

(1)

Growth of Cells (microbial, plant & animal)

Batch growth - culturing cells in a vessel with initial charge of medium.

CLOSED SYSTEM - Nothing is added or withdrawn during cultivation

Quantification of cell concentrations

Essential to establish kinetics & stoichiometry.

Methods of measurement → Direct
↓ Indirect

Cell mass or cell number - Both desirable

Preferred, easy!

Haemocytometer - Direct cell counting, no. of cells

in calibrated grid is counted using a microscope
At least 20 grids should be counted & Avg reported

Depth & Area known. Volume can be calculated

Precautions - culture medium should not contain suspended particles.

Stains can be used to distinguish dead & live cells

Methylene blue for m.o. Tryphan blue for animal cells.

Method useful for non-aggregated cells, plant cells / molds can not be counted.

Plate count method - Plates gelled with agar are used for counting of viable cells.

Culture sample diluted 1:10, 1:100, 1:1000 & spread on agar surface & plates incubated colonies counted on agar surface following incubation (CFU), suitable for bacteria & yeast not for molds. Time consuming

Alternative method - Agar gel medium is placed on a small ring mounted on a microscope slide. Cells are spread on this miniature culture dish. After incubation for few doubling time slide is examined with a microscope to count cells

COLONY HAVING LIMITED REPRODUCTION

ARE VISUALIZED

Plant cell "dye" used for viability - Triphenyl Tetrazolium chloride
Animal cell - Tryphan Blue Microbial cell - Methylene blue

Ref:-
 Biochemical
 Engineering
 Fundamentals
 Bailey &
 ollis.

