BIOCHEMCIAL 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

Market size determined by problem solved

Selling price is Fixed by utility And competition

$$PROFIT = VF_M(S_P S_A - C_M)$$

Market share is a function of proprietary position:

- Patents
- Marketing
- Distribution
- Innovation speed

Specific Activity of Product

Manufacturing cost

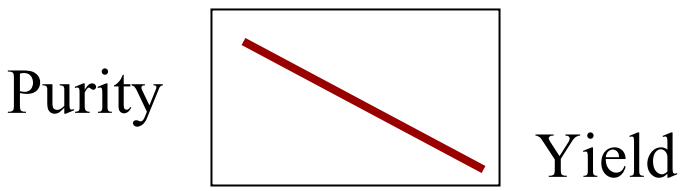
Sensitivity of Profit to S_p, S_p & C_m

Sa	Sp	Cm	Profit
(Units/lb)	($/unit)	($/lb)	$ MM
200	0.45	55	210
200	0.45	35	330
200	0.35	55	90
500	0.45	55	1020
2000	0.45	55	5070

V= 30 b lb sugar & Fm = 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

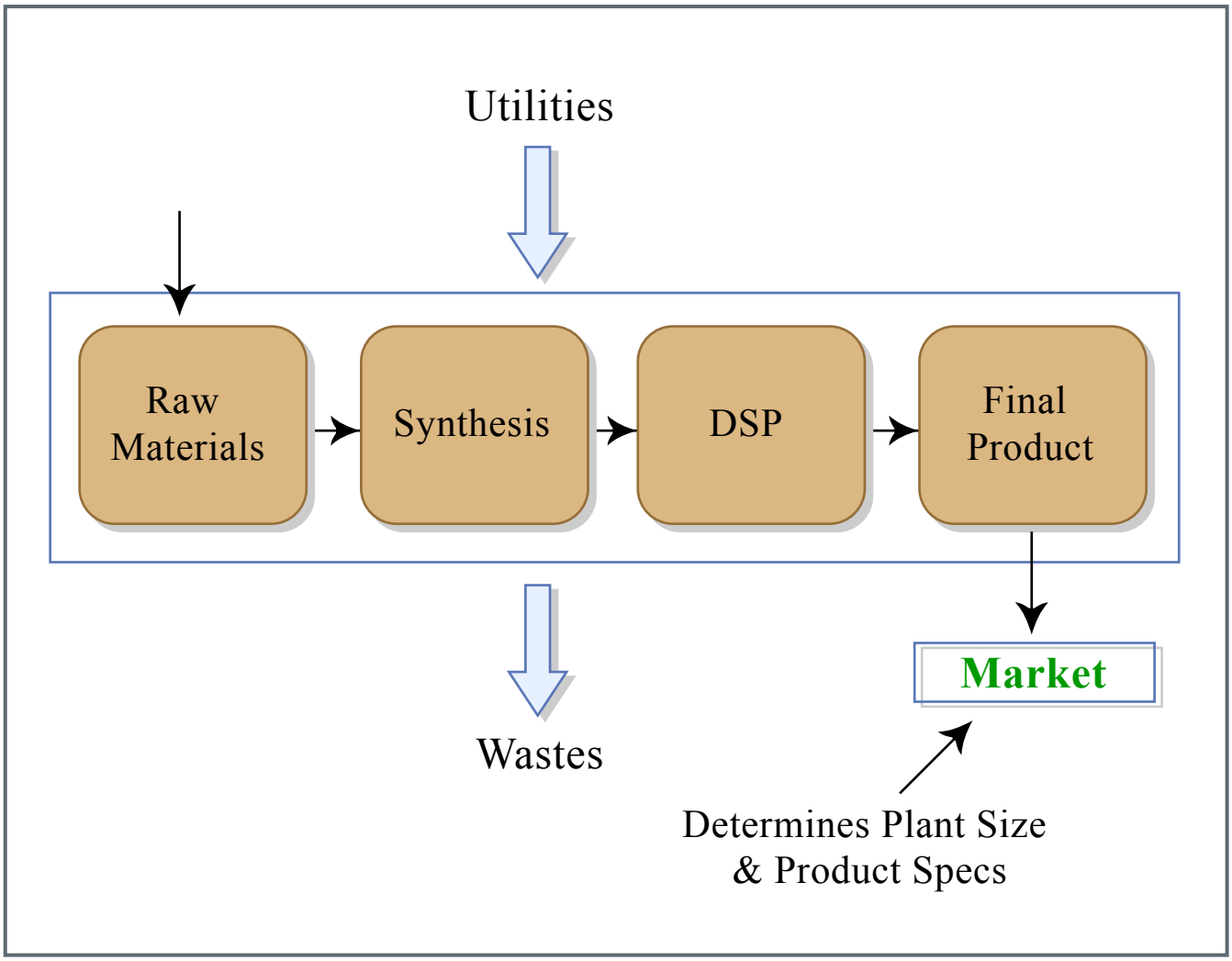


Figure by MIT OCW.

$$\text{Manufacturing Plant Size} = \frac{(Market)(MarketShare)}{(RecoveryEfficiency)(Titer)(SpecficAcitivity)}$$

MANUFACTURING BY FERMENTATION

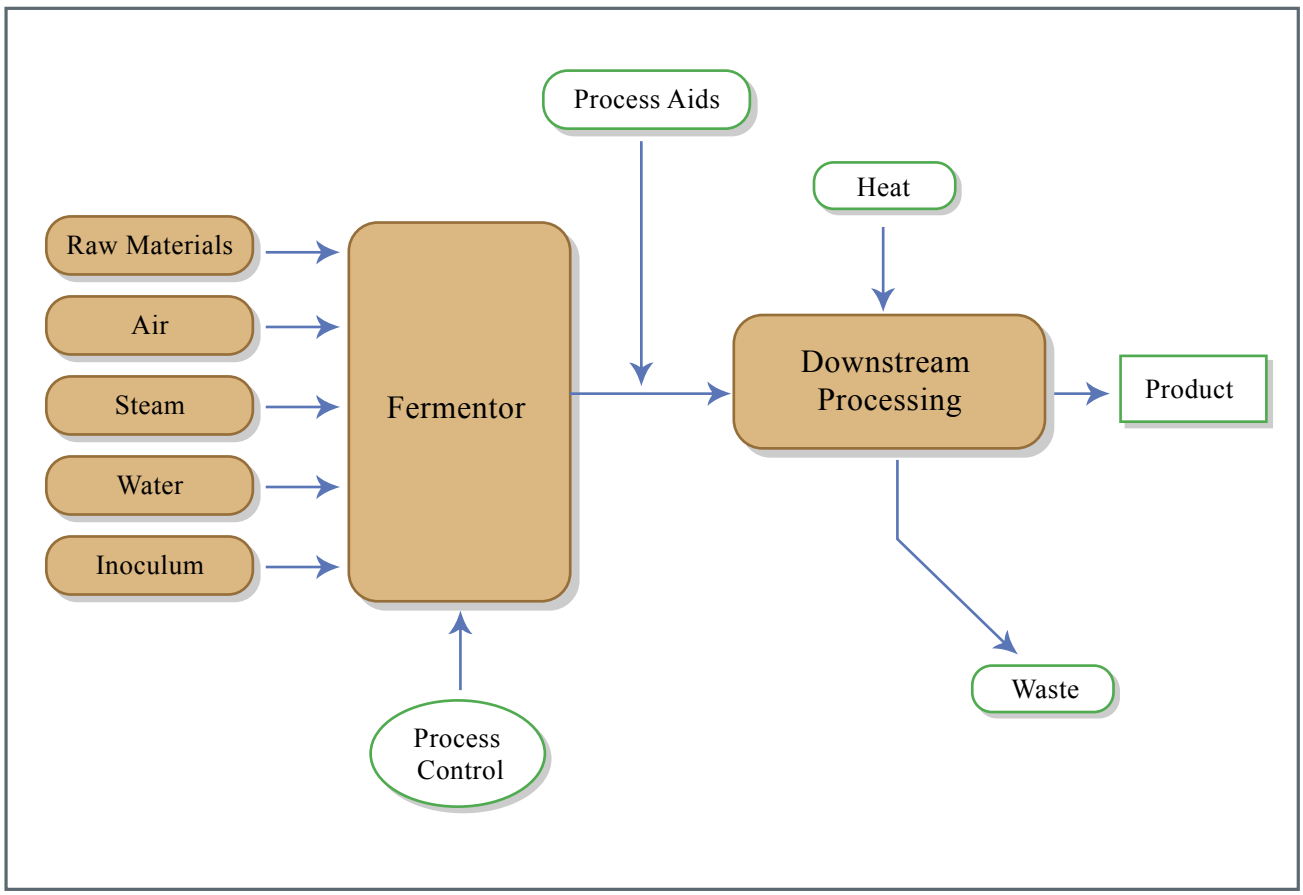


Figure by MIT OCW.

