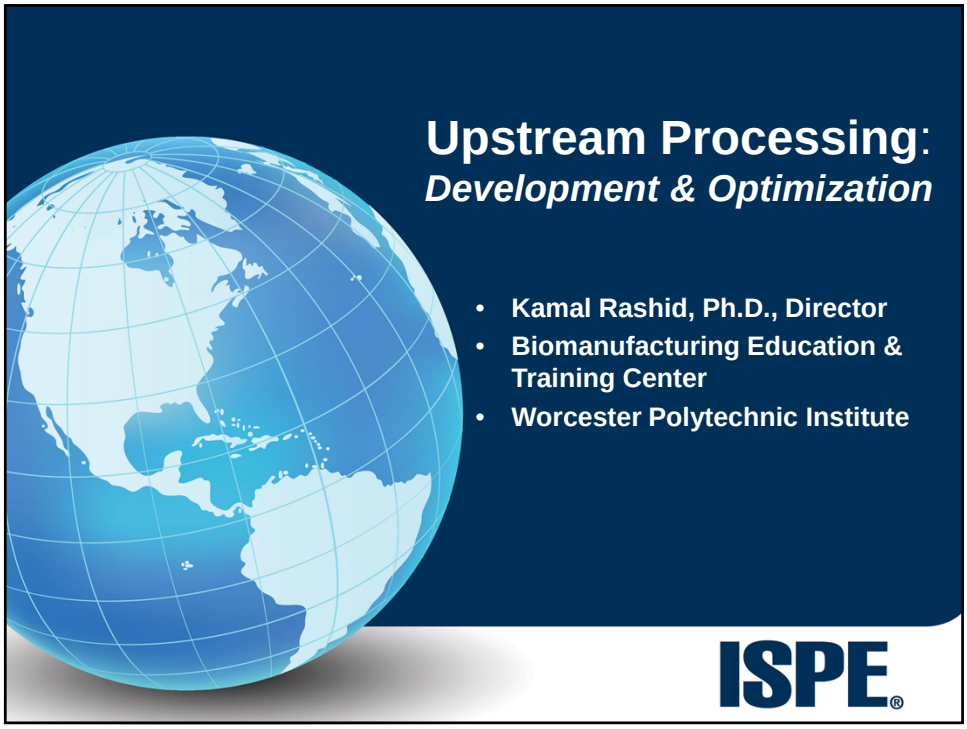
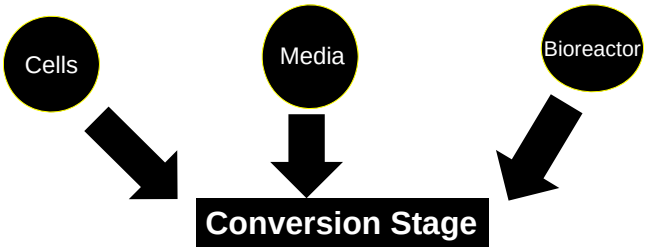


Outline

- Introduction to Upstream processing
- Microbial *vs.* Mammalian Systems
- Cell Line Optimization
- Media Development
- Process Scale-up
- Continuous Upstream Processing

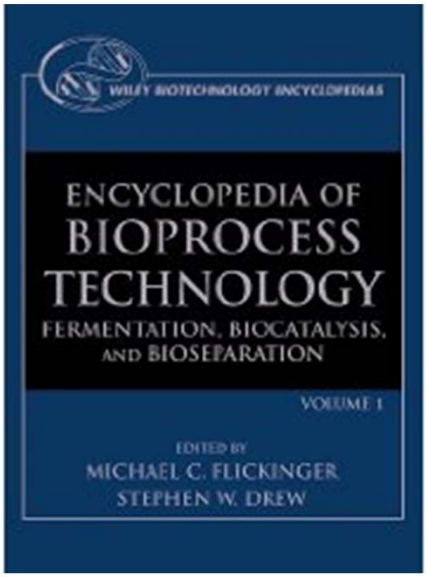
Bioprocessing

- Combining living matter with nutrients under specific conditions to make a desired product



Dissolved O_2 pH Temp
Agitation Aeration Asepsis

Product Separation & Purification



The Basics:

Fermenter: For Microbial Growth

Bioreactor: For Animal Cell Growth

Similarities and Differences

- **Fermentor** – A cell culture system designed for growing highly aerobic bacteria, yeast and fungi
- **Bioreactor** – A cell culture system designed for growing mammalian and insect cells

Fermentors

- Used for growing cells that
  - are fairly robust (because they have a cell wall)
  - grow rapidly (therefore produce a great deal of heat due to a high rate of metabolism)
  - require a lot of oxygen (because of their rapid rate of growth)
- Used for fairly short processes

Bioreactors

- Used for growing cells that are:
  - very fragile (they are highly susceptible to breakage due to shear since they lack a cell wall)
  - grow slowly (therefore do not generate very much heat due to metabolism)
  - do not require a lot of oxygen

Growth

- Growth is defined as an increase in cellular constituents resulting in an increase in the organism size, population number or both.
- In a closed system, the exponential phase of growth remains for only a few generations before entering stationary phase.
- In an open system with adequate nutrient supply and waste removal, the exponential phase can be maintained for a long time.

Bioprocessing Deals with Living Cells

- Microbial Cells
- Animal Cells
- Insect Cells
- Plant Cells

Microbial Cells/Fermentation

- The term fermentation is derived from the Latin verb *fervere,* to boil,
- The process for the production of a product by mass culture of microorganisms
  (microbiologists)
- An energy generating process in which organic compounds act as both electron donors and acceptors (Biochemists)

Choice of Microbial Cell System

- Most Common
  - Bacteria
    - E. Coli
    - Lactobacillus
    - Bacillus
  - Yeast
    - Saccharomyces
    - Pichia

Growth of a typical microorganism

- Lag Phase
- Log or exponential Phase
- Deceleration Phase
- Stationary or Plateau Phase
- Death Phase
  - Nutrient Depletion
  - Accumulation of toxic metabolites
    - This situation is applicable in batch cultures
    - Growth can be extended by addition of fresh medium
      (Continuous culture)

Factors Affecting Microbial Growth

- Availability of nutrients
- Nutrient quality
- Temperature
- pH
- Accumulation of toxic metabolites
- Rate and nature of mixing usually change with every 10 fold increase
- Oxygen demand

Strain Improvement

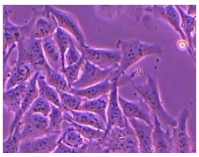
- Screening
- Classical Mutagenesis
- Recombinant DNA and genetic engineering
- Some think that screening and classical mutagenesis offer a significant advantage over r-DNA.
- Why?
  - Minimal start-up time
  - Sustaining the gains over the years

Fermentation: Industrial

- Microorganisms convert raw materials into useful products
  - A set of steps to produce a product
  - The process utilize well characterized cells
    - Wild type
    - Mutant
    - Genetically engineered
  - The crude product after a set of well defined purification steps, leads to a consistent product

Animal cells and Bioprocessing

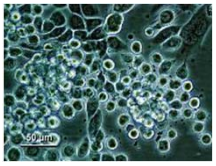
- BHK21
- Vero
- HEK 293
- CHO
- HeLa
- 3T3 Cells
- Hundreds of other cell lines



$1 invested in vaccine production saves $10 in future costs of health care

Insect Cell Culture and Bioprocessing

Sf9 Cell Line
Common name: Fall Armyworm



Cell Growth: *Potential Problems*

- Concerns with viability
- Concerns with Stability
- Concerns with media: *Composition Preparation and Sterilization*
- Concerns with bio-waste containment

Microbial *vs Mammalian*

- **Microbial:**
  - Shorter doubling time
  - Shear resistant
  - Suspension culture
  - Product conc. High
  - Product value can be low to moderate
  - Cell density very high
  - Genetic stability not a problem
- **Mammalian:**
  - Longer doubling time
  - Shear sensitive
  - Anchorage/Suspension
  - Product conc. Low
  - Product value high to very high
  - Cell density low
  - Genetic stability often a problem

Why Animal Cells ?