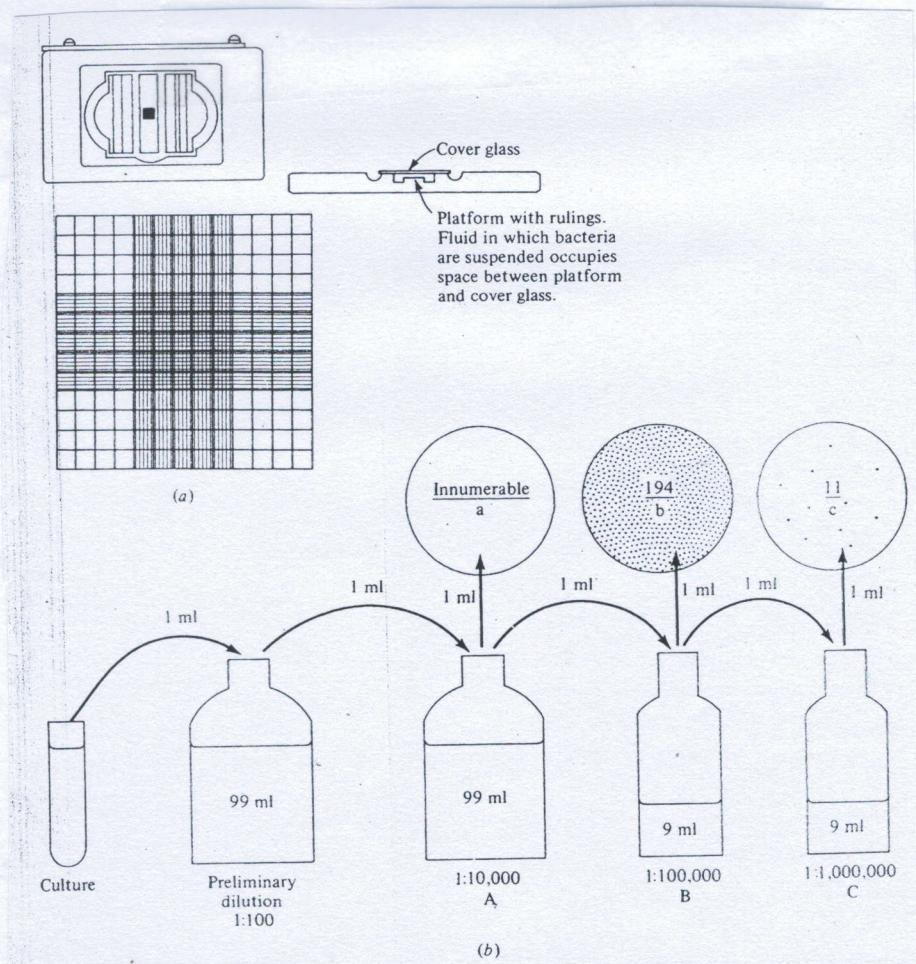


Figure 7.1 Techniques for measurement biomass concentration. (a) In the hemacytometer a grid facilitates cell counting. (b) In the colony-count method, greatly diluted samples from the original culture are plated on nutrient agar in Petri dishes, where the number of colonies indicates the number of viable cells in the diluted sample. (c) Measurement of turbidity of a cell suspension provides a measure of population density. [Reprinted by permission from M. Frobisher, "Fundamentals of Microbiology," 8th ed., pp. 46, 47, 49, W. B. Saunders Co., Philadelphia, 1968; (a) also courtesy of Arthur H. Thomas Co., Philadelphia.]

Disadvantages - ① Direct count method does not distinguish between live & dead cells.

COULTER COUNTER -

- Principle - Relatively high electrical resistance of cells. Employs two electrodes & electrolyte.
- one electrode is in the tube containing orifice. A vacuum is applied to the inner tube which causes an electrolyte solution containing the cells to be sucked through the orifice
 - An electric potential is applied across the electrodes. As the cell passes through the orifice electric resistance increases which causes pulses in electrical voltage.
 - No. of Pulse is a measure of no. of particles.
 - Particle count is calculated as the counter is activated for a predetermined sample volume
 - Height is a measure of cell size
- Disadvantage - No distinction between cells & particles
- different orifice sizes are used for different cells.

Ref -

Fermentation &
Enzyme Technology
by Wang, Cooney
Deonair, Dunkill
Humphrey & Lilly

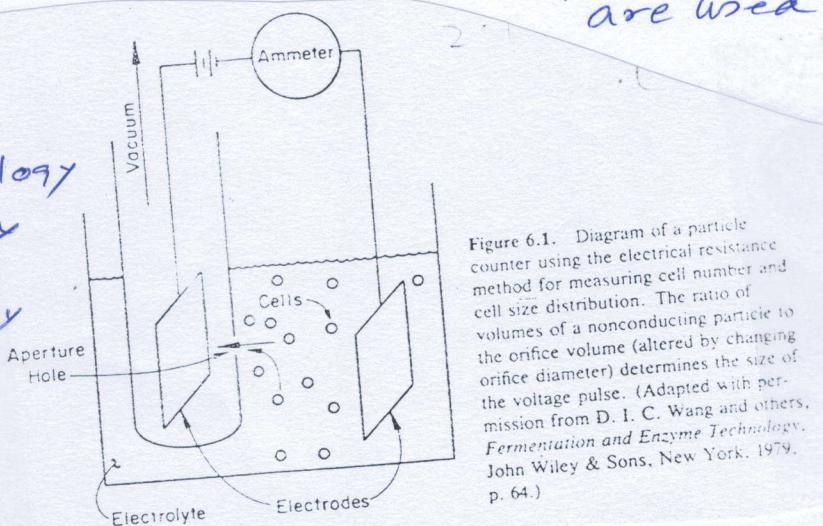


Figure 6.1. Diagram of a particle counter using the electrical resistance method for measuring cell number and cell size distribution. The ratio of volumes of a nonconducting particle to the orifice volume (altered by changing orifice diameter) determines the size of the voltage pulse. (Adapted with permission from D. I. C. Wang and others, *Fermentation and Enzyme Technology*, John Wiley & Sons, New York, 1979, p. 64.)

Cell mass :-

Dry cell weight : Samples of whole culture broth collected, centrifuged, washed with buffer/water & then dried at 80°C for 24 hours or at 110°C for 8 hours

Disadvantage - Non cellular solids e.g. Calcium Carbonate molasses solids, corn steep liquor cellulose or soy bean meal are not removed during washing & may contribute to final dry weight.

Turbidity - OD or Turbidity of a culture broth is proportional to the cell mass. Method is simple & inexpensive to estimate cell mass on a fast- & routine basis. $\lambda = 600 - 750 \text{ nm}$ Blank with air or water & the ratio of I to I_0 is known as transmittance & depends on path length of the light through the solution. This is expressed as Lambert's law

Lambert's law — When a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as the length of the medium increases.

A similar law by Beer relates the transmittance to the conc' of the solution, which states Beer's law — "When a ray of monochromatic light passes through an absorbing medium, the intensity decreases exponentially as the concentrations of the absorbing medium increases."

