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Principles of Cell Disruption Methods

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SUMMARY

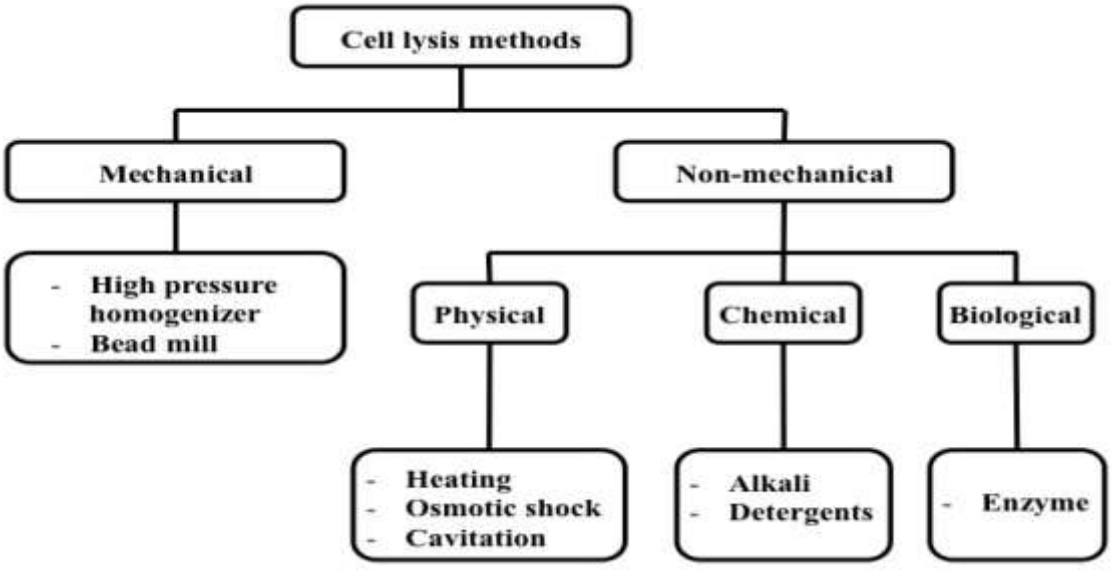
Cell disruption is a crucial component in biotechnology and the subsequent steps in the production of biological products. Because cell disruption greatly improves the recovery of biological products, it is important to disrupt cells in order to extract and retrieve the desired products. Cell disruption cannot be seen as a standalone process since it alters the physical characteristics of the cell slurry, which has an indirect impact on subsequent processes. Because each cell has a unique structure, it requires a distinct approach to disruption. Yeast cells are extremely difficult to disrupt because their cell walls prevent solvents from accessing the desired products, acting as additional disruption deterrents. Bacterial cells, moulds, plant cells, mammalian cells, and ground tissue are other cell types that need to be disrupted. Depending on whether they are gramme positive or gramme negative, bacterial cells may be disrupted using various techniques since the quantity of peptidoglycan and the existence of an envelope influence the whole procedure. In contrast to plant cells, which are more challenging to disrupt, mammalian cells are the most easily damaged since they do not have a cell wall. Due to the fact that biological products might be extracellular, intracellular, or periplasmic, there are several sorts of cell disruption techniques. The sensitivity of the cells to disruption, the product stability, the ease of extraction from the cell debris, the speed of the procedure, and the cost of the method are some factors that affect the choice of disruption method.

INTRODUCTION

In order to liberate intracellular fluids containing molecules or particles of interest, such as proteins or viruses, cell disruption, also known as cell lysis, involves destroying the cell wall and/or membrane. Cell disruption's main objective is to lyse suspended cells and retrieve as much of the molecules or particles of interest as is feasible(Gomes et al., 2020).

Cell disruption methods

Mechanical and non-mechanical cell disruption techniques can be classed. There are two types of mechanical procedures: solid shear methods and liquid shear methods. Physical, chemical, and enzymatic processes comprise the non-mechanical categories(Shehadul Islam et al., 2017).



Source:(Shehadul Islam et al., 2017)
Fig 1:- Classification of cell disruption methods

Mechanical physical methods

The primary idea behind mechanical disruption techniques is that the cells are put under a lot of stress either by pressure, abrasion with fast bead agitation, or ultrasound. Cavitation, shearing, impingement, or a combination of them are a few disruption techniques.