- Post translational modifications and folding of complex proteins are not shared by microorganisms.
- Vaccines are only produced in animal cell cultures.
- Antibody production is purely cell culture
    - At least at the present time

Cell Line Optimization

Once a primary culture is established, successful sub-culture is usual

It allows for:

- Cloning
- Characterization
- Preservation
- Greater uniformity

Cells from vertebrate cultures are usually finite

In order to overcome this finite life span a transformation event must take place.

Cell Bank: Master Cell Bank (MCB) and Master Working Cell Bank (MWCB)

- **MCB**: Multiple aliquots of viably preserved cells, originating from a single homogeneous pool, created immediately prior to preservation.
- **MWCB:** Derived from one or more vials of the MCB. Sub cultured to a passage number selected by the manufacturer and approved by FDA.

QC of Production Cultures: Cell Culture Media

- Composition and source records.
- Serum certified to be free from contaminants.
- Additives are free of contaminants.
- Trypsin is free from adventitious should not be present in production cell cultures.
- Effective methodology for inactivation or elimination of viruses

Quality control testing

- Tests for the presence of bacteria and fungi.
- Tests for the presence of mycoplasma.
- Tests for the presence of viruses.

Biological Products Characteristics

- Derived from living sources.
  - *Animal, Plants, microorganisms*
- Complex mixtures of proteins
  - *Not easily identified, characterized*
- Heat sensitive
  - *Controlled temp. required during production, packaging and storage*
- Susceptible to microbial contamination.

Critical Biological Product Parameters (required by FDA)

- Safety.
- Potency.
- Consistency.
- Purity.

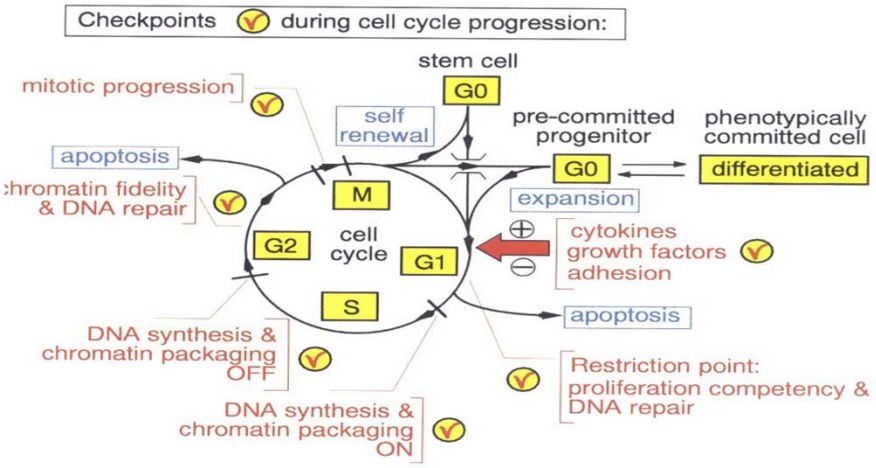
Personnel Qualifications

- Appropriate education, training and experience.
- Ongoing training in cGMP.
- Adequate number of qualified personnel.

Tests for Endogenous Retroviruses

- Plaque Assay.
- Biochemical Assay: *Reverse-Transcriptase*
- Transmission Electron Microscopy

Cell Cycle and Productivity Improvement



Media Issues

- There are more than 200 individual components (amino acids, trace metals, vitamins, growth factors, carbon sources, etc.) found in various commercial growth media formulations.
- Some of these may be critical for cell growth or productivity, others may be toxic at certain levels, and many may be involved in complex interactions in the same or competing pathways within the cell.

