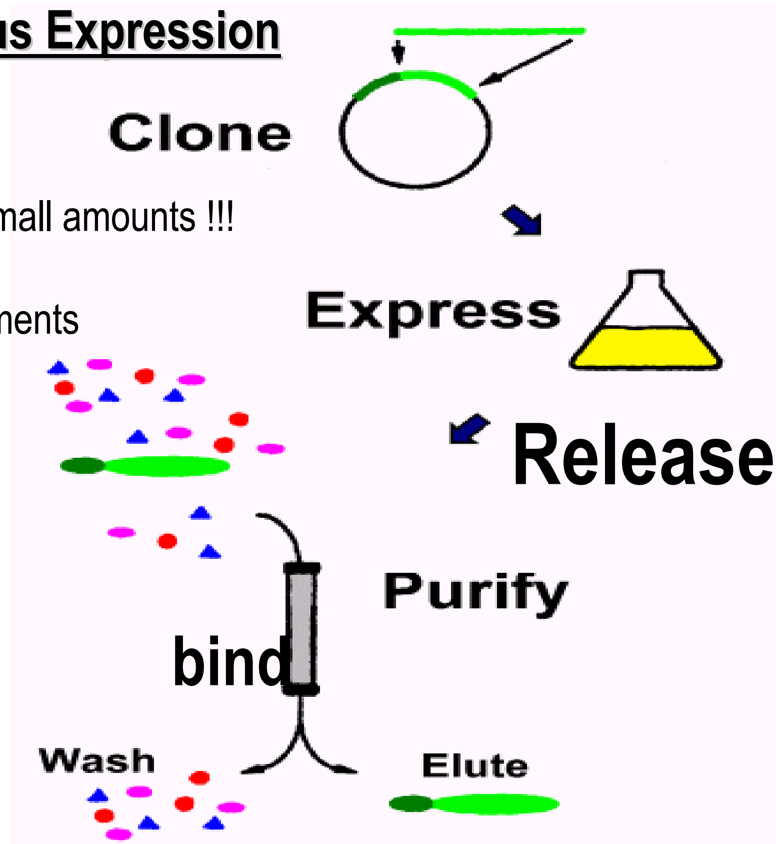
BIOC4004 - Industrial Biochemistry
Lecture 09 - Mon Feb 02, 04

Topics for the Day:

- producing protein !!!
- scaling-up production
- fermentors and bio-reactors

Small Scale Heterologous Expression



- sometimes you only need small amounts !!!
- grow in small flask
- can be used for pilot experiments

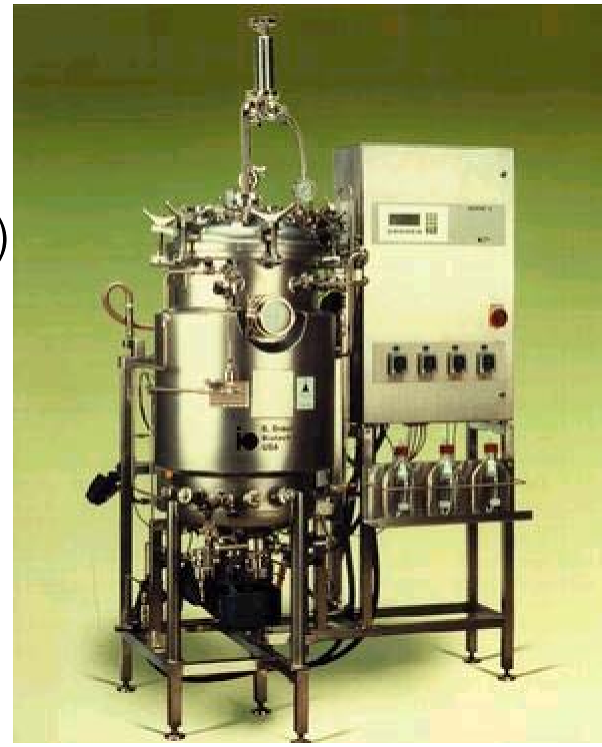
Fermentors and Bioreactors….why ???

Fermentors: bacteria and yeasts
Bioreactors: insect cells, plant cells, mammalian cells

- Time, money, space, efficiency, effort….
- For example: one 5-L bioreactor
  - shown to yield the equivalent of hybridoma cells as 150 X 250-mL T-flasks
    - 4-fold increase in efficiency
    - HUGE decrease in space and technician time required

- Factors to consider
  - Yields required(mg vs. gm vs kg)
  - What's the product ? Biomass vs Protein vs Metabolite
  - Animal vs Microbial host
  - Purification of the product

Scaling-up Production



Method of scale-up

- many small units (parallel scale-up)
- one large system (large bio-reactor)

Design Strategy

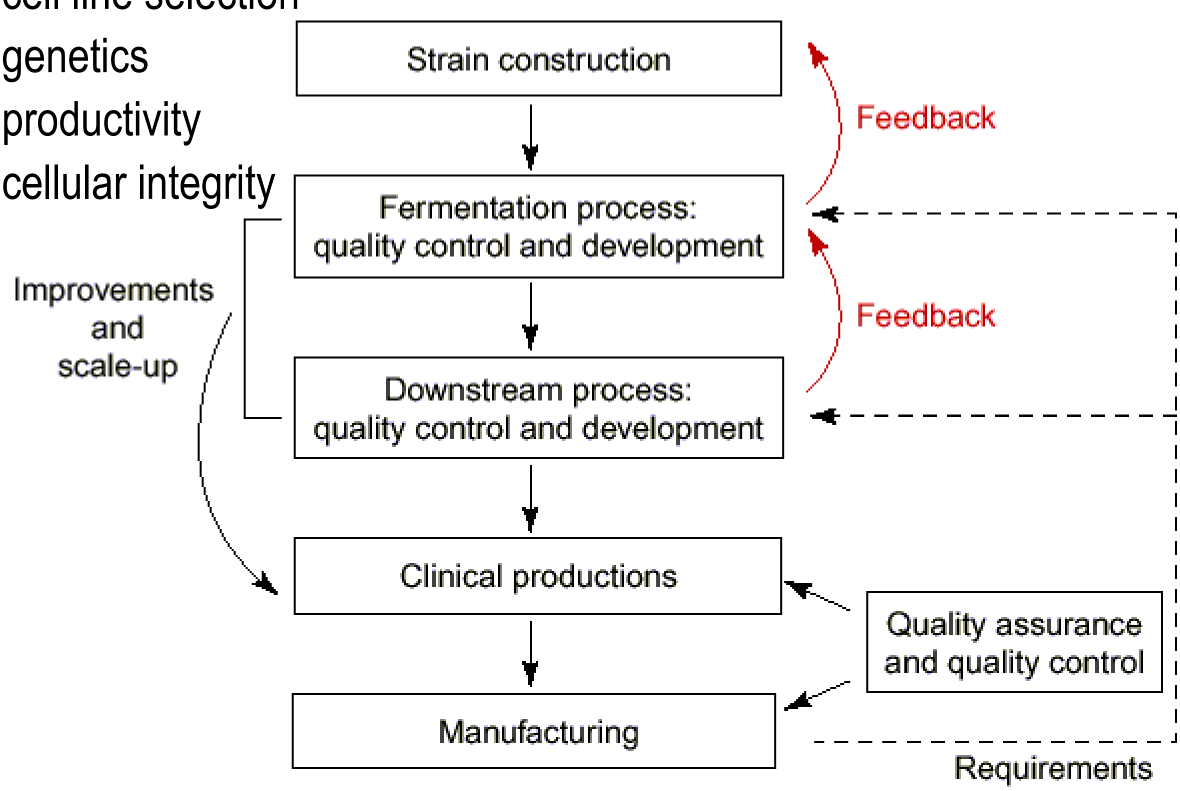
- stirred tank (most common)
- rotor-bottles
- hollow fibers

Design Strategy

- batch
  - one big batch carried to completion
  - cells and products collected at the end
  - start from scratch
- fed batch
  - same as batch but extra nutrients are added along the way
- continuous or perfusion
  - inflow of medium= outflow of medium
  - steady state: cell concentration & product concentration

Things to consider during scale-up (pt.1)

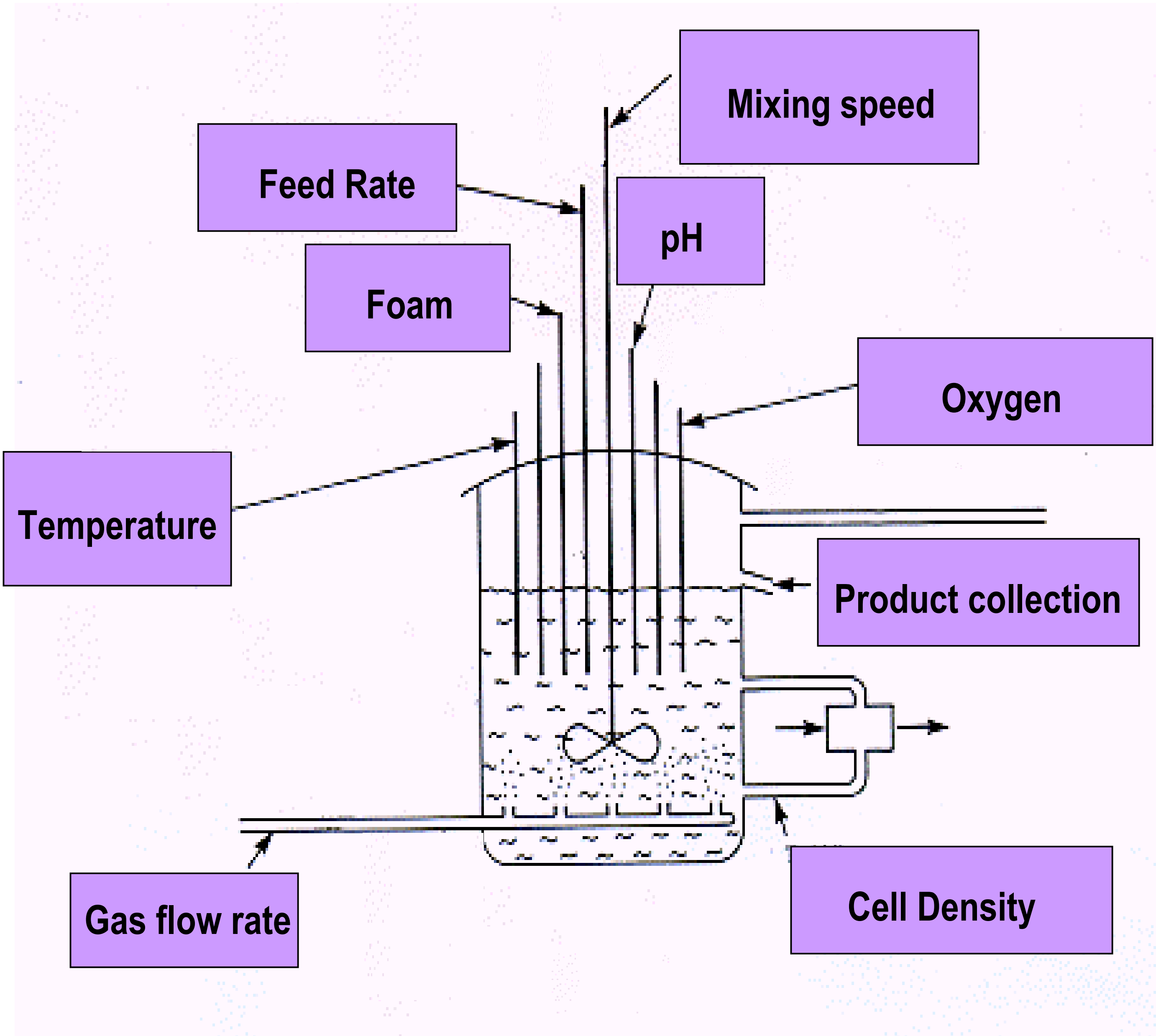
- Physical Factors:
  - Mixing (air, temperature, nutrient conentration, cells, solids, immiscible liquids)
  - Sparging (ie. aerating the cells; avoiding CO_2 build-up)
    - oxygen is often a limiting factor
  - Temperature control
  - Feed rate (ie. adding carbon source and other nutrients)
- Chemical Factors:
  - media formulation
  - pH
  - build-up of nasty by-products or inhibitors
- Biological Factors:
  - cell line selection
  - genetics
  - productivity
  - cellular integrity



Things to consider during scale-up (pt.2)

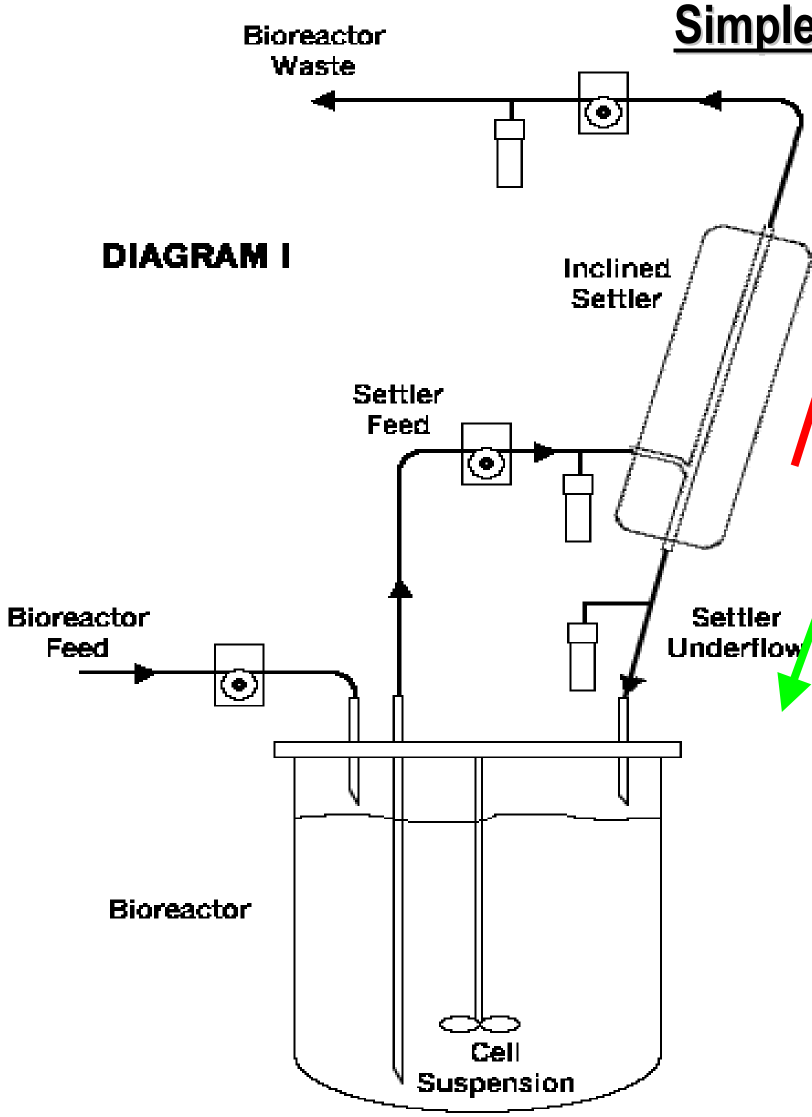
- Bioreactor Design Considerations must take into account the host-cell type
  - Growth rate
    - microbial: minutes to hours - good for batch mode
    - mammalian: hours to days - good for perfusion
  - Temperature
    - microbial: very variable optima - need not be as precise ± 1 deg
    - mammalian: 37 to 42 deg - need high precision ± 0.1 deg
  - pH
    - microbial: very variable, quite resistant - direct addition of base or acid
    - mammalian: fussy !!!!! - CO_2 to lower pH; N_2 to neutralize low pH
  - Oxygen
    - microbial: lots of growth, lots of oxygen sparging
    - mammalian: less growth, less sparging (more precise)
  - Shear-force sensitivity
    - microbial: very resistant, high mixing is OK
    - mammalian: very sensitive, low mixing necessary
  - Foaming
    - microbial: high sparging, high mixing, high protein means lots of foam
      - anti-foaming agents usually added
    - mammalian: not usually necessary

A simplified view of a fermentor



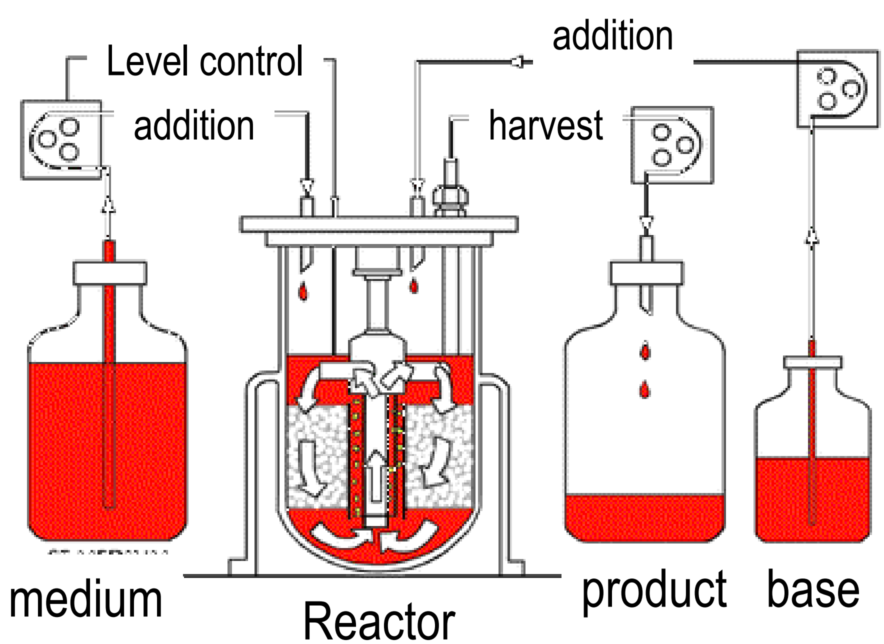
All of these various parameters must be monitored !!!

