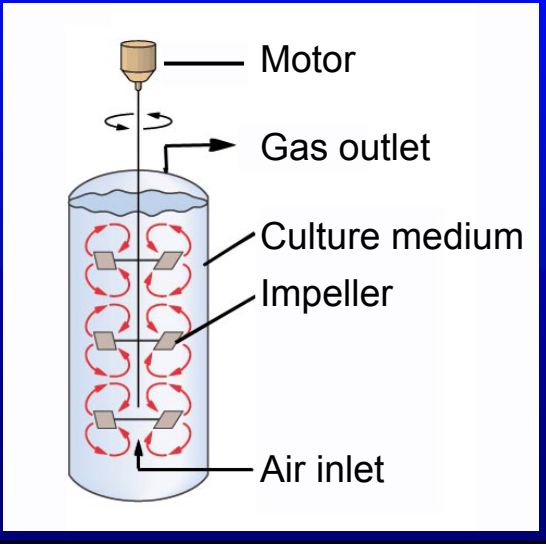
Bioreactors

- Stirred Tank bioreactor
- Bubble column
- Airlift reactor
  - internal-loop
  - external-loop

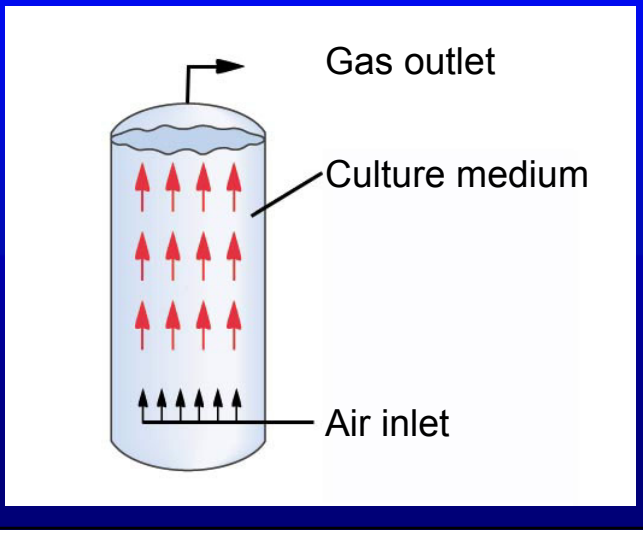
Stirred-Tank Bioreactor



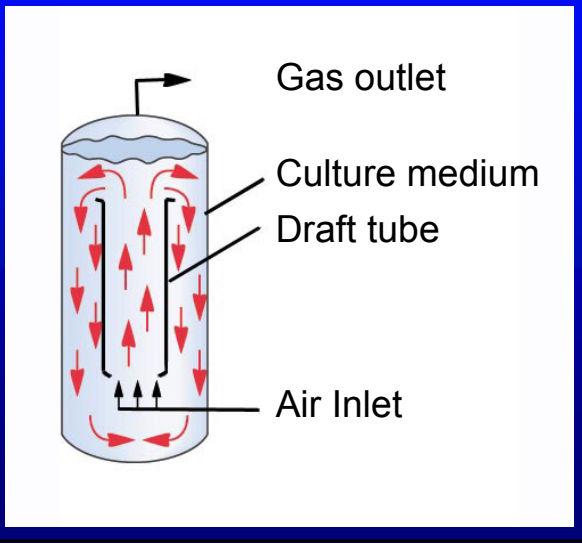
Advantages of Stirred Tank

- highly flexible operating conditions
- readily available commercially
- provides efficient gas transfer to cells
- history of use with a variety of microorganisms

