Introduction to Bioprocess Engineering SQBI2513

Basic downstream processing

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Types of bioproducts

- three major categories of bioproducts:
  (i) cells,
  (ii) intracellular products, and
  (iii) extracellular products

Example: whole cells as bioproduct

- Single-cell protein (sources of mixed protein extracted from pure/ mixed cultures of _____, _____,_____ or bacteria used as substiture for protein-rich food in human and animal feeds.
- baker's _____
- animal feed supplements derived from yeast fermentations.
- glucose isomerase enzyme entrapped in inactivated microbial cells and formulated as catalyst pellets
- Competent cells for transformation
- Cells strains for industrial applications.
  - Cells are either used directly or dried and packaged with only minimal further purification.

Example: Intracellular bioproduct

- Active intracellular proteins—_____enzyme
- Active compounds in plants or cells
- Proteins in _____ bodies from recombinant bacterial

Example: extracellular bioproducts

- Proteins/enzymes secreted by signal peptide (or other secretory pathways).
- antibiotics
- organic acids
- alcohols secreted during microbial fermentations or cell culture.

Practical questions to think…

- Are bioproducts easy to purify? Why?
- Can bioproducts be purify/recover in a single steps?

We now move on to the methods to separate solid and liquid….

- Think for a while….
  - Why do we need to separate solid and liquid?
  - Can you give a example?

SEPARATION OF INSOLUBLE PRODUCTS.

Examples of insolubles:

- The cells
- Unfinished materials in the media/ substrate
- Precipitated compounds produced after the reaction
- Protein inclusion bodies inside the cells, and etc.

SEPARATION OF INSOLUBLE PRODUCTS.

General methods used for solid-liquid separation

(i)sedimentation,

(ii)centrifugation and/or

(iii) filtration.

Sedimentation

- Sedimentation can only be done if:
  - the particle _____ is large
  - _____is significantly different from the others

- Sedimentation processes can increase settling velocity by manipulating the environment of the particles so that they aggregate and increase their particle size.
  - Sometime a chemical called Alum (hydrated aluminuum potassium sulfate) is used to precipitate the particles

3 min Group discussion (3-4 persons)

- The efficiency of sedimentation is low. Can you think of other options to increase the efficiency?

Any better unit operation compare to sedimentation?

Filtration….

Limitation of membrane

- Can membrane clog?
- Why membrane can clog?

The scientific term where membrane clogged is called “foul” (membrane fouling…)

Bioprocess challenge

- How to reduce membrane fouling?
- Cheap material?
- Will our product bound permanently onto membrane?
- Increase efficiency?

Dead end filtration	Cross flow filtration
Example:	Example:
Limitations:	Limitations:
Advantages:	Advantages:

Define

Term	Definition
Membrane	
Cake	
Retentate	
Filtrate/permeate	

GENTLE LYSIS METHODS

Cell disruption method	Application	General procedure
Osmotic lysis This very gentle method is well suited for applications in which the lysate is to be subsequently fractionated into subcellular components.	Blood cells, tissue culture cells	Suspend cells in a hypoosmotic solution.
Freeze-thaw lysis Many types of cells can be lysed by subjecting them to one or more cycles of quick freezing and subsequent thawing.	Bacterial cells, tissue culture cells	Rapidly freeze cell suspension using liquid nitrogen, then thaw. Repeat if necessary.
Detergent lysis Detergents solubilize cellular membranes, lysing cells and liberating their contents	Tissue culture cells	Suspend cells in lysis solution containing detergent
Enzymatic lysis Cells with cell walls can be lysed gently following enzymatic removal of the cell wall.	Plant tissue, bacterial cells, fungal cells	Treat cells with enzyme in isoosmotic solution

Harsh LYSIS METHODS

Cell disruption method	Application	General procedure
Sonication Ultrasonic waves generated by a sonicator lyse cells through shear forces. Complete shearing is obtained when maximal agitation is achieved, but care must be taken to minimize heating and foaming.	Cell suspensions	Sonicate cell suspension in short bursts to avoid heating. Cool on ice between bursts.
French pressure cell Cells are lysed by shear forces resulting from forcing cell suspension through a small orifice under high pressure.	Microorganisms with cell walls (bacteria, algae, yeasts)	Place cell suspension in chilled French pressure cell. Apply pressure and collect extruded lysate.

Harsh LYSIS METHODS— cont.

Cell disruption method	Application	General procedure
Grinding Some cell types can be opened by hand grinding with a mortar and pestle.	Solid tissues, microorganisms	Tissue or cells are normally frozen with liquid nitrogen and ground to a fine powder. Alumina or sand may aid grinding.
Mechanical homogenization Many different devices can be used to mechanically homogenize tissues. E.g blender.	Solid tissues	Chop tissue into small pieces if necessary. Add chilled homogenization buffer (3–5 volumes to volume of tissue). Homogenize briefly. Clarify lysate by filtration and/or centrifugation.
Glass bead homogenization The abrasive actions of the vortexed beads break cell walls, liberating the cellular contents.	Cell suspensions, microorganisms	Suspend cells in an equal volume of chilled lysis solution and place into a sturdy tube. Add 1–3 grams of chilled glass beads per gram of wet cells. Vortex 1 minute and incubate cells on ice 1 minute. Repeat vortexing and chilling two to four times.

