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High-Pressure Homogenization a Technique for the Microbial Cell Disruption

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Abstract: Cell disruption is a crucial step to obtain cellular components for various biological studies. There exists a wide spectrum of reliable methods for cellular disintegration ranging from enzymatic digestion of the cell envelope to high-pressure disruption. High-pressure homogenization is a favourite method of microbial cell disruption applicable at large scale, because of its effectiveness and rapidity to handle various sample volumes at low cost. Microbial cells are lysed by shear forces resulting from forcing the suspension through a small orifice under the high pressure. Since high-pressure homogenization causes microbial cell disruption, it can cause reduction of microbial population as well.

Keywods: Cell disruption, High-pressure homogenization, Microbial cells

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1. Introduction

Microbial cells present particular difficulties in the application of standard extraction methods because of their thick and chemically refractile cell walls. The microbial cell envelope has often a decided propensity to behave as a molecular filter, allowing the translocation of molecules in or out according to their sizes even when the cell membrane integrity has been destroyed completely [1]. Moreover, while providing rigidity, the microbial cell wall acts as a barrier, protecting the protoplast from physical or osmotic injury. Thus, the microbial cell wall is extremely difficult to eliminate compared with mammalian cell membrane [2].

Cell disruption (lysis) is a crucial step to obtain intracellular components for various research studies in biology (extraction of nucleic acids for subsequent purification, isolation of intracellular enzymes, retrieval of recombinant proteins produced in cell and tissue cultures, proteomic analyses). It constitutes an essential step in downstream processing, as it has a considerable influence not only on the total quantity of the material recovered, but also on its biological activity, association with other cellular components, and the possible interference of proteolytic degradation, oxidation or contaminants that may influence the subsequent purification steps [3, 4].

2. Cell disruption methods

A wide variety of methods can be used to break up microbial cells, each one having its advantages and disadvantages. Vigorous mechanical treatments reduce intracellular extract viscosity but can result in the inactivation of labile proteins by heat or oxidation. On the other hand, gentle treatments may not release the target protein from cells, and resulting intracellular extracts are extremely viscous [4, 5]. The choice of disruption method depends on the cell type/origin and the cell wall composition. Gram positive bacteria are easily disrupted by enzymatic treatment alone. In Gram negative bacteria, the outer lipid bilayer must be permeabilized first - to expose the cell wall peptidoglycan to enzymatic digestion. The detergent lysis reagents have proved to be extremely effective for outer membrane permeabilization [3, 5]. Yeasts are more difficult-to-disrupt than bacteria. Their thick complex cell walls compose up to 25% of the cells' dry weight. Typical components are glucans, cellulose, mannoproteins, and chitin interconnected by covalent, disulfide, hydrogen, and hydrophobic bonds. Cell disruption technology includes two basic groups of methods non-mechanical and mechanical [2, 5].

Non-mechanical methods include chemical permeabilization (cationic/anionic detergents), physical disruption (osmotic shock, pressure release, freezing/thawing), enzymatic permeabilization (use of lyticase, zymolyase, proteinase K) [2]. These methods are generally applied when the sample of interest consists of easily lysed cells (tissue cultures, blood cells, some microorganisms) or when only a particular subcellular fraction is to be analyzed (cytoplasmic proteins, intact organelles e.g. mitochondria) [6].

Mechanical methods include ultrasonic disruption (sonicator), mechanical agitation (homogenizer, mortar and pestle, blender, glass beads) and pressurized disruption (French press) [2]. These methods are employed when cells are less easily disrupted (cells in solid tissues, cells with tough cell walls). More vigorous lysis methods obviously result in complete disruption of cells [6].

Mechanical methods of high-pressure homogenization and bead milling are favourite methods for the use at large scale, because of their effectiveness, rapidity to handle various sample volumes and low cost. Ultrasonication, chemical reagents including detergents, enzymatic treatments, freeze-thaw cycles, enzymatic methods combined with chemical or physical techniques are also very effective and are used more frequently in laboratory, especially in microscale [5].

3. High-pressure homogenization

High-pressure disruption has been effectively applied for disruption of microorganisms, plant and animal cells for decades and the equipment and mechanisms involved have been described in detail in the literature [5, 7 9]. This technique belongs to time consuming methods compared e.g. with ultrasonication. Since the heat generation is negligible, the biochemical integrity of the sample is preserved. The method is very effective as it leads to nearly complete breakage of cells and minimal sample loss compared with other methods such as grinding. The only drawback is the cost of the equipