

ChEn 5751 Bioseparation Overview

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Characteristics of Bioseparations

- Starting Materials
 - Fermentation broth (bacteria and yeasts, mycelial fungi and streptomycetes, mammalian or insect cell cultures) or defined media and complex media
 - Biological materials (blood, plant and animal tissues or organs)
 - Product concentration is usually dilute
- Properties utilized in bioseparations
 - size, density, solubility, partitioning, mobility, charge, hydrophobic interactions, biological molecular interactions, etc.
- Quality of products
 - activity, purity, contaminants
 - For biologics, consistency of products
 - The "structure" or "composition" of the product is not very well defined, e.g. virus, glycoprotein. The sugar of protein has a lot of heterogeneity

Concentrations of product in the effluent streams from fermentors

	Molecular weight	Concentration	Price
Ethanol	46	70-100 g/l	40¢/lb
Amino Acids	110 (Avg)	20-120 g/l	\$2/kg
Vitamin B12	1355	2-5 g/l	
Cellulase	~300K	15 g/l	
Penicillin		30 g/l	\$50/kg
Monoclonal Antibody	180K	50-150 mg/l	\$2000/g
Recombinant Interferon		25 g/l	
tPA		50 mg/l	\$400/mg
Polysaccharide (xanthan gum)		10 g/l	

In general concentration is low, product value varies widely.

Fermentation Broth of MSG Production

Monosodium Glutamate

Molasses (45% solid)	50% (w/w)
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NH ₄ OH	
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After Fermentation

Residual unfermented molasses	10% (w/w)
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Cell mass	10 g/l
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Glutamic acid	120 g/l
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NH ₄ OH	
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The product stream from complex medium fermentation can be very complex.

Molecular components of an <i>E. coli</i> cell		
	Percent total weight	Number of each kind
Water	70	
Proteins	15	~3000
Nucleic acids		
DNA	1	1
RNA	6	~1000
Carbohydrates	3	~50
Lipids	2	~40
Building block molecules and intermediates	2	~500
Inorganic ions	1	12

Some rDNA proteins are produced inside E coli cells (intracellular).

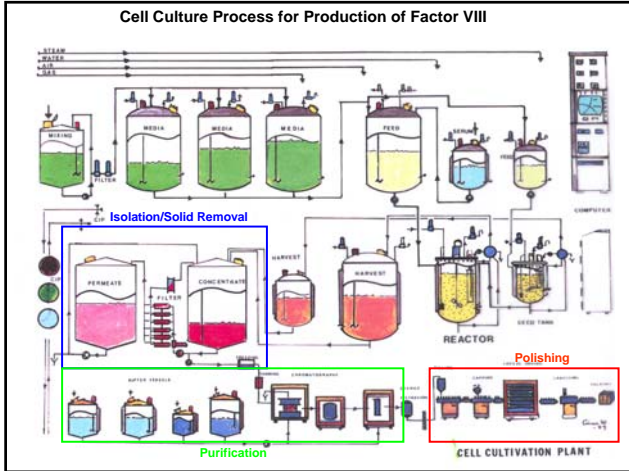
Recombinant interferon in <i>E. coli</i> as inclusion bodies		
Medium	Glucose	45 g/l
	NH ₄	10 g/l
	Other salts	
Product	Dry Cell mass	20 g/l
	Interferon	0.1 g/g cell mass
	Other salts	

Typical Operations of Bioseparation

- **R**emoval of solids
 - Filtration, centrifugation, microfiltration
- **I**solation of product (volume reduction)
 - Cell disruption, extraction, adsorption, ultrafiltration, precipitation
- **P**urification
 - Adsorption, elution chromatography, ultrafiltration, electrophoresis, precipitation, crystallization
- **P**olishing
 - Crystallization, drying, auxiliary process, solvent recovery, water preparation

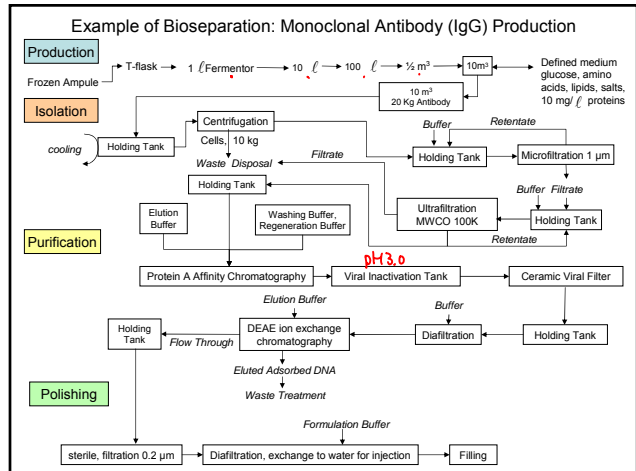
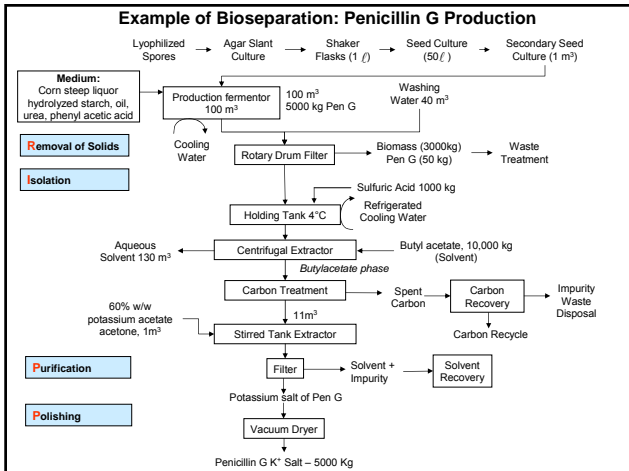
Common Stages of Bioseparation

- **R**emoval of solids
- **I**solation (volume reduction)
- **P**urification
- **P**olishing



Example of a recombinant protein purification (see flow chart of recombinant antibody production)

Step	Volume of output stream (liter)	Antibody in output stream (g)	Total Protein in output stream (g)	Specific antibody (g antibody/g total protein)	Total DNA (ng)	Specific DNA (ng/mg protein)
Cell Culture Broth	1000	1500	1600	0.93	-	ND
Centrifugation	950	1450	1500	0.97	-	ND
Microfiltration	1200	1420	1470	0.97	-	ND
Ultrafiltration	60	1420	1430	0.99	286	0.2
Affinity Chromatography	18	1380	1390	0.99	278	0.2
Diafiltration	20	1380	1390	0.99	-	ND
Gel permeation chromatography	80	1200	1200	>0.99	240	0.2
Ion exchange	100	1150	1150	>0.99	230	0.2
Diafiltration	20	1150	1150	>0.99	11.5	0.01