Media Issues

- Media optimization studies:

Experiments with different medium ingredients that may have an impact on the growth process and productivity

- Optimum medium composition
- Optimal process parameters
- Proper Statistical analysis – complex interactions
- Do not forget downstream processing

Scale Up

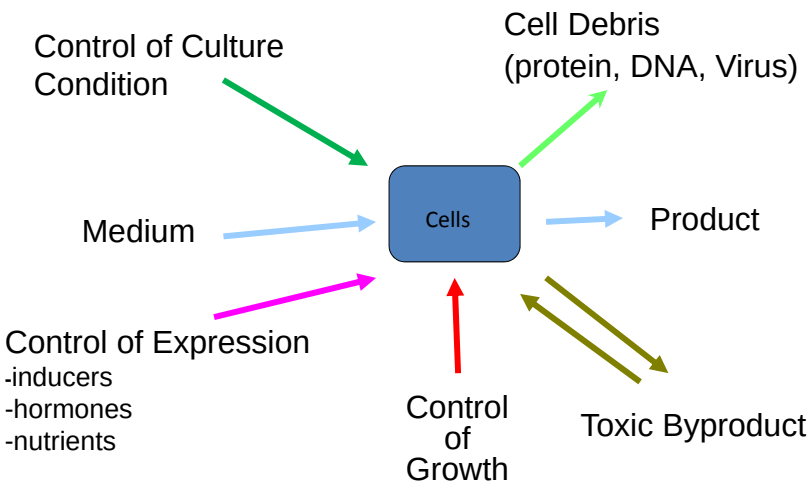
From mg  Kg

- Parameters change
- Performance remain constant

Successful Scale-Up

- A process has been designed.
- There is a predictable increase in the production capacity.
- Product potency is as predicted.
- No unexpected entities are found in the end product.
- The process is validated at production scale.
- Documents are available for regulatory process.

The Cell is a Factory



Scale-Up Challenges

- It involves living organisms
- The organisms are highly selected and/or modified
- The organism's environment is altered in a very complex manner
- Alterations lead to variable interactions
- Interactions often cause unexpected results

Batch Failure

- Cost of failures are estimated to be around $1-2 Billion (Junker, Merck Research Labs)
- How to manage failures?
  - Risk management program
  - Strong training program
  - Investing in right process equipment

Bioreactor Design Elements

- To provide oxygen and other gases without creating a high shear environment
  - Low gas flow regulatory and monitoring devices
  - Appropriate controller for three or four gas control (air, oxygen, nitrogen and carbon dioxide)
  - Low flow rate or shielded spargers
  - Low shear impellers for mammalian cell systems

Probes

What is a Probe ?

Basically a biosensor

Common types of probes in bioreactor systems:

- Dissolved Oxygen Probe
- pH Probe
- Temperature probe

Optimization Studies / Parameters

- Growth parameters need to be analyzed and monitored for enhanced yield:
  - pH
  - Dissolve Oxygen
  - Glucose up-take
  - Lactate production
  - Ammonium concentration
  - Growth rate – Cells
  - Product titer

Bioreactor Design Impact on Optimization

- Accurate measurement of the key process parameters.
- Reproducibility of these accurate measurement from batch to batch.
- Determination of process variables to cell growth and productivity.
- Keeping the operation as such to enable automatic or real time adjustments to the process to keep the production at high levels.
- Aseptic automatic or manual sampling.