Simple Perfusion Bioreactors



Dead cells !!!
- smaller
- settle slowly
- removed

Live cells !!!
- larger
- settle faster
- back into system



Fermentation

The process of generating **product** by a process of mass production using cell growth carried out inside a **fermentor**.
Note: in most cases, a misnomer, because fermentation implies anaerobic growth

Products:

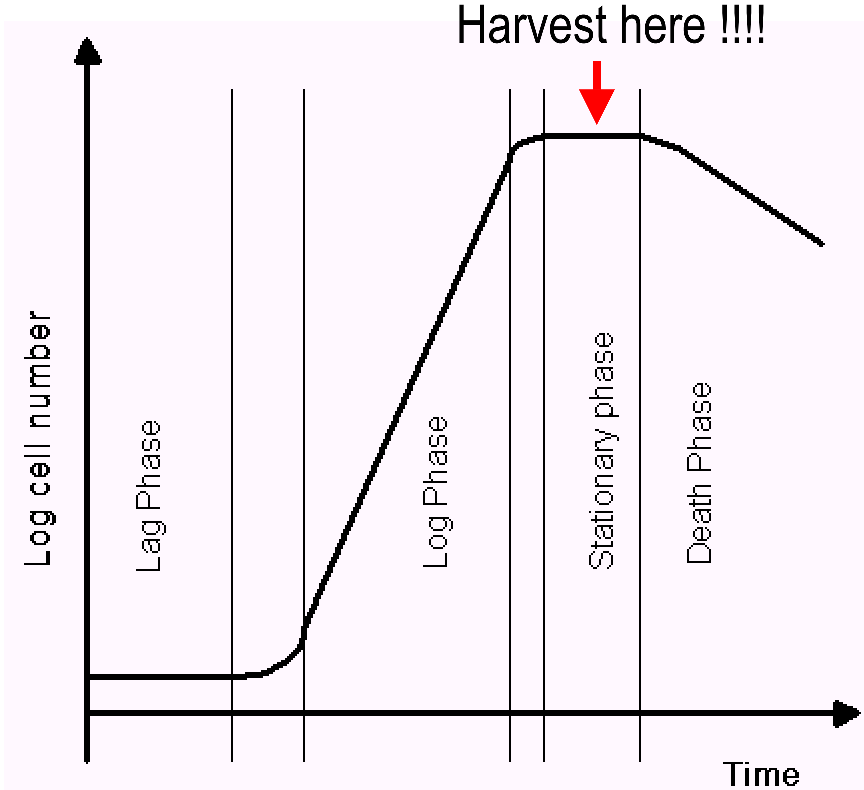
- Biomass (ie. the cell itself)
- "Native" metabolites
    - amino acids
    - antibacterials, antifungals
    - carbohydrates, lipids, nucleotides
    - enzymes
    - toxins
- Engineered products
    - enzymes
    - therapeutics
    - "non-native" metabolites

- Fermentation usually performed using:
    - bacteria (*E. coli*, *B. subtilis*, etc.)
    - yeasts (*S. cerevisiae, P. pastoris*)

Bacterial Fermentation

- bacterial fermentations usually carried in **batch mode** because of the fast doubling time
    - at t=0, sterile nutrient solution, inoculation occurs
    - no further nutrients added
        - oxygen if aerobic
        - antifoam
        - acid / base for pH regulation

Harvest here !!!!