Bead mill

Originally utilised in the paint industry, bead mills have been modified for cell disruption in both small- and large-scale manufacturing. Given the variety of patterns that have been created, it is an effective technique to disrupt various microbial cells. The fundamental idea calls for a jacketed grinding chamber with a centrally located rotating shaft. The shaft is equipped with agitators, which provide the tiny beads in the chamber dynamic energy. The beads start to slam against one another as a result. Due to the increased bead-to-bead contact, the number of beads used increases the degree of disruption(Jahanshahi et al., 2002). The following process factors are included: agitator speed, bead size, bead proportion, cell suspension concentration, and agitator disc design. The main problems with bead mills include high temperatures that grow as the amount of beads increases, inadequate scale-up, and most significantly, a high risk of contamination.

Ultrasound

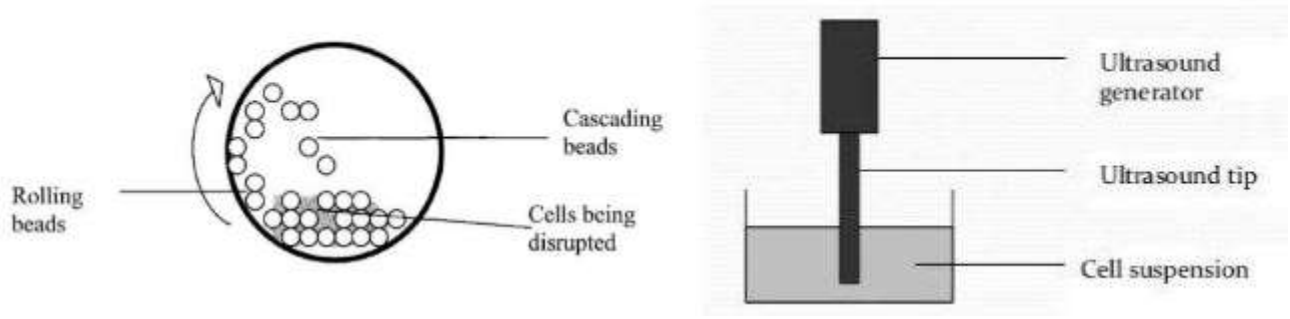


Fig 2 :- Schematic representation cell disruption method (a) Bead mill (b) ultrasonic disruption

Ultrasonic vibrators, which generate high frequency sound with a wave density of roughly 20 kHz/s, are the source of ultrasonic disruption. A titanium probe submerged in the cell solution is used as a transducer to transform the waves into mechanical vibrations. Cell disruption with this technique is done on both bacterial and fungal cells(Halim et al., 2012). Cell disruption in bacteria takes 30 to 60 seconds, while it takes yeast 2 to 10 minutes. This technique is typically combined with a chemical technique, most often lysis(Sheng et al., 2012) . When used on a small scale, sonication can be quite productive, but it performs horribly when scaled up. It requires a lot of energy and poses serious health and safety risks because of the noise it makes. It also not continuous procedure.

French press and high pressure homogeniser

The cell suspension is fed via a valve and into a pump cylinder in a French press, also known as high pressure homogenization. Then, it is pushed through a small annular gap and discharge valve at a pressure of up to 1500 bar, where the pressure then lowers to atmospheric. Due to the discharge's quick reduction in pressure, which makes the cells explode, cell disruption is achieved. One of the most well-known and often employed approaches is this one. Yeast cells are its main usage. It is a crucial component for homogenising milk in the dairy manufacturing business. In order to achieve the necessary level of disruption, the press can be operated at greater pressures while requiring fewer passes through the slurry. However, the inactivation of some heat-sensitive proteins may restrict the operating pressure and increase the necessary passageways (Engler & Robinson, 1981). As a result, a variety of variables, including temperature, operating pressure, the placement of the enzymes inside the cell, and the number of passes, affect protein release. The concentration of biomass affects the process. The homogenizer may be used for large-scale manufacturing, whereas the French press is a small-scale approach. Homogenizers come in a variety of designs and can include up to 50% of the feed in solids. Additionally, a lot of heat is produced—1.5^{o}C/1000 psi(Samarasinghe, 2012).

