Fermentation :
Some Basic concepts

The Objectives

Grow cells in a controlled environment that support growth and production of product

Factors to consider

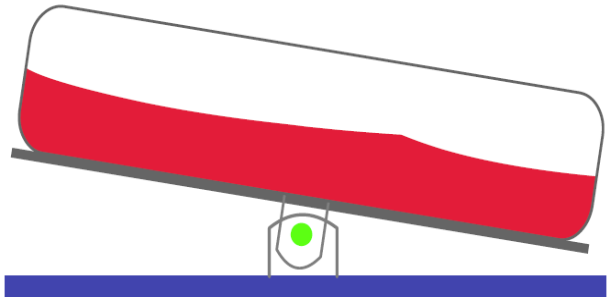
- Nutrients – composition and supply
- Oxygen transfer / CO_2 levels
- Mixing capacity
- Heat transfer
- pH control
- Foaming

Spinner

Characteristics and uses

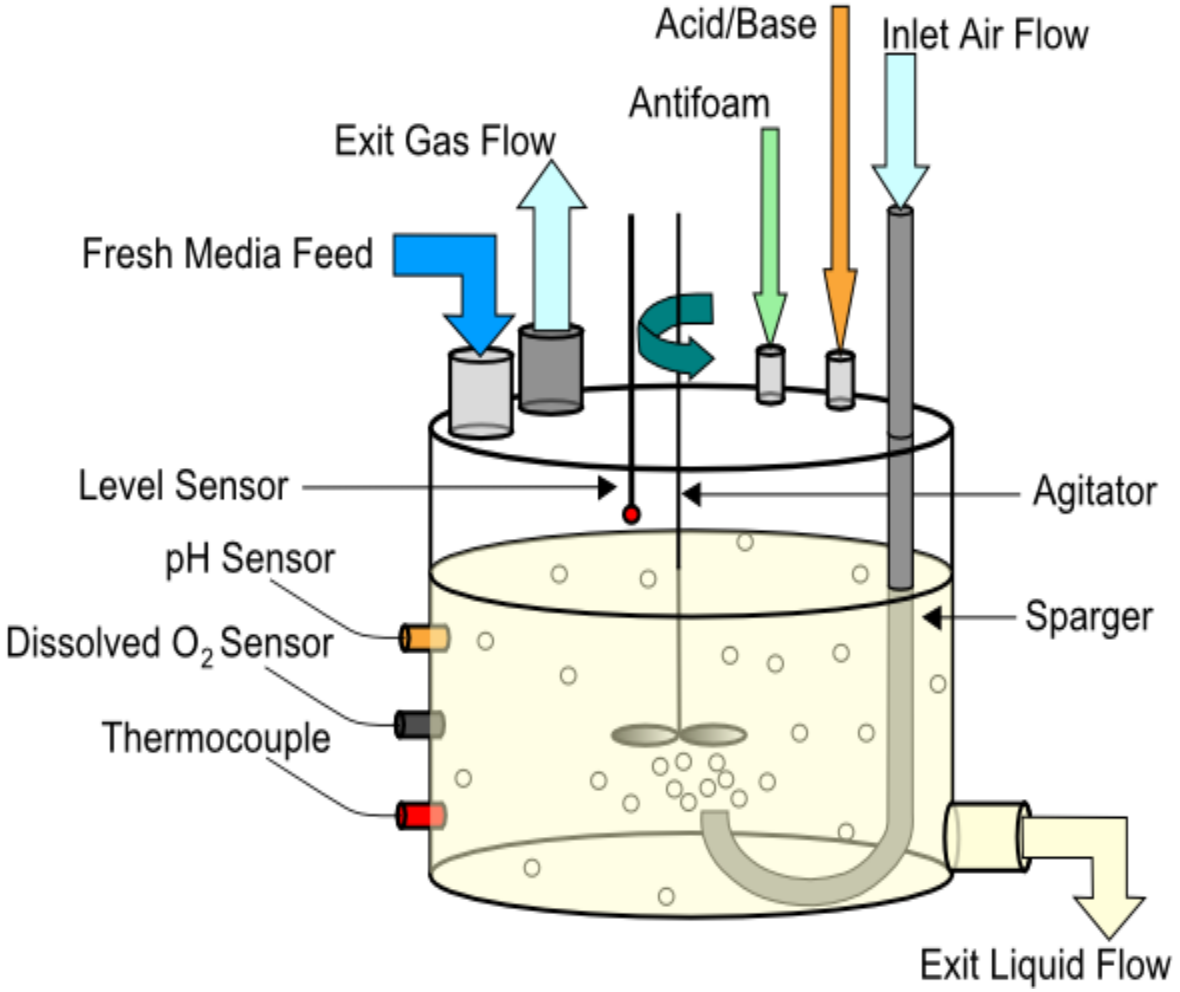
- used for small scale cultures
- limited controls- temp and agitation
- simple setup
- cheaper than using a fermentor, but more likely to fail in large scale

Wave Bag technology



Roller bottles

Elements of a Bioreactor (fermentor)



