BCHE 4520

Engineering and Design of Biochemical Separations Processes

http://cmbe.engr.uga.edu/bche4520

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"BioSeparations"

Key Points

- Survey nature of the course
- Textbook(s)
- Grading Policy – 4 tests
- “Final Exam” = Presentation
- BE HERE AT 8:00 A.M. (~~8:02 A.M.?~~)

Section 1 INTRODUCTION

A) Classification of bioproducts

1. By Size

- small molecules
- large molecules
- particles (e.g., whole cells or insoluble super molecules)

2. By Type

- uncharged solvents (saccharides, ethanol...)
- charged solutes (vitamins, citric acid...)
- macromolecules (proteins, nucleic acids...)
- particles

3. By Necessity for Purity

- Industrial Grade “Technical”
- Food Grade
- U.S. Pharmacopeia (USP)
- Analytical Standard
- Injectible Grade

NOTE: Essentially same compounds might be any of these grades

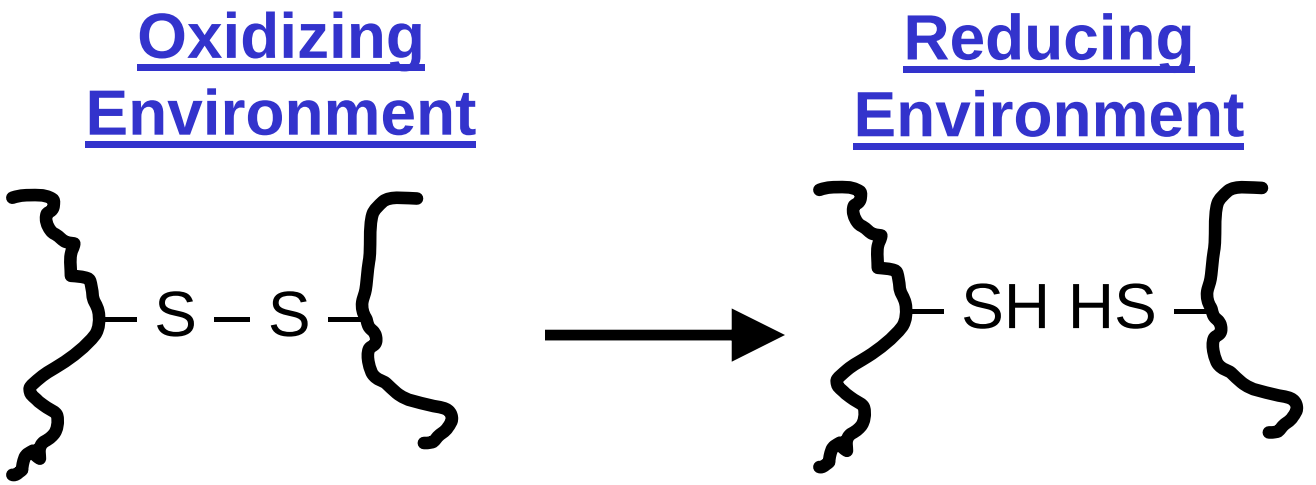
4. By Stability

- Stable
- Unstable – Redox, thermal, pH, water activity

B) Proteins – Special Considerations

Proteins usually require a specific tertiary structure in order to be active.

The process of protein folding is complicated.



