if supplied with appropriate conditions and nutrients
- The culture process allows single cells to act as independent units, much like bacterium or fungus
- The cells are capable of dividing
- Animal cell culture was successfully established in 1907

TECHNIQUES OF CULTIVATING ANIMAL CELLS

- Excise tissues from specific organs of animals (lung, kidney) under aseptic conditions.
- Transfer tissues into a growth medium containing serum and antibiotics in small T-flasks.
- These cells form a primary culture that usually attach onto the glass surface of flask in monolayer form.
- The cells growing on support surfaces are known as anchorage-dependent cells.
- Some cells grown in suspension culture and are known to non-anchorage-dependent cells.
- Then a cell line appears from the primary culture and is known as secondary culture.
- Remove the cell from the surface of flasks using trypsin and add serum to the culture bottle.
- The serum containing suspension is then used to inoculate secondary cultures.
- Many secondary lines can be adapted to grow in suspension and are non-anchorage dependent.

BIOREACTOR CONSIDERATIONS FOR ANIMAL CELL SUSPENSION CULTURES:

Properties of animal cell that set constraints on design of animal cell bioreactor

-cells are large (10-20μm)

-more fragile

-grow more slowly than most bacteria and fungi

-toxic metabolites e.g. ammonium & lactate produced during growth Common features of animal cell bioreactor:

1)Reactor should be gently agitated and aerated. Agitation speed ≈20rpm.Bubble-column & airlift reactor operating at high aeration may cause damage of cells

2)Supply of CO_2-enriched air

3)Removal of toxic products from metabolism e.g. lactic acid, ammonium

Require gentler culture condition and control systems that are optimized for lower metabolic rates.

REQUIREMENTS FOR A BIOREACTOR FOR ANIMAL CELL CULTURE

1)well-controlled environment (T, pH, DO, nutrients, and wastes)

2)supply of nutrients

3)gentle mixing (avoid shear damage to cells)

4) gentle aeration (add oxygen slowly to the culture medium but avoid the formation of large bubbles which can damage cells on contact).

removal of wastes

BIOREACTORS FOR ANIMAL CELL CULTURE:

- Static flask and roller bottle
- Spinner flask
- Rotary perfusion bioreactor
- Rotating wall bioreactor
- Compression bioreactor

SCALE- UP

- Small volume reactors
  [T flask, shaker flask(5-25ml)]
- Intermediate scale
  [Small, highly controlled bioreactors(1-5L)]
- Production scale
  [Large reactor(1000L)]

5.1.2 PLANT CELL BIOREACTORS

BIOREACTOR

A bioreactor refers to any manufactured device or system that supports a biologically active environment. In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms.

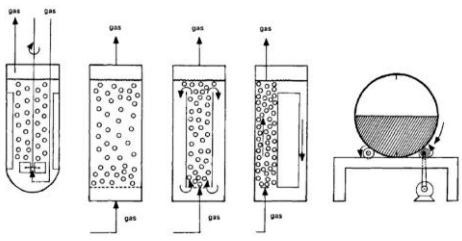
BIOREACTOR CONSIDERATION

All modes of cultivation can be adopted to plant cells. Plant cells are often found in groups which can alter in size during the cell growth. Degree of aggregate formation is influenced by the degree of mixing. The large size, rigid cellulose-based cell wall, and large vacuole make the plant sensitive to shear stress.

BIOREACTOR TYPES FOR PLANT CELL SUSPENSION CULTURE

Bioreactors are a vessel or device in which biological reaction takes place and they maintain a sustainable environment for cell growth or product formation. Schematic presentation of different types of bioreactors used for plant cell suspension culture.

Source: Plant Tissue Culture: Theory and Practice (1983) by S. S. Bhojwani and M. K. Razdan. Plant cell culture bioreactors are different from microbial fermenters because of the differences in cellular properties of plants and microbes.



CHARACTERISTICS OF PLANT CELL BIOREACTORS

Despite some differences in the bioreactors, some characteristics should be followed by all the cell culture bioreactors which are explained below:

- Aeration and low shear mixing
- Adequate dispersion of gas
- Homogenous mixing
- Easy handling

PLANT CELL TISSUE BIOREACTORS:

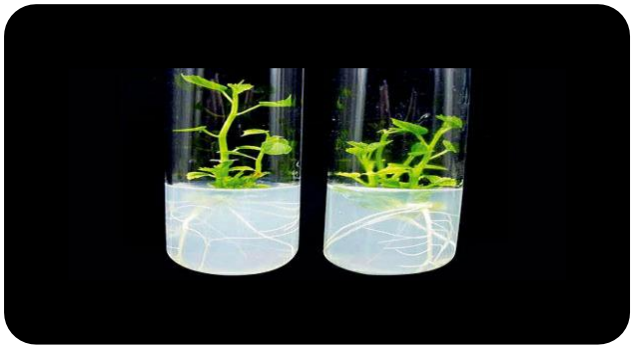
- Many thousands of chemicals are produced only in plants.
- Only few % of the world's plants have been scientifically named and only few compounds have been screened to produce novel & useful compounds.
- Around 120 drugs are derived from plants.
- In western world around 25% of pharmaceuticals are derived from extraction of plants.