WHEN SELECTING UNIT OPERATIONS THERE ARE CHOICES AND DECISIONS MUST BE MADE

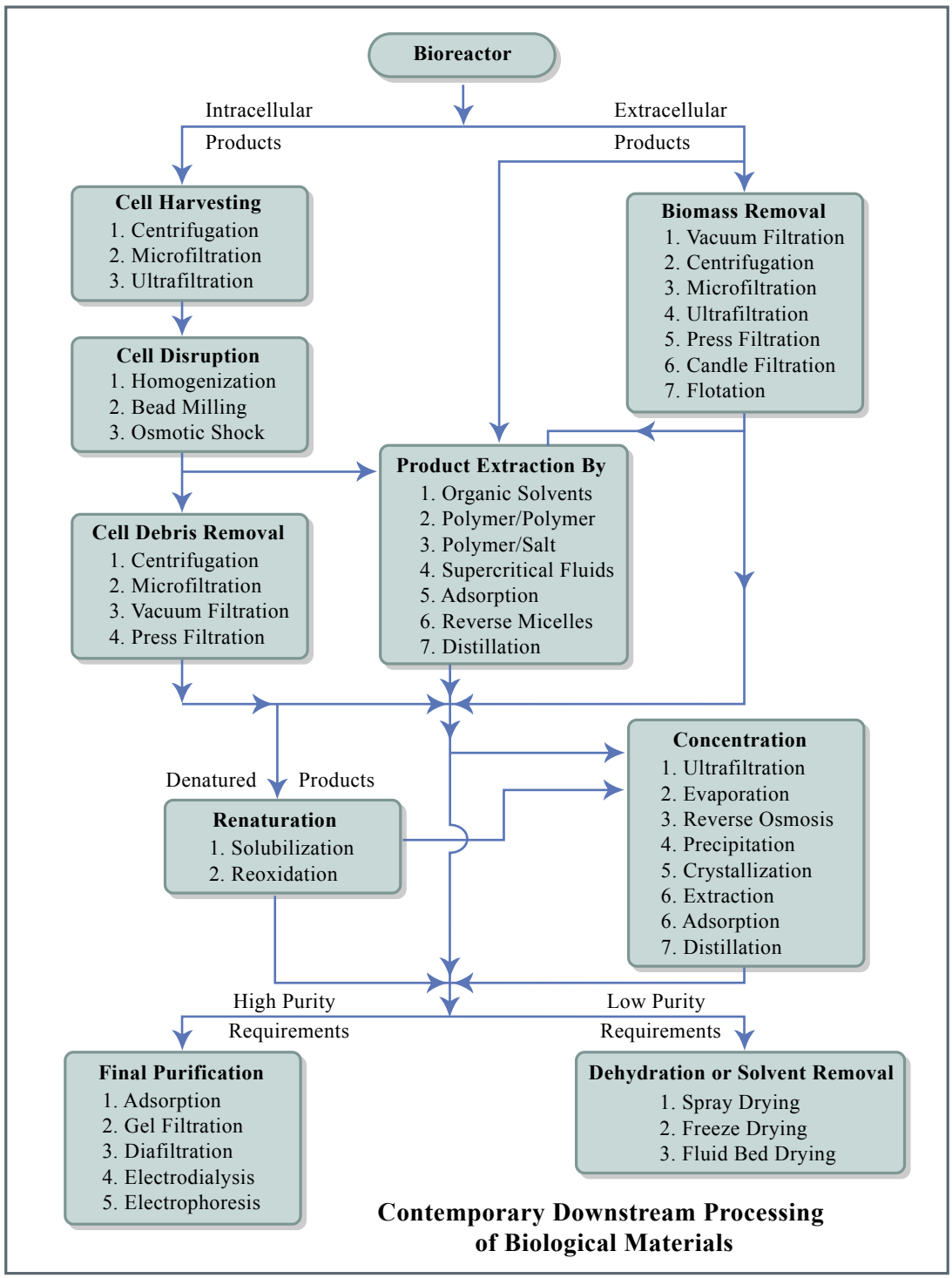
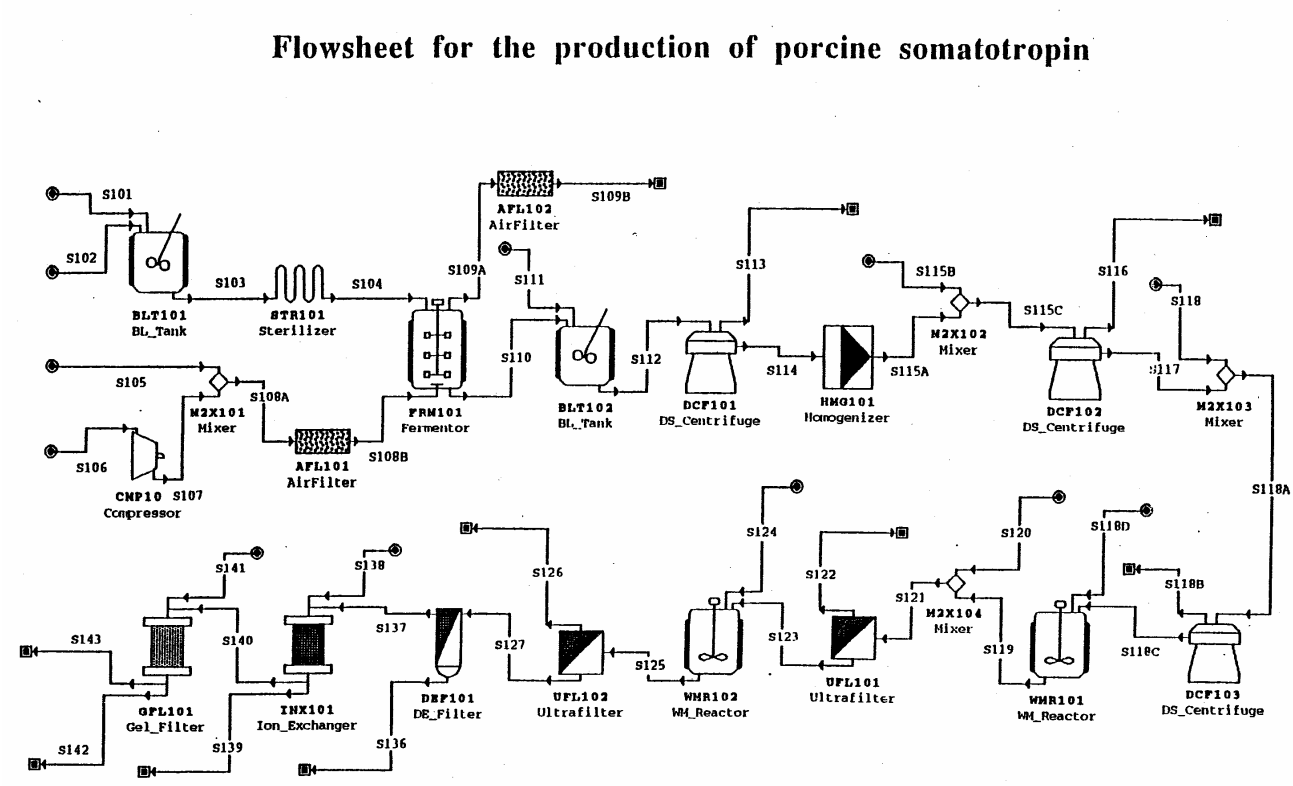