Process takes time

Prokaryotic cells package large amounts of protein into inclusion bodies

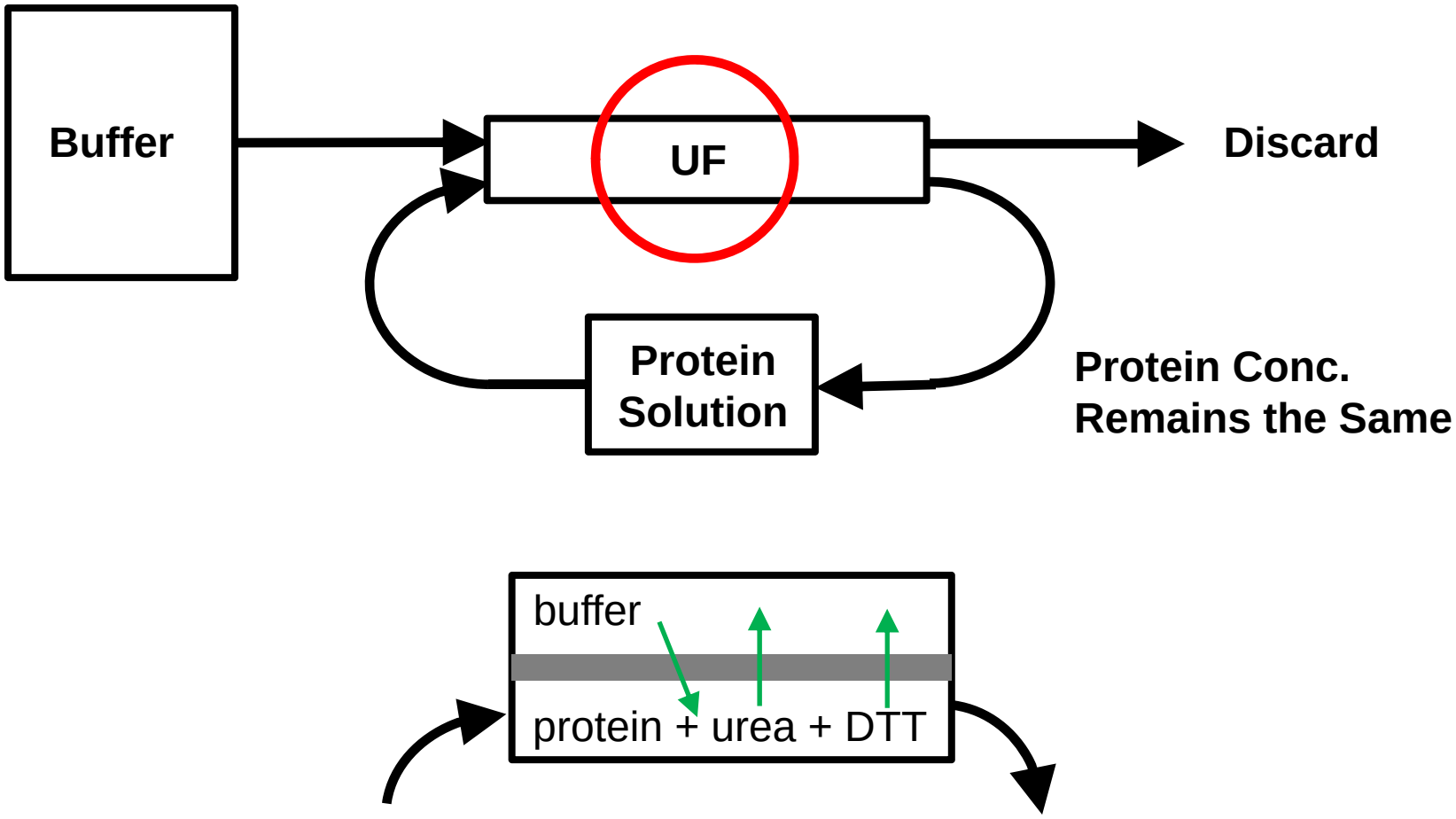
80% of the cases where protein is overproduced in *E. coli*

Inclusion bodies must be resolubilized following a recipe using denaturation agent (urea, guanidine) + reducing agent (dithiothreitol)

Example

1. Lyse cells on ice with lysozyme, tris, EDTA
2. Centrifuge
3. Pellet is washed — **Separation**
4. Pellet is dissolved in 6M guanidine HCl (denaturated agent) + 0.1 mM dithiothreitol (reducing agent)
5. Renature protein slowly with buffer until diluted by a factor of 60 — **Dilution**
6. Precipitate (impurities) formed is collected by centrifugation
7. Purification by anion exchange chromatography — **Purification**
8. Purification by size exclusion chromatography

Diafiltration can be used to renature proteins slowly.



C) Stages of Downstream Processing

1. Liquid-Solid Separation

- relatively easy to separate insoluble particles from soluble particles
- generally does not affect volume

2. Product Isolation – Separation

- remove materials having widely different properties (e.g., acids from amines, proteins from nucleic acids)
- can increase or decrease volume

3. Purification

- remove materials having similar properties
- may not be necessary if crude product is acceptable
- can increase or decrease volume

4. Polishing

- reduce volume
- put in correct form (hydration, crystal, morphology)

5. Formulation

- mix with ingredients necessary for use of biochemical
- excipients (stability)
- adjuvants (irritants)

Water is an important component to be removed.
Advantage in reducing volume as soon as possible.

D) Unit Operations

<u>Definition:</u> A single process and associated equipment that performs a separation or purification operation.