WHAT IS PLANT TISSUE CULTURE IN BIOTECHNOLOGY?

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.

HOW IS THE TISSUE CULTURE OF PLANT CELL USED?

Tissue culture involves the use of small pieces of plant tissue (explants) which are cultured in a nutrient medium under sterile conditions. Using the appropriate growing conditions for each explant type, plants can be induced to rapidly produce new shoots, and, with the addition of suitable hormones, new roots.



WHICH MEDIA IS USED IN PLANT TISSUE CULTURE?

Murashige and Skoog (MS) medium Skoog in 1962 while the two scientists were working on the discovery of plant growth regulators. It is the most commonly used medium in the tissue culture lab.

PLANT CELL TISSUE CULTURE

Plant research often involves growing new plants in a controlled environment. These may be plants that we have genetically altered in some way or may be plants of which we need many copies all exactly alike. These things can be accomplished through tissue culture of small tissue pieces from the plant of interest. Tissue culture involves the use of small pieces of plant tissue (explants) which are cultured in a nutrient medium under sterile conditions. Using the appropriate growing conditions for each explant type, plants can be induced to rapidly produce new shoots, and, with the addition of suitable hormones, new roots. These plants can also be divided, usually at the shoot stage, to produce large numbers of new plantlets. The new plants can then be placed in soil and grown in the normal manner.

OBJECTIVES

1)To understand a procedure that is often used to propagate many plants of the same genetic background.

2)To understand the importance of sterile techniques.

MATERIALS

- 1 vial of Murashige Skoog (MS) medium. (If you wish to make up your own growing medium you could use the recipe for the Murashige medium given at the end of this section.)
- 1 L sterile distilled water
- 8 g of agar/L
- 30 g sucrose/L
- 1.5-L or 2-L container in which to prepare the growth medium
- Small amounts of 1M NaOH and 1M HCl to adjust the pH of the medium
- 30 flat-bottom culture tubes with closures
- Glass aquarium or box lined with plastic
- Plastic sheet to cover the top of the aquarium

- Adhesive tape
- 10% bleach in a spray bottle
- 70% alcohol in a spray bottle
- Forceps or tweezers
- Gloves
- Cutting equipment such as a scalpel blade or razor blade
- 2 bottles of sterile distilled water (purchase at the grocery store)
- Pressure cooker
- Your chosen plant (cauliflower, rose, African violet, or carnation)
- Paper towel for cutting on, or sterile petri dishes if available
- 2 or 3 beakers or containers of sterile water
- A well-lit area away from direct sunlight or use full-spectrum grow-lights
- Hormones such as BAP (benzyl aminopurine) and NAA (naphthalene acetic acid) stimulate growth and root development, respectively. (Commercial rooting hormone solutions and powders are also available from hardware stores.)

PROCEDURE

PREPARATION AND STERILISATION OF GROWING MEDIUM

These steps will make 1 L of growth medium, which is enough to prepare about 65 growing tubes.

- Dissolve the MS mixture in about 800 ml of distilled water. Stir the water continuously while adding the salt mixture. Add 30 g sugar and stir to dissolve. Adjust pH to 5.8 using 1M NaOH or 1M HCl as necessary while gently stirring. Add distilled water to make the total volume up to 1 litre
- Weigh out 8 grams of agar and add it to the MS solution. Heat the solution gently while stirring until all the agar has dissolved.
- Pour the still warm medium into the polycarbonate tubes to a depth of about 4 cm which will use about 15 ml of medium per tube.
- Place the tubes (with lids on the tubes but not tightened) in a pressure cooker and sterilize for 20 minutes. Allow the pressure cooker to cool, then remove the tubes and tighten the lids. Alternatively, the tubes can be placed in boiling water for 30 minutes, but make sure that none of the water is able to enter the tubes.

PLANT PREPARATION

Your plant material must first be surface sterilized to remove any bacteria or fungal spores present. We aim to kill all microorganisms, but at the same time does not cause any adverse damage to the plant material.

- Cauliflower should be cut into small sections of florets about 1 cm across. If using a rose or other cutting, cut the shoots into about 5- to 7-cm lengths. Whole African violet leaves can also be used.
- Wash the prepared plant material in a detergent-water mixture for about 20 minutes. If trying hairy plant material, scrub with a soft brush (toothbrush). This will help remove fungi etc., and the detergent will help wet the material and remove air bubbles that may be trapped between tiny hairs on a plant.
- Transfer the washed plant material to the sterilizing Clorox solution. Shake the mixture for 1 minute and then leave to soak for 10-20 minutes. Carefully pour off the bleach solution using the lid to keep the plant tissue from coming out and then carefully cap the container.