These two laws are conveniently combined in the "Beer-Lambert law" so that if ℓ is the path length & 'c' is the conc' of the solution

$$\text{then } T = \frac{I}{I_0} = e^{-k'c\ell}$$

$$\text{or } \log_e \frac{I_0}{I} = k'c\ell$$

$$\text{or } \log_{10} \frac{I_0}{I} = k c \ell$$

$\log_{10} \frac{I_0}{I}$ is known as the extinction or absorbance. The extinction is sometimes called as optical density. If the Beer-Lambert law is obeyed then a plot of extinction against conc' gives a straight line passing through origin.

The photoelectric colorimeter. A diagram of the basic arrangement of a typical colorimeter is given in Fig. 4.1. White light from a tungsten lamp passes through a slit then a condenser lens to give a parallel beam.

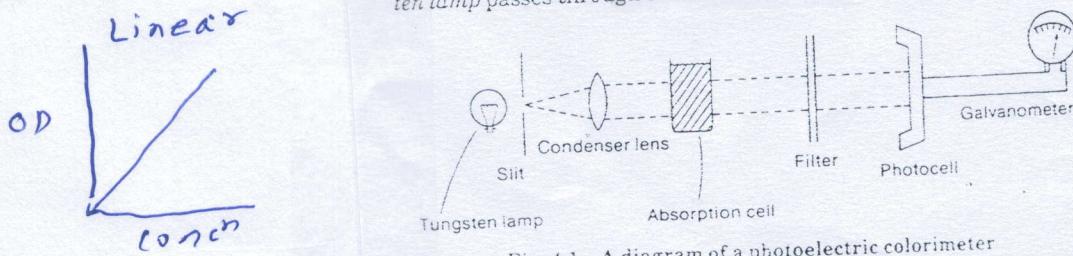
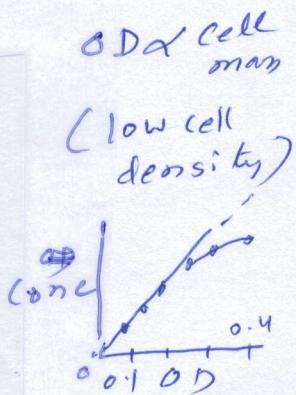


Fig. 4.1 A diagram of a photoelectric colorimeter



With the help of standard curve the conc' of unknown sample can be readily determined. If 'l' is 1cm & 'c' expressed as g/mol/l then the absorbance = K , the molecular extinction coefficient (which is a characteristic of the compound).

Precautions — Beer-Lambert law only applies for mono chromatic light & providing there is no change in ionization, dissociation or solvation of solute with concentration.

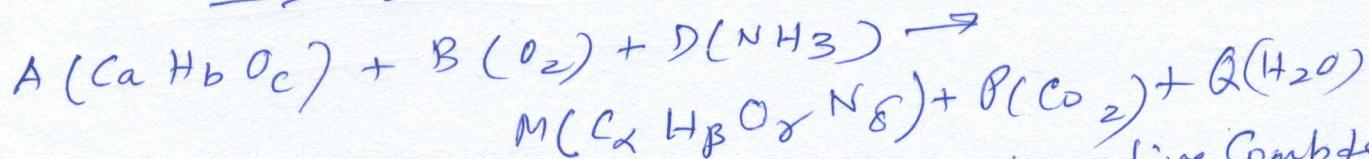
Estimation of cell mass: "Indirect methods"

(4)

The cell mass measurement methods described above are not applicable to mould or plant cells etc. It is necessary to resort of indirect methods to assess growth wherein following stoichiometry applies.

C-source + N source + phosphate + O_2

→ Cell mass + CO_2 + H_2O + product + heat

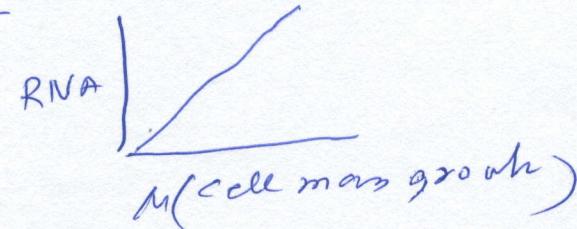


A, B, D, P & Q are mols of respective Compts
 $M \rightarrow$ mols of a cell unit

Nutrient Uptake & growth

Phosphate sulfate, Mg

Product formation



DNA & protein are also correlated to growth.

