BIOCHEMICAL PROCESSING: OVERVIEW

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DOWNSTREAM PROCESSING COURSE
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THE CHALLENGE OF DOWNSTREAM

It is difficult to efficiently and economically recover a high purity biochemical product from a complex mixture of related and functional molecules, impurities and contaminants which have similar physical and chemical properties.
YOUR GOAL

IF YOU DON’T KNOW WHERE YOU ARE GOING AND YOU DON’T HAVE A MEANS OF MEASURING WHERE YOU ARE THEN YOU WON’T KNOW WHEN YOU ARRIVE

Ama Dablam
22,275 ft
THE DIVERSE BIOCHEMICAL PROCESS INDUSTRY

• PRODUCTS AND SERVICES FOR MULTIPLE MARKETS
  Food & Beverage
  Health Care
    Therapeutics
    Diagnostics
    Device
  Specialty Chemical
  Commodity Chemicals
  Waste Treatment

• MANUFACTURING BY MULTIPLE SYNTHETIC & EXTRACTIVE TECHNOLOGIES
  Biosynthetic - Microbial, Animal, Plant
  Extractive – Animal, Plant
  Chemical Synthesis
THE DIVERSE BIOCHEMICAL PROCESS INDUSTRY (Continued)

• PRODUCTS BELONG TO MULTIPLE CLASSES
  – Small Molecules
  – Proteins
  – Nucleic Acids
  – Carbohydrates
  – Catabolites & Anabolites
  – Cells And Viruses

• PRODUCTS & PROCESSES REGULATED
  – FDA, EMEA
  – EPA
  – OSHA
Where is the Leverage?
Relationship of Profit to Price

\[
\text{PROFIT} = VF_M (SPSA - CM)
\]

Market size determined by problem solved

Selling price is Fixed by utility And competition

Market share is a function of proprietary position:
- Patents
- Marketing
- Distribution
- Innovation speed

Specific Activity of Product

Manufacturing cost

Sensitivity of Profit to \( S_p \), \( S_p \) & \( C_m \)

<table>
<thead>
<tr>
<th>( S_a )</th>
<th>( S_p )</th>
<th>( C_m )</th>
<th>Profit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Units/lb)</td>
<td>($/unit)</td>
<td>($/lb)</td>
<td>$ MM</td>
</tr>
<tr>
<td>200</td>
<td>0.45</td>
<td>55</td>
<td>210</td>
</tr>
<tr>
<td>200</td>
<td>0.45</td>
<td>35</td>
<td>330</td>
</tr>
<tr>
<td>200</td>
<td>0.35</td>
<td>55</td>
<td>90</td>
</tr>
<tr>
<td>500</td>
<td>0.45</td>
<td>55</td>
<td>1020</td>
</tr>
<tr>
<td>2000</td>
<td>0.45</td>
<td>55</td>
<td>5070</td>
</tr>
</tbody>
</table>

\( V = 30 \, \text{b lb sugar} \) & \( F_m = 0.2 \)
POINTS TO CONSIDER IN DOWNSTREAM PROCESSING

- DSP begins with Raw Material selection “Garbage in means garbage out”
- There are trade-offs, e.g. between purity and yield “No Free lunch”
- Mass and Energy are conserved, “What goes in must come out somewhere and in some form. There may be transformation in form
- There are Impurities and Contaminants
- You will be watched, e.g. by customers (internal and external) and the FDA. Therefore be sure to define metrics and appropriate analytical methods
- Regulation includes FDA, EPA, and OSHA
- Design:
  - Target – the Spec Sheet
  - Path – the PFD
  - Measure – Analytical
- Murphy’s Law
  - Contaminants – need control
  - Lost material – need robustness
SUPPLY CHAIN IN BIOPROCESSING

Manufacturing Plant Size = \frac{(Market)(MarketShare)}{(RecoveryEfficiency)(Titer)(SpecificActivity)}
MANUFACTURING BY FERMENTATION

Figure by MIT OCW.
WHEN SELECTING UNIT OPERATIONS THERE ARE CHOICES AND DECISIONS MUST BE MADE

**Bioreactor**

**Intracellular Products**
- **Cell Harvesting**
  1. Centrifugation
  2. Microfiltration
  3. Ultrafiltration
- **Cell Disruption**
  1. Homogenization
  2. Bead Milling
  3. Osmotic Shock
- **Cell Debris Removal**
  1. Centrifugation
  2. Microfiltration
  3. Vacuum Filtration
  4. Press Filtration

**Extracellular Products**
- **Biomass Removal**
  1. Vacuum Filtration
  2. Centrifugation
  3. Microfiltration
  4. Ultrafiltration
  5. Press Filtration
  6. Candle Filtration
  7. Flotation
- **Cell Disruption**
  1. Homogenization
  2. Bead Milling
- **Cell Debris Removal**
  1. Centrifugation
  2. Microfiltration
  3. Vacuum Filtration
  4. Press Filtration

**Product Extraction By**
- **Product Extraction By**
  1. Organic Solvents
  2. Polymer/Polymer
  3. Polymer/Salt
  4. Supercritical Fluids
  5. Adsorption
  6. Reverse Micelles
  7. Distillation

**Renaturation**
- **Renaturation**
  1. Solubilization
  2. Reoxidation

**Concentration**
- **Concentration**
  1. Ultrafiltration
  2. Evaporation
  3. Reverse Osmosis
  4. Precipitation
  5. Crystallization
  6. Extraction
  7. Adsorption
  8. Distillation