Fermentor Controls and Parameters

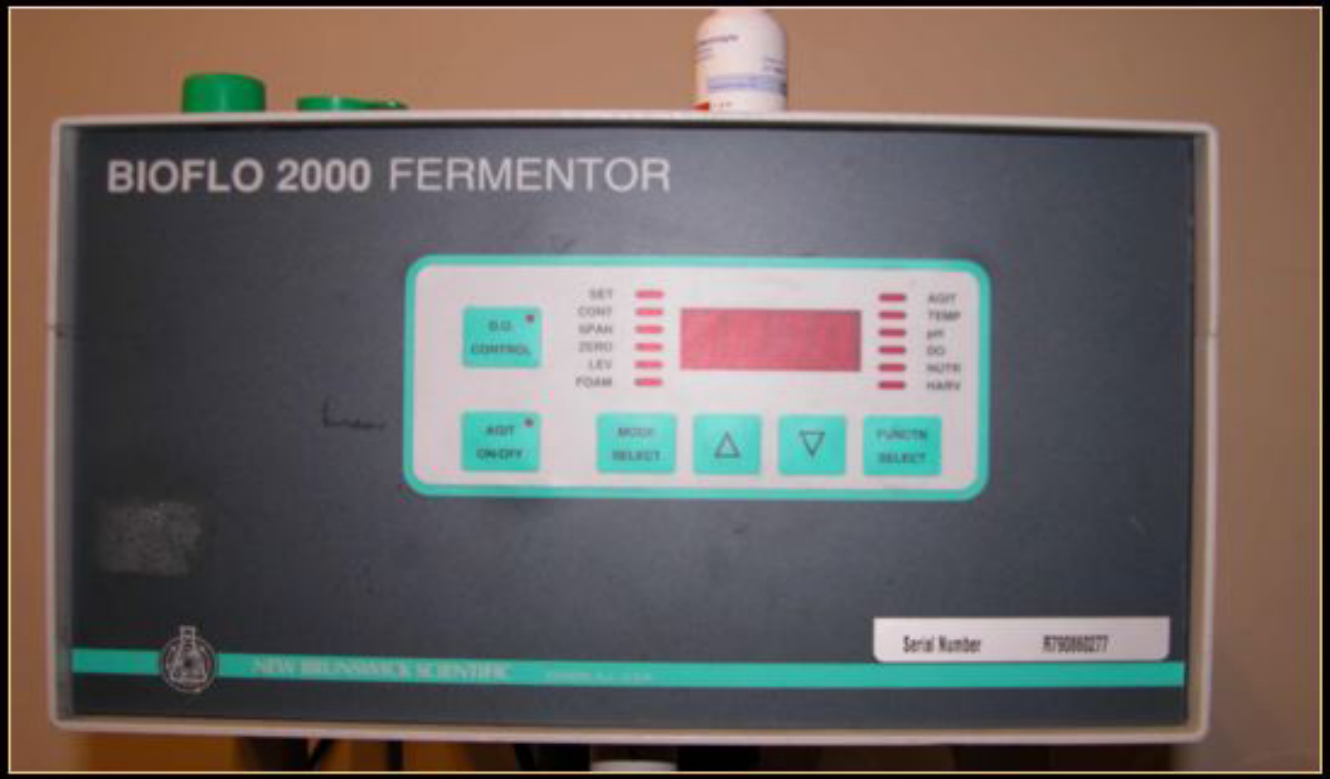
- Temperature
- Pressure
- pH (both acid and base)
- Sparging gasses
- Vent exhaust
- Innoculation
- Media additions
- Sampling
- DO (dissolved oxygen)

Fermentor Controls

Headplate

- Multiple ports for everything you need to add in, take out, or put probes in

Fermentor Controls



Programmable Logic Controller (PLC)

- Program set-points
- Includes: Temperature, pH, DO, and many other key parameters, agitation
- Parameters stated in Batch Record

Fermentor Controls

PUMP and DO Controllers

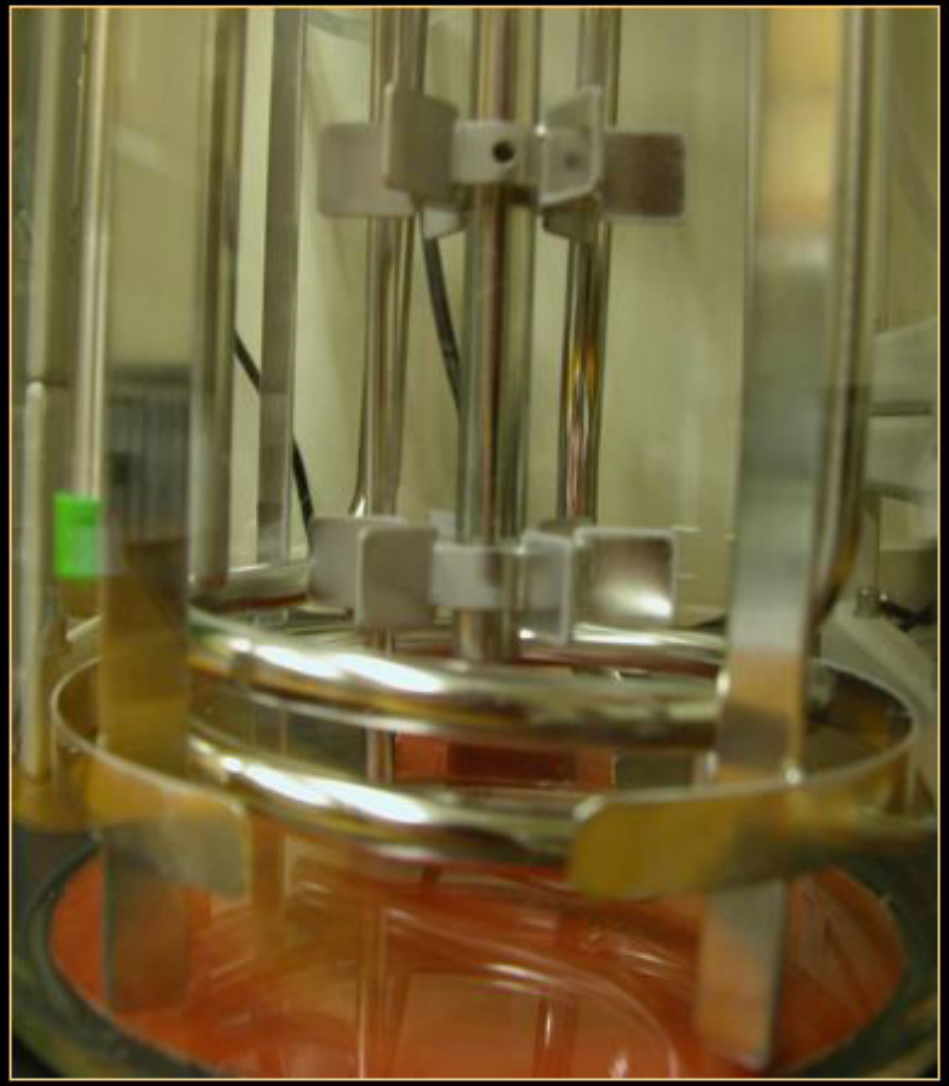
- Probes measure for pH and DO levels
- Pumps transfer fluides or gases to control pH and DO

