



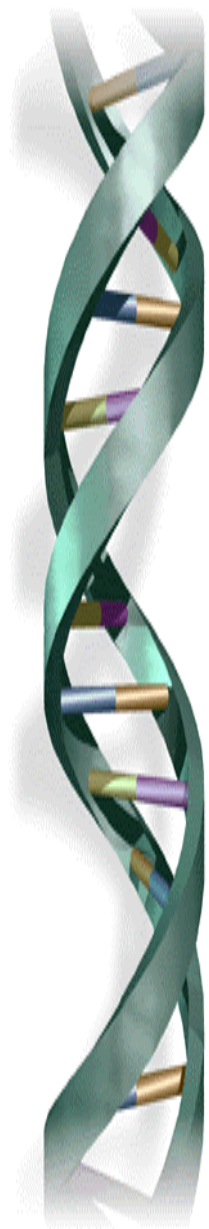
Sensors in Bioprocess Control

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Harvard's Law

Under the most rigorously controlled conditions of pressure, temperature, volume, humidity, and other variables, the organism will do as it darn well pleases

Translation ...

You put the organisms into the tanks and pray



Presentation Outline

- ◆ The needs for process control
- ◆ Industrial bioprocesses
- ◆ Cell and its environment
- ◆ Measurements
- ◆ Sensor requirements
- ◆ Examples
- ◆ Conclusion and future directions
- ◆ Acknowledgments



The Need for Process Control

- ◆ To maintain consistent process performance (productivity, quality) throughout the development cycle (R&D to Manufacturing).



Consistent Process Performance

◆ R&D

- ❖ Experiment to Experiment
- ❖ Scale to Scale

◆ Manufacturing

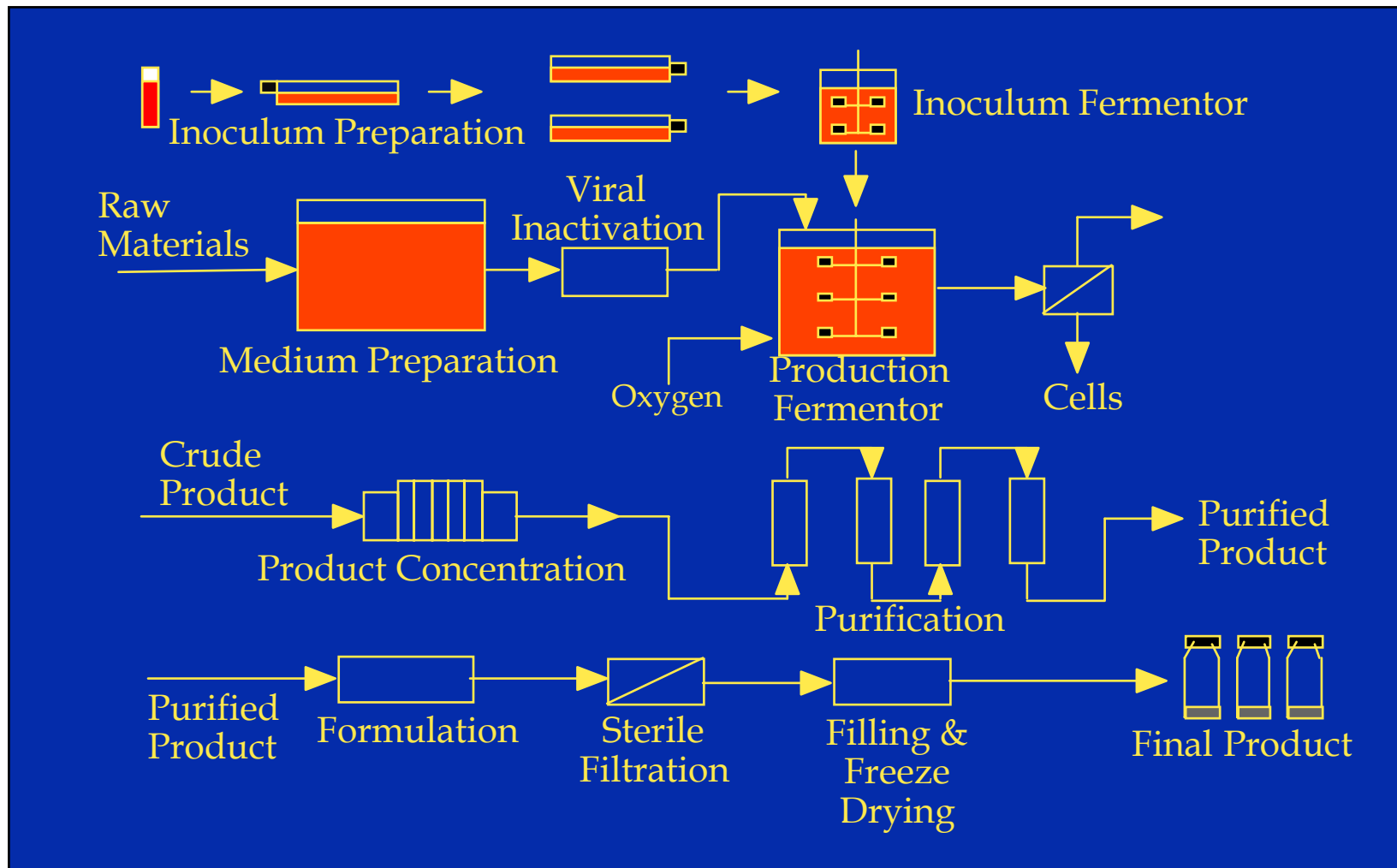
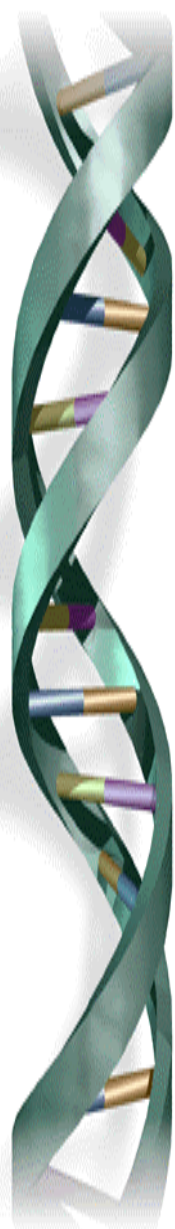
- ❖ Thaw to Thaw
- ❖ Run to Run
- ❖ Campaign to Campaign
- ❖ Plant to Plant