1. **Principles used to analyze unit operations**
   - **material balance**
   - **heat balance**
   - **chemical equilibria, reaction kinetics, thermodynamics**
   - **transport phenomena**

2. Quantification of Process & Product Quality

$$\text{Purity} = \frac{\text{Qty Product}}{\text{Qty Everything}}$$

$$\text{Fold Purity} = \frac{\text{Purity at stage i}}{\text{Purity initially}}$$

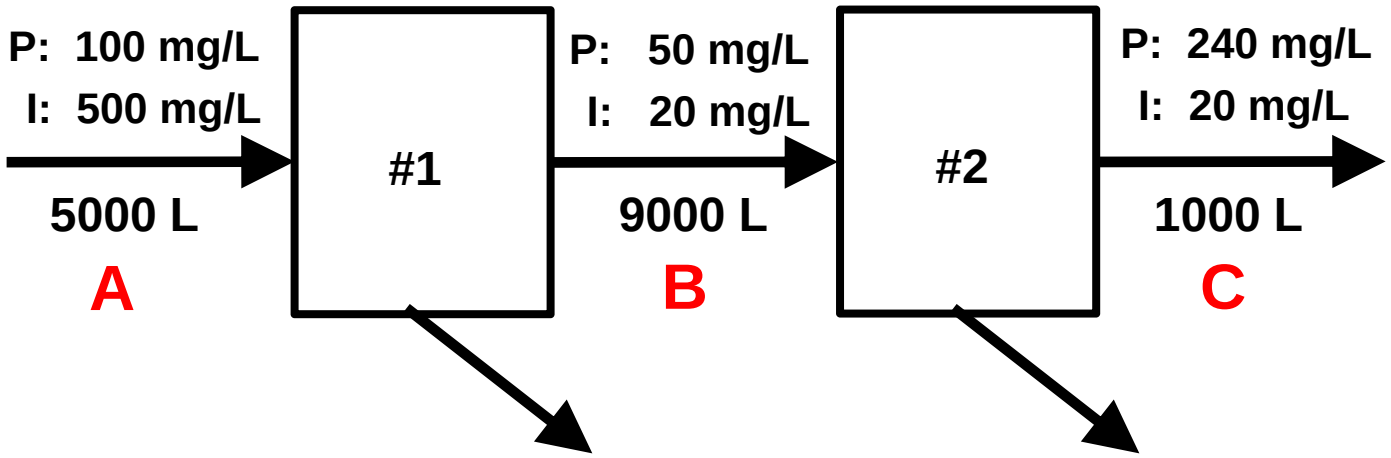
$$\text{Specific Activity} = \frac{\text{Units of Activity}}{\text{Mass Material}}$$

$$\text{Process Yield} = \frac{\text{Product in Effluent}}{\text{Product in Feed}}$$

$$\text{Reaction Yield} = \frac{\text{Product in Effluent}}{\text{Reactant in Feed}}$$

Process Yield is different from Reaction Yield. ProcessYield gives an indication of loss during a separation/purification process (with no reaction).

Example



	A	B	C
Purity*	16.7%	71.4%	92.3%
Fold Purification	—	4.28	5.52
Quantity Product	500 g	450 g	240 g
Yield	—	90%	53.3%

* Based on Solvent-Free Mixture

E) Categories for Unit Operations

1. Solid/Liquid Separations

- centrifugation
- sedimentation
- filtration
- microfiltration

2. Liquid Concentration

- ultrafiltration
- reverse osmosis

3. Solids Concentration

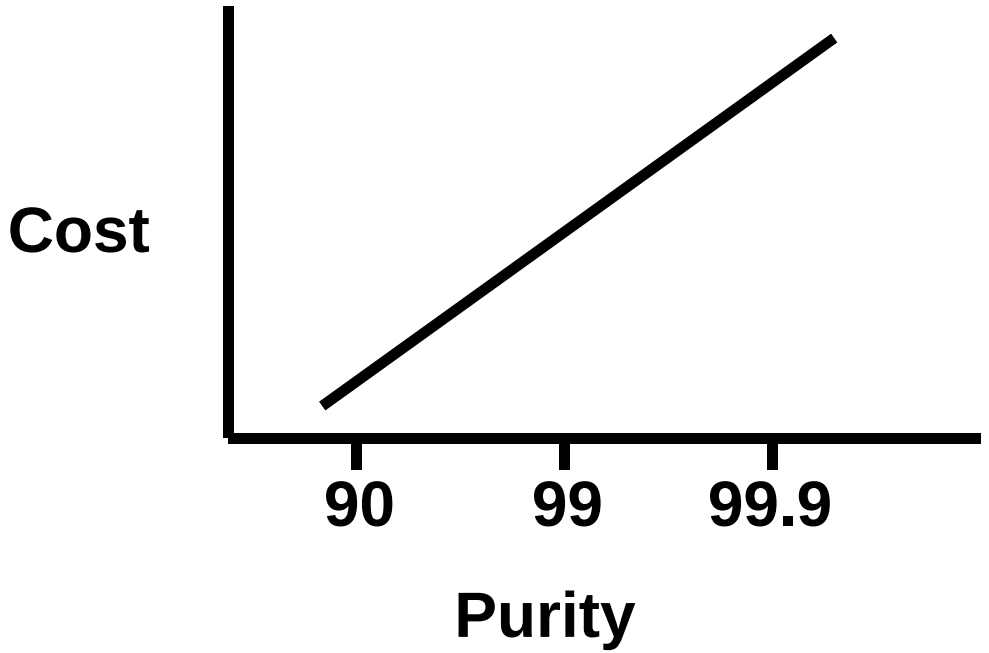
- crystallization
- precipitation
- extraction