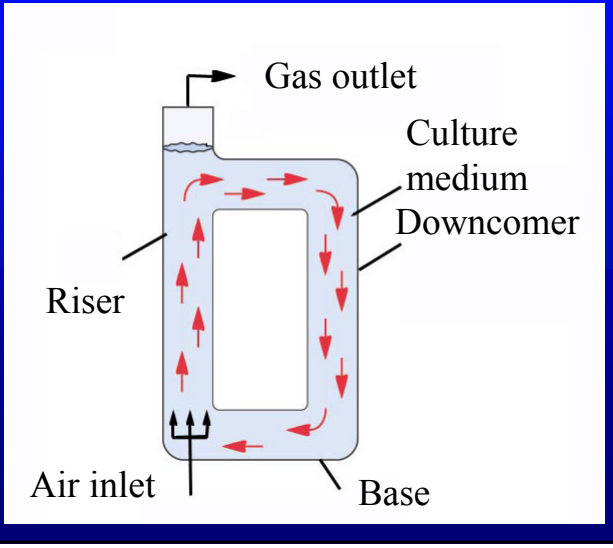
Bubble Column Bioreactor



Internal-loop Airlift Bioreactor



External-loop Airlift Bioreactor



Advantages of Bubble Column and Airlift

- more energy efficient
- no mixer shaft, one less potential site for contamination
- lower shear environment than stirred tank

Benchtop Bioreactors

1.3 - 14 liters
pH probe
dissolved O_2 probe

new brunswick scientific
http://www.nbsc.com/

Bioreactors

40 to 120 liters
mobile pilot plant
probes for:
- pH
- dissolved O_2
- temperature
- agitation

new brunswick scientific
http://www.nbsc.com/

Bioreactors

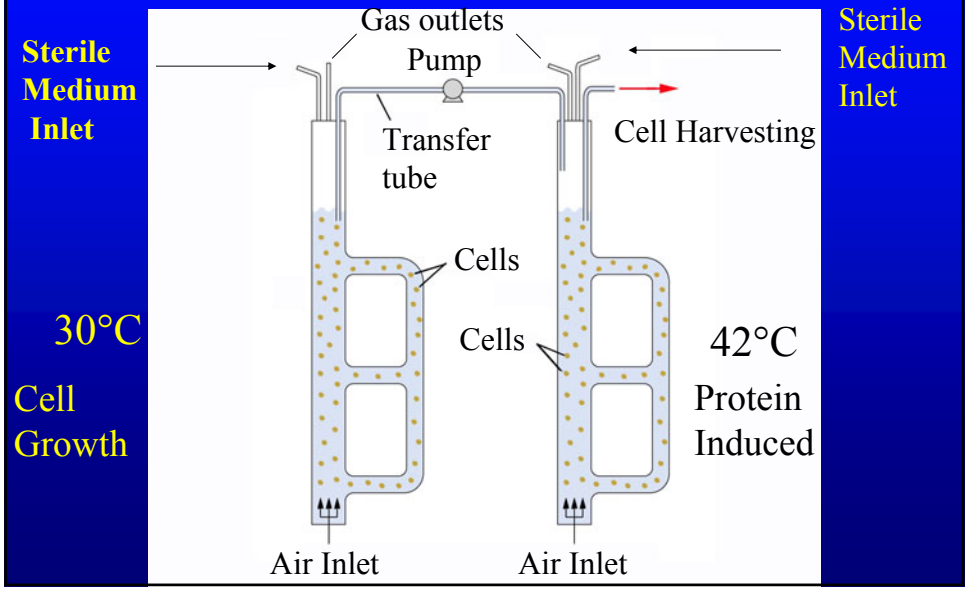
75 to 500 liters

new brunswick scientific
http://www.nbsc.com/

- Microbial Growth Kinetics
- Maximizing Fermentation Efficiency
- Bioreactors
- Large Scale Fermentation Examples
- Harvesting Cells
- Disruption of Microbial Cells

- Production of T4 DNA Ligase
  - P_L promoter of phage l
  - Regulated by temperature sensitive cI repressor protein of phage l
    - 30°C permissive temp - no expression
    - 42°C restrictive temp - expression