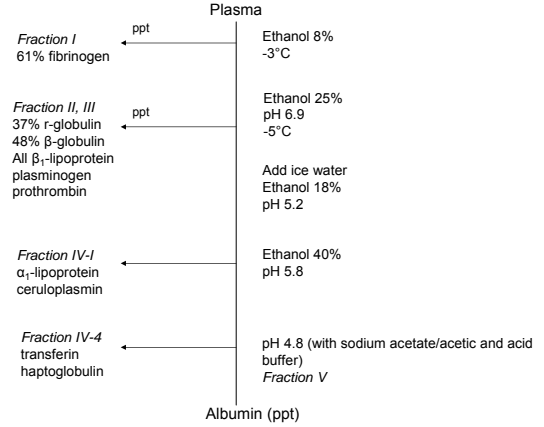


Example: Fractional Precipitation

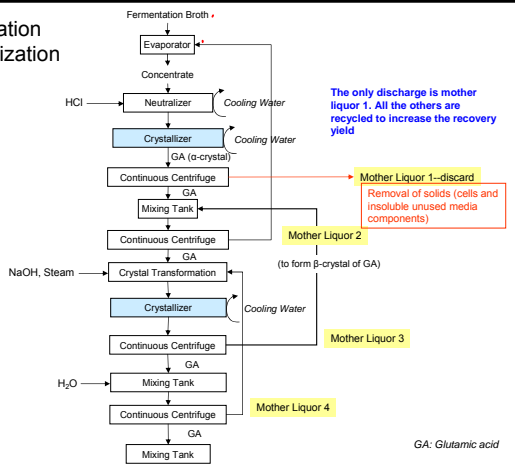
- Ethanol fractionation of human plasma protein factors: pH, temperature, ionic strength & protein concentration change
- Cohn's method 6:
 - primary means of preparing albumin, γ -globulin, fibrinogen

Effectively combine pH, temperature, solvent and salt concentrations that affect the solubility of different components of plasma to separate them.

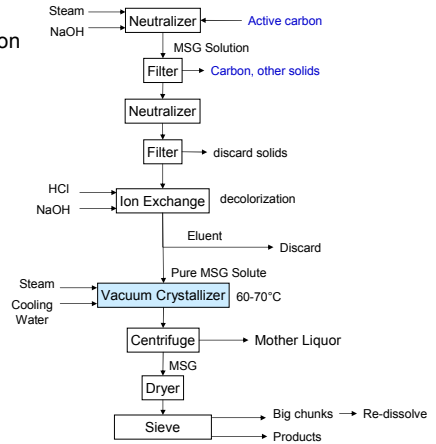
Cohn Fractionation of Blood Proteins



MSG Isolation & Crystallization



MSG Isolation & Crystallization (con't)



Unit Operation	Properties Used in Separation	Application
Filtration	Size	Solid removal
Centrifugation	Size, density	Solid removal
Microfiltration	Size	Solid Removal
Extraction		
<i>Solvent Extraction</i>	Partition	Isolation
<i>Aqueous Two-Phase Extraction</i>	Partition	Isolation
Adsorption		
<i>Ion Exchange Chromatography</i>	Charge	Isolation, Purification
<i>Affinity Chromatography</i>	Molecular interaction	Purification, Isolation
<i>Hydrophobic Interaction Chromatography</i>	Protein-ligand interaction	Purification, Isolation
<i>(Transition) Metal Ion Chromatography</i>	Sequence-specific tag-metal interaction	Purification, Isolation
Elution Chromatography, (Liquid Chromatography, HPLC)		
<i>Gel Permeation Chromatography</i>	Size and shape of molecules	Purification
<i>Reverse Phase Chromatography</i>	Size, molecular interaction	Purification
<i>Chromatofocusing</i>	Charge, Mobility	Purification
<i>Displacement Chromatography</i>	Molecular interactions	Purification
Electrophoresis	Charge, Mobility	Purification
Ultrafiltration	Size	Isolation, Purification
Reverse Osmosis	Size, Molecular diffusivity	Isolation, Purification
Precipitation	Solubility	Isolation
Crystallization	Solubility, Molecular interactions	Purification, Polishing

Unit Operation	Properties Used in Separation	Application	Major Characteristics
Filtration	Size	Solid removal	Produces relatively dry cake
Centrifugation	Size, density	Solid removal	Produces paste or cell concentration
Microfiltration	Size	Solid Removal	Retentate solid content, not very high
Extraction			
<i>Solvent Extraction</i>	Partition	Isolation	
<i>Aqueous Two-Phase Extraction</i>	Partition	Isolation	
Adsorption			
<i>Ion Exchange Chromatography</i>	Charge	Isolation, Purification	
<i>Affinity Chromatography</i>	Molecular interaction	Purification, Isolation	
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Reverse Osmosis	Size, Molecular diffusivity	Isolation, Purification	
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