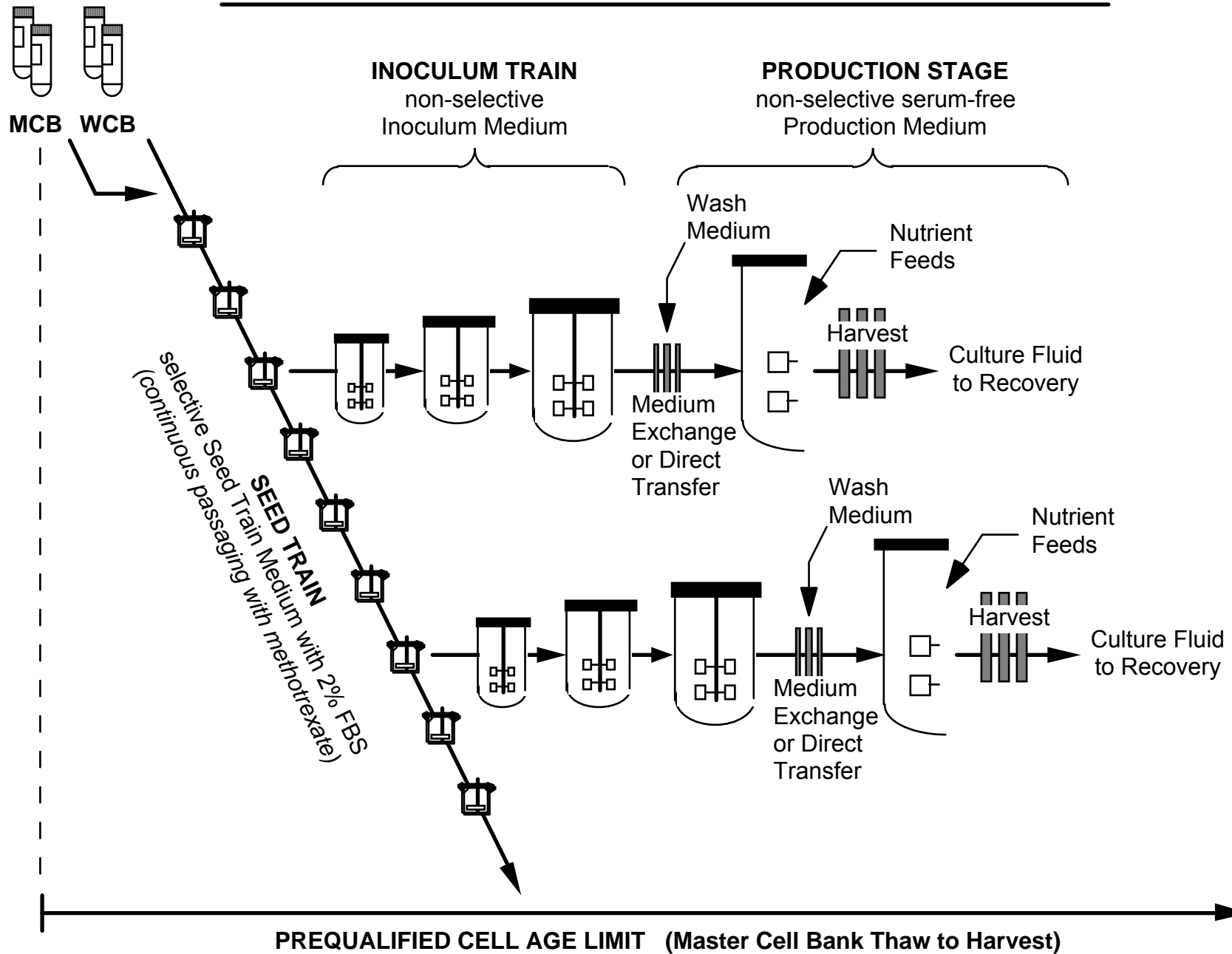
Sensors for Bioprocess Control

- ◆ Measurements for process or system analysis
 - ❖ Search for underlying functional relationships
 - ❖ In depth analysis of the interaction of the organisms with their environment
- ◆ Provide capabilities for process control
 - ❖ Setting up and maintaining the optimum environmental conditions for growth and/or formation of product
- ◆ Large-scale versus laboratory bioreactors

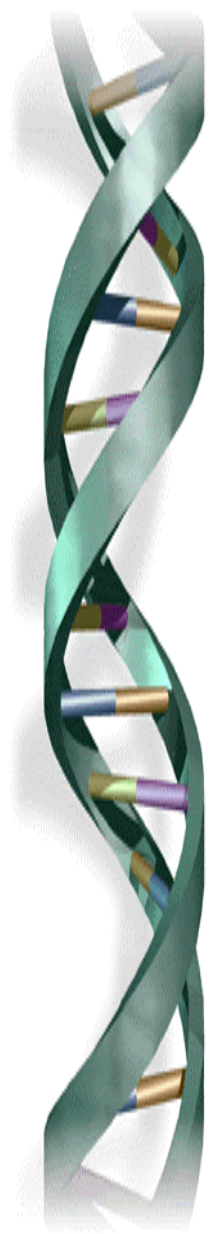
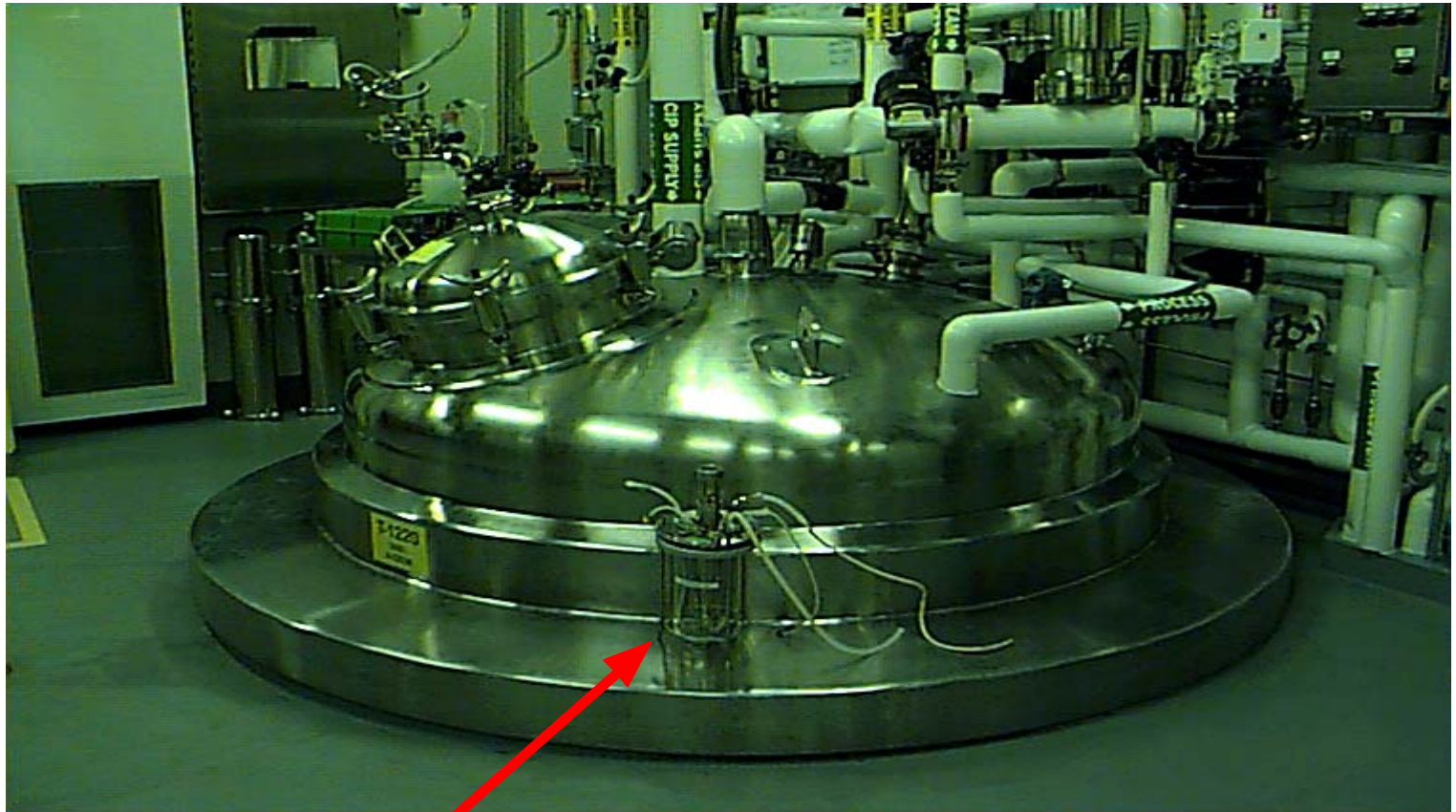
Generic Process Flow Diagram for Protein Pharmaceutical