Figure by MIT OCW.

Process Flow Diagram for *E. coli* Recombinant Porcine Somatotropin



Fermentation Process Development

A +B --> C

A + C ---> D +E

Set of Enzymes and Reactions

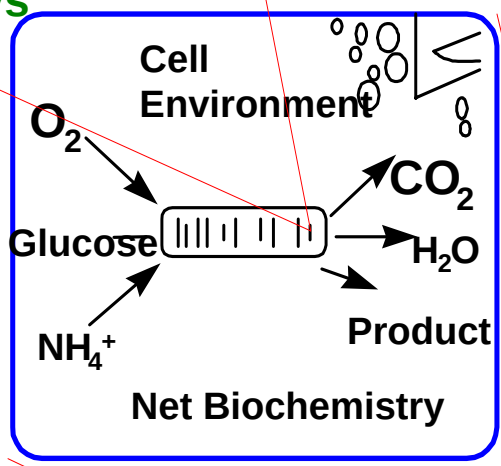
Molecular Biology

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



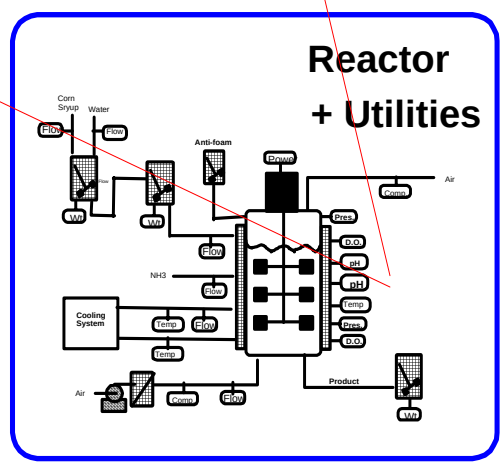
Elemental balances

Solubility & Equilibria

Performance Assessment

Growth Rate
Product Concentration
By-Product Concentration
Raw Materials Utilization

Mass transfer
Water balance
Equipment correlations



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 PO_4 catabolite repression**
- **Specific precursors, inducors, 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 REQUIUREMENTS

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

MEDIA DESIGN

MEETING THE REQUIREMENTS FOR GROWTH A PRODUCT FORMATION
(continued)

4. REGULATORY CONSTRAINTS

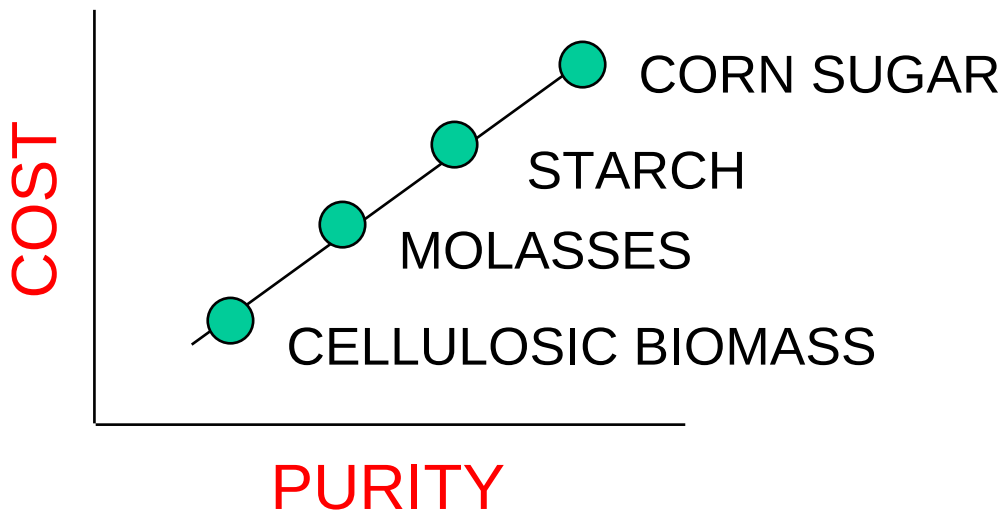
Qualification of vendors
Multiple sources
Traceability
Potential impurities or contaminants
Consistency

5. TECHNO-ECONOMIC CONSTRAINTS

Cost
Materials availability
Product recovery
Environmental impact

FERMENTATION MEDIA

NUTRIENT	RAW MATERIAL	PRETREATMENT
CARBON SOURCE		
GLUCOSE	CERELOSE	HYDOLYZED FROM STARCH
	MOLASSES	INVERSION
		(SUCROSE TO FRUCTOSE AND GLUCOSE)
	STARCH	SOLUBILZIATION
	CELLULOSE	GRINDING AND HYDROLYSIS
FATS/OILS	SOYBEAN OIL	
	COTTONSEED OIL	
NITROGEN SOURCE		
	AMMONIA	
	PROTEIN HYDROLYSATES	ACID OR ENZYME CATALYZED
		HYDROLYSIS