TRANSFER OF PLANT MATERIAL TO TISSUE CULTURE

Use the sterile gloves and equipment for all of these steps.

Place the plant material still in the Clorox bleach sterilizing container, the containers of sterile water, the sterilized forceps and blades, some sterile paper towel to use as a cutting surface and enough tubes containing sterile medium into the sterile aquarium. The gloves can be sprayed with a 70% alcohol solution and hands rubbed together to spread the alcohol just prior to placing hands into the chamber.

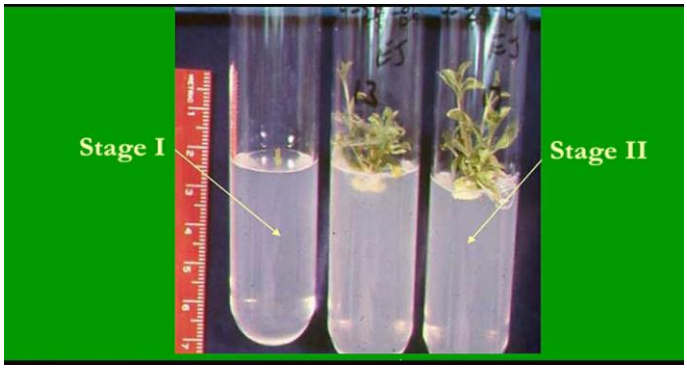
1)Carefully open the container with the plant material and pour in enough sterile water to half fill the container. Replace the lid and gently shake the container to wash tissue pieces (explants) thoroughly for 2-3 minutes to remove the bleach. Pour off the water and repeat the washing process 3 more times.

2)Remove the sterilized plant material from the sterile water, place on the paper towel or sterile petri dish. Cut the cauliflower into smaller pieces about 2 to 3 mm across. If using rose, cut a piece of stem about 10 mm in length with an attached bud. The African violet leaf

can be cut into small squares about 1-1.5 cm across. Be sure to avoid any tissue that has been damaged by the bleach, which is apparent by its pale colour.

3)Take a prepared section of plant material in sterile forceps and place into the medium in the polycarbonate tube. Cauliflower pieces should be partly submerged in the medium, flower bud facing up. Rose or other cuttings should be placed so that about 4-5mm at the base of the cutting is inserted into the medium so that it will remain upright, with the bud facing upwards. The African violet leaf pieces should be laid directly onto the medium surface.

4)Replace the cap tightly on the tube.



GROWIMG PLANTS

1)The tubes containing plant sections may be placed in a well-lit area of the classroom although not in direct sunlight. The shoots will probably grow more quickly if the explants are placed under fluorescent or grow-lights to provide at least 12 hours of light per day. The aquarium can be used as a growth chamber with the lighting about 20-25 cm (8-10") overhead.

2) Roots can appear within 6 weeks on cauliflowers. The rose, African violet, or other cuttings will need to be moved into the rooting medium for roots to properly develop

3) Working inside the sterile aquarium chamber, remove the cap from the culture tube. There will usually be several shoots that have arisen from each explant. These shoots should be carefully separated by gently removing the whole explanation from the medium with sterile forceps and then separating the shoots by gently pulling them apart using two pairs of forceps.

POTTING THE CLONES

Once roots are well formed the plants are ready to be transferred into soil.

1)Each plant should be carefully removed from its tube of medium and planted into a small pot containing a clean light potting mix. Gently wash off all of the agar medium prior to planting. The plants will still need to be protected at this stage since they are not acclimated to the drier air of the classroom when compared to the moist environment of the tube.

2)Place all of the pots onto a tray and cover loosely with a plastic dome or tent. Place the plants in an area with 12-16 hours of light (either natural or artificial) but not direct sunlight.

3)After a week, the cover can be gradually removed, and the plants acclimated to stronger light and drier atmospheric conditions.

4)You now have a collection of plants in your classroom that are genetically exactly the same. You could use these plants to carry out other experiments knowing that one common source of variation in the experiment has been eliminated.

5.2 DETERMINATION OF K_La

MEASUREMENT OF K_La

- It is extremely difficult to measure both ’K_L‘and 'a' in a fermentation and, therefore, the two terms are generally combined in the term K_La, known as the volumetric mass-transfer coefficient,
- The units of K_La, are reciprocal time (h^{-1}).

- The volumetric mass-transfer coefficient is used as a measure of the aeration capacity of a fermenter.
- The larger the K_La, the higher the aeration capacity of the system.

The K_La value will depend upon the design and operating conditions of the fermenter and will be affected by the variables such as

–aeration rate,

–agitation rate

–impeller design.

DETERMINATION OF KLa

Determination of K_La value is done by following method:

→ Sulphite oxidation technique
→ Gassing-out techniques

-- Static gassing out method

--Dynamic gassing out method

→ Oxygen balance technique

SULPHITE OXIDATION TECHNIQUE

- The oxygen transfer rates is determined by the oxidation of sodium sulphite solution
- This technique does not require the measurement of dissolved oxygen concentrations
- Based on the rate of conversion of a 0.5 M solution of sodium sulphite to sodium sulphate in the presence of a copper or cobalt catalyst.