Solid-liquid separation: An Example

A heterogeneous protein that is cloned into E. coli system has a few advantages such as high expression yield. However, inclusion body is a common problem. Inclusion body is aggregated proteins that are translated/expressed but is inactive due to improper folding. Inclusion body is located inside the cells. Sometime, the Inclusion body can undergo a series process of refolding to make it active again. The process of protein refolding is done outside the cells by adding certain kinds of chemicals and reagents.

QUESTION: Say you are a bioprocess engineer/biologist in a manufacturing company. From a fermentation broth, you are ask to get the inclusion bodies and pass it to the lab for protein refolding process, how are you going to solve the problem?

Problem solving strategy:

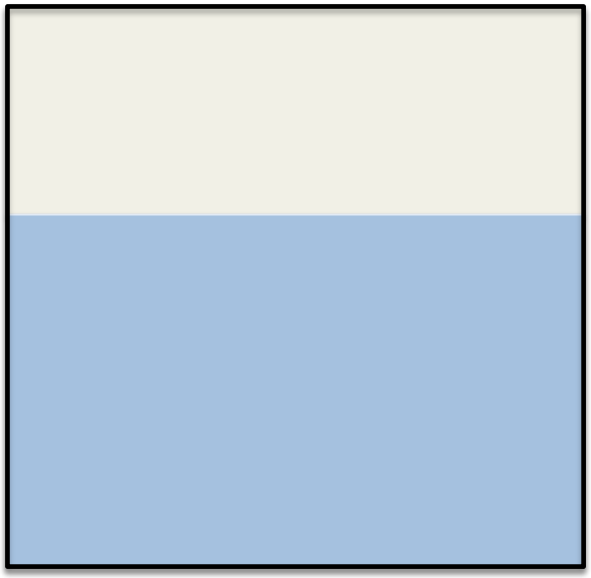
1. Identify statement of problem:

   _____

2. Create a flow method/flow sheet (how many steps do you need?):

Liquid-liquid extraction

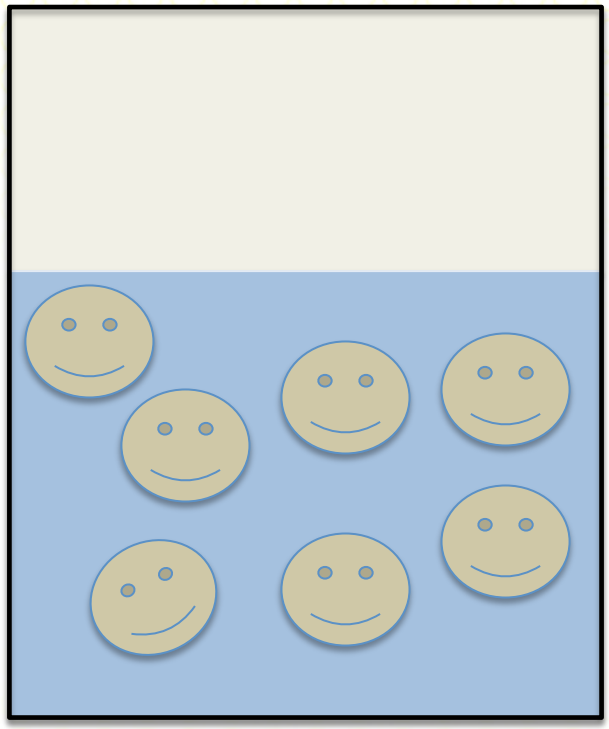
- Do oil and water mix?
- What happen after we put water into oil and leave it for a long time?



- Two layers = Two phase (fasa)
- When oil and water meet, they are **immiscible liquids**

ADVANCE PRODUCT RECOVERY:

Liquid-liquid extraction

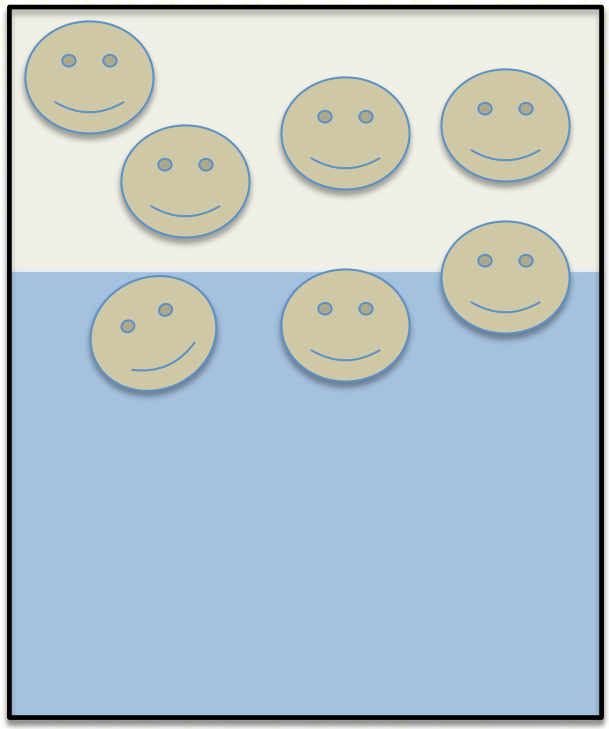


- Extraction is a process in which two phases come into contact with the objective of:
  - transferring a solute or particle from one phase to the other.

After some time

ADVANCE PRODUCT RECOVERY:

Liquid-liquid extraction



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