OVERVIEW OF MEDIA DESIGN

Raw Material Options

There is a critical need for analytical support

Fermentation Process

Downstream Process

Waste Treatment

Product Specifications

5C's

- 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

High Impurity Content

- STREPTOMYCIN
  - 2.5% Cerelose
  - 4% Soybean Oil

Multiphase Components

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

Variable Quality

- BACITRACIN
  - 3% Corn Steep
  - 3% Glucose

- Baker's Yeast
  - Molasses

PROCESS CONSTRAINTS

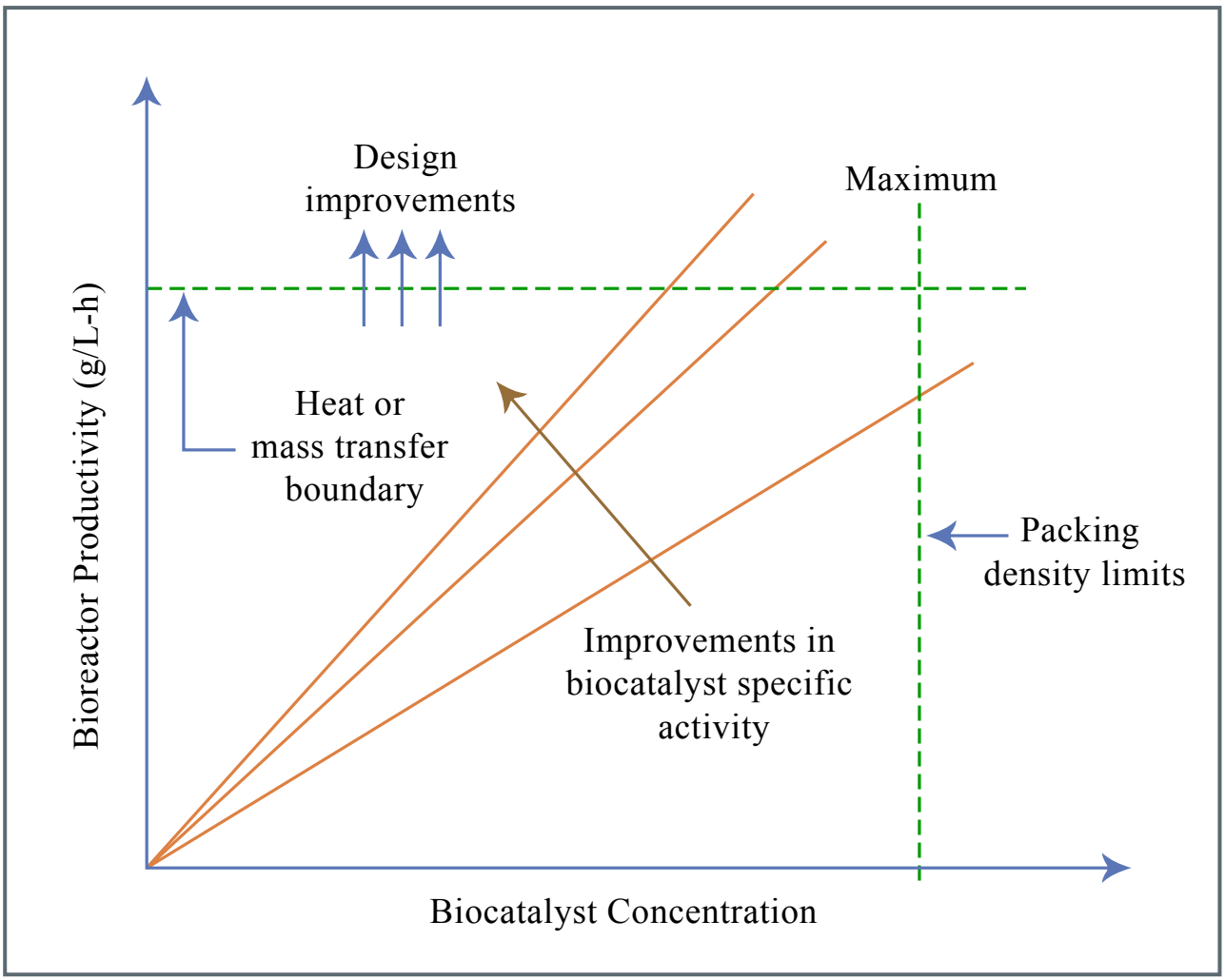


Figure by MIT OCW.

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