Two stage airlift bioreactor with temperature induction of protein expression

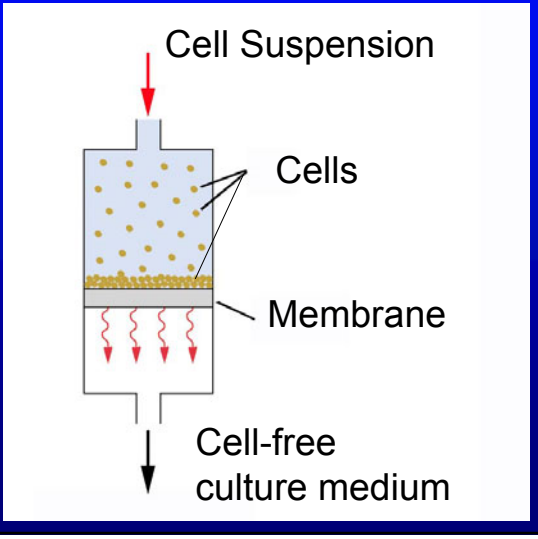


- Microbial Growth Kinetics
- Maximizing Fermentation Efficiency
- Bioreactors
- Large Scale Fermentation Examples
- Harvesting Cells
- Disruption of Microbial Cells

Harvesting Cells

- Separate cells from culture medium
- Two methods
  - High Speed semicontinuous centrifugation
  - Filtration
    - Dead end
    - Cross flow

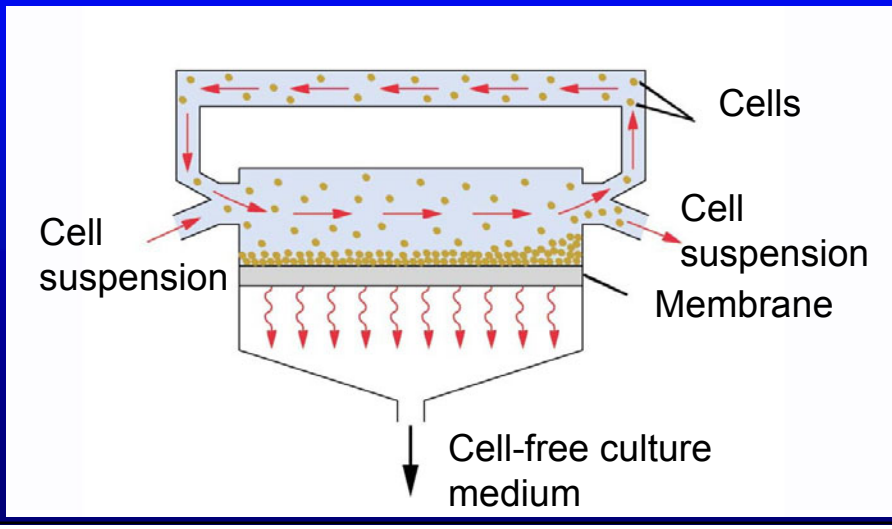
Dead End Filtration System



Simple system
Widely used

Filtration ends
when membrane
is clogged with cells

Cross-flow Filtration



- Microbial Growth Kinetics
- Maximizing Fermentation Efficiency
- Bioreactors
- Large Scale Fermentation Examples
- Harvesting Cells
- Disruption of Microbial Cells

Methods for Disruption of Microbial Cells

- Method will vary depending on the type of cell and its particular cell wall
- Regardless of the method
  - Disruption must be effective
  - Method cannot be too harsh - product must remain in an active form

Cell Walls of Diverse Microbes

- Gram(+)
  - thick peptidoglycan cell wall
  - external to cytoplasmic membrane
  - N-acetylglucosamine and N-acetyl muraminic acid residues linked by peptides
- Gram(-)
  - Outer and cytoplasmic membranes
  - Separated by a thin peptidoglycan layer

Cell Walls of Diverse Microbes

- Yeast
  - thick layer of partially phosphorylated mannans and β-glucan
- Lower fungi
  - multilayered cell walls
  - composed of α- and β-glucans, glycoproteins, chitin, etc.