Fermentor Controls

pH Controller

- Probe measures pH
- The two pumps push a base or acid
- Prime vs Auto

Fermentor Controls



Temperature Control

- Water temperature controlled

- Temperature Coil

Fermentor Controls

Agitator

- Keeps culture in suspension
- Measured in RPM

Fermentor Controls

dO Controller

- Oxygen Tanks and step down regulators
- DO flow regulator

Fermentor Controls

Sample Port

- Pulls required samples
- Can pull specific volumes

Considerations for Media Design

- Optimal cell growth and product synthesis
- Media needs to provide Carbon and nitrogen sources, growth factors and oxygen transfer.
- Many cell types or organism may lack enyzmes to process the substrates to either break down nutrients or to synthesize key components for growth
- Media should be able to maintain the highest cell growth possible
- Maintain pH control
- Osmolality limits
- Stability of components (precipitation of key components)
- Cost

Nutrients

Complex or Undefined media

- Complex medias include a variety of carbon and nitrogen sources from organic materials such Casein digest, tryptone digest , yeast extract , Fetal calf serum
- Mammalian cell culture often requires additional growth factors such as insulin, transferrin, fetuin , albumin, folic acid
- Serum is often added in early stages of development when specific growth factors are not known
- Complex media can make purification difficult, all known components must be validated for their removal

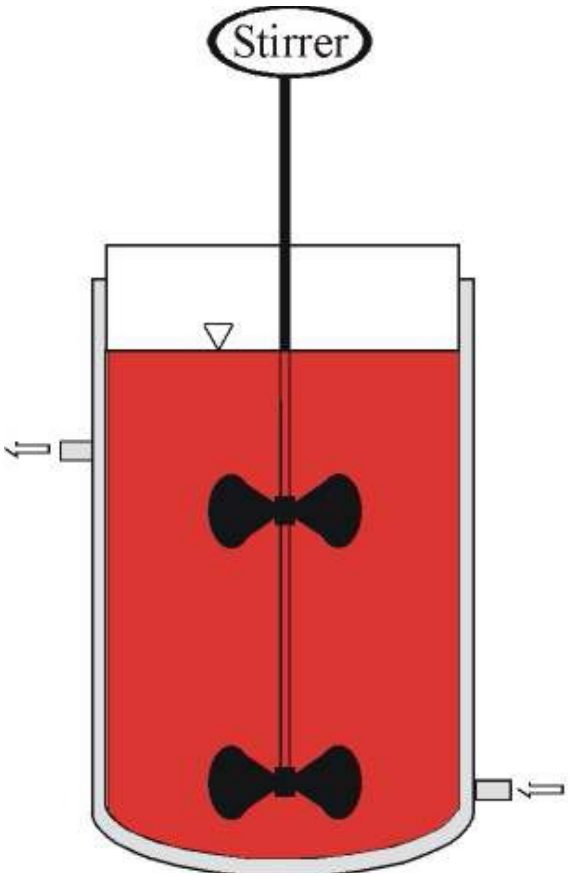
Nutrients/ defined

- Defined medias are those with simple compounds such as defined sugars ie glucose, mannitol, defined nitrogen sources such as ammonium salts or nitrates as inorganic nitrogen sources with few additional growth factors that may be trace metals
- Organisms that grow in defined media represent a lower cost and a more expeditious down stream process

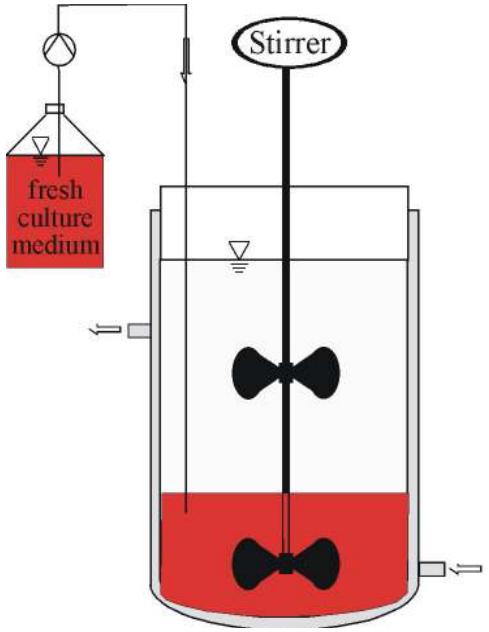
Modes of Nutrient Supply

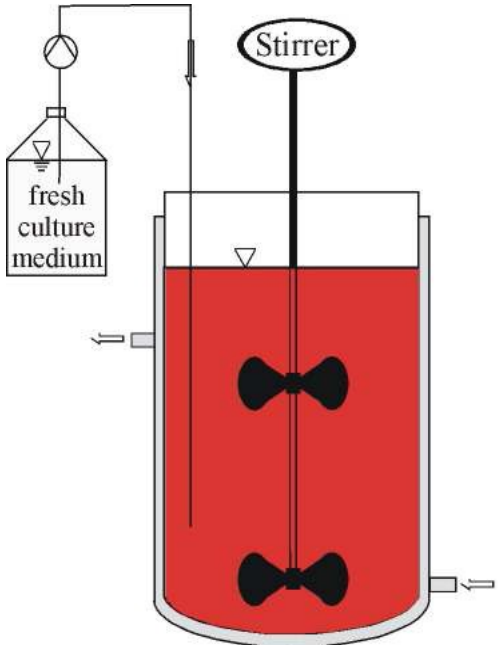
- **Batch** – single batch no additions are made to the vessel during the fermentation run
- **Fed Batch-** key components such as single amino acids , or carbon sources such as glycerol or glucose are added throughout the run to maintain the rate of growth
- **Solera batch-** a proportion of the fermenter volume is replaced periodically and replaced with an equal media volume of fresh
- **Continuous culture-** fermenation broth is removed at the same rate as media addition to mainatin a steady state in terms of cell density within the fermenter