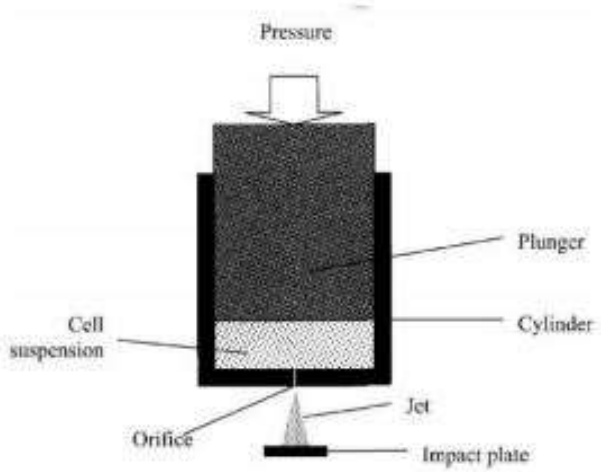


Fig 3 : -Schematic representation of the basic principle of a French press

Non-mechanical physical methods

Thermolysis

Large-scale production has the ability to use thermolysis more frequently. When cells in G(-) bacteria are heated to 50^{o}C, periplasmic proteins are liberated. At 90^{o}C, E. coli may release its cytoplasmic proteins in under 10 minutes. Short high temperature shocks have been shown to improve protein release compared to extended temperature exposures at lower values. Unfortunately, the results are rather inconsistent since variations in temperature affect how soluble proteins are. The creation and melting of ice crystals during freezing and thawing of a cell slurry might result in the rupture of the cells. Larger crystals can grow as a result of gradual freezing, which can seriously harm the cell(Zhu et al., 2006). This approach, when used in conjunction with cell grinding, has shown excellent results. However, it is only available in small-scale facilities and is quite expensive. Enzyme activity decrease has also been reported in some instances.

Osmotic shock

The proper operation of cellular activities often necessitates a set of tightly controlled chemical conditions. This means, for instance, that the internal pH or salt concentrations of that cell shouldn't depart materially from the ideal levels. Specific to each species are the ideal conditions and the capacity to endure less-than-ideal conditions. Although cells have the ability to actively govern their internal environment, abrupt and significant changes in the cell's environment may cause a severe shock that causes cell death and disruption. Osmotic shock is a method that may be used to lyse cells in biotechnological applications. Cells are initially exposed to either a high or low salt concentration with this method. Then, the circumstances are abruptly reversed, causing osmotic pressure and cell lysis (figure 4). Water moves fast from environments with low salt concentrations to environments with high salt concentrations, which explains why. So, if the cells are first exposed to a solution with a high salt concentration, water enters the cell following exposure to a solution with a low salt concentration(Mutanda et al., 2011). As a result, the cell explodes due to an increase in internal pressure. In contrast, water leaks out of the cell when it is exposed to a high salt concentration (1 molar solution) after being exposed to a low concentration, which causes cell disruption.

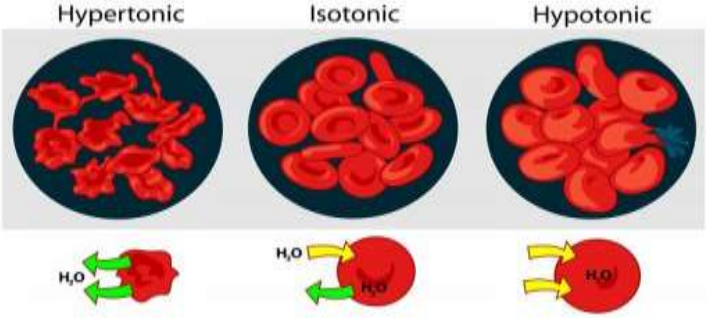


Fig 4 :- Osmotic shock. Exposure of cells to either high or low salt concentration causes cell disruption.

Due to its poor efficacy, osmotic shock is not a frequently utilised approach for cell disruption. For example, enzymatic pre-treatment to weaken the cells would typically be necessary for an effective disruption. Additionally, this method uses a lot of water and calls for the addition of large amounts of salt. Additionally, diluted products might cost more to process downstream.

Non-mechanical chemical and enzymatic methods

For cell disruption, there are a number of chemical techniques available in addition to physical and mechanical ones. These techniques rely on the use of chemicals or enzymes during the disruption process. There are many different ways that techniques work, but the ones that are most frequently employed act by rupturing the cell wall via enzymes, osmotic pressure, or by interfering with or precipitating cell wall proteins. In order to attain the necessary efficiency, a number of disruption techniques might be combined. Below, we go over the other tactics in greater depth.

Detergents

Anionic, cationic, and non-ionic detergents are the three categories of cell-disrupting detergents. All detergents have one thing in common: they immediately destroy the cell wall or membrane, which causes the intracellular substance to leak out. Sodium dodecyl sulphate (SDS), one of the most widely used anionic detergents, disrupts protein-protein interactions and reorganises the cell membrane (Thermo Fisher Scientific | Detergents for Cell Lysis and Protein Extraction). Triton X100, a non-ionic detergent, is another substance that is frequently employed for cell lysis. It works by causing membrane proteins to dissolve. In addition to these chemical substances, cell disruption can also be achieved using cationic detergents such ethyl trimethyl ammonium bromide. It is hypothesised that it affects the phospholipids and lipopolysaccharides in cell membranes.

Detergents interact with the components of cell membranes, which causes the membrane to separate. Many proteins will be denatured during cell lysis, which is a drawback of utilising detergents(Sharma et al., 2012). Additionally, detergents may interfere with later downstream processing stages. Cell lysis may thus need to be followed by a further purification step, which restricts their application in large-scale 13 operations. However, after DNA, RNA, or proteins are recovered from cells, detergents are frequently utilised for cell lysis in laboratories.

