Biotechnology: *The Challenge for Tomorrow*

Technology Progress

<table>
<thead>
<tr>
<th>Chemistry &amp; Physics</th>
<th>First Half of 20\textsuperscript{th} Century</th>
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<tbody>
<tr>
<td>Engineering, Electronics, Computer and Information Technology</td>
<td>Second Half of 20\textsuperscript{th} Century</td>
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<tr>
<td>Biotechnology, Bioprocessing, Biomanufacturing, Genomics, Proteomics and the rest of the “ics”</td>
<td>Dominant Economic Force for the 21\textsuperscript{st} Century</td>
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</tbody>
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### Biotechnology: Historical Development

- **Pre Pasteur Era** Before 1865
  - Alcoholic beverages, dairy products
- **Pasteur Era** 1865-1940
  - Ethanol, acetone, glycerol, citric acid
- **Antibiotic Era** 1940-1960
  - Penicillin, Viral vaccines
- **Post Antibiotic Era** 1960-1975
  - Amino acids, enzymes
- **Era of New Biotechnology** 1975- Tomorrow
  - Genetic engineering and recombinant DNA
  - Hybridoma technology
  - Human genome project and gene therapy
  - Genetically modified organisms
  - The “omics” era

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### Biotechnology

- Improve public health
- Improve the quality of life
- Contribute to economic growth
Biotechnology

- rDNA Technology
- Monoclonal Antibody Technology
- Bioprocess Technology

United States Biotech Clusters

Source: Elaine Johnson-Bio-Link
So What is Bioprocessing?

Why use Biological Systems?

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

Cells → Media → Bioreactor → Conversion Stage

Dissolved $O_2$
Agitation

pH
Aeration

Temp
Asepsis

Product Separation & Purification
Bioprocessing Products

• Biopharmaceuticals
  – Insulin
  – Growth hormones
• Antibiotics
• Vitamins
• Biomass- Biofuels
• Increased agricultural productivity
  – Food Bioprocessing
    • Lipases for cheese flavor and texture
    • Pectinases for clarification of wines and juices
    • Amylase for high glucose corn syrup

Amino Acid Production
Example: Monosodium Glutamate

Fermentor Size:
63,420 Gallons
(240,360 liters)

100 Ft High.
Bioprocessing deals with living Cells

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

Choice of Microorganism

- Most Common
  - Bacteria
    - E. Coli
    - Lactobacillus
    - Bacillus
  - Yeast
    - Saccharomyces
    - Pichia
- Strain Improvement
Microorganisms and Bioprocessing

- **Ethanol production vs. amino acid production:**
  - **Ethanol** is a by product of catabolic pathways. These are waste products.

  - **Amino acids** are building blocks that the cells synthesize at the expense of energy. This process is highly regulated.

Microbial Metabolites

- **Primary metabolites:**
  - Ethanol
  - Glutamic Acid & Citric Acid
  - Lysine
  - Polysaccharides

- **Secondary metabolites:**
  - Produced mostly from intermediates and end products of primary metabolites:
    - Penicillin
    - Cephalosporin
    - Streptomycin
    - Cyclosporine
Animal cells and Bioprocessing

- BHK21
- Vero
- HEK 293
- CHO
- HeLa
- 3T3Cells
- 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
Baculovirus Expression Vector System (BEVS)

Since the development of the baculovirus expression vector system (Smith et al., 1983), hundreds of proteins been produced in insect cells. Virtually every cell line is a potential or actual source of material for molecular biologists to use in a wide range of studies. Well known products produced using BEVS:
- tPA
- Influenza Vaccine

Recombinant Microorganisms

- Organisms which contain foreign genetic material, generally but not always extra chromosomal, that has been introduced deliberately as opposed to having been acquired naturally.
- Genetically Engineered
- Designed to carry out process efficiently
  - Overproduction of a protein not normally made by the host
  - Heavily utilized by the biotechnology industry
Recombinant Microorganisms

• Recombinant microorganisms are often more susceptible to shear stress than wild type.

• Why?

• The extra metabolic burden of synthesizing a foreign protein. This process often weakens the cell wall and increases sensitivity to shear effects.

Recombinant Microorganisms

• Cellular response to shear effects is a decrease in protein synthesis including the desired recombinant protein.

• Shear forces can cause stress and alter physical and chemical properties of the cell.
  – Example: Surface polysaccharide production may increase in the cell thus, making down stream processing more difficult.
Recombinant Proteins

Who is involved in the production?
Cell Biologists Chemical Engineers

- Gene Isolation *Large Scale production
- Gene characterization *Optimize conditions
- Gene modifications *Provide maximum yield
- Create cells that effectively express genes
- Use for industrial production of proteins

Research Areas of Bioprocessing

- Bioreactor Design
- Process Monitoring
- Biocatalysis
- Separation and Purification
**Bioreactor Design**

- The design of an appropriate bioreactor in which the environment can be controlled so that a bioprocess can be carried out efficiently is essential.
- Such a design requires a basic understanding of molecular, genetic, metabolic and cellular functions involved in the growth of cells and the expression of cellular products.

**Biocatalysis**

- Biocatalysis is the development of specialized enzymes and catalytic antibodies for particular uses.
- A basic understanding of these biocatalysts at the molecular level is essential.
- Examples:
  - Thermodynamic studies to understand realizable yields.
  - Studies involving x-ray diffraction and nuclear magnetic resonance spectroscopy to elucidate atomic structure.
Process Monitoring and Control

• Design of new and advanced methodologies for monitoring and controlling bioprocesses is essential.
• Recent development in these methodologies include:
  – Capillary electrophoresis for rapid analytical techniques.
  – Designing of biosensing devices and control networks

Separation and Purification

• There is a real need to improve the present methods of separating and purifying cellular products (downstream processing).
• Examples:
  – Development of industrial scale chromatographic separations.
  – Development of disposable industrial filtration technology
  – Cost effectiveness
Typical Biologic Process Flow

Cell Culture/Clarification

Cell bank vial → Spinner or Shake Flask → Seed Bioreactors → Production Bioreactor → Centrifugation → Depth Filtration → Clarified Harvest

Product Capture

Affinity Chromatography → ion pH Viral inactivation

Fine Purification

Ion Exchange Chromatography → Hydrophobic Interaction Chromatography → Viral Filtration → UF/DF → Bottling

Biologic Definition

• Any virus, therapeutic serum, toxin or analogous product applicable to the prevention, treatment or cure of diseases or injuries in man.

• Types of Biologics:
  • Classical: Vaccines, Blood and serum products
  • Recombinant: proteins, peptides, MABs, Recombinant vaccines
  • Others: Gene transfers, transgenics
Hybridoma Technology

Antigen

Cells fuse to make hybridomas

Cancerous plasma cells

Antibody-producing plasma cells

Hybridoma cells grow in culture

Individual hybridoma cells are cloned

Hybridomas are kept alive in mouse

Desired clones are cultured and frozen

Monoclonal antibodies are purified

Questions?

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