$Na_2S0_3 + 0.5 0_2 = Na_2S0_4$

- As oxygen enters solution it is immediately consumed in the oxidation of sulphite, so that the sulphite oxidation rate is equivalent to the oxygen-transfer rate.
- Since the dissolved oxygen concentration, is zero then the KLa may then be calculated from the equation:

$dC_L / dt = OTR = K_La \cdot C^*$ (i)

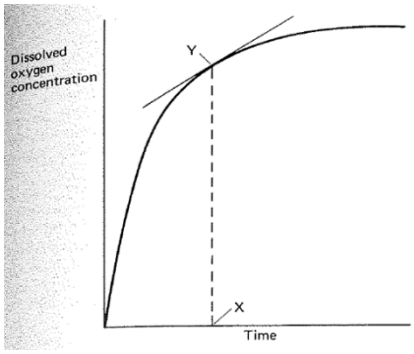
$K_La = OTR / C^*$

where OTR is the oxygen transfer rate

- The volumes of the thiosulphate titrations are plotted against sample time and the oxygen transfer rate may be calculated from the slope of the graph.

GASSING OUT TECHNIQUES

- The estimation of the KLa of a fermentation system by gassing-out techniques depends upon monitoring the increase in dissolved oxygen concentration of a solution during aeration and agitation.
- The oxygen transfer rate will decrease during the period of aeration as CL approaches C* due to the decline in the driving force (C* - CL).
- The oxygen transfer rate, at particular time, will be equal to the slope of the tangent to the curve of values of dissolved oxygen concentration against time of aeration.



The increase in dissolved oxygen concentration of a solution over a period of aeration. The oxygen transfer rate at time X is equal to the slope of the tangent at point Y.

- To monitor the increase in dissolved oxygen over an adequate range it is necessary first to decrease the oxygen level to a low value
- Two methods have been employed to achieve this lowering of the dissolved oxygen concentration

---the static method and

---the dynamic method

STATIC GASSING OUT

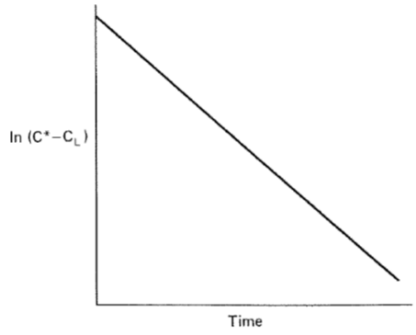
- First described by Wise (1951),
- The oxygen concentration of the solution is lowered by gassing the liquid out with nitrogen gas, so that the solution is 'scrubbed' free of oxygen.
- The deoxygenated liquid is then aerated and agitated and the increase in dissolved oxygen monitored using some form of dissolved oxygen probe.

----The increase in dissolved oxygen concentration is given by

$dCL / dt = KLa(C^*-CL)$ (ii)

--- Taking logarithm after Integration of equation (ii) we have

$\ln(C^*-CL) = - K_La.t$



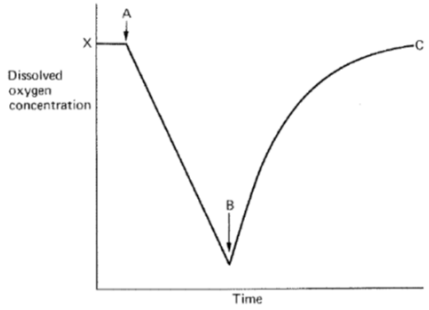
A plot of the In(C* - CL) against time of aeration, the slope of which equals -KLa.

Dynamic gassing out method

$OTR = dC_L / dt = K_La(C^*-C_L) – xQO_2$ -----(iii)

Where,

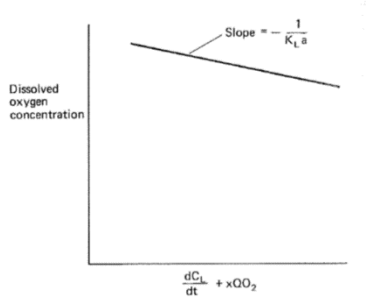
- x is the concentration of biomass and
- QO_2 is the specific respiration rate (mmoles of oxygen g-l biomass h- I).
- The term xQO_2 is given by the slope of the line AB
- Dynamic gassing out for the determination of KLa .Aeration was terminated at point A and recommenced at point B.