TYPICAL LARGE-SCALE CELL CULTURE PROCESS



Scaling Up From 2-L to 12,000-L

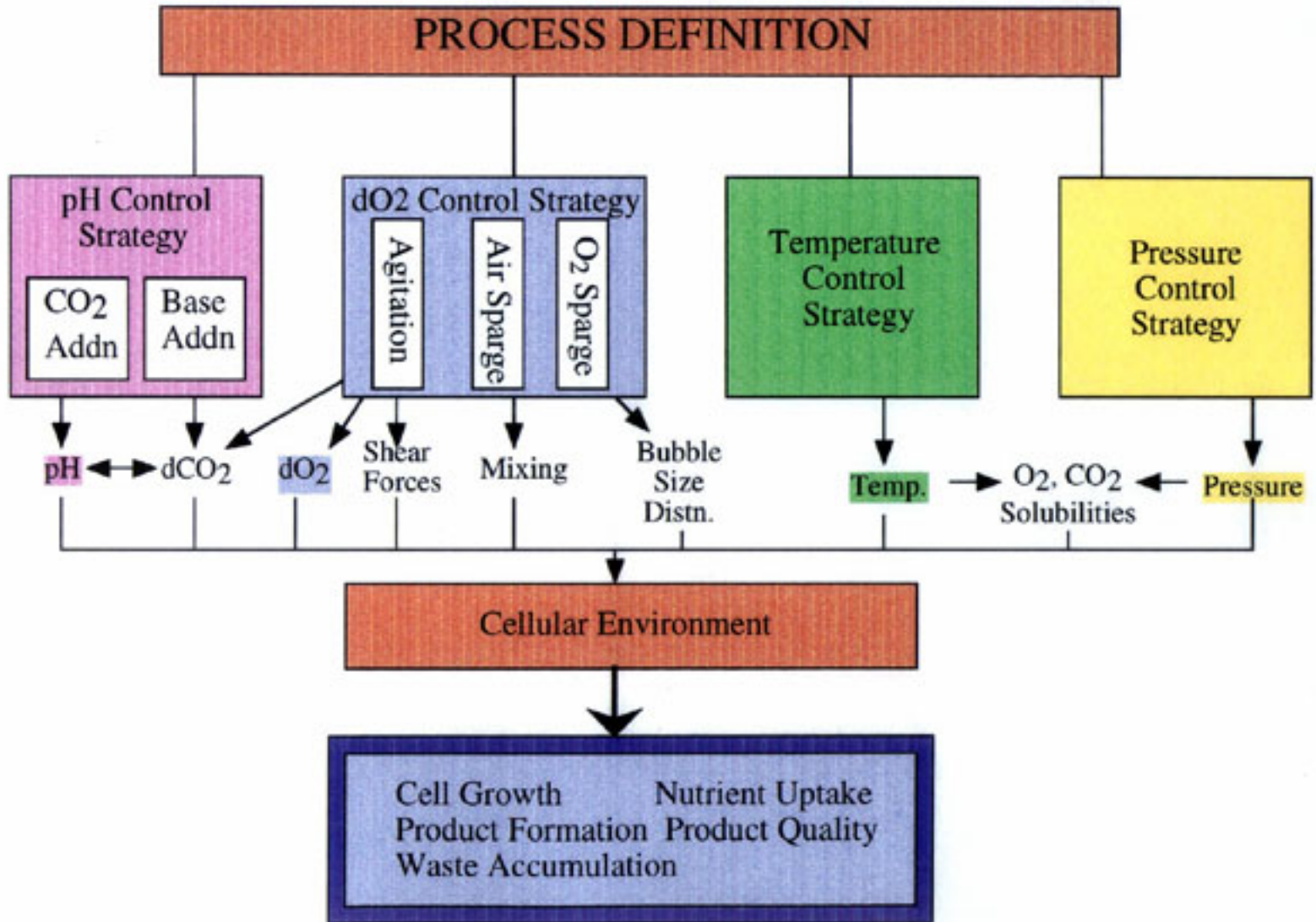




Cell and Its Environment

- ◆ Cells are isolated from complex multicellular organisms
- ◆ Homeostasis is maintained in these organisms by many specialized organs and tissues working synergistically.
- ◆ Therefore mammalian cells do not have the capacity to maintain homeostasis by themselves
- ◆ Cell culture is not a natural environment for mammalian cells.

Interactions Between Cell Culture Environmental Controls





Measurements

- ◆ Biological
- ◆ Chemical
- ◆ Physical



Biological Measurements

- ◆ Cell Density
- ◆ Cell Viability
- ◆ Cell Size
- ◆ Morphology
- ◆ Cellular assays
- ◆ Molecular/Genetic Assays



Chemical Measurements (cont.)

- ◆ Product
 - ❖ Concentration
 - ❖ Quality
- ◆ By-products
 - ❖ Organic acids – acetate, lactate, etc
 - ❖ Proteins
 - ❖ Ammonia
- ◆ Chemical environment
 - ❖ pH
 - ❖ Dissolved gases (dissolved O₂, pO₂, pCO₂)
 - ❖ Osmolality
- ◆ Off-gas
 - ❖ O₂ (OUR)
 - ❖ CO₂ (CER)



Physical Measurements

- ◆ Temperature
- ◆ Agitation
- ◆ Pressure
- ◆ Level (volume)
- ◆ Weight
- ◆ Broth density
- ◆ Viscosity



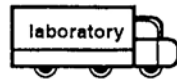
Important Criteria for Sensors

- ◆ Reliability, Accuracy, Reproducibility
- ◆ Long-term Stability
- ◆ Specificity
- ◆ Response time
- ◆ Dynamic behavior
- ◆ Ability to be repeatedly cleaned and sterilized
- ◆ Ease of operation
- ◆ Ease of maintenance
- ◆ Size
- ◆ Cost

Modes of Bioprocess Monitoring

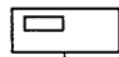
off-line

e.g. ELISA, plasmid stability



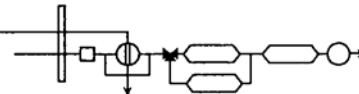
in situ

e.g. O₂, pH



on-line

e.g. FIA (glucose, lactate)



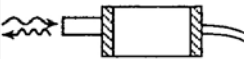
at line

e.g. HPLC, biomass,
mass spectrometry



non-invasively

e.g. culture fluorescence



Callis JB, Illman DL, Kowalski BR. Process Analytical Anal Chem 1987;59:624A-637A.