Batch fermentations



- Advantages:
- • easy to perform
- • simple upscaling possible
- Disadvantages:
- • low cell densities
- • low productivity
- • often product degradation





Fed-batch / Solera fermentations

Advantages:

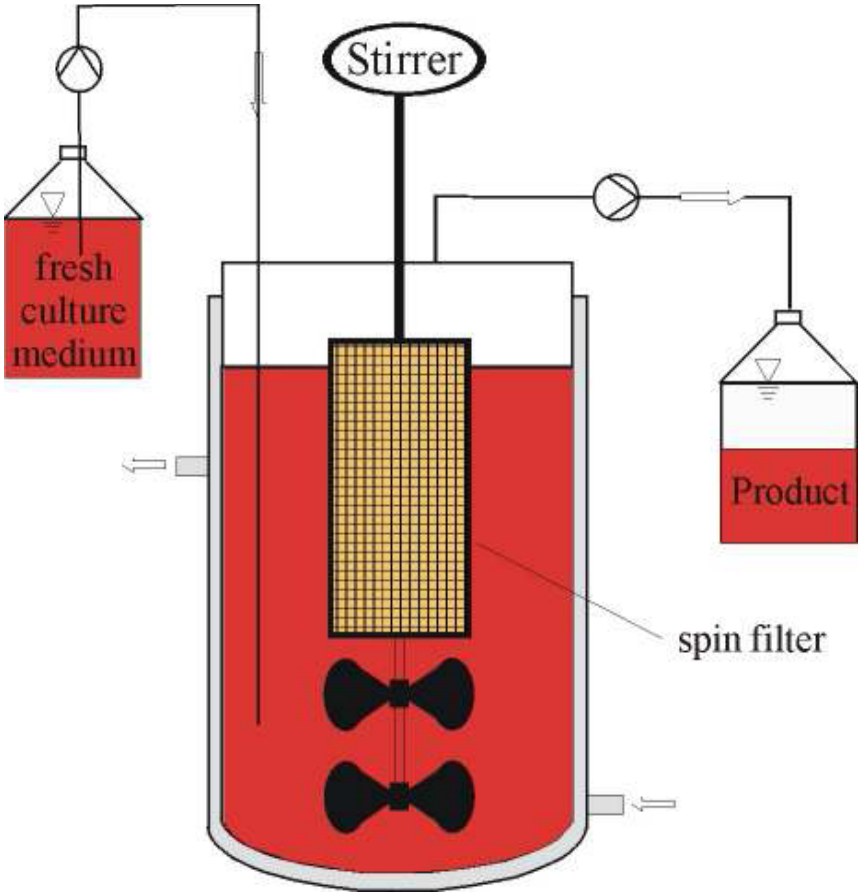
- easy to perform
- simple upscaling possible
- prolonged cell growth and production

(compared to batch mode)

Disadvantages:

- low cell densities
- low productivity
- often product degradation

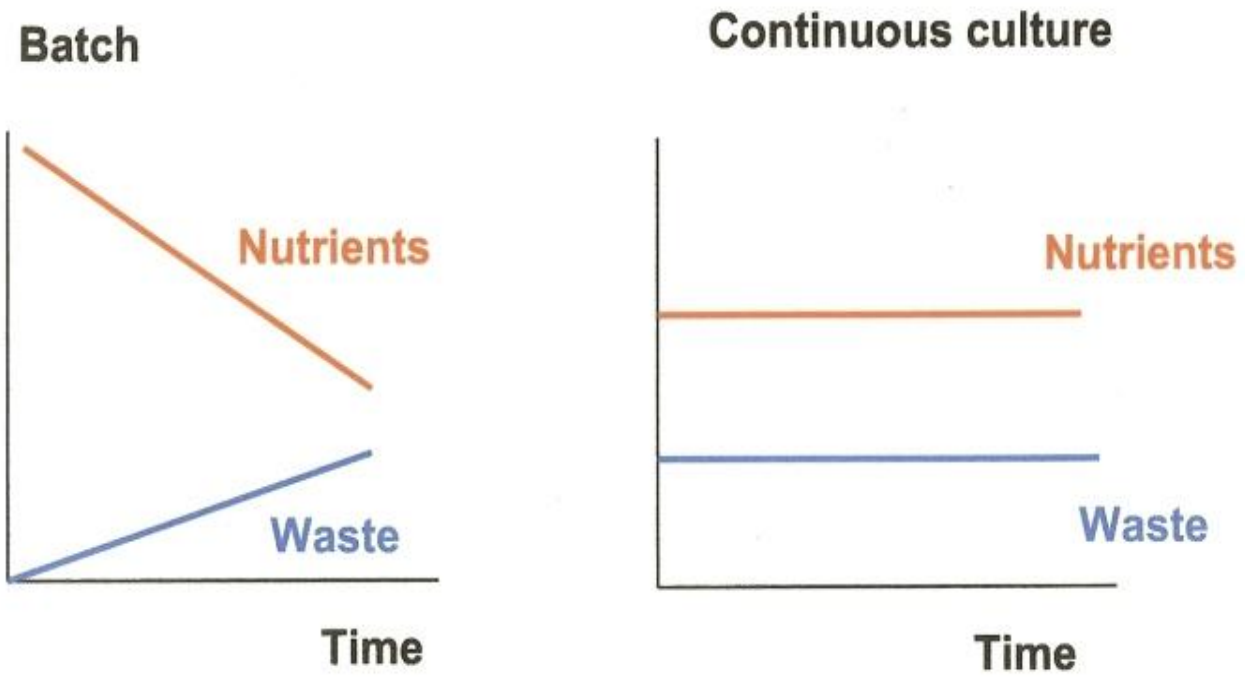
Continuous fermentations



Advantages:
- high cell densities
- high productivity
- no limitation / inhibition
- good product quality