"NADH" can be used as online indicator of Biomass. Excitation wavelength 340 nm Emission 460 nm
 - NADH fluorescence = $f(pH, Temp, Medium, fluor, Biomass)$

Advantages - No noise $\rightarrow \downarrow$ const H

lag in measurement & cell metabolic events in bioreactor

measured by IR analyzer Broth Fluor - medium fluor

- CO_2 production \uparrow of growth

- Utilization of NH_3 & growth

- drop in pH & growth in mycelial growth Alkaloid & growth Biomass

- Polysaccharide production & growth (Xanthum, Pullulan)

- Viscosity increase & growth

Heat Evolution - Major product of growth is heat

Heat of combustion of m.o. is const H (5 kcal/g)

Heat evolution is dependant on efficiency with

which the C-source is utilized Heat & growth.

Measurement in fermenters by energy balance on cooling water & dynamic calorimetry (Cooney 1968)

$$Q_{acc} = Q_f + Q_{ref} + Q_{evap} + Q_{sent} + Q_{surr}$$

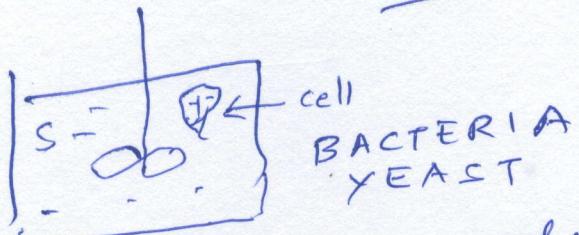
Packed cell volume :- particularly for Plant cell cultures (5)

- Centrifuge whole broth under standard conditions of rotation & time in tapered graduated tube. Volume occupied by solids \rightarrow cell mass. Cell sp. gravity is 1.05 - 1.1. The above procedures can be used when suspended solids are present in broths as they settle more rapidly than cells. Their volume can be visually detected & subtracted from total.

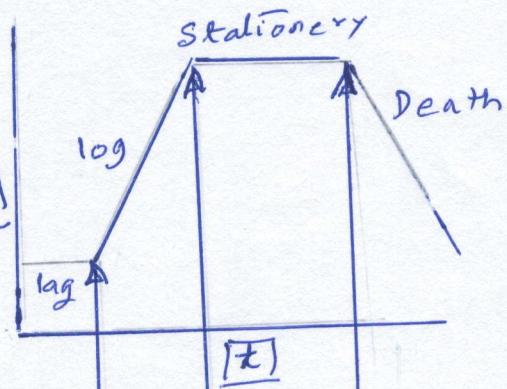
Viscosity measurements - Useful for mycelial growth

or polysaccharide formation. Viscosity increases during fermentation. Most broths exhibit Non Newtonian characteristics (Shear stress is not \propto to shear rates) However apparent viscosity at a fixed shear rate can be correlated to cell or product concn.

BATCH FERMENTATION



Growth kinetics needed $\log N$
for the design and operation
of reactors.

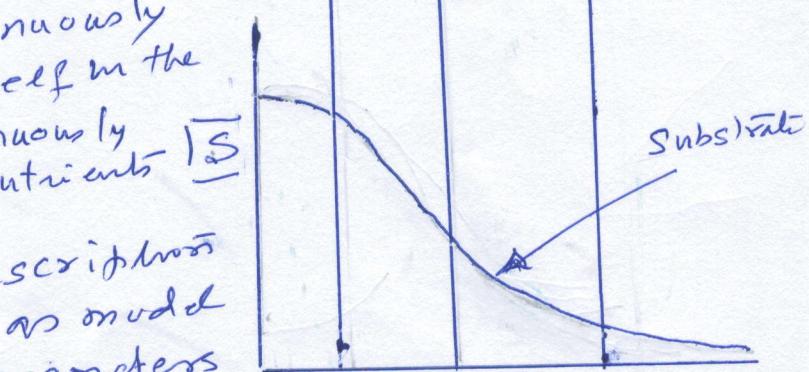


Young & old cells continuously changing & adapting itself in the media environment, continuously adapting in the existing nutrients available in the reactor

Accurate mathematical description (Model) - not possible as model becomes too complex, parameters impossible to determine

Assumptions - Unstructured, Distributed cell is represented by x (Cell \downarrow on model uniformly throughout the culture)

True only under
Balanced growth
conditions.