Sampling Considerations

- ◆ Representative sample
- ◆ Sample size
- ◆ Sterility requirements
- ◆ Utilities considerations
- ◆ Disposal considerations
- ◆ Liquid versus vapor samples
- ◆ Sample preservation



Requirements for in Situ Sensors

- ◆ Fully cleanable and sterilizable
 - ❖ Good thermal stability and compressive strength, no temperature hysteresis
- ◆ Long-term stability and accuracy
- ◆ Fast response
- ◆ No flow dependence
- ◆ No interference
 - ❖ Air bubbles (O₂ and CO₂) or by microbes
 - ❖ Complex media
- ◆ No fouling
- ◆ Low maintenance
- ◆ Small size



Sterile Sampling Designs

◆ Sample location

- ❖ Sample withdrawal position
- ❖ Method of connection
- ❖ fluid velocity profile
- ❖ Containment considerations

◆ Sampler and container design

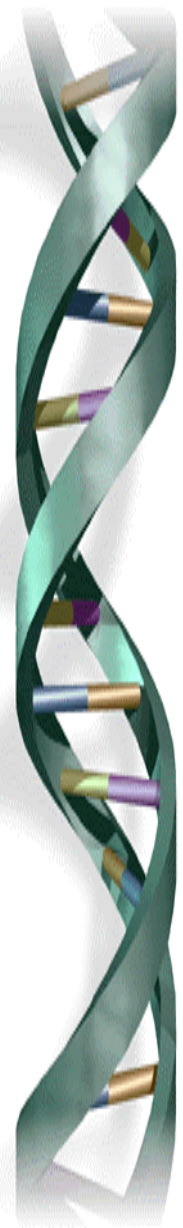
- ❖ Materials of construction
- ❖ Process and sample variables
 - ↑ Temperature
 - ↑ Pressure
 - ↑ Slurry/two phases
 - ↑ viscosity



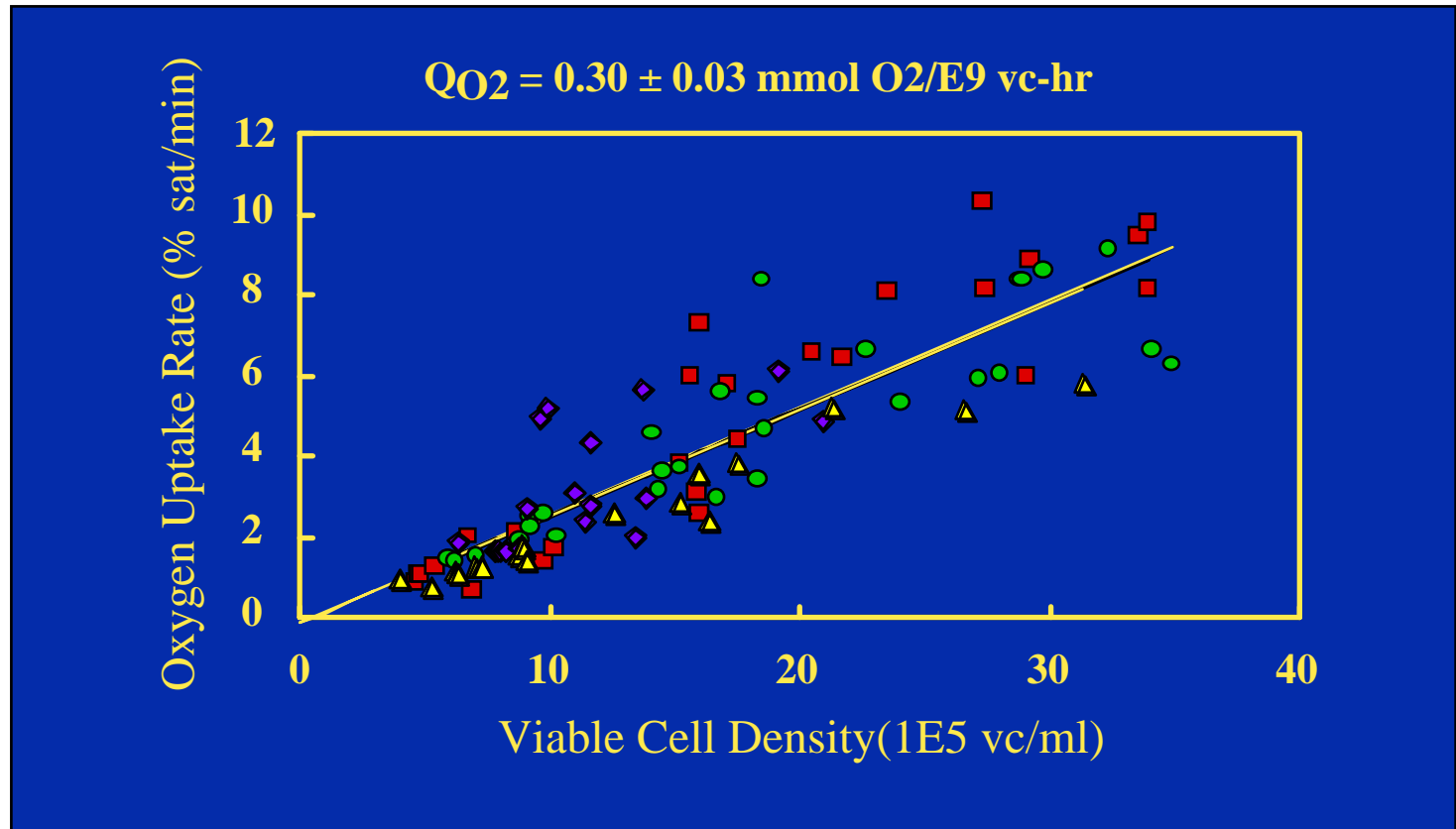
Sampling Designs (Cont.)

- ◆ Sterilization options
- ◆ Purging considerations
- ◆ Venting considerations
- ◆ Containment
- ◆ Design options

Cell Density and Viability Measurements

- 
- ◆ Need to know the viable and nonviable cell density to evaluate growth rate, death rate and specific productivity
 - ◆ Direct - Count the cells
 - ❖ Hemocytometer with Trypan Blue staining - total viable and total nonviable cells
 - ❖ Coulter counting - total cell number
 - ◆ Indirect - Measure a factor which correlates with cell number
 - ❖ Packed Cell Volume (PCV) - correlation with cell number
 - ❖ Optical Density (OD)
 - ❖ Dry weight
 - ❖ Total DNA
 - ❖ Total protein
 - ❖ Cellular enzymatic activity
 - ❖ Cellular metabolic activity