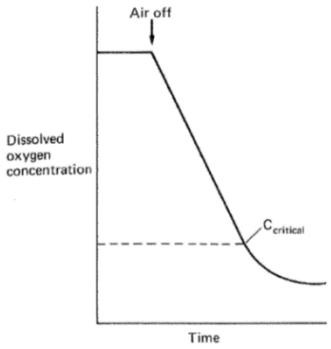
- Equation (iii) may be rearranged as:

$C_L = -1/K_La\{(dC_L / dt)+ xQO_2\}+C^*$ ----(iv)

- Now from equation (iv), a plot of C_L versus dCL/dt + xQO_2 will yield a straight line, the slope of which will equal $-1/K_La$
- The dynamic method for determination of KLa values. The information is gleaned from Fig. 9.7. by taking tangents of the curve, Be, at various values of CL'



- The occurrence of oxygen limitation during the dynamic gassing out of a fermentation.



ADVANTAGES

- The dynamic gassing-out method has the advantage over the previous methods ofdetermining the KLa during an actual fermentation and may be used to determine KLa values at different stages in the process.
- The technique is also rapid and only requires the use of a dissolved-oxygen probe, of the membrane type.

LIMITATIONS

- A major limitation in the operation of the technique is the range over which the increase in dissolved oxygen concentration may be measured.
- It may be difficult to apply the technique during a fermentation which has an oxygen demand close to the supply capacity of the fermenter
- Both the dynamic and static methods are also unsuitable for measuring K_La values in viscous systems.

OXYGEN-BALANCE TECHNIQUE

- Use to measure KLa during fermentation process.
- The amount of oxygen transferred is determined, directly into solution in a set time interval

The procedure involves measuring the folIowing parameters:

- The volume of the broth contained in the vessel,VL (dm3).
- The volumetric air flow rates measured at the air inlet and outlet, Qi and Qo' respectively (dm3 min~ 1).
- The total pressure measured at the fermenter air inlet and outlet, Pi and Po, respectively (atm. Absolute).
- The temperature of the gases at the inlet and outlet, 1; and To, respectively (K).
- The mole fraction of oxygen measured at the inlet and outlet, Yi and Yo' respectively.

The oxygen transfer rate may then be determined from the folIowing equation (Wang et al.,1979):

- $OTR = (7.32 \times 1Q^5/V_L)(QiPiyi/Ti - QoPoyo/To)$ -----(v)

- Where 7.32 X 10^5 is the conversion factor equalIing (60min h ~l) [mole/22.4 dm3 (STP)] (273 K/l atm).
- These measurements require accurate flow meters, pressure gauges and temperature-sensing devices as welI as gaseous oxygen analysers.
- The ideal gaseous oxygen analyser is a mass spectrometer analyser which is sufficiently accurate to detect changes of 1 to 2%.

The KLa may be determined, provided that CL and C* are known, from equation(1)

$dC_L / dt = K_La(C^*-C_L)$

Or $OTR = K_La (C^*-C_L)$

Or $K_La = OTR/(C^*-C_L)$

- The oxygen-balance technique appears to be the simplest method for the assessment of KLa and
- Has the advantage of measuring aeration efficiency during a fermentation.

FACTORS AFFECTING KLa VALUES IN FERMENTATION VESSELS

- A number of factors have been demonstrated to affect the KLa value. Such factors include

→ the air-flow rate employed in vessels
→ the degree of agitation inside vessels
→ the degree of agitation inside vessels
→ the presence of antifoam agents.

5.3 CORRELATION FOR K_La

THE CORRELATION OF KLa AND CELL GROWTH RATES DURING CULTIVATION

By determining kLa theoretically or experimentally in model media, an obviously assumption is that higher kLa will ensure higher biomass growth rates. Although, in real applications

different phenomena can manifest, which can negatively affect the process performance depending on applied mixing and aeration regimes. In such cases, theoretically ‘better’ bioreactor designs will not always guarantee higher biomass growth rates at a higher predetermined kLa values.

The following phenomena can potentially drive the deviations of the predicted vs. real biomass growth rate in respect to kLa:

1. RHEOLOGICAL PROPERTIES OF THE FERMENTATION MEDIA

If the viscosity of the cultivation media and the related parameters (for example, pseudo plasticity) increases during the process, the distribution of the introduced mixing energy in the reactor volume changes. For example, in the case of relatively small viscosities, most effective mixing will be achieved using standard Rushton turbines. However, at higher viscosities, other mixer rotor designs will be more suitable. The latter can be explained by the fact that Rushton turbines generate pronounced locally intense flux zones and the agitation energy value falls lower faster near the reactor walls at higher viscosities. As a result, the decrease of OTR using standard Rushton turbines can be more pronounced in this case.

2. THE MORPHOLOGICAL CHANGES OF THE MICROORGANISMS BY MIXING

Some types of microorganism cells are sensitive to intense mixing. This regard, for example, to mammalian and plant cells, mycelial microorganisms and algae cultures. Too intense mixing can induce changes to the cell morphology, resulting in a decrease in growth rate. Although, the mentioned growth rate decrease is attributed to a decrease in OUR. In such cases, mixers and their operating modes must be selected which do not yet induce critical shear stresses to the cells. For shear sensitive cultures, standard Rushton turbines are usually not the best choice. In such cases, the use of shear sensitive mixers, such as pitched-blade impellers, propellers or similar are recommended.