Factors Affecting the Composition and Strength of the Cell Wall

- Culture conditions
- Cellular growth rate
- Phase of the growth cycle
- Storage of the concentrated cells
- Whether cell was overexpressing a cloned gene

Methods for Disruption of Microbial Cells

- Chemical
- Biological
- Physical

- Regardless of the method
  - Disruption must be effective
  - Method cannot be too harsh - product must remain in an active form

Methods for Disruption of Microbial Cells

- Chemical
  - Alkali
  - Organic solvents
  - Detergents

Methods for Disruption of Microbial Cells

- Biological
  - Enzymes
    - specific for the major cell wall constituent of the organism

Methods for Disruption of Microbial Cells

- Physical
  - Non-mechanical
    - osmotic shock
    - freeze-thaw cycles

Methods for Disruption of Microbial Cells

- Physical
  - Mechanical
    - Sonication
    - Wet milling
    - High pressure homogenization
    - Impingement

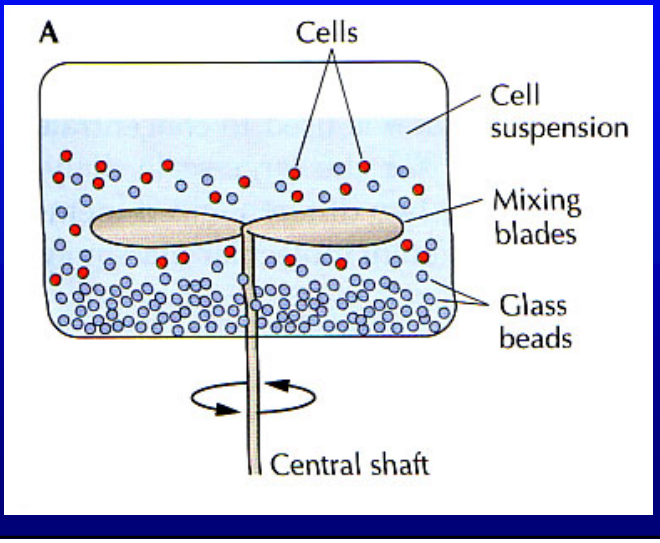
Methods for Disruption of Microbial Cells

- Sonication
  - High pressure sound waves
  - cell disruption by shearing and cavitation
  - useful only for small volumes

Methods for Disruption of Microbial Cells

- Wet-milling
  - Concentrated cell suspension mixed with small glass beads (<1mm) in mill chamber
  - Mill agitates cells and beads, creating shear forces which lyse cells
  - Useful for a number of organisms
  - High concentration of cells; large volumes

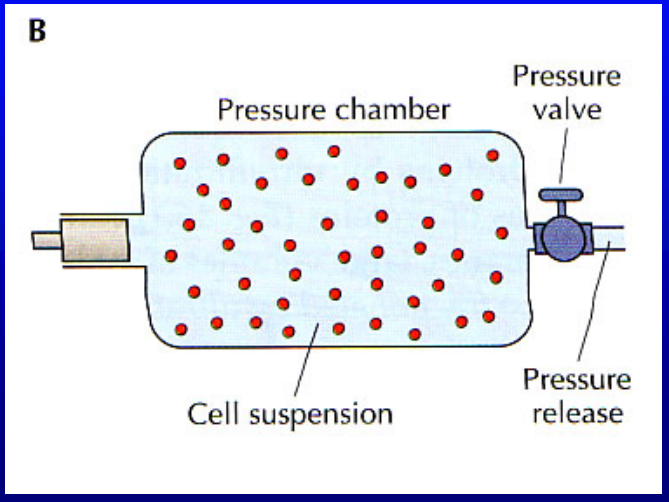
Wet Milling for Cell Disruption



Methods for Disruption of Microbial Cells

- High-pressure homogenization
  - concentrated cells pumped into a valve assembly
  - put under pressure
  - pressure released suddenly, cells lyse
  - Small volumes of concentrated cells