CHO Cell Biomass Estimation via Oxygen Uptake Rate Measurement

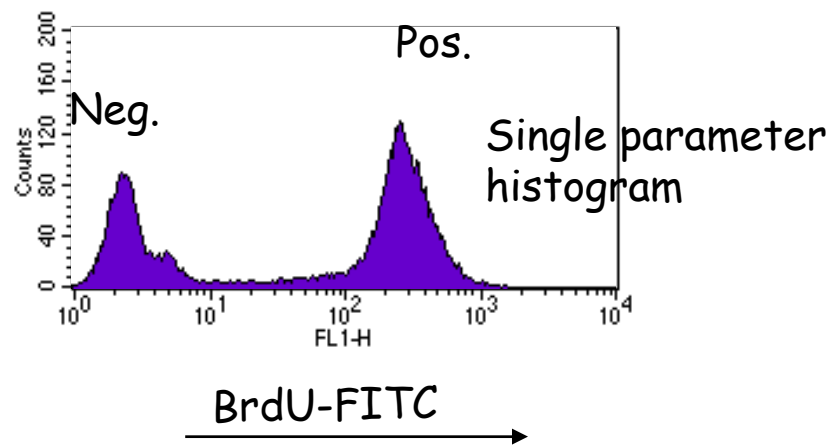
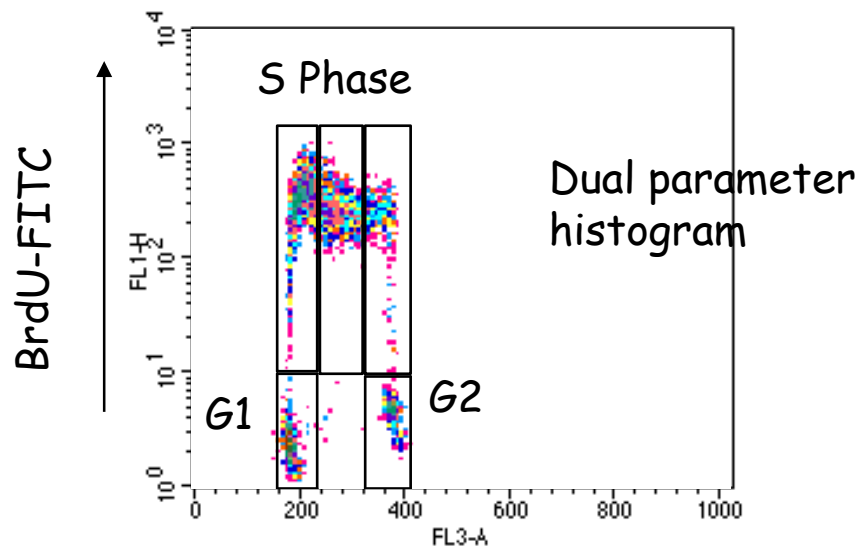
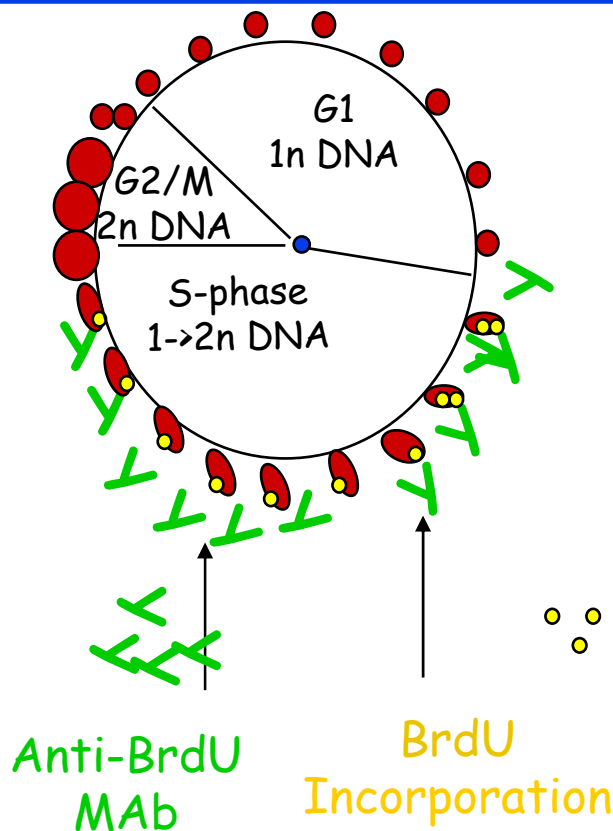
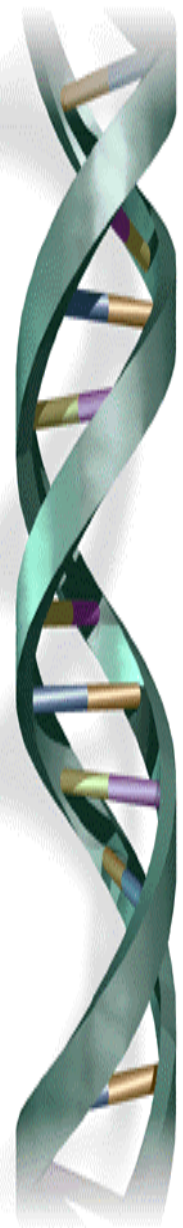




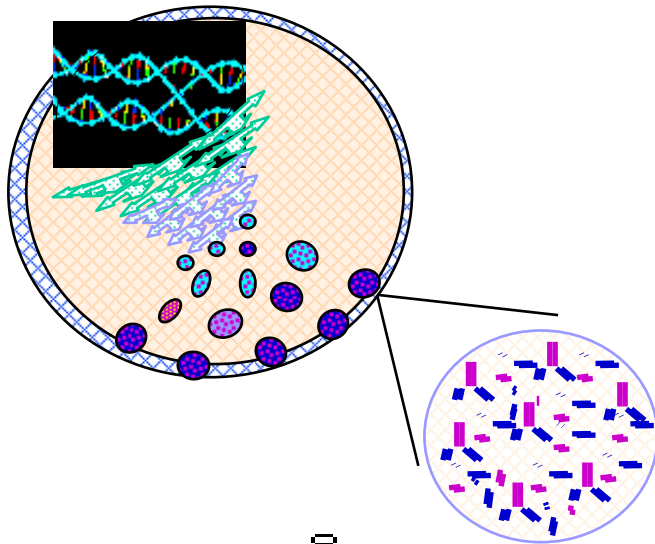
Cellular Assays

- ◆ Measure physiological or expressed parameters and will be useful in measuring “How” cell lines differ.
 - ❖ Bromodeoxyuridine (BrdU) cell cycle
 - ❖ Fluorescent methotrexate (F-Mtx) binding
 - ❖ Specific productivity
 - ❖ Cell tracer

Cell cycle analysis with anti-BrdU and flow cytometry



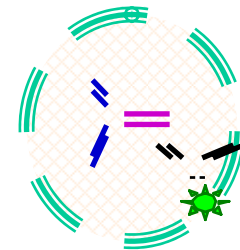
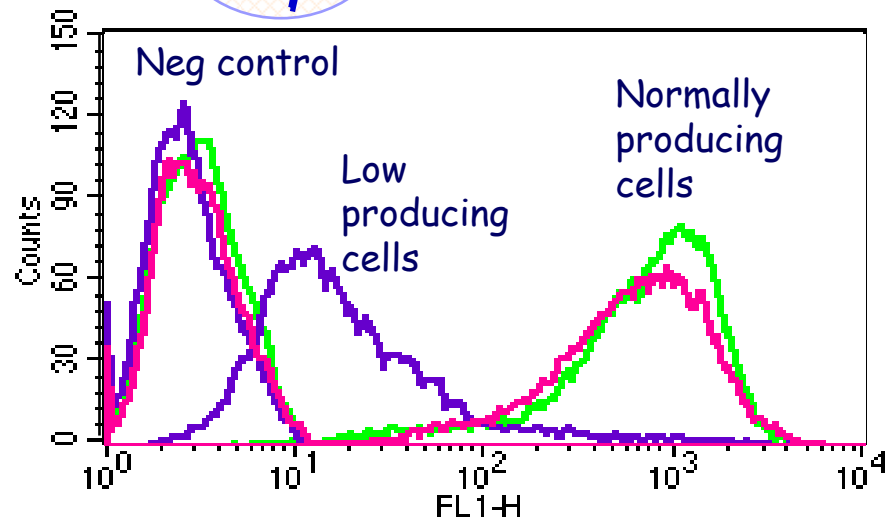
Measurement of intracellular product