**Final Purification**
- **Final Purification**
  1. Adsorption
  2. Gel Filtration
  3. Diafiltration
  4. Electrodialysis
  5. Electrophoresis

**Dehydration or Solvent Removal**
- **Dehydration or Solvent Removal**
  1. Spray Drying
  2. Freeze Drying
  3. Fluid Bed Drying

**Low Purity Requirements**
**High Purity Requirements**

**Contemporary Downstream Processing of Biological Materials**

Figure by MIT OCW.
Process Flow Diagram for *E. coli* Recombinant Porcine Somatotropin

Flowsheet for the production of porcine somatotropin
Fermentation Process Development

Molecular Biology
- Expression system
- Plasmid design and copy number
- Control of metabolism

Experimental Parameters
- Host cell selection
- Expression system
- Media design
- Fermentation conditions
- Aeration strategy
- Cell harvesting strategy

Metabolic pathways
- Process kinetics

Set of Enzymes and Reactions

\[ \text{A} + \text{B} \rightarrow \text{C} \]
\[ \text{A} + \text{C} \rightarrow \text{D} + \text{E} \]

Elemental balances

Solubility & Equilibria

Performance Assessment
- Growth Rate
- Product Concentration
- By-Product Concentration
- Raw Materials Utilization

Mass transfer

Water balance

Equipment correlations

Cell Environment
- Glucose
- \( \text{CO}_2 \)
- \( \text{NH}_4^+ \)
- \( \text{O}_2 \)
- \( \text{H}_2\text{O} \)
- Product

Net Biochemistry

Reactor + Utilities
- Air
- Wt
- Power
- Comp.
- pH
- D.O.
- Pres.
- Flow
- Corn Syrup Water
- Flow
- NH3
- Flow
- Anti-foam
- Temp
- Temp
- Temp

Experimental Parameters
- Host cell selection
- Expression system
- Media design
- Fermentation conditions
- Aeration strategy
- Cell harvesting strategy
STRATEGIES FOR MEDIA DESIGN

- Selection of media from literature
- Analogy with medium for another organism
- Rationale design from cell and product needs and process demands
- Experimental design

Who should be involved in media design?
- Microbiologist
- Purchasing
- Analytical chemist
- Process engineering
MEDIA DESIGN

MEETING THE REQUIREMENTS FOR GROWTH AND PRODUCT FORMATION
A Systematic Approach to Media Design

1. FERMENTATION PROCESS OBJECTIVES

Cell mass vs. Product synthesis
Substrate allocation model
Physiological Model
  Avoid C, N, S or PO4 catabolite repression
  Specific precursors, inducers, or repressors

2. NUTRITIONAL REQUIREMENTS

Elemental requirements
Specific nutrients, e.g. vitamins, minerals, amino acids, etc.
Energy requirements - Carbon source and Oxygen
  Growth
  Product Synthesis
  Maintenance

3. ENVIRONMENTAL REQUIREMENTS

pH profile
Temperature profile
Dissolved oxygen profile
Catabolite repression
Physiological constraints, e.g. ionic strength, product inhibition
MEDIA DESIGN

MEETING THE REQUIREMENTS FOR GROWTH
PRODUCT FORMATION
(continued)

4. REGULATORY CONSTRAINTS

Qualification of vendors
Multiple sources
Traceability
Potential impurities or contaminants
Consistency

5. TECHNO-ECONOMIC CONSTRAINTS

Cost
Materials availability
Product recovery
Environmental impact
# Fermentation Media

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Raw Material</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbon Source</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>Cerelose</td>
<td>Hydrolyzed from starch</td>
</tr>
<tr>
<td>Molasses</td>
<td></td>
<td>Inversion (Sucrose to Fructose and Glucose)</td>
</tr>
<tr>
<td>Starch</td>
<td></td>
<td>Solubilization</td>
</tr>
<tr>
<td>Cellulose</td>
<td></td>
<td>Grinding and Hydrolysis</td>
</tr>
<tr>
<td>Fats/Oils</td>
<td>Soybean Oil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cottonseed Oil</td>
<td></td>
</tr>
<tr>
<td><strong>Nitrogen Source</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Hydrolysates</td>
<td>Acid or Enzyme Catalyzed Hydrolysis</td>
<td></td>
</tr>
</tbody>
</table>
There is a critical need for analytical support.

Fermentation Process

Downstream Process

- Cost of raw material
- Cost of fermentation efficiency
- Cost of downstream efficiency
- Cost of waste treatment
- Customer demands for product quality
FERMENTATION MEDIUM COMPONENTS

• PENICILLIN
  Molasses
  0.2% Soybean Oil
  1% Cottonseed Flour

• STREPTOMYCIN
  2.5% Cerelose
  4% Soybean Oil

• LACTIC ACID BACTERIA
  Phosphate buffer
  0.5% Tryptone
  0.5% Yeast Extract

• BACITRACIN
  3% Corn Steep
  3% Glucose

• Baker’s Yeast
  Molasses
PROCESS CONSTRAINTS

- Maximum Biocatalyst Concentration
- Bioreactor Productivity (g/L-h)
- Packing density limits
- Design improvements
- Heat or mass transfer boundary
- Improvements in biocatalyst specific activity

Figure by MIT OCW.
DISCUSSION POINTS

• Where does DSP begin?
• Where does DSP end? How pure does the product need to be?
• The problem of trade-offs
• Mass and energy are conserved
• Mass can be transformed
• You will be watched
• Regulation by FDA, EPA, OSHA
• Design goals
  – Target → the specifications
  – Path → the PFD
  – Metrics → analytical tech’s
• Murphy’s law