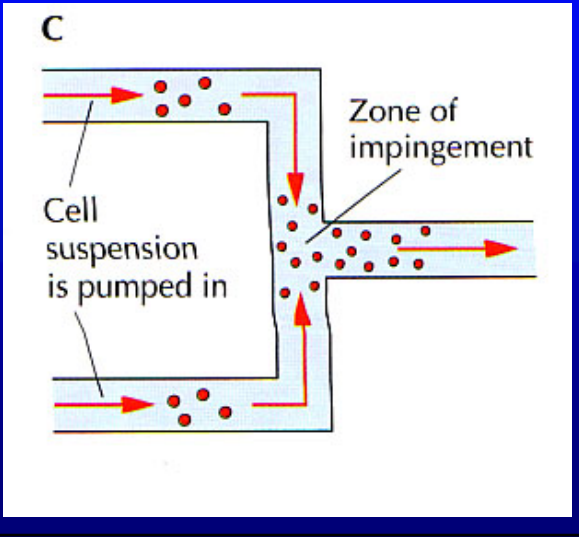
High-pressure Cell Lysis



Methods for Disruption of Microbial Cells

- Impingement
  - high velocity stream of suspended cells hits a stationary surface or second stream
  - Shear forces are created at point of contact
  - Dilute suspensions and large volumes

Cell Disruption by Impingement

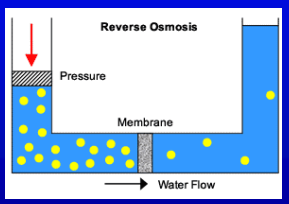


Further downstream processing

Separation of solids from solution

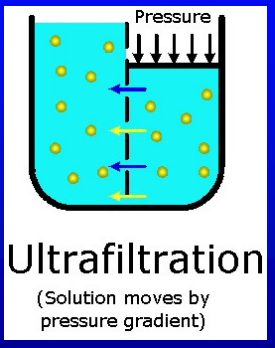
Reverse osmosis

Pores < 1000 dalton



ultrafiltration

Pores > 1000 dalton



Further purification

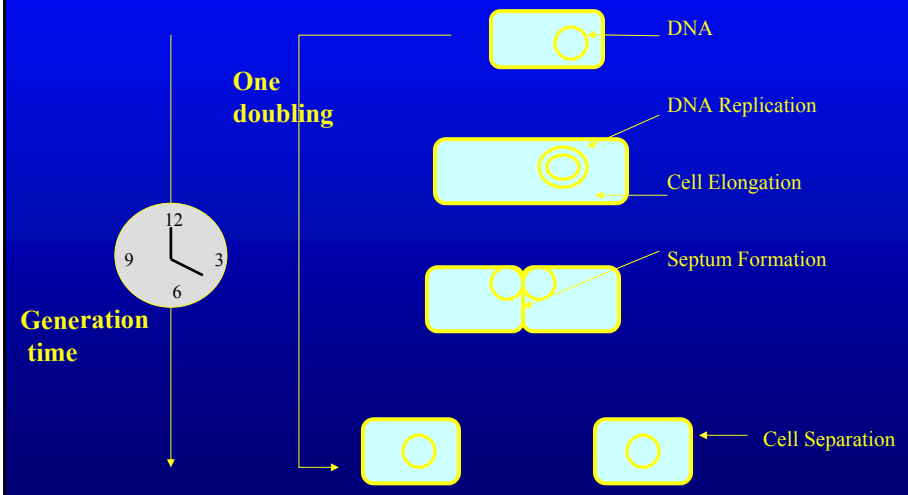
Adsorption chromatography (activated charcoal)