Besides medium is formulated in such a way that only "one component" may be limiting the reaction rate. All other components are at high concn (always!).

① Strategies to minimize the lag phase

- The inoculating culture should be as active as possible & the inoculation should be carried out in the exponential growth phase
- The culture medium used to grow the inocula should be as close as possible to the final full scale fermentation composition
- Use of reasonably large inocula (order of 5% of the new medium volume) is recommended to avoid undue loss by diffusion of required intermediates or activators.

② Log phase

The log phase is characterised by a straight line on semilog plot of $\ln x$ vs time. This is a period of balanced or steady growth during which μ remains constant.

Balanced growth - is observed when all the properties of cell are increasing by same ratio if biomass is doubling the RNA & DNA should double during the same time only under balanced growth conditions cell can be represented as cell mass, cell no. or conc' of protein, DNA or RNA

every \leftarrow extensive property of growing system increases by same factor during that interval. It is also important that medium is formulated in such a way that one component may be limiting the reaction rate & all other components are at high conc' (Synthetic & Complex medium)

Generally $\frac{dx}{dt} \neq \frac{dn}{dt}$ However Engineers

$$\text{assume } \frac{dx}{dt} \propto \frac{dn}{dt}$$

$$C_N = C_{N_0} 2^N \quad \left\{ \begin{array}{l} N = \text{No. of divisions} \\ N_0 = \text{Initial No. of cells} \end{array} \right.$$

Binary fission

In exponential growth phase

$$\text{X} \quad \mu(t-t_0) \quad r_n = \frac{dn}{dt} = \mu C_n \quad \text{or} \quad \frac{dx}{dt} = \mu x$$

$$\frac{C_n}{C_{N_0}} = e^{\mu t}$$

$$\frac{2}{1} = e^{\mu t_d}$$

$$t_d = \left[\frac{\ln 2}{\mu} \right]$$

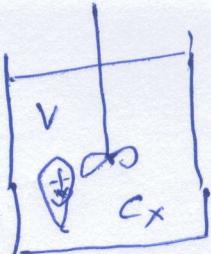
t_d = doubling time

$$\text{or} \quad \int_{C_{N_0}}^{C_N} \frac{dc_n}{C_n} = \int_{t_0}^t \mu dt \quad \text{At } t=t_0 \quad C_n = C_{N_0} \quad t=t \quad C_n = C_N$$

$$\left[\ln \frac{C_n}{C_{N_0}} \right]_{C_{N_0}} = \mu (t - t_0)$$

$$\left. \frac{C_n}{C_{N_0}} = e^{\mu(t-t_0)} \right\} \text{or} \quad \frac{C_n}{C_{N_0}} = e^{\mu t} \quad \text{if } t_0 = 0$$

(7)



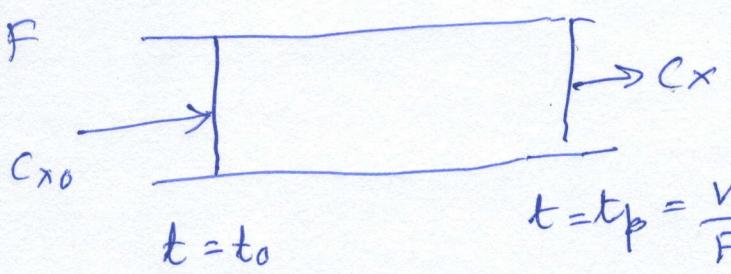
Batch

Contents Uniform
in composition at all
times

$$\text{Batch } \frac{dx}{dt} = r_x = \mu C_x$$

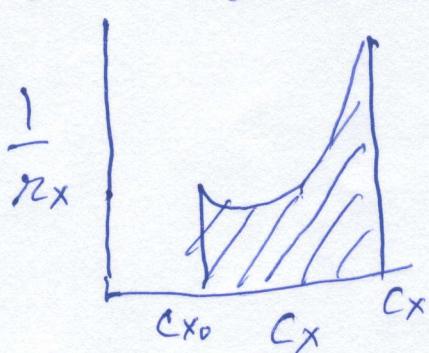
$$\text{or } \frac{dx}{dt} = \mu x \quad | \quad \int_{C_{x0}}^{C_x} \frac{dx}{r_x} = \int_{t_0}^t dt = (t - t_0) \quad (1)$$

't' is not the time when culture was inoculated but when culture starts to grow. Steady state PFF is mode. Cell concn of an ideal batch fermenter after time t will be the same as that of steady state PFF at a longitudinal location where residence time $\tau = t$



Plug flow -

- Nutrient & m.o. enter from one end of cylindrical tube
- cell grow while they pass
- No complete mixing
- Properties vary in longitudinal & radial directions
- radial variations less.
- ideal tubular flow fermenter with out radial variations is called P.F.F.