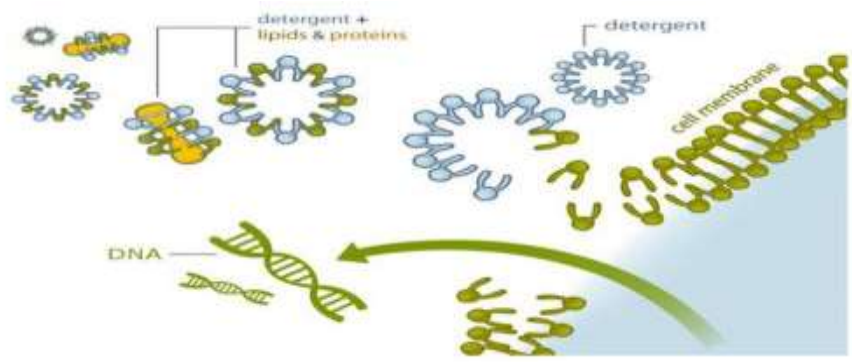


Fig 5:- Cell disruption with detergents. Detergents interact with cell membrane compounds which will lead to disassembly of cell membrane.

Solvents

Utilising chemical solvents is another technique for disrupting chemical cells. Examples of solvents that can be used to lyse cells include certain alcohols, toluene, methyl ethyl ketone, and dimethyl sulfoxide. These solvents remove the lipid components of the cell wall, which causes the release of intracellular components. Numerous producing organisms can be employed with this technology, although some denatured proteins may be an issue. The benefit is that by selecting the solvent, it may be feasible to choose the desired product. Large-scale processes do not often use this strategy (Benavides & Ritopalomares, 2006). Cell lysis can also be accomplished by hydrolyzing the cell wall using an alkali chemical (pH 10.5–12.5), in addition to solvents. The method's drawback is that it requires expensive chemical solutions to neutralise alkali. Additionally, the product could not be stable under alkaline circumstances.

Enzymes

Utilising digestive enzymes that break down the microbial cell wall is another method for achieving cell lysis. The type of cell walls and membranes present in various cell types and strains determines the type of enzyme that is utilised. Figure 6 depicts the mechanism for disruption of cell by enzyme. For instance, lysozyme is a typical enzyme used to break down gramme positive bacteria's cell walls. The peptidoglycan's -1-4-glucosidic linkages are hydrolyzed by lysozyme(Show et al., 2015). Lysozyme is less effective in the case of gramme negative cell walls because gramme negative bacteria have a different cell wall than gramme positive bacteria.

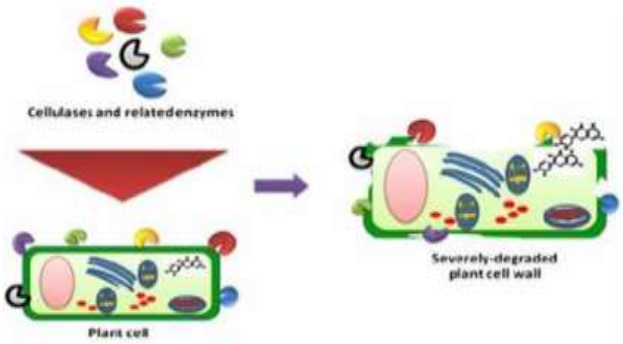


Fig 6:- Enzymatic cell disruption concept. Enzymes degrade the cell wall components which will lead to release of intracellular compounds.

One of the least harsh procedures is the cell lysis process that uses enzymes. However, the use of the enzyme in large-scale operations is constrained by their high cost and scarce availability. Additionally, the additional enzyme can make downstream processing more difficult (such as purification). However, immobilisation of enzymes might reduce these problems.

CONCLUSION

There are different ways to disrupt cells, each with advantages and downsides of its own. Although relatively easy and efficient, mechanical procedures including high pressure homogenization, bead mills, and grinding may cause mechanical stress on cells and lead to the loss of shear-sensitive biomolecules. Contrarily, chemical techniques, such as the use of detergents or solvents, are more-mild but might not be appropriate for all cell types or biomolecules. Physical procedures that disrupt cells quickly and effectively include sonication and osmotic shock, although they may also produce heat and oxidative damage. These techniques, however, are also prone to contamination by cell or particle fragments. In conclusion, the technique of cell disruption used will depend on the kind of cell, the biomolecule being extracted, and the particular demands of the experiment. It is crucial to thoroughly weigh the benefits and drawbacks of each technique before choosing the one that will best help you reach your goals.

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