Ion exchange chromatography

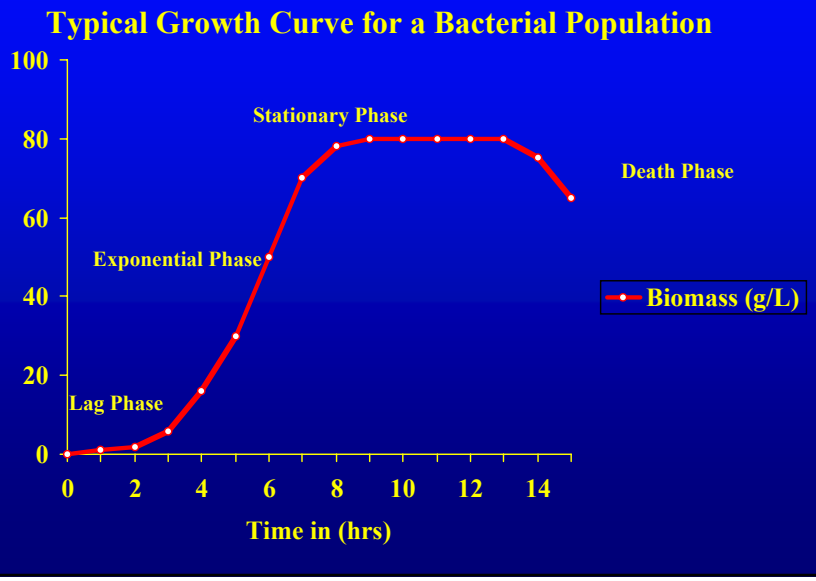
Affinity chromatography

Growth kinetics in continuous systems

Doubling Time:
Time required for a cell population to double



Generation time: The average time for one cell cycle



For a batch process, the rate of cell growth in the exponential phase is given by:

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

x is the concentration of cells (biomass in g/L)

μ is the specific growth rate of the cells

t = time in hrs

This equation is valid under conditions of balanced growth, which is when the cell composition remains constant. During the exponential growth phase, cell growth is not limited by nutrient concentrations and μ equals μmax. However, during the deceleration phase the specific growth rate of the cells depend on the concentration of limiting substrate. In this case, μ can be calculated using the Monod expression:

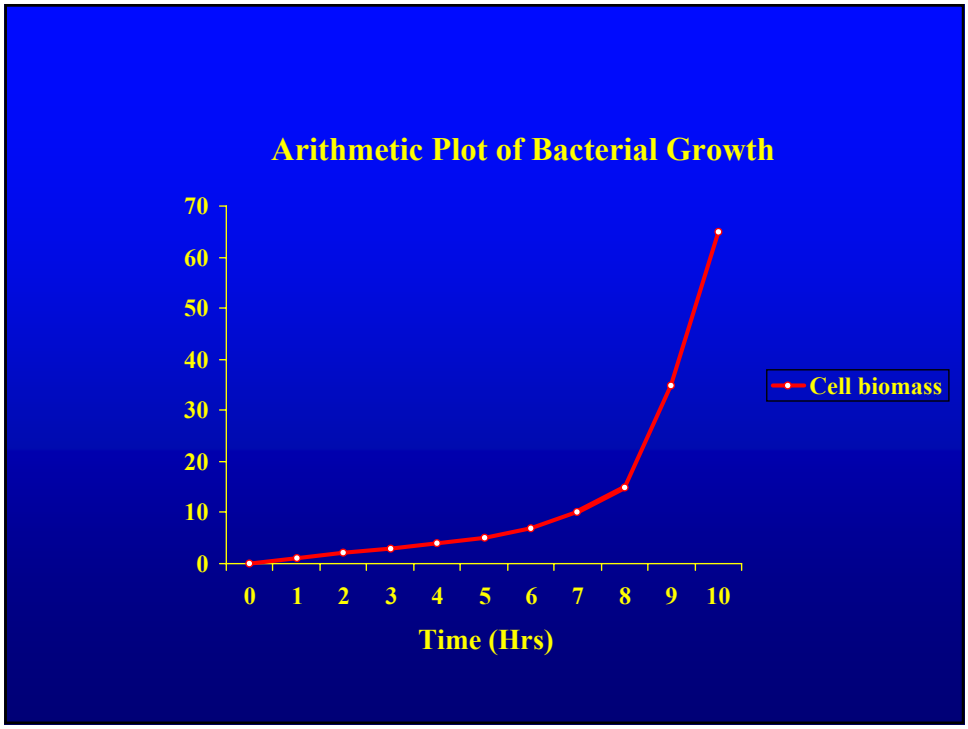
On Integration

$$\mathbf{x_t = x_0 e^{\mu t}}$$

x_0 = original biomass concentration
x_t = biomass concentration after time t
e = base of the natural logarithm