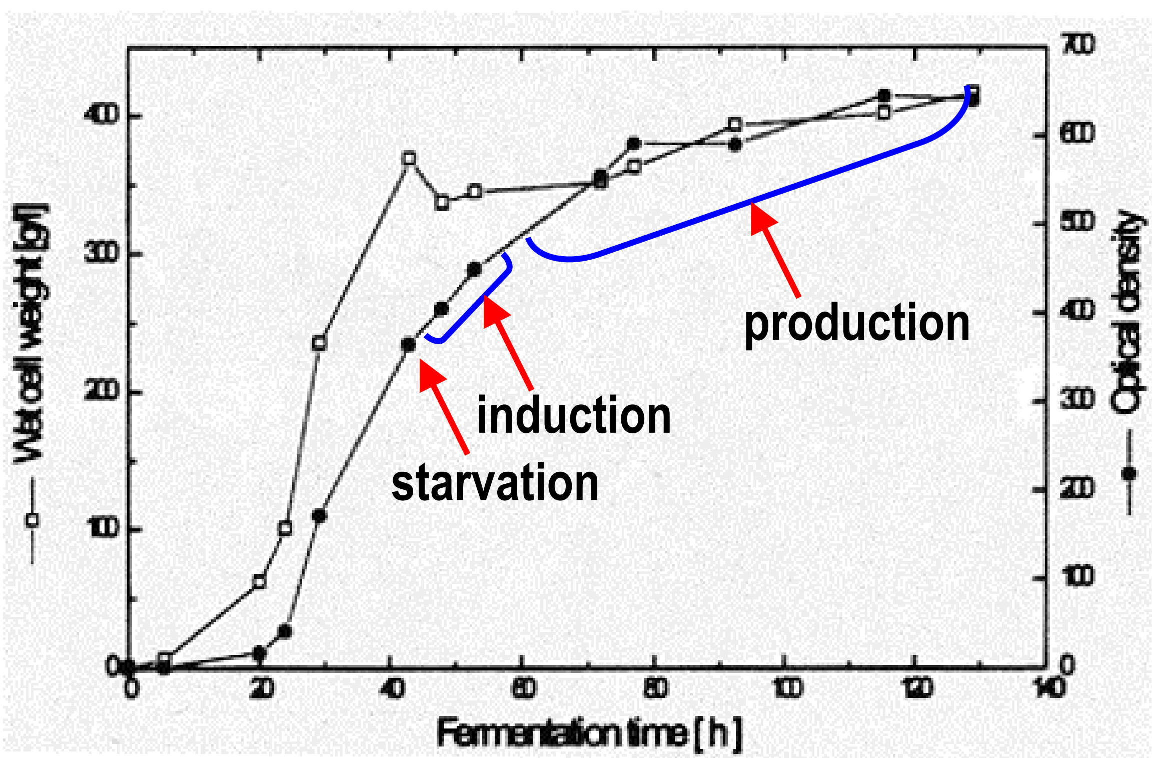


Many important metabolites produced at the end of log phase or early stationary phase

- **batch-fed mode:** extra nutrients are added along the way
    - can lead to better yields
    - can slow down accumulation of nasty metabolites
- **continuous mode**: nutrients are added continuously but an equal amount of converted medium (and cells) is removed in a steady-state fashion.
    - **Chemostats** remove converted medium so that one substrate is in equilibrium
    - **Turbidistats** remove converted medium so that cell density is in equilibrium

An example of a *Pichia pastoris* fermentation

TIME (Hrs)	STAGE	MODE	FEED SUBSTANCE*	FEED RATE (ml/L/hr)
0-20	Growth	Batch	None	N.A.
20-42.5	Growth	Fed-Batch	50% Glycerol	24**
42.5-43	Starvation	Batch	None	N.A.
43-49	Induction	Fed-Batch	100% Methanol	1-10.9***
49-97	Production	Fed-Batch	100% Methanol	15
97-141	Production	Fed-Batch	100% Methanol	2



- Two concerns
  - oxygen: fast growth means oxygen depletion
    - need lots of aeration
  - temperature: fast growth means heat accumulation
    - protein production ceases at 32 deg
    - need good temp regulation

Mammalian Cells and Bioreactors

- Shear and Mechanical sensitivity:
  - improved bioreactor design (surfaces, propellers, etc..)
  - additives to protect against sparging sensitivity

- Apoptosis and Necrosis
  - cells with impaired apoptotic pathways
  - reducing mechanical and physical stresses

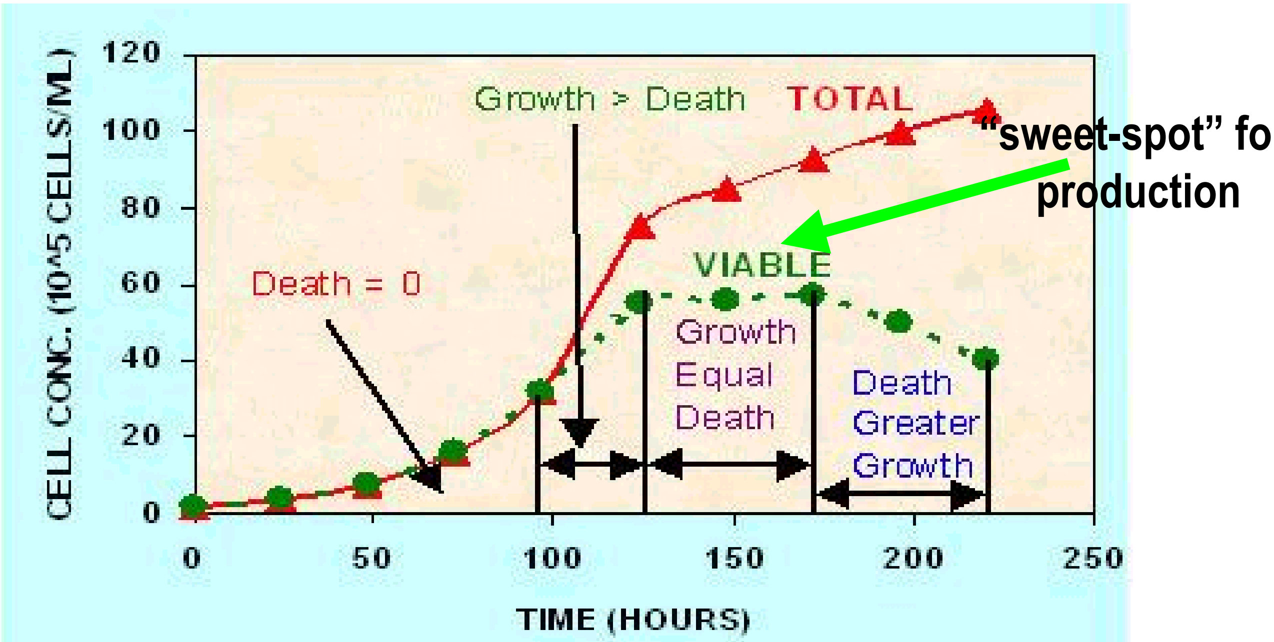
- Need for growth factors, serum : viruses, prions
  - development of serum-free media

- Toxic byproducts: ammonia, oxy-radicals
  - cells engineered to produce less ammonia
  - anti-oxidants

- Maximizing production:
  - high density of viable cells
  - low growth rate
  - high productivity
  - Fed-Batch: higher short-term production
  - Continuous-Perfusion: lower but longer production; higher overall yield