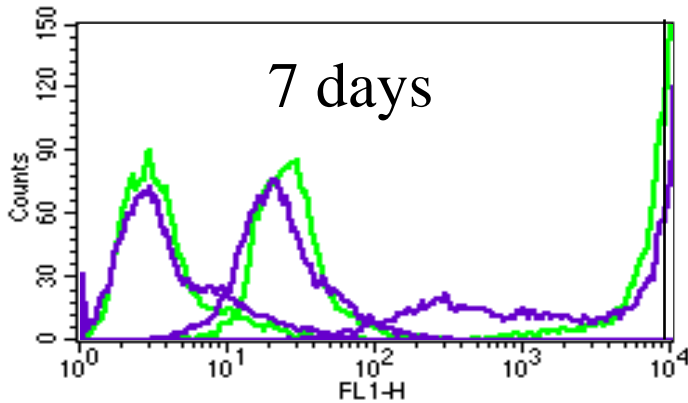
Cells are


- Formaldehyde-fixed
- Detergent permeabilized


- Product is detected with FITC conjugated F(ab')₂




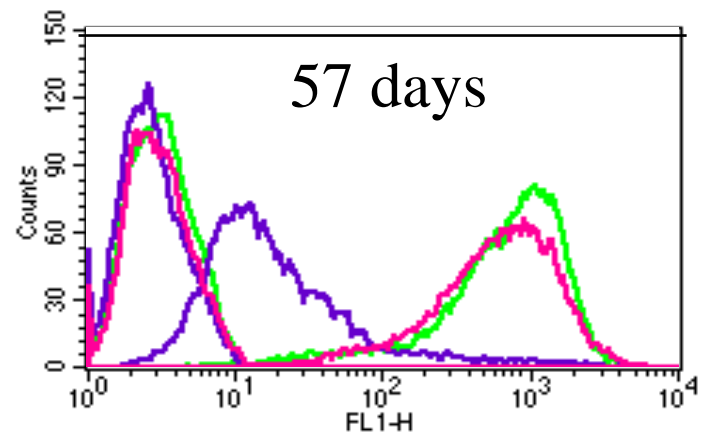
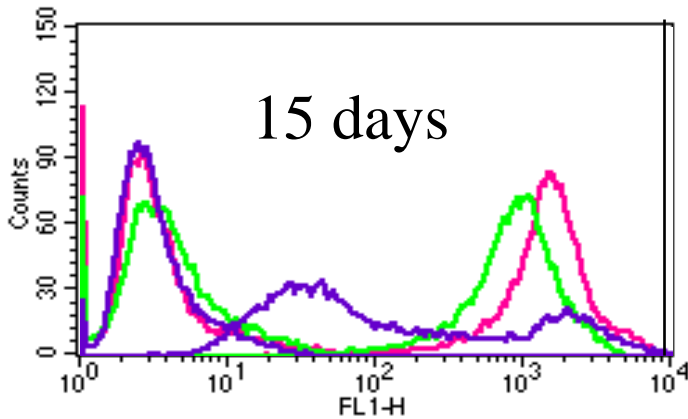
Rapid Loss of Intracellular Product Over Time



C line 

B line 

A line 





Cell Culture pH Control

glucose, amino acids, vitamins, O_2 , ...

----> Cells, CO_2 , Lactate, NH_3 , H_2O , Product ...

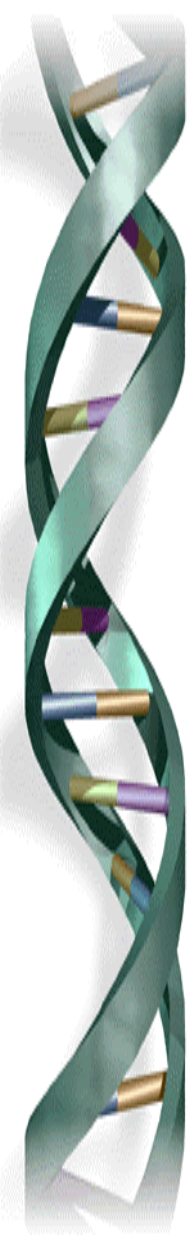
◆ Control Objectives

- ❖ Maintains desired pH with minimum osmolality rise
- ❖ Ensure consistent pH profiles from run to run

◆ Typical Means of Control

- ❖ Use acid source (CO_2 gas) and base source (Na_2CO_3)
- ❖ Apply gap/deadband controller (± 0.03 pH units from setpoint)

Control of Dissolved Gases in Cell Cultures

- 
- ◆ During aerobic growth, cells require O_2 and produce CO_2
 - ◆ Cells sensitive to extremes of dissolved gas concentrations
 - ❖ Hypoxia (<1% of air saturation?)
 - ❖ Hyperoxia (>100% of air saturation?)
 - ❖ CO_2 required for synthesis/energy metabolism reactions
 - ❖ Excess CO_2 can inhibit respiration reactions, change intracellular pH
 - ❖ Minimum required levels unknown
 - ❖ CO_2 levels may influence product characteristics
 - ❖ Control of culture pH, dO_2 , dCO_2 , pressure all inter-related



Theoretical Nutrient Depletion Times in *E.coli* Fermentation

◆ Oxygen

- ❖ At 30% of air saturation, $[O_2] \sim 0.075$ mM
- ❖ With $OUR = 5$ mmoles/L-min
- ❖ Depletion Time = 1.8 seconds