On taking natural logarithms

$$\mathbf{\ln x_t = \ln x_0 + \mu t}$$



Monod Equation

- The decrease in growth rate and cessation of growth may be described by the relationship between m and the residual growth limiting substrate

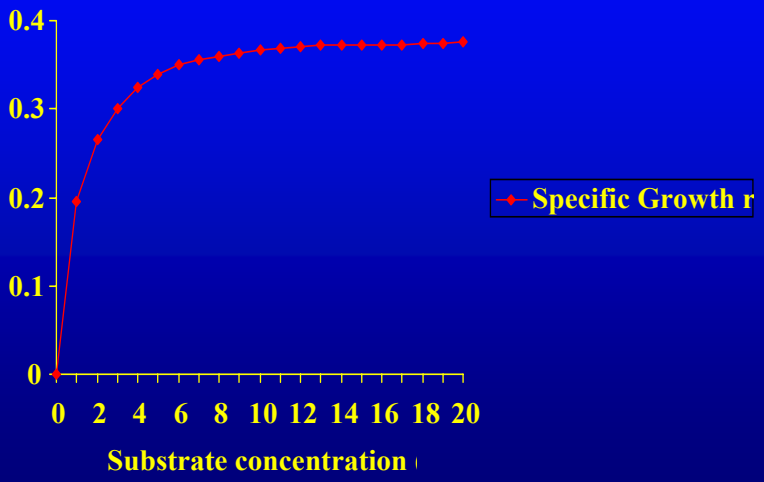
$$\mu = \frac{\mu_{\max} s}{K_s + s}$$

s = residual substrate concentration (g/L)

K_s = substrate utilisation constant when m is half μ max (g/L)

μ max = maximum specific growth per hour

The relationship between substrate concentration and specific growth rate



Yield Coefficient

- Important in optimising batch fermentations
  Defined as

$$x = Y_{x/s}(S - S_r)$$

x = biomass concentration (g/L)
$Y_{x/s}$ = yield coefficient (g biomass/g substrate utilised)
S = initial substrate concentration (g/L)
S_r = residual substrate concentration (g/L)

Continuous Growth Kinetics

- Start as batch fermentations but exponential growth can be extended by addition of fresh broth
- Reactor is continuously stirred and constant volume is maintained
- Steady state conditions exist
- The rate of addition of fresh broth controls growth

Continuous Growth Kinetics

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

D = dilution rate (per hour)

F = flow (L/h)

V = reactor volume (L)

Continuous Growth Kinetics

Under steady state conditions

$$\frac{dx}{dt} = \text{Rate of growth In rector (washout)} - \text{Rate of loss From reactor}$$

or

$$\frac{dx}{dt} = \mu x - Dx$$

Under steady state conditions
rate of growth = rate of loss
hence $dx/dt = 0$
therefore $\mu x = Dx$
and $\mu = D$

Continuous Growth Kinetics

• At fixed flow rates and dilution rates the specific growth rate is dependant on the operating dilution rate

• For any given dilution rate under steady-state conditions the residual substrate concentration in the reactor can be predicted by substituting D for μ in the Monod equation