Process Optimization Strategy - Up Stream

- The aim of optimization studies are:
  - Maximize productivity of the process
  - Minimize the cost of production
    - Minimize the volume of the media
    - Design cost effective process
    - Maintain cell viability
    - Characterize vital factors affecting the transport of nutrients to the cells.
    - Use modern statistical designs to understand interactions between variables.
    - Optimize the mass transfer of gases

Process Optimization - Up-Stream

- Medium design and feeding strategies
  - Use of chemically defined media
  - Reduce risk of introducing adventitious agents
- In vaccine production pay attention to:
  - The time of infection – 4-5 day post seeding
  - Harvest time- 2-3 days post infection
  - Virus input- MOI

Process Optimization - Up-Stream

Scalability in mammalian systems

- Is the cell line scalable successfully
- Is the cell line growing at the same level of population doubling from bench scale to pilot scale to production scale in the optimized medium

Process Optimization - Up-Stream

- In summary the key focus areas for up-stream process development include:
  - Cell line and clone
  - Expression vector design
  - Medium optimization
  - Bioreactor conditions
    - Constant agitation and aeration
    - Constant agitation and variations in aeration
    - Variations in agitation with constant aeration
    - Variation in both variables

Process Optimization - Up-Stream

- Batch-to-Batch Variability:
  - Collect data on cell count and viability from 3-5 batches
  - Grown at the following time intervals:
    - 0 hour
    - 12 hour
    - 24 hour
    - 36 hours
    - 48 hours
    - 60 hours

Scale-Up

- Scale-up is a fundamental component of process development especially in the biotechnology industry.
- Scaling up a mammalian cell culture process requires consideration to the following factors:
  - mixing time
  - oxygen transfer,
  - carbon dioxide removal.

Scale - Up

- Process parameters change, performance stays constant.
- How to determine that ?
  - Study and validate each step at several scales to insure yielding of a constant product.
- Parameters affected by scale up:
  - Biological:
    - Strain stability Growth Pattern
    - Growth kinetics Oxygen requirements
  - Equipment related:
    - Aseptic operation
    - Agitation and aeration

Scale – Up/ Animal Cells

- Cell:
  - Anchorage dependent
  - Suspension
- Product:
  - Secreted
  - Non secreted
- Process:
  - Batch
  - Continuous

Scale - Up

- Scale up is a serious matter and involves careful planning and careful execution:
  - A process has been designed
  - There is a predictable increase in production capacity
  - Product potency is not changed
  - No unexpected entities are found in the product
  - The process can be validated at the production scale

Scale - Up

- Success or failure of scale up depends on how close rate, yield, and purity at larger scale matches those results at the bench scale.
- The scale up process consist of:
  - Media selection, preparation and sterilization
  - Inoculum development
  - System sterilization and media addition (Asepsis)
  - Selection of parameters
  - Selection of process: batch - continuous
  - Monitor and control
  - Harvest
  - Cleaning

Scale-Up Challenges

- Asepsis
  - Volume of inputs
    - Media
    - Water
    - Air
    - Other gases
    - Sterilization
- Containment
  - Aerobic
    - Flanges Connectors
    - Pressurization Gas compressors

Scale-Up Challenges

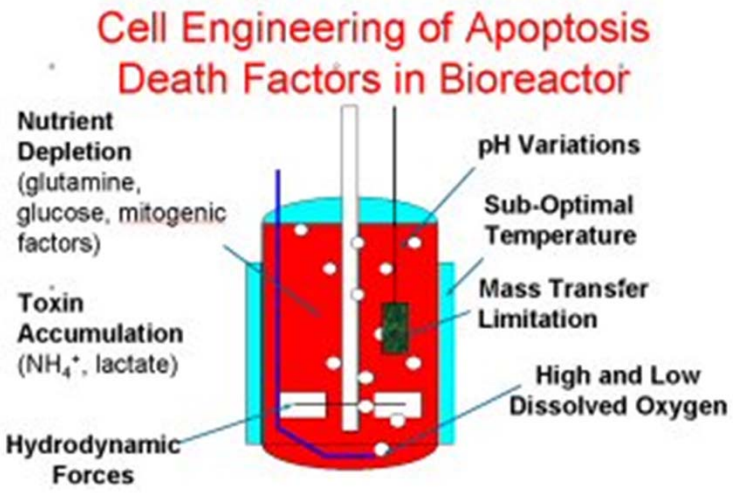
- **Cleaning**
  - Fouling
  - Scale formation
  - Equipment reuse
  - Equipment inspection
  - Agents for use and disposal
- **Time**
  - Transfer time Lag phases
  - Heating/cooling cycles Holding time