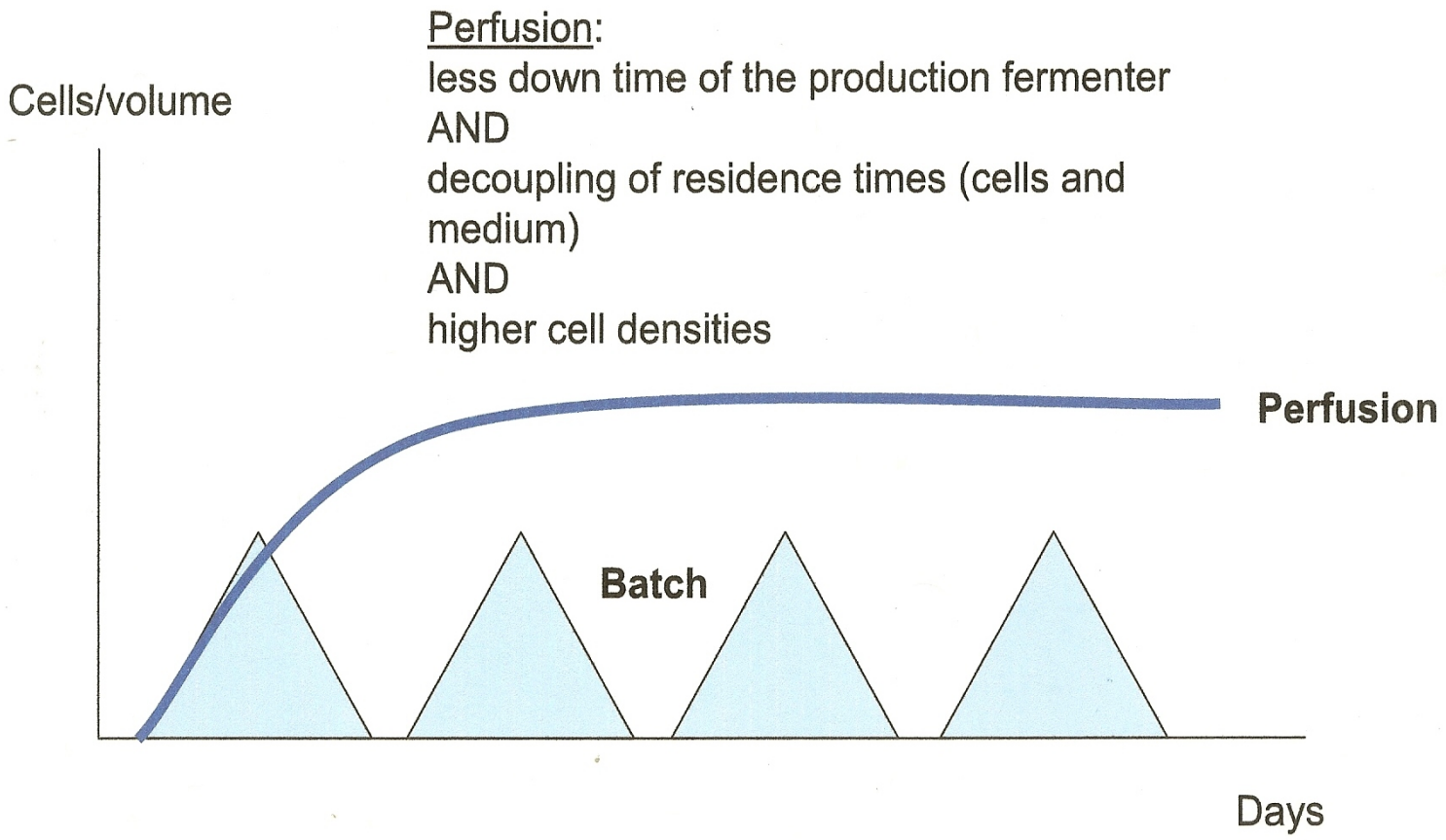
Disadvantages:
- high technical demands

Batch/fed-batch vs. Continuous culture

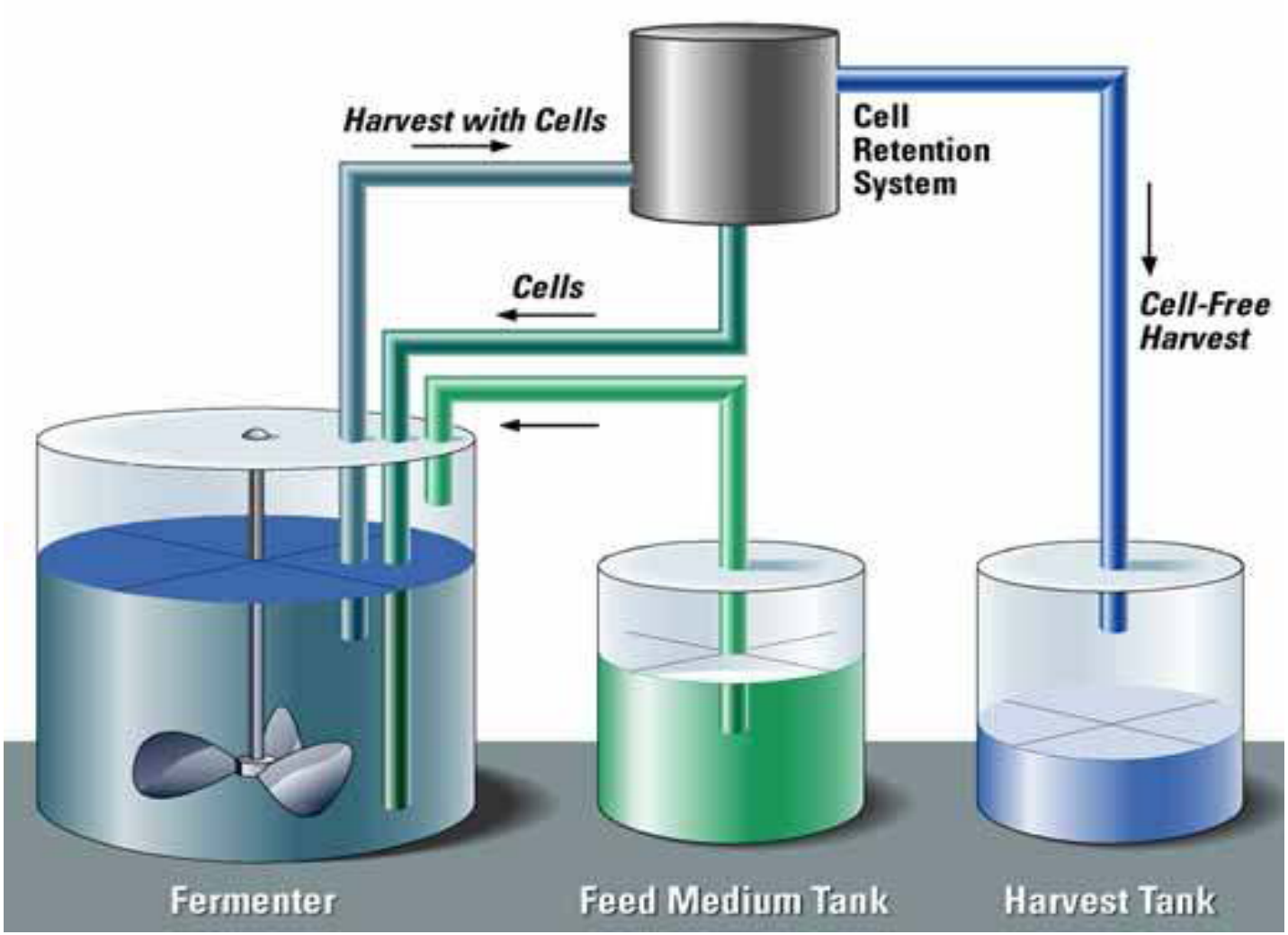


Continuous culture: nutrients and fermentation by-products stay at the same concentration. Continuous culture mimics the blood stream in the body

Batch/fed-batch vs. Continuous perfusion culture



Perfusion culture



pH Control

- Most Eukaryotes require a near neutral environment
- Metabolism of sugars such as glucose results in formation of acids and CO2
- Metabolism of Nitrogen sources results in release of amines
- pH excursions inhibit growth and may become toxic to cells
- pH control through buffering salts such as tris, hepes, Mes (Goode Buffers biological compatibles)
- pH control through use of auto titration systems with Ammonuim hydroxide and a low molarity acid

Heat transfer

- During the metabolism of carbon sources , heat is released
- Mechanical systems such as agitation and sparging also generate heat

- Convection exchange for small vessels
- Heat transfer loops within bioreactors
- Jacket exchangers

Control of Oxygen

- The most efficient metabolism is achieved aerobically