3. FOAMING

The high degree of aeration and agitation required in fermentations frequently gives rise to the undesirable phenomenon of foam formation. The presence of foam may also have an adverse effect on OTR. From the other side, all antifoams are surfactants and are expected to have some effect on OTR. Antifoams tend to decrease OTR, it also causes a collapse of bubbles in foam but they may favour the coalescence of bubbles with the liquid phase,

resulting in larger bubbles with reduced surface area to volume ratios and hence a reduced OTR.

OXYGEN UPTAKE RATE

During biomass growth, the microorganism oxygen demand increases. This demand is characterized by the volumetric oxygen uptake rate OUR. This is described by the term:

$OUR = QO2 \cdot X.$

Where

QO2 – specific oxygen uptake rate (mmoles O2/g biomass, h-1).

X – biomass concentration, g/L.

QO2 depends on the microorganism strain and applied substrate. In the following table the specific oxygen uptake rates QO2 for different microorganisms and substrates are summarized:

Type of microbial culture	Carbon source	QO2, (mmol/g·h)
Aspergillus niger	Glucose	1.6
Beneckea natriegens	n-Propanol	12
Penicillium chrysogenum	Lactose	1.2
Saccharomyces cerevisiae	Ethanol	10
Streptomyces aureofaciens	Corn starch	7.0
Streptomyces coelicolor	Glucose	7.4

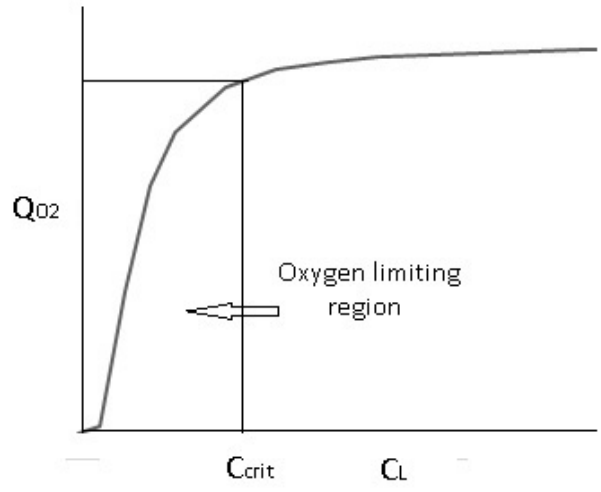
Streptomyces griseus	Meat extract	4.1
Xanthomonas campestris	Glucose	4.5

The task of the bioreactor is to ensure the supply of oxygen to the cells until no other growth limiting factors come into play. This characterizes the volumetric oxygen transfer rate OTR:

$OTR = kLa \cdot (C^* - CL)$

Where the designations are according to equation.

This means that until OTR > OUR, the growth of microorganisms will continue. If the OTR value is higher, then higher biomass yields will be possible to achieve. By analysing equation, it can be seen that OTR will be proportional to kLa and (C* - CL). By applying equation, it is necessary to take into account the cell specific oxygen consumption QO2 dependence on CL. When CL is less than Ccrit, QO2 decreases



Relation between the specific oxygen consumption QO2 and

dissolved oxygen concentration CL.

Ccrit can differ depending on microorganism strain and process conditions. Often Ccrit is about 10-20% from the equilibrium oxygen concentration C*. C* typically varies in range 6 – 9 mg/l.

By knowing kLa, C* and Ccrit, the maximum achieved biomass concentration (or in this case called critical biomass concentration Xcrit) can be determined. In opposite, if we know what biomass concentration Xmax must be achieved in the certain cultivation process, then the necessary kLa of the bioreactor can be evaluated by maximal operating values of mixer rotation speed n and gas flow rate Q (of course, if another limiting factor does not comes into play).

Taking into account the above-mentioned information, it can be concluded that the maximal biomass concentration will be achieved, if OTR = OUR and CL = Ccrit.

$kLa \cdot (C^* - Ccrit) = QO2 \cdot Xcrit$

and from equation (20) follows:

$Xcrit = kLa \cdot (C^* - Ccrit) / QO2$

The biomass concentration calculated by equation is, of course, a theoretical maximum and it will be achieved only if all other cultivation conditions are maintained optimal.

The calculation example using equations of OUR

A strain of Streptomyces coelicolor is cultivated in a 2000 litre bioreactor according to the example mentioned above using glucose as the substrate. Equilibrium oxygen concentrations C* is 8 mg/L. Ccrit is 20 % from C*.

WHAT IS THE MAXIMUM BIOMASS CONCENTRATIONS THAT CAN BE ACHIVED IN THIS CASE?