4. Preliminary/Final Purification

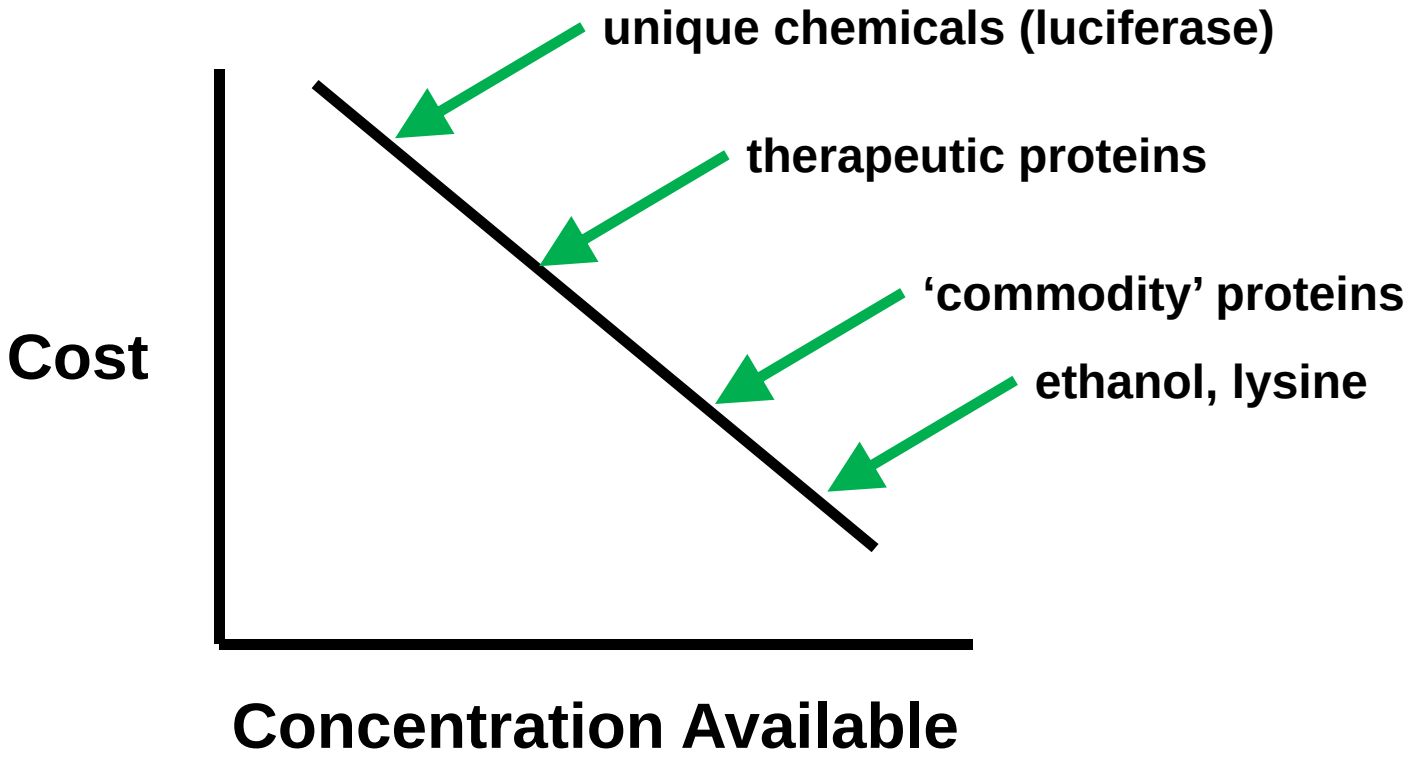
- adsorption
- chromatography
- affinity chromatography

F) Other Comments

1. Cost is related to purity of final product



2. Cost is related to product concentration available in process



Tendency for cost to decrease with time...

- increased capacity
- process improvements
- competition