- Transfer of oxygen via sparging : small bubbles represent large surface area for introducing O2
- Agitation at high rates decreases bubble sizes
- Cells sensitive to shear forces cannot be sparged , O2 is introduced through silicone diffuser tube
- Media composition temperature can affect the amount of O2 that can be saturated into the media

What does DO tell us?

- Aerobic respiration requires the consumption of oxygen
- O_2 solubilty is limited
- When carbon source is limited metabolism slows down and O_2 demand declines and DO levels rise
- When DO levels rise this indicates that the Carbon source may be depleted

Foaming

- Foam is caused by air bubbles that are stabilized by proteins in the fermentation broth
- Interferes with the surface interface gas exchange hindering $O_2/C0_2$ control
- Fouls exit filters and interferes with vessel pressure control
- Control by addition of silicon oils, propylene glycol, octanaol (agents that lower surface tension)
- Antifoam agents need to be validated for removal.

Monitoring Growth

Cell mass

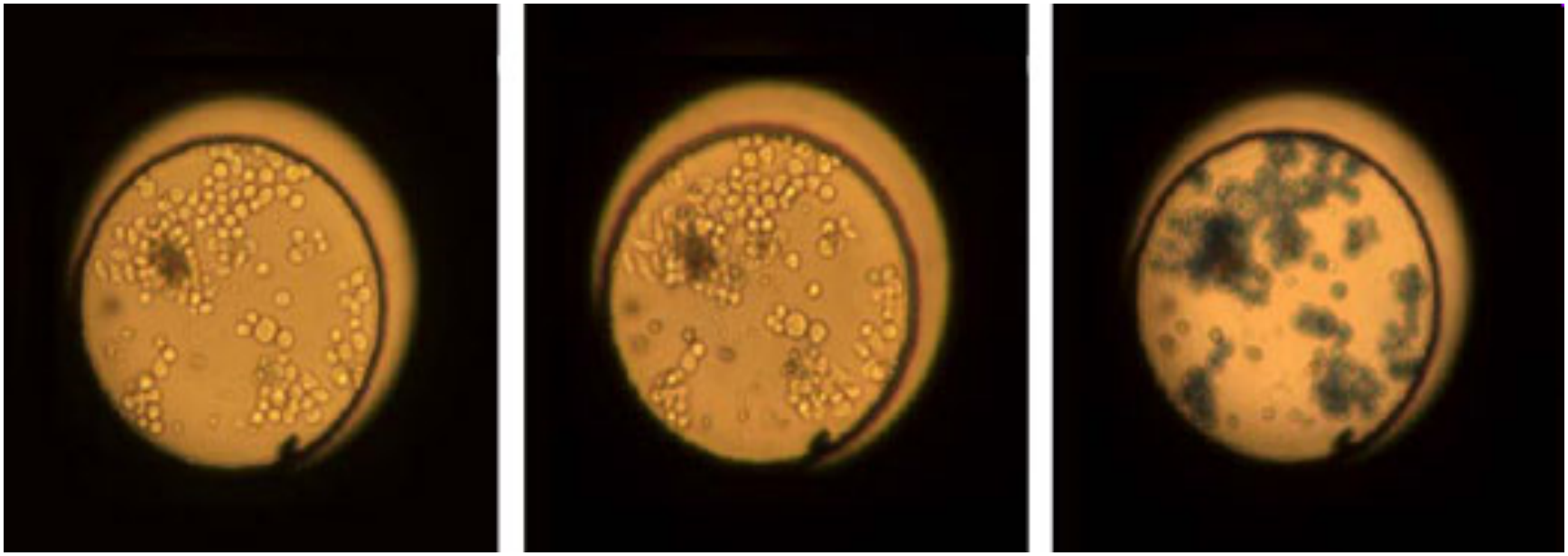
- Optical density
- Cell counts
- Hemacytometer Coulter counter Plate counts
- Dry mass weight