$$D = \frac{\mu_{max} S_r}{K_s + S_r}$$

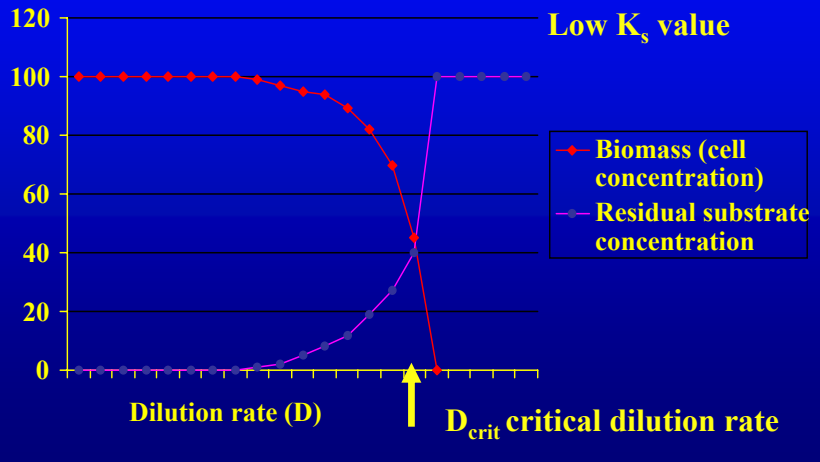
where S_r is the steady-state residual concentration in the reactor at a fixed dilution rate

Critical dilution rate

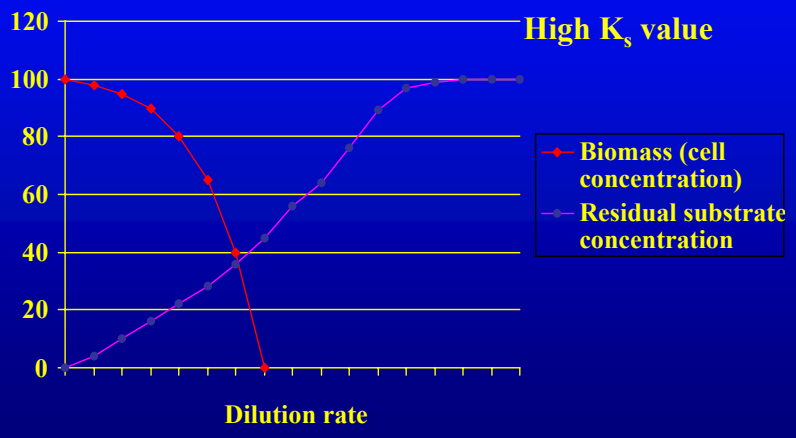
- The dilution rate at which x = zero is termed the critical dilution rate D_{crit}
- D_{crit} is affected by the constants μ_{max} and K_s and the variable S_r,

  the larger S_r the closer D_{crit} to μ_{max}

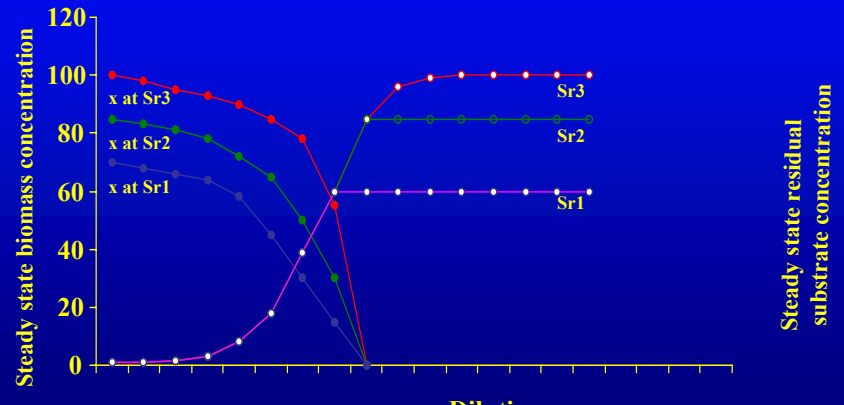
Growth of a microorganism in continuous chemostat culture



Growth of a microorganism in continuous chemostat culture



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



X = steady state cell concentration
s = steady state residual substrate concentration

Sr = Initial substrate concentration

$$D = \frac{\mu_{max} s_r}{K_s + s_r}$$

Rearranging gives:

$$D\,(K_s + S_r) = \mu_{max}\, S_r$$

dividing by S_r then gives:

$$\frac{DK_s}{S_r} + D = \mu_{max}$$

Hence:

$$s_r = \frac{DK_s}{\mu_{max} - D}$$

Consequently, the residual substrate concentration in the reactor is controlled by the dilution rate