Scale-Up Considerations

- Bench – to – Pilot Scale
  - Understanding the process control
  - Meeting the regulatory needs
  - Producing product for clinical trials
    - Quantity
    - Quality
- Pilot – to – Production Scale
  - More understanding the process control
  - Dealing with financial pressures
    - Batch failure

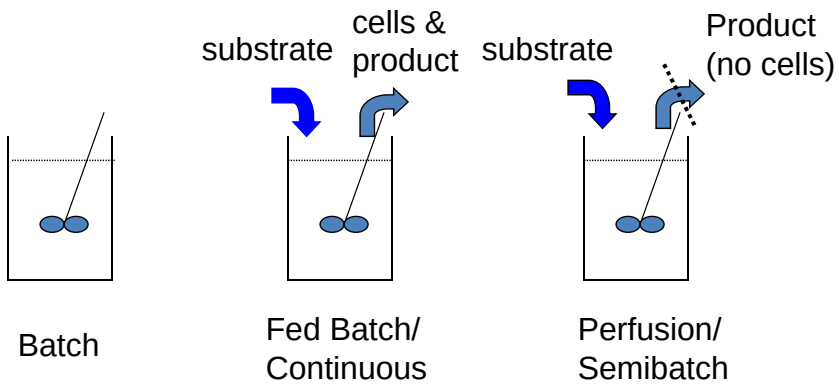
Scale – Up Considerations

- Pilot scale experiments are required for a successful scale-up to determine:
  - Product titer at harvest
  - Reactor productivity (mg product/L/day)
  - Maximum cell growth rate
  - Shear effects
  - Oxygen mass transfer



56

Modes of Operation- Fermentation

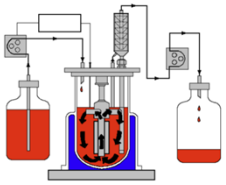


Batch Culture in a Stirred Tank Bioreactor

- In batch reactors the idea is to put all the reactants in a vessel via a combination of mixing, heating, and cell growth to make a product
- It can operate also as semi-batch where at least one reactant is gradually added over a period of time as different reactions take place (Fed Batch Culture)

Continuous Culture

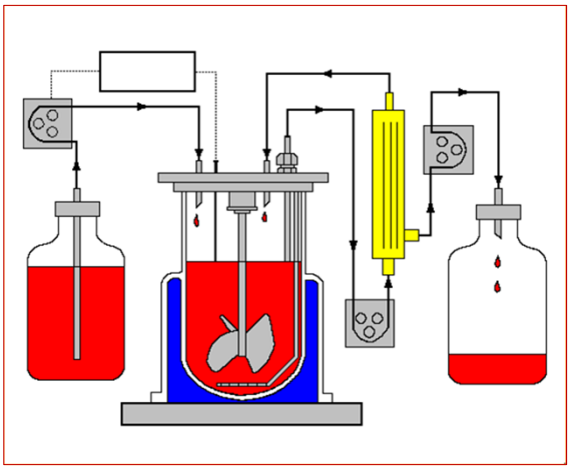
- The idea is to maintain constant and uniform conditions within the reactor.
- Control of the reactor is by constant feed of properly proportioned reactants with temperature control and continuous removal of products at a rate matching the input of feed materials



Continuous Culture

- Continuous reactors offer higher productivity than a comparable size batch reactor
- It may require only one tenth the size of a corresponding batch reactor

Perfusion Techniques



Thank You