Viability (trypan blue test)

Estimation of specific productivity (pg/ cell / day)

Nutrient consumption

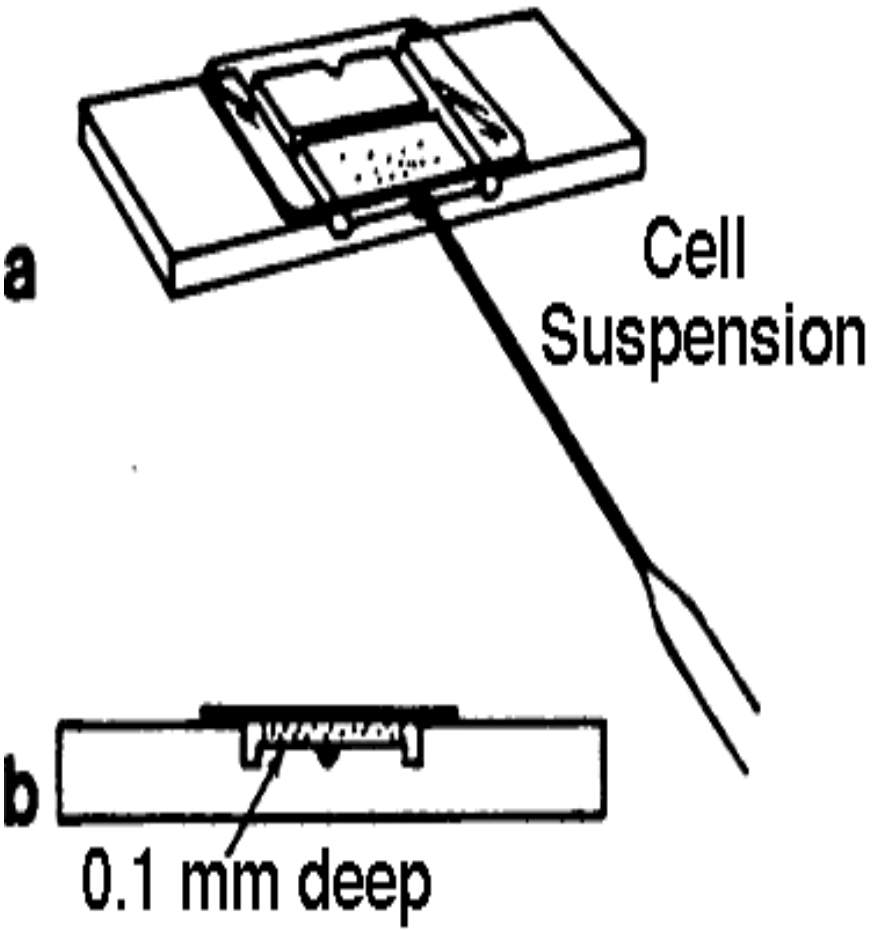
Illustration of Trypan Blue test

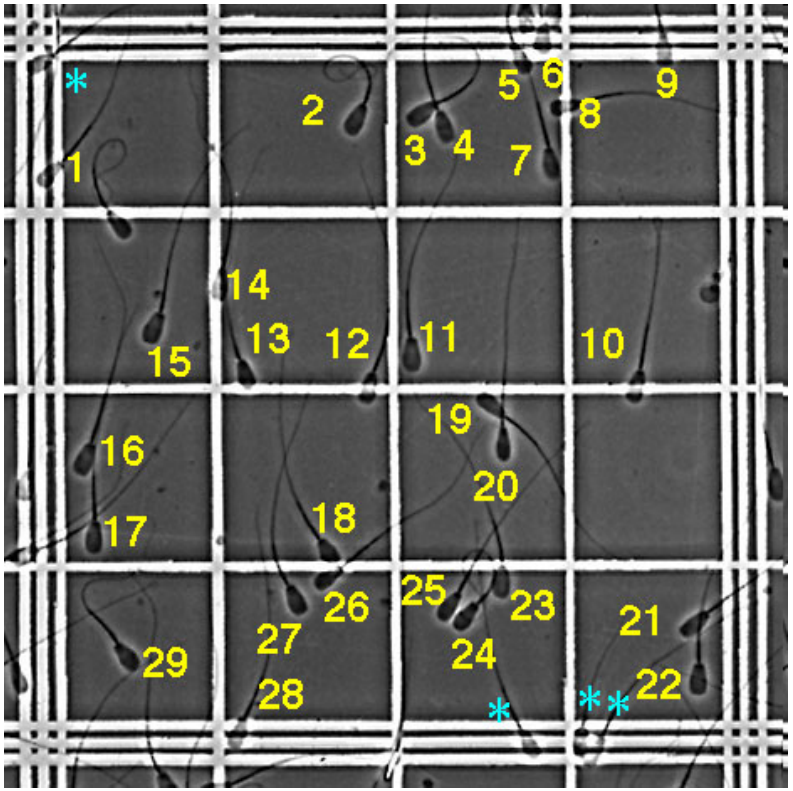


Time lapse images of trypan blue cell (Sigma Aldridge Trypan Blue Solution) viability study performed on drosophila KC cells treated with Valinomycin.