'U' curve is observed for autocatalytic reactions.

$S + X \rightarrow X + X$
rate for above reaction is slow at the start because 'x' is less if Monod's kinetics apply

$$\text{Then } \int_{C_{x0}}^{C_x} \frac{K_s + C_s}{M_m C_s C_x} dC_x = \int_{t_0}^t dt \quad (2)$$

$$\gamma_{X/S} = \frac{\Delta C_x}{\Delta C_s} = - \frac{C_x - C_{x0}}{C_s - C_{s0}}$$

$$-(C_s - C_{s0}) = \frac{1}{\gamma_{X/S}} (C_x - C_{x0})$$

$$C_s = C_{s0} - \frac{1}{\gamma_{X/S}} (C_x - C_{x0})$$

Substitute C_s in Eqn (2) & integrate

$$(t - t_0) M_{max} = \left(\frac{K_s \gamma_{X/S}}{C_{x0} + C_{s0} \gamma_{X/S}} + 1 \right) \ln \frac{C_x}{C_{x0}} + \frac{K_s \gamma_{X/S}}{C_{x0} + C_{s0} \gamma_{X/S}} \ln \frac{C_{s0}}{C_s}$$

Yield Concept

Growth yield is defined by $Y = \frac{\Delta x}{\Delta s}$

Where Δx is the increase in biomass by utilization of Δs amount of substrate

$$Y = -\frac{dx}{ds}$$

Growth yield is an important parameter & it expresses the qualitative nutrient requirement of an organism. If x_0 & s_0 are the initial biomass & substrate concn respectively & x, s are the biomass & substrate concn after time 't'

then during growth

$$(x - x_0) = Y(s_0 - s)$$

$$\text{or } Y = -\frac{(x - x_0)}{(s - s_0)}$$

Similarly for product

$$Y_{P/x} = \frac{\Delta P}{\Delta x} = \frac{P - P_0}{x - x_0} \quad P_0 = \text{very small} \approx 0$$

$$Y_{P/s} = \frac{\Delta P}{\Delta s} = \frac{\Delta P}{\Delta x} \cdot \frac{\Delta x}{\Delta s} = Y_{P/x} \cdot Y_{x/s}$$

$$\boxed{Y_{P/s} = Y_{P/x} \cdot Y_{x/s}}$$

Maintenance

Energy — The energy source which is used other than growth of cell is called maintenance energy

m = coefficient.

$$\left(\frac{ds}{dt}\right)_m = mx$$

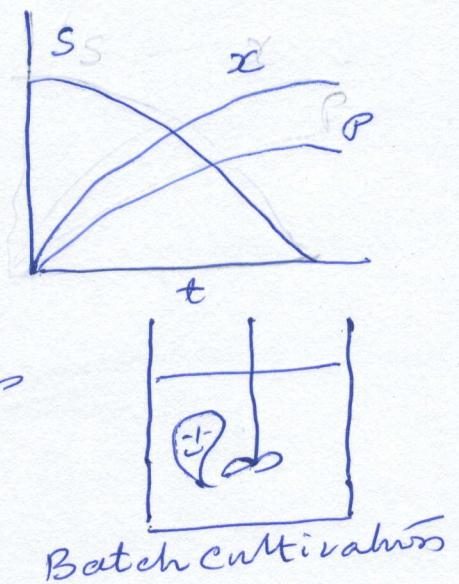
The total energy source consumed Δs_e is supposed to contribute a ration of growth Δs_g plus a ration of energy for maintenance Δs_m . Maintenance of cell requires energy for many purposes

(1) For turn over of cell material

(2) Preservations of right ionic composition & intracellular pH.

(3) Motility

(4) Maintenance of large intracellular pool of metabolites against a concentration gradient.



Batch cultivation

From Definition we can write -

$$Y_E = \frac{\Delta x}{\Delta S_E} = \frac{\Delta x}{\Delta S_G + \Delta S_m}$$

Δx = Amount of biomass formed

where maintenance Energy = 0 ie $\Delta S_m = 0$
we have true growth yield

$$Y_{EG} = \frac{\Delta x}{\Delta S_G}$$

Y_{EG} is the maximum possible value of growth yield for the energy source.