- Necessity for substrate attachment
  - microcarrier beads
  - Novel bioreactor designs:
    - roller bottles
    - hollow-fiber

Kinetics of Cell Growth and Cell Death



- protein production is maximized when cells are not dividing too vigorously
- energy does not get diverted into cell division
- energy devoted to protein production !!!!
- Need to do controlled feeding

Typical Protein Production Rates

q_P = SPEC. PROD. SYNTHESIS RATE

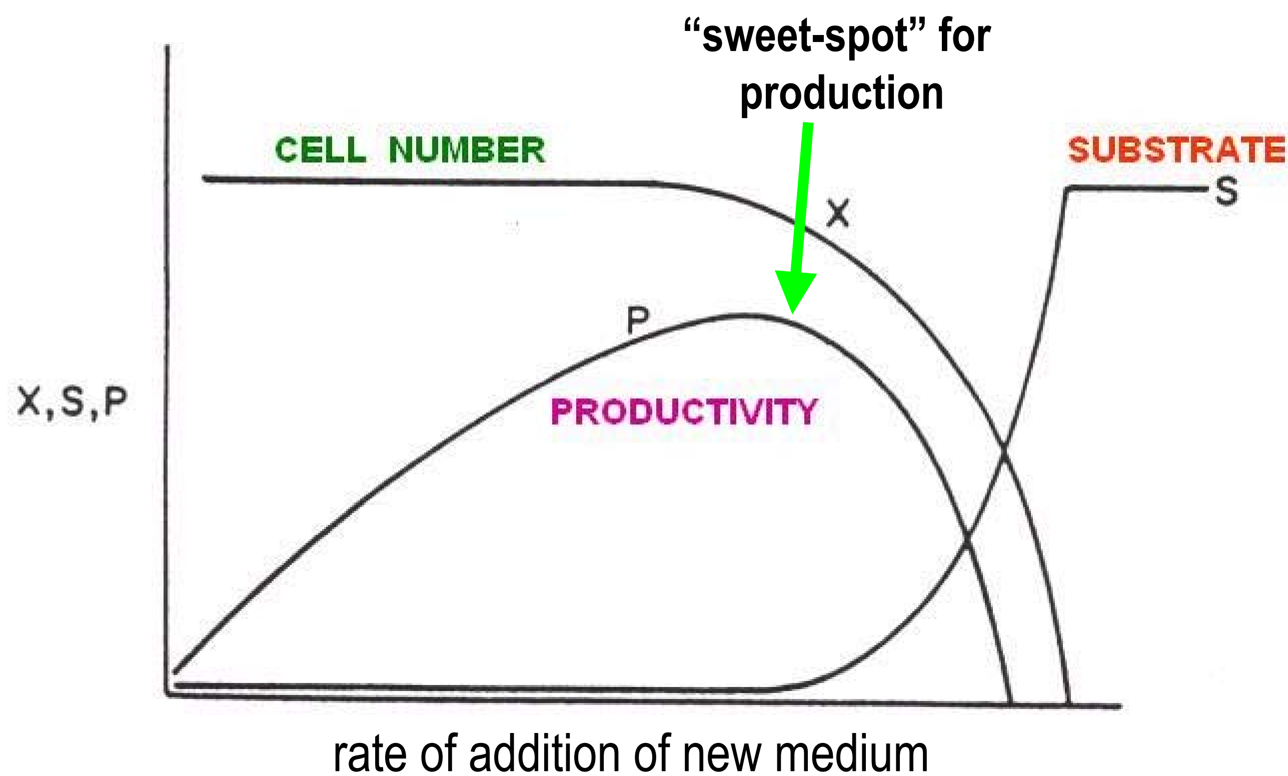
RECOMBINANT PROTEINS:

$$q_P = \frac{\text{10 to 50 Picogram } (10^{-12}\text{ gm})}{\text{CELL - DAY}}$$

MONOCLONAL ANTIBODIES:

$$q_P = \frac{\text{50 to 300 Picogram}}{\text{CELL - DAY}}$$

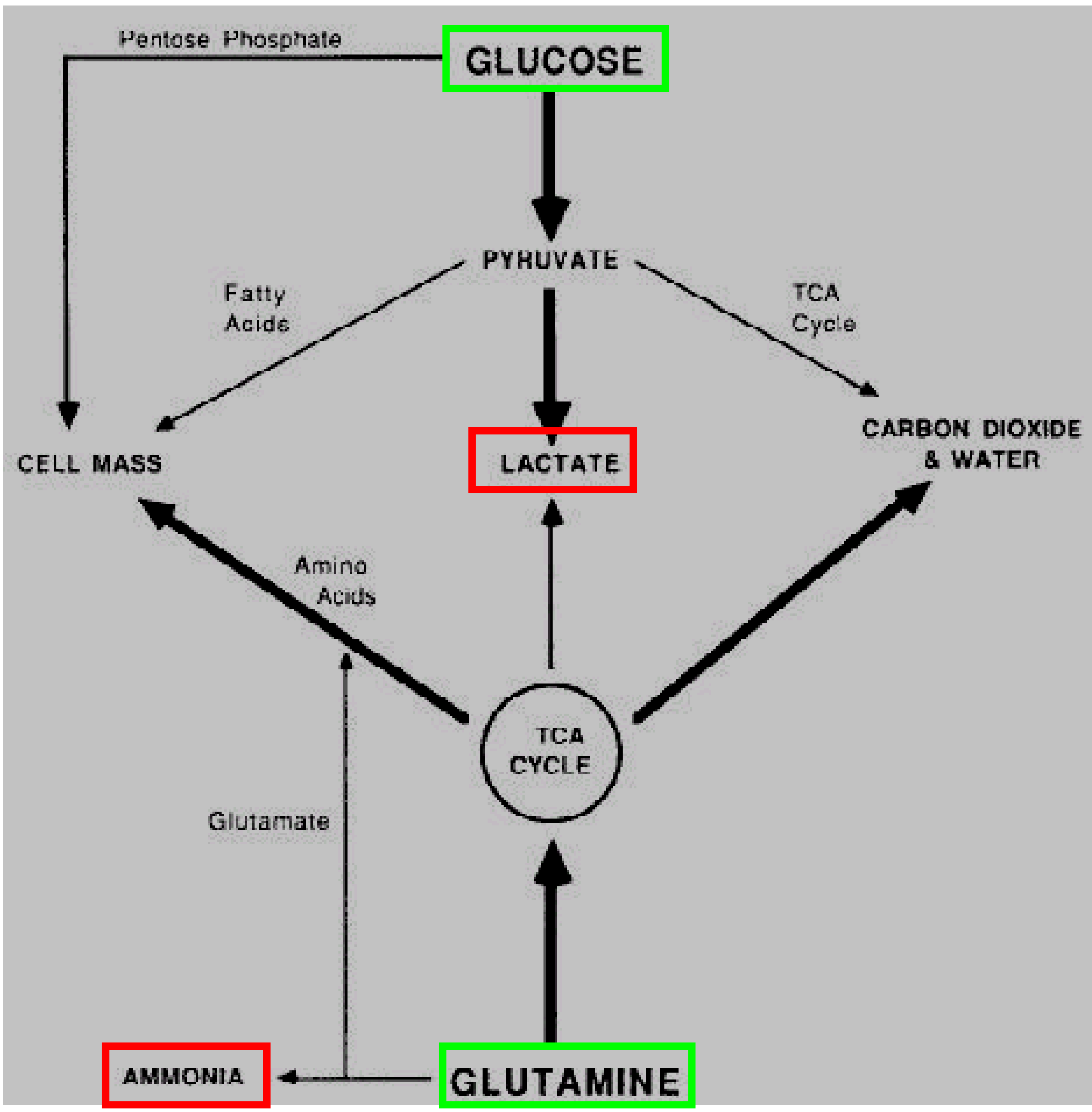
Steady-state Relationships of a Continuous Culture



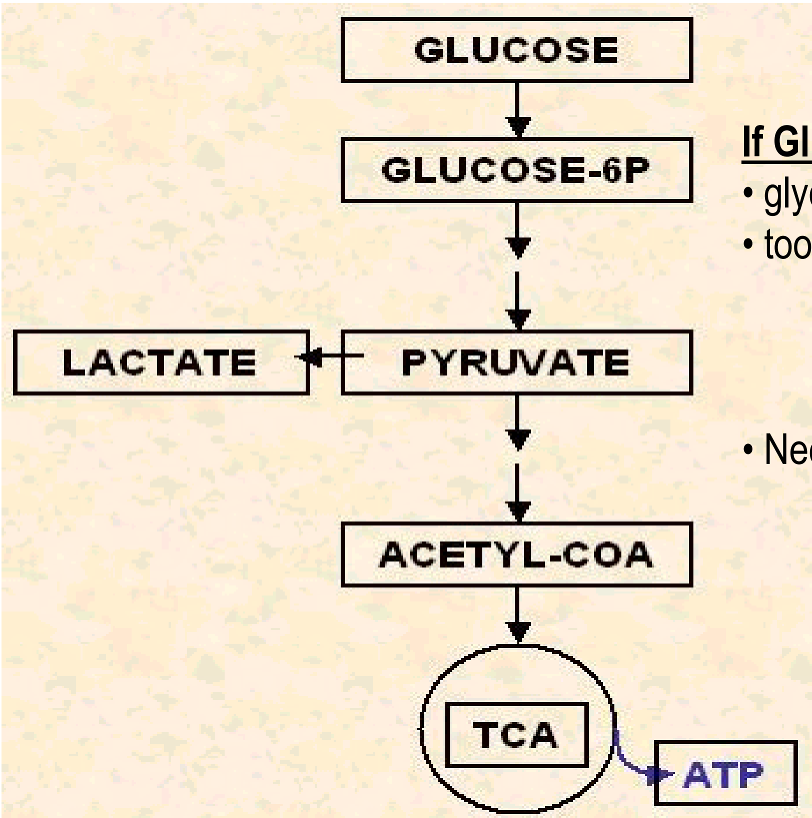
- At low nutrient rates, the growth rate of the culture outstrips the addition of new nutrients
    - not enough nutrients to maximize production
    - too much accumulation of nasty metabolites
- Too high a nutrient rate leads to
    - CELL DIVISION !!! (which is evil...)
        - energy "wasted" on cell division
        - cell removal
    - accumulation of substrate

Too Much of a Good Thing : the problem with Glucose and Glutamine

- two limiting substrates: glutamine (N) and glucose (C)
- two inhibitory products: lactate(L) and ammonia (A)