www.nanopointimaging.com/.../cell-viability.html

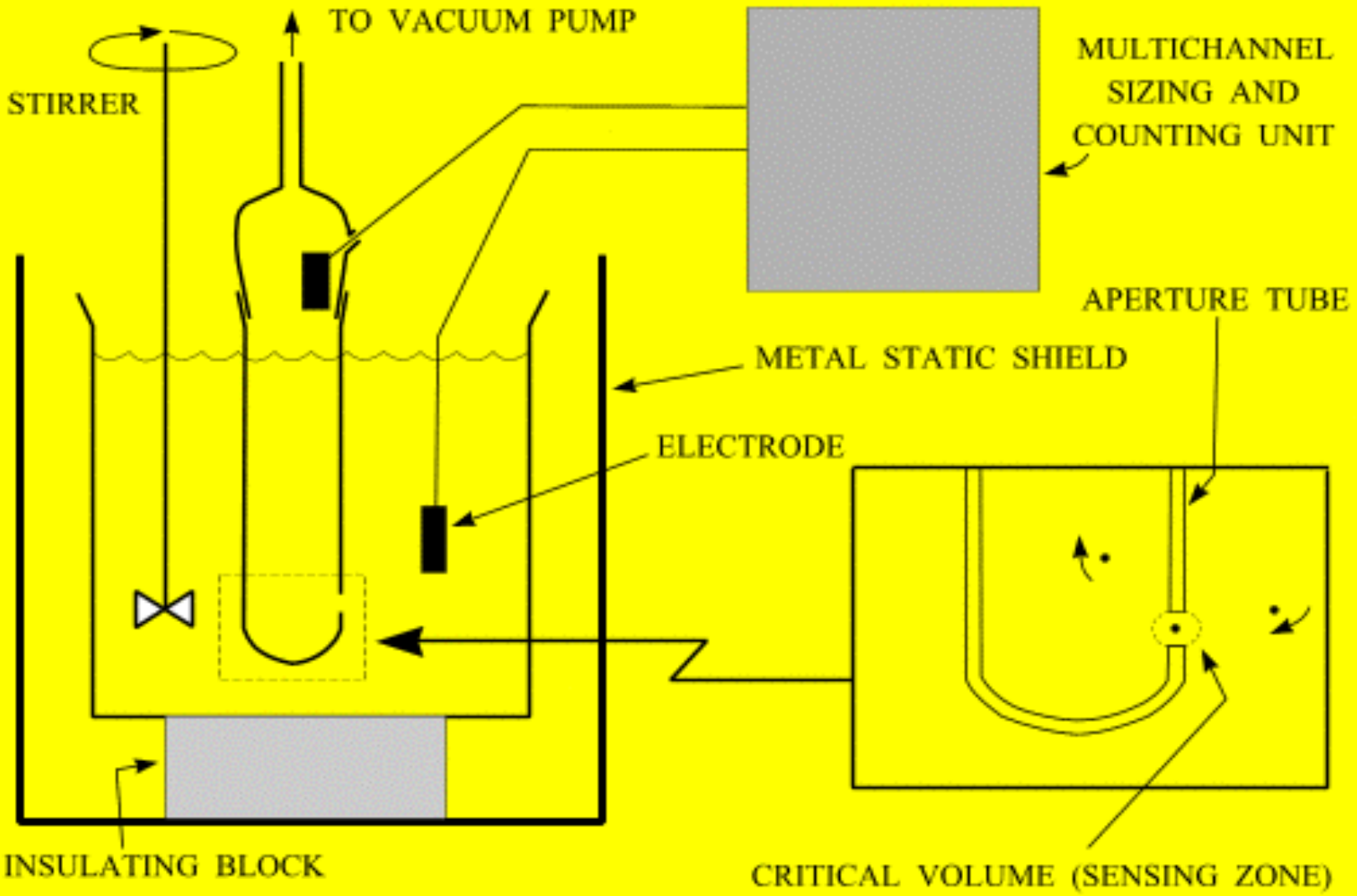
hemacytometer





http://www.ansci.wisc.edu/jjp1/ansci_repro/lab/procedures/hemacytometer/Hemocytometer%20use.html

BASIC ELECTRO-RESISTANCE MULTICHANNEL PARTICLE-SIZE ANALYZER



Modified from McCave and Syvitski (1991)

Choosing which organism: E.coli

Merits:

- Very fast growth- large batches possible in very short time
- Culture are easily maintained
- Can be grown in simple media (Luria broth)

Disadvantages:

- inherent high endotoxin load
- Proteins are often expressed in refractile bodies – inclusion bodies need to be renatured
- Very high Cell densities are difficult to achieve
- Proteins cannot be glycosylated

Choosing Which Organism: Yeast

Merits:

- Easily grown at lower temperatures (30C)
- Grows to very high density with short division time
- Proteins are expressed , secreted and soluble
- Glycosylation can be engineered to be human –like
- Minimal media- simple economical growth media
- Tends to secrete few cellular proteins
- Protease knockouts prevent degradation of secreted proteins

Disadvantages:

Some proteins may not fold correctly

Pichia and Saccharomyces expression systems covered by patent issues.

Although glycosylation occurs many variants can be highly antigenic

Choosing which Organism : Mammalian cells

Merits:

- Expresses folded soluble proteins
- Glycosylation is “ correct”
- Some cell lines can be grown to very high densities

Disadvantages:

- Doubling time is slow compared to Yeasts or bacteria (days vs hours)
- Specific productivity may be low
- Media optimization can be difficult
- May require long bioreactor run to harvest target