Overall Balance for energy source utilization is given by

$$\text{Total rate of substrate consumption} = \frac{\text{Rate of substrate consumption for growth}}{\text{Rate of substrate consumption for maintenance}}$$

$$\left(\frac{ds}{dt} \right)_{\text{total}} = \left(\frac{ds}{dt} \right)_G + \left(\frac{ds}{dt} \right)_m$$

$$\left(\frac{ds}{dx} \cdot \frac{dx}{dt} \right)_T = \left(\frac{ds}{dx} \cdot \frac{dx}{dt} \right)_G + \left(\frac{ds}{dt} \right)_m$$

$$\left(\frac{1}{Y_E} \cdot \mu x \right) = \left(\frac{1}{Y_{EG}} \cdot \mu x \right) + m x$$

$$\frac{1}{Y_E} = \frac{1}{Y_{EG}} + \frac{m}{\mu}$$

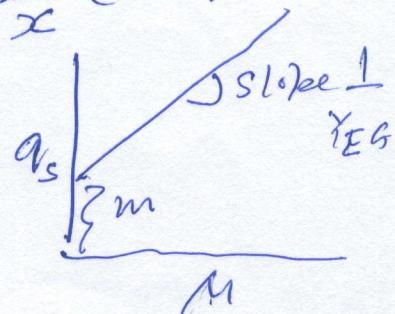
Also $\frac{ds}{dt} = \frac{1}{Y_{EG}} \cdot \mu x + m x$

from above Equations

divide by $\frac{dx}{dt}$

$$\frac{1}{x} \frac{ds}{dt} = \frac{1}{x} \left(\frac{1}{Y_{EG}} \cdot \mu x \right) + \frac{1}{x} (m x)$$

$$q_s = \frac{\mu}{Y_{EG}} + m$$



Growth Models

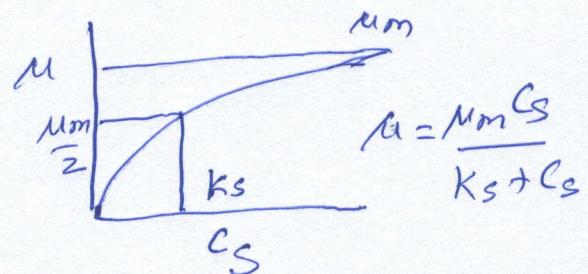
(a) Monod's model

Assumptions -

- (1) Microbial cells are like particles
- (2) Homogeneously distributed in a bioreactor

Then $\mu = \frac{\mu_m C_s}{K_s + C_s}$ only under

True
balanced growth
conditions & when inhibitory toxic metabolites
are present in low concns.



Other Growth Models

$$\mu = \mu_m (1 - e^{-C_s/K_s}) \quad \text{Tessier}$$

$$\mu = \mu_m (1 + K_s S^{-1})^{-1} \quad \text{Moser}$$

$$\mu = \frac{\mu_m S}{B x + S} \quad \text{Contois}$$

where $B = \text{Apparent Michaelis' const}$
of x (biomass concⁿ)

Algebraic
solution of
the eqns.
much more
difficult
than Monod

Modifications of Monod's model

Monod's model says if S is significantly high μ does not increase further which is not true. Some times high ' S ' inhibits also. Some growth models include a term ' K_e ' which accounts for the maintenance of cells

$$\mu = \frac{\mu_m C_s}{K_s + C_s} - K_e \quad \text{This gives}$$

better fit of
data - but difficult
to solve.

Addition of Product concⁿ terms to Monod's model

$$\mu = \mu_m \left(\frac{S}{K_s + S} \right) \left(\frac{K_1}{K_2 + P} \right)$$

$$\mu = \frac{\mu_m S}{K_s + S} (1 - K_i I)$$

$$\mu = \left(\frac{\mu_m S}{K_s + S} \right) \left(\exp(-K_i I) \right)$$

$$\mu = \left(\frac{C_s}{K_s + C_s} \right) \left(\frac{K_P}{K_P + C_P} \right)$$

$$\mu = \mu_m \left(\frac{C_s}{K_s + C_s} \right) \left(1 - \left(\frac{C_P}{C_{Pm}} \right)^n \right)$$