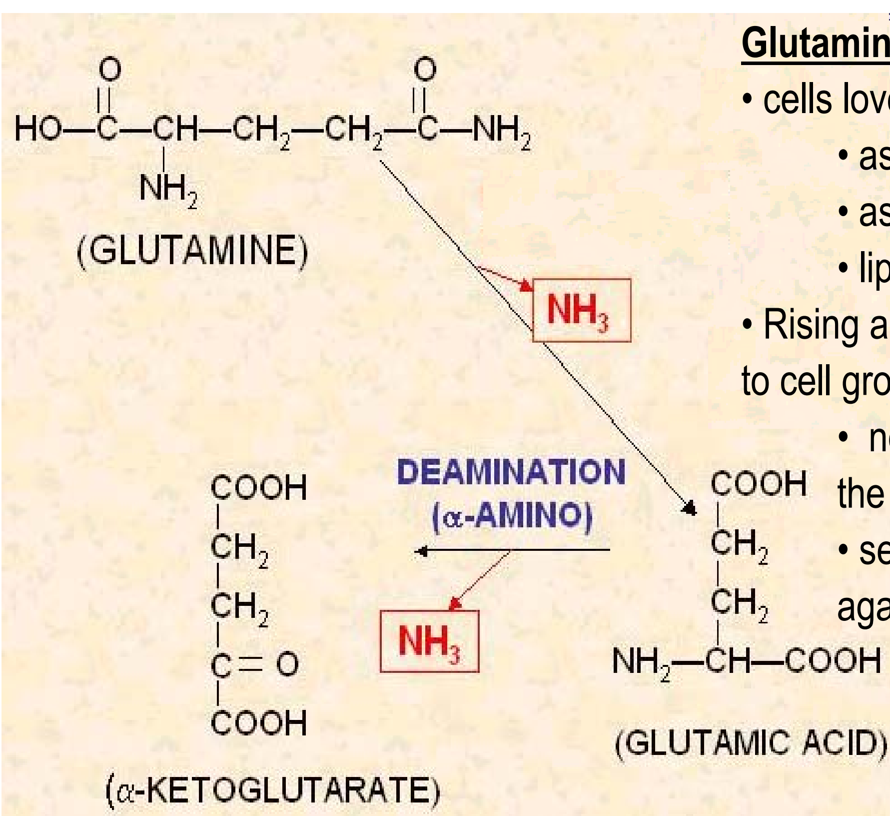


Too Much of a Good Thing : the problem with Glucose and Glutamine



If Glucose levels too high:

- glycolytic flux too high
- too little TCA cycle
    - inefficient Glucose utilization
    - too much Pyruvate made
        - too much Lactate made
- Need to control glucose levels !!!!
    - Less lactate
    - more ATP production
    - more protein

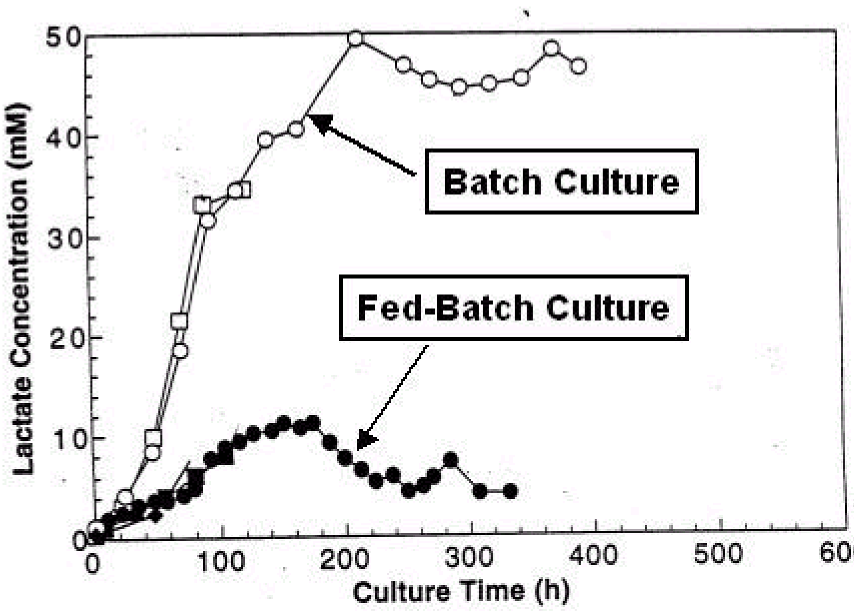


Glutamine and Ammonia production

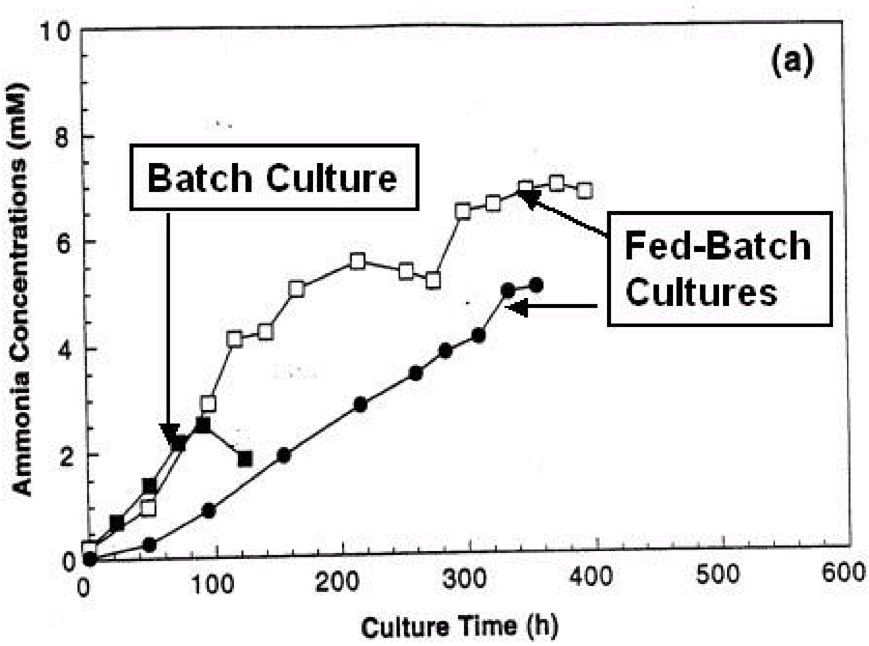
- cells love glutamine
    - as a protein building block
    - as an energy source (TCA)
    - lipid and nuclotide biosynth
- Rising ammonia levels are quite toxic to cell growth
    - need to remove ammonia from the medium
    - serum offers some protection against ammonia toxicity

The Effect of Culture Method on Lactate and Ammonia Levels

Lactate Production in a Hybridoma Culture



Ammonia Production in a Hybridoma Culture



- Lactate levels can be regulated by regulating Glucose addition
- Ammonia levels best controlled by continuous removal