Title: The Cell disruption method for various tissue

By: Klbrom Fitwi

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Outline

- Questioning
- The objective cell disruption
- Various method of cell disruption
- Evaluation of cell disruption
QUESTION

- What is cell disruption? Definition
- Why is required? Its application and objective
- What are the different types of cell disruption methods?
- What factors to consider during cell disruption?
- How do we know whether the cell is disrupted or not?
Cell disruption is the breaking apart of cell wall or cell membrane to effect the release of intracellular product.
Why is cell disruption required?

- To extract biological products of interest that is not secreted from cells

- First step towards protein purification.
CLASSIFICATION OF DISRUPTION TECHNIQUES

LARGE-SCALE DISRUPTION

MECHANICAL
- Bead Mill
- Homogenizer
- Microfluidizer
- Sonicator
- French Press

NON-MECHANICAL
- PHYSICAL
  - Decompression
  - Osmotic Shock
  - Thermolysis
  - Freeze-thaw
  - Desiccation

- CHEMICAL
  - Antibiotics
  - Detergents
  - Chaotropes
  - Chelating Agents
  - Solvents
  - Hydroxide & Hypochlorite

- ENZYMATIC
  - Autolysis
  - Lytic Enzymes
  - Cloned-Phage Lysis
**CHOICE OF DISRUPTION METHODS**

Methods will vary depending on the type of cell and its particular cell wall structure.

**Type of cell disruption method:**

**Enzymatic method**

The use of enzymatic methods to is well established for preparing cells for disruption or for preparation of protoplasts (cells without cell walls) for other uses such as introducing cloned DNA or sub-cellular organelle isolation.
Disadvantages include:

- **Not always reproducible:**
  In addition to potential problems with the enzyme stability, the susceptibility of the cells to the enzyme can be dependent on the state of the cells.

- **Not usually applicable to large scale.**
  Large scale applications of enzymatic methods tend to be costly and irreproducible.

- The enzyme must be removed (or inactivated) to allow cell growth or permit isolation of the desired material.
Mechanical method: Bead Method

- Mechanical method for cell disruption uses glass or ceramic beads and a high level of agitation.

- The beads are added to the cell suspension in a tube and the sample is mixed on a common laboratory vortex mixer or electric motor to provide vigorous agitation.
Disadvantages include:

→ Limited ability to scale to larger samples.

→ Variability in product yield and purity

→ Occasional problems with foaming and sample heating, especially for larger samples
Sonication:

- Mechanical method for cell disruption applies **ultrasound** (typically 20-50 KHz) to the sample (sonication).
- In principle, the high-frequency is generated electronically and the mechanical energy is transmitted to the sample via a metal probe that oscillates with high frequency. The probe is placed into the cell-containing sample and the high-frequency oscillation causes a localized high pressure region breaking open the cells.
Disadvantages include:

- Heat generated by the ultrasound process must be dissipated.
- High noise levels (most systems require hearing protection and sonic enclosures).
- Yield variability
- Free radicals are generated that can react with other molecules.
Homogenizer
The main disruptive factor in this process is the **pressure applied on the sample** and consequent pressure drop across the valve. This causes the impact and shear stress on the cells making them to break which are proportional to the operating pressure.

**French press technology:**

Uses an external **hydraulic pump to drive a piston within a larger cylinder that contains the sample.** The pressurized solution is then squeezed past a needle valve. Once past the valve, the pressure drops to atmospheric pressure and generates shear forces that disrupt the cells.
Disadvantages include:

- Not well suited for larger volume processing.
- Awkward to manipulate and clean due to the weight of the assembly (about 30 lbs/14 Kg).
Physical method

Decompression method:
A fourth laboratory-scale system for cell disruption is rapid decompression or the “cell bomb” method. In this process, cells in question are placed under high pressure (usually nitrogen or other inert gas up to about 25,000 psi) and the pressure is rapidly released. The rapid pressure drop causes the dissolved gas to be released as bubbles that ultimately lyse the cell.
Disadvantages include:

- Only easily disrupted cells can be effectively disrupted (stationary phase *E. coli*, yeast, fungi, and spores do not disrupt well by this method).
- Large scale processing is not practical.
- High gas pressures have a small risk of personal hazard if not handled carefully.
La radiofrecuencia entra en todas las capas para estimular el colágeno, este por su parte tiene un efecto de restablecimiento interno e impulsando desde abajo desaparece o minimiza la hendidura, mejorando así la calidad de la piel.
Osmotic shock

Cells rupture due to an osmotic pressure gradient. Osmotic pressure is the hydrostatic pressure produced by a difference in concentration of solutes between a semipermeable membrane. When the extracellular solute concentration becomes small, the osmotic gradient causes water to flow through a cell membrane, which will eventually burst the cell.
Freezing thaw:

Repeated freezing and thaw of bacterial cells disrupts them because of the repeated formation of sharp ice crystals - many beginning to grow in the inside of the cells. Sort of like burst in balloon by using needles from the inside!
Chemical treatment:

A variety of chemical treatments can be used to “permeabilize” or lyse cells. For example, non ionic surfactants can be used to break the plasma membranes in animal cells. Organic solvents like toluene have been used to lyse yeast.
MEASUREMENT METHODS

DIRECT
  - MICROSCOPY
  - PARTICLE SIZE ANALYSIS
  - CELL ViABILITY
  - CELL COUNTING

INDIRECT
  - OPTICAL DENSITY
  - PROTEIN ESTIMATION
Optical microscopy of the yeast cells at various stages of disruption
Reference

- http://www.eng.uwo.ca/people/amargaritis/Margaritis%20CBE%20403a%20Fall%202007/CBE%20403a%20Course%20Notes%203%20(cell%20disruption%20methods)%20Fall%202007.pdf
Thank you