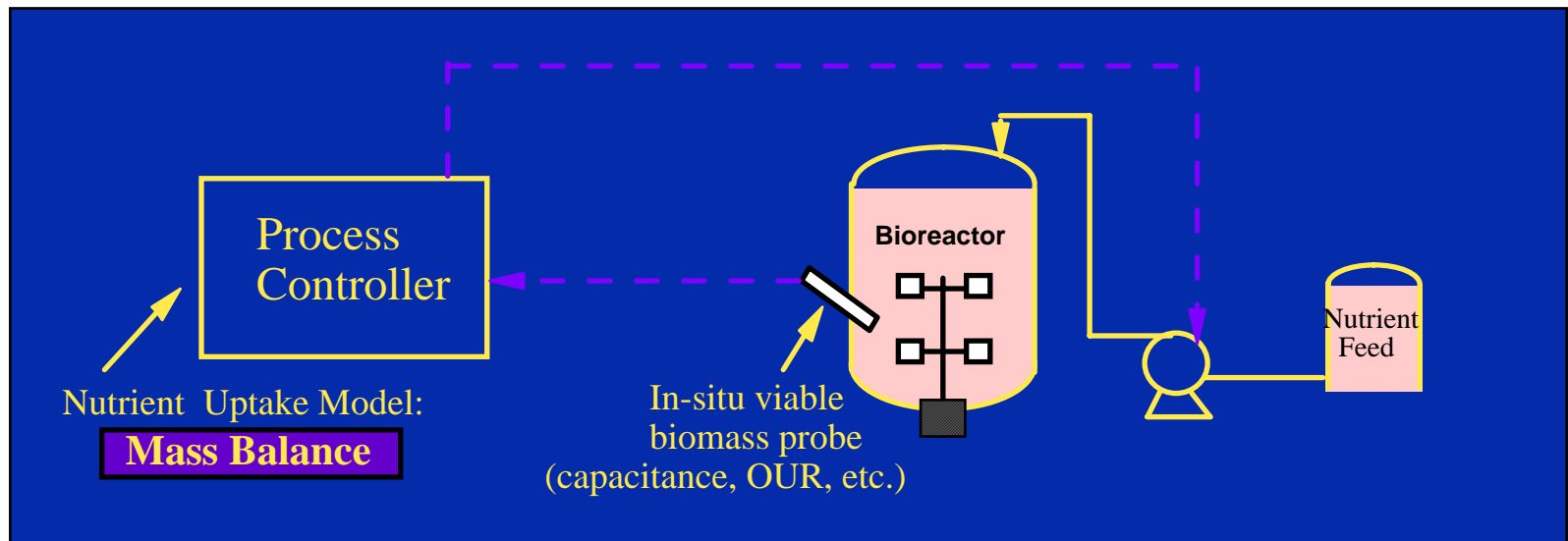
◆ Glucose

- ❖ To avoid acetate formation, $[glucose] \sim K_s, gluc$
- ❖ $K_s, gluc \sim 20 \mu M = 3.6$ mg/L
- ❖ During growth, glucose uptake rate ~ 2 mmoles/L-min
- ❖ Depletion Time ≤ 0.6 seconds

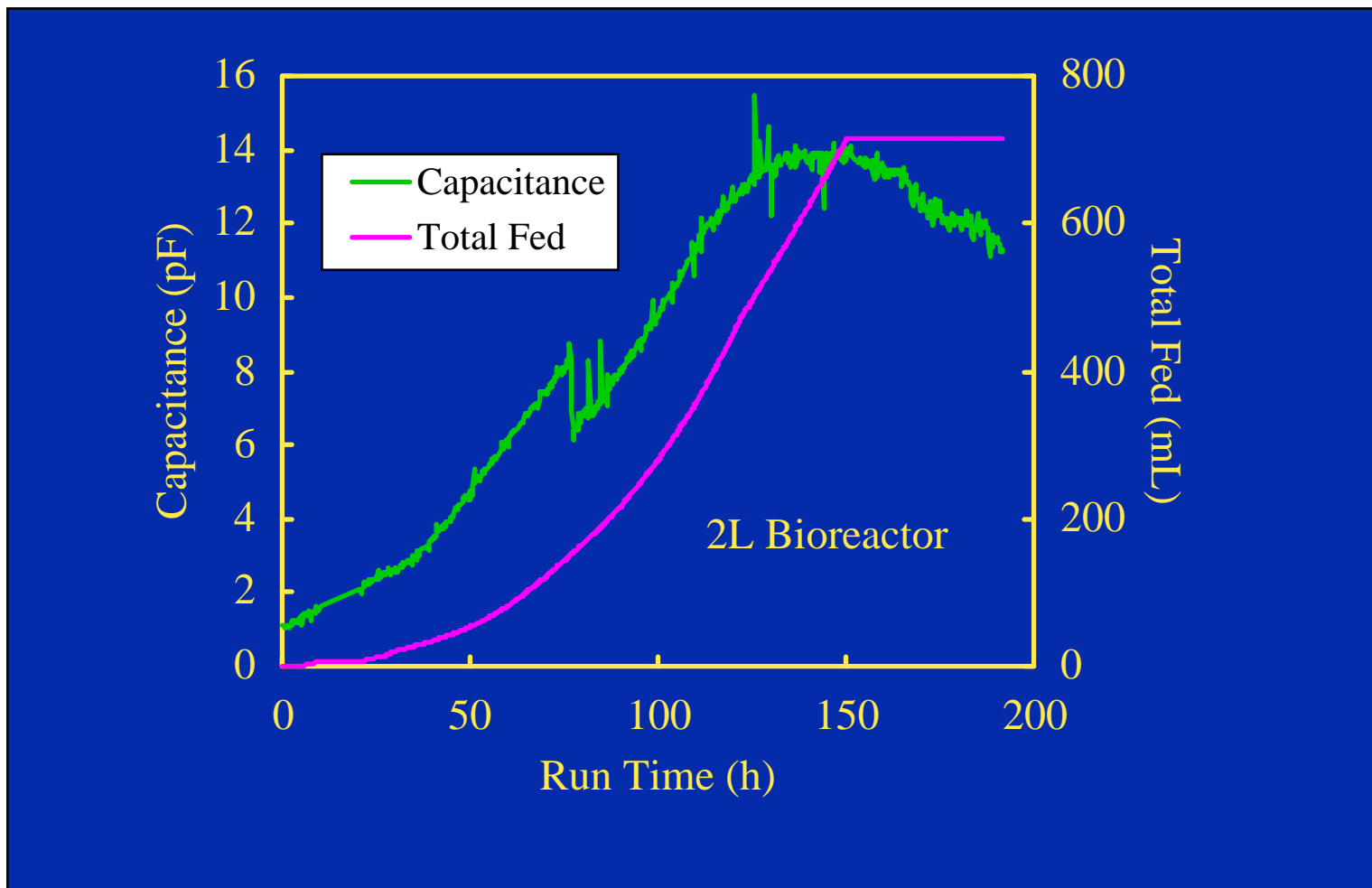
◆ Typical mixing times = 12 to 50 seconds