Let’s assume that Xmax = Xcrit. Firstly, all parameters are to be defined in SI units:

$kLa = 0.074\ s^{-1}$

C* = 8 mg/L; (mg/L = 10 [kg/106mg] / 1 [m3/103 L] = 103/106 = 10^{-3} kg/m3); = $8 \cdot 10^{-3}$ kg/m3

$Ccrit = 0.2 \cdot 8 = 1.6 \cdot 10^{-3}$ kg/m3

QO2 according to the table seen above for S. coelicolor is 7.4 mmol/g·h.

1 mol O2 = 32 g O2; 1mmol O2 = 32 · 10-3 g O2; 1mmol O2 = 32 · 10-6 O2 kg

1 g=10-3 kg; 1h=3.6 · 103 s.

QO2 = 7.4 mmol/g·h = (7.4 · 32 · 10-6)/ (10-3 · 3.6 · 103) = 65.8 x 10-6 kg/kg·s

Xcrit = (0.074· (8-1.6) · 10-3)/ (65.8 · 10-6) = 7.2 kg/m3 = 7.2 g/L. This means that the maximal biomass concentration of 7.2 g/L can be achieved during Streptomyces coelicolor cultivations in the defined bioreactor.

CONCLUSIONS

From the information presented above, the following conclusion can be drawn regarding kLa as a bioreactor efficiency parameter:

1. The theoretical calculation equations of kLa with satisfactory accuracy are applicable to popular mixer rotor types (Rushton turbine, pitched blades, etc.) and for bioreactor volumes of up to 3000 litres.

2. Experimental methods are labour-intensive, but if the mixing system consists of different or not widely applied mixers rotors, then it is recommended to determine kLa experimentally for scale-up purposes.

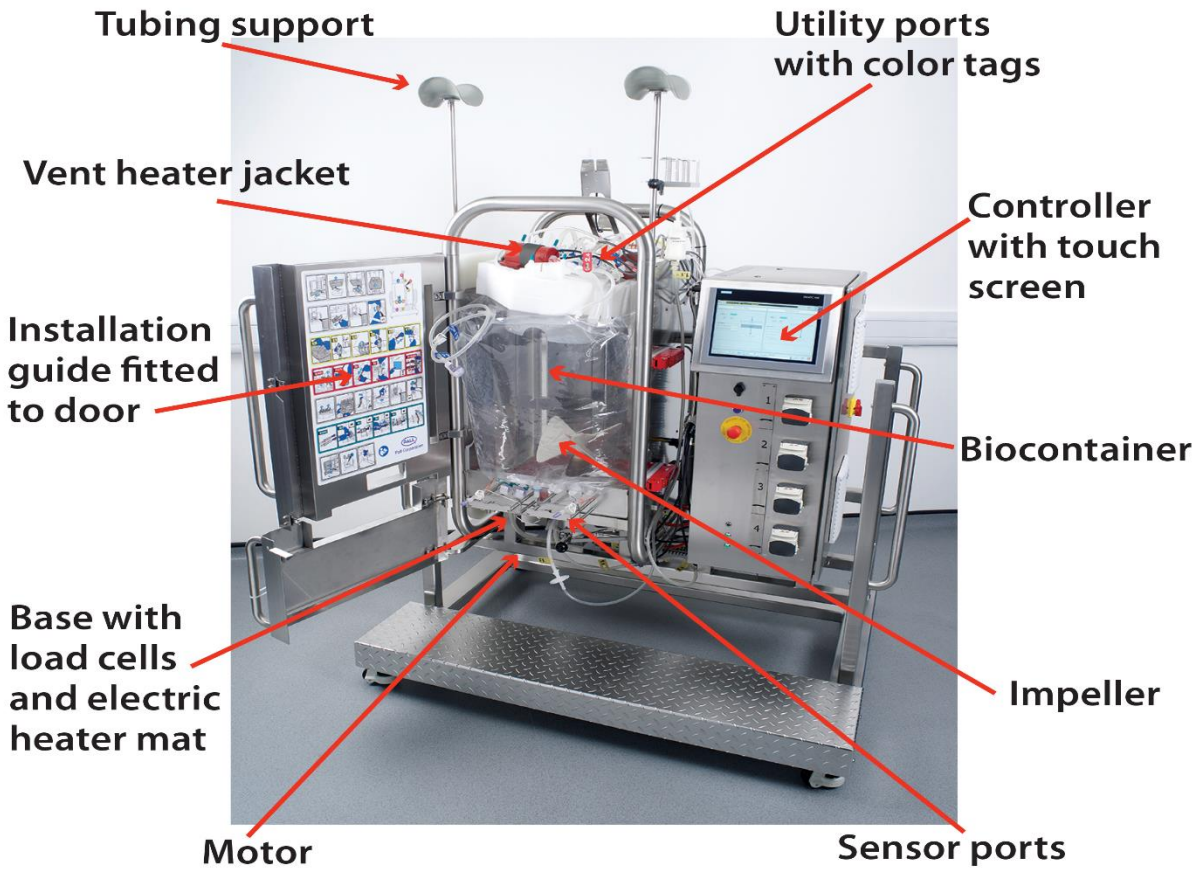
3. By using experimental methods it is possible to determine kLa during running cultivation processes (dynamic gassing-out and oxygen balance methods).

4. The determined kLa usually correlates sufficiently with the growth of biomass during the cultivation process, as long as it is not significantly affected by cultivation-related phenomena (rheology, morphology, foaming).

5. Oxygen uptake rate differs for different cultivation processes depending on the microorganism type and applied carbon source. This means that when selecting the scale-up bioreactor and it`s parameters for a particular process, it is desirable to know QO2 of the applied microorganism strain. The latter will dictate what kLa bioreactor should be chose to provide sufficient OTR for obtaining targeted biomass/product yields.

5.4 INTRODUCTION TO SINGLE USE BIOREACTORS

A single-use bioreactor or disposable bioreactor is a bioreactor with a disposable bag instead of a culture vessel. Typically, this refers to a bioreactor in which the lining in contact with the cell culture will be plastic, and this lining is encased within a more permanent structure, either a rocker or a cuboid or cylindrical steel support.



In general, there are two different approaches for constructing single-use bioreactors, differing in the means used to agitate the culture medium.

They are:

1. Stirrer type

2. Rock motion type

Some single-use bioreactors use stirrers like conventional bioreactors, but with stirrers that are integrated into the plastic bag. The closed bag and the stirrer are pre-sterilized. In use the bag is mounted in the bioreactor and the stirrer is connected to a driver mechanically or magnetically.

Other single-use bioreactors are agitated by a rocking motion. This type of bioreactor does not need any mechanical agitators inside the single-use bag.

Both the stirred and the rocking motion single-use bioreactors are used up to a scale of 1000 Litres volume.

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Types of Single Use Bioreactors

Stirrer Type

Rocking Motion Type

MEASUREMENT AND CONTROL

Measurement and control of a cell culture process using a single-use bioreactor is challenging, as the bag in which the cultivation will be performed is a closed and pre-sterilized system. Sensors for measuring the temperature, conductivity, glucose, oxygen, or pressure must be built into the bag during the manufacturing prior to sterilization. The sensors can't be installed prior to use of the bioreactor as in the conventional case. Consequently, some challenges must be taken into consideration. The bag is assembled, delivered and stored dry, with the consequence that the usual pH-electrodes cannot be used. Calibration or additional assembly is not possible. These constraints have led to the development of preconfigured bags with new types of analytical probes.

ADVANTAGES OF SINGLE USE BIOREATORS

• Eliminates validation issues because the cleaning process is avoided.

• Shortens downtime and turnaround time because no cleaning is required.

• Lowers risk of cross-contamination with use of new bags for each run.

• Offers multiple advantages based on the elimination of the cleaning process.

• Decreased operating costs and capital investment: savings on start-up capital costs as well as utilities, space, and labour requirements.

• Cost savings from reduced cleaning

• Validation is reduced because systems are less complex.

• Ease of installation

• Ease of moving when empty.

DISADVANTAGES OF SINGLE USE BIOREACTOS

• Limitation in liquid transfer.

• Scalability is an issue.

• Expensive to use.

• Performance is not completely proven.

• Slight increase in variable costs per run.

• Leachable and inability to store hot liquids.

• Potential for puncture.

• Difficulty in moving when full.

• Pressure and temperature sensitivity.

• Disposal costs.

ENVIRONMENTAL ASPECTS OF SINGLE USE BIOREACTORS

Environmental aspects for single-use bioreactors are important to consider due to the amount of disposable material used compared with conventional bioreactors. A complete life cycle assessment comparing single-use bioreactors and conventional bioreactors does not exist, but many ecological reasons are supporting the concept of single-use bioreactors. For a complete life cycle assessment not only the manufacturing, but also the repeated use needs to be considered. Even the main part of a single-use bioreactor is not a disposable but will be continuously reused.

The plastic bag that is used instead of a culture vessel is a disposable, as well as all the integrated sub-assemblies like sensors, tubing, and stirrers. The bag and all its parts are mainly made from plastics that are derived from petroleum. Current recycling concepts are mainly focused on incineration, to recover the energy originated from the petroleum as heat and electricity. Most of the petroleum would be burned anyway in power plants or automobiles (citation required). Burning of the single use components of bioreactors creates a detour through biochemical engineering during their life cycle that does not have a big influence.

PROGRESS FOR THE SINGLE USE BIOREACTORS

• Wide acceptance of bioprocess bags

• Single use bioreactors are scalable, and performance is comparable to Stainless Steel bioreactors.

• 1000L Bioreactor scales can open up opportunities in commercial applications.

COMMERCIALLY AVAILABLE SINGLE USE BIOREACTOR SYSTEMS

- XDR - Xcellerex
- Mobius - Millipore
- CelliGen BLU - Eppendorf
- Hyclone S.U.B - Thermo Scientific
- Single Use Bioreactor - Applikon