Galactosylation Control via Controlled Nutrient Feeding

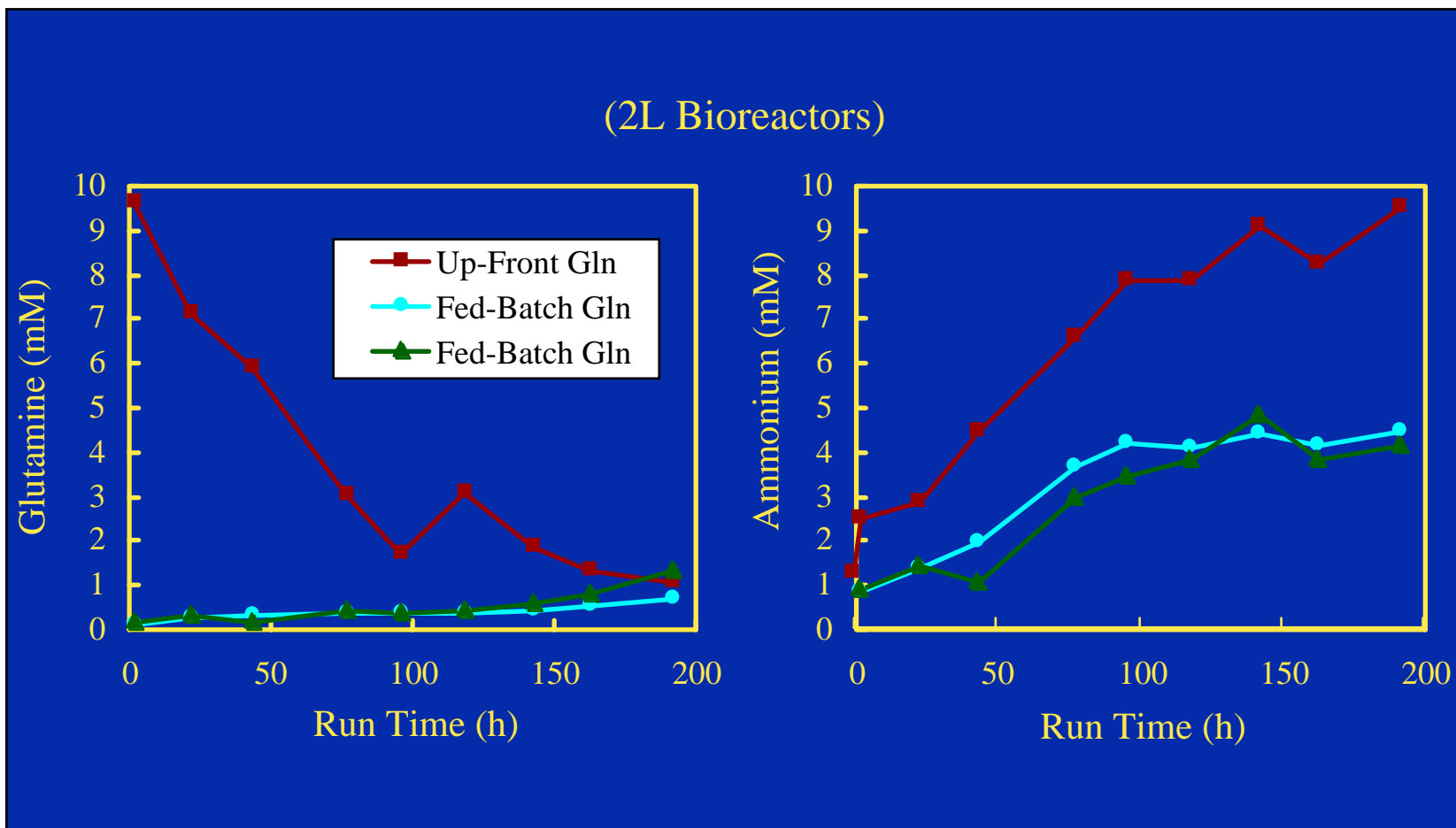
- ◆ NH_3 can influence glycosylation efficiency by increasing intracellular pH
- ◆ Reduce waste product accumulation (i.e., NH_3) by controlled feeding of nutrients which generate NH_3
- ◆ Use on-line biomass estimation to generate feed profile



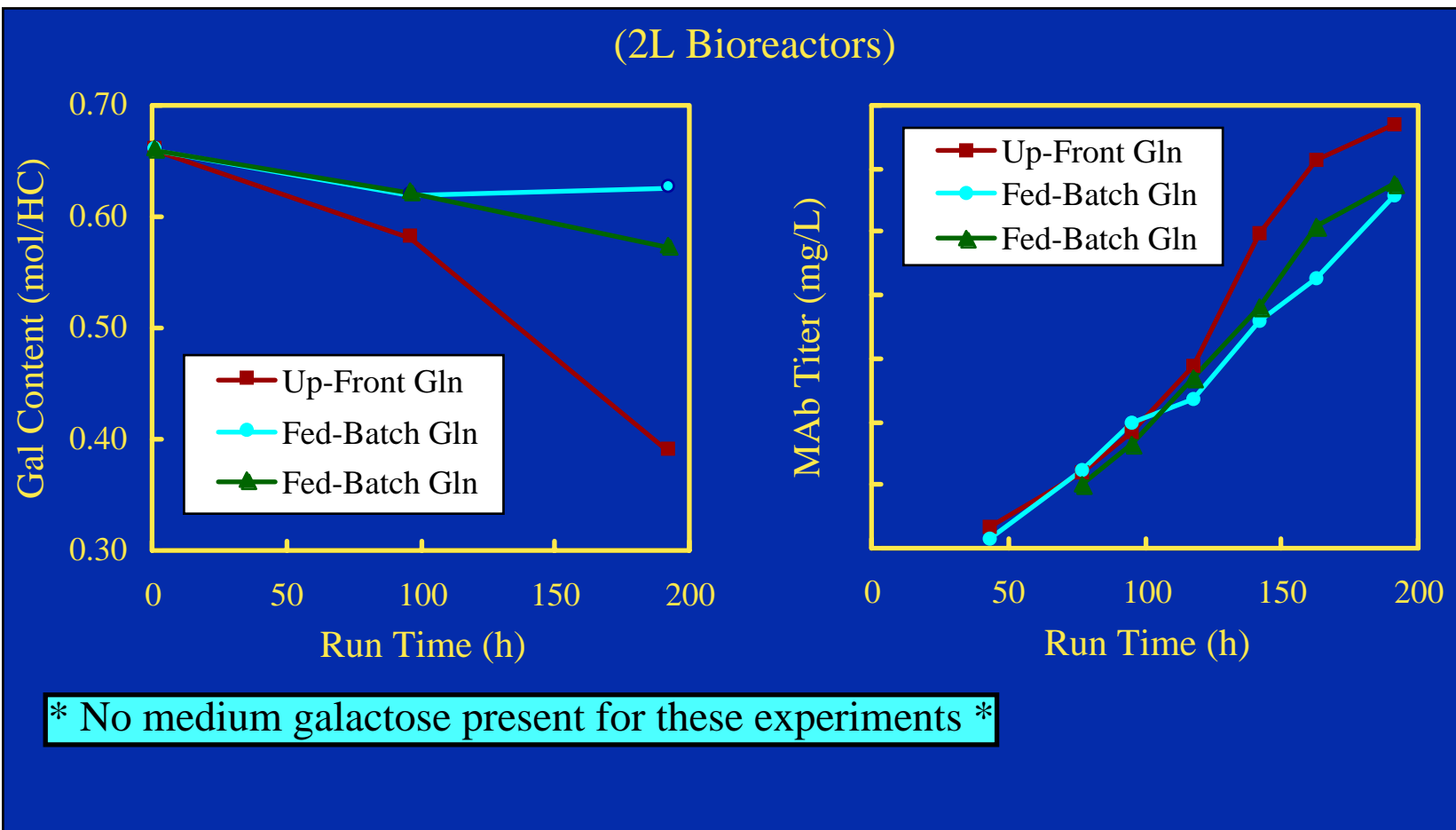
Nutrient Feed Rate Based on Estimated Viable Cell Population



Controlled Nutrient Feed Results in Improved Galactosylation



Controlled Nutrient Feed Results in Improved Galactosylation



* No medium galactose present for these experiments *



Immediately Applicable But Still Missing...

- ◆ On-line in situ glucose (and other critical metabolites) sensors
- ◆ On-line viability measurement for cell culture
- ◆ Simpler (for manufacturing use) population assessment tools
- ◆ Soft sensors
- ◆ Analysis tools for retrospective modeling, troubleshooting and optimization



Conclusion and Future Directions

- ◆ Improved sensor technology can provide the basis for new control strategies
- ◆ Increase our ability to run more consistent and more productive bioprocesses
- ◆ The challenge is to develop sensors which can be readily implemented for more effective process control



Acknowledgments

- ◆ John Frenz
- ◆ Cynthia Hoy
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- ◆ Bob Kiss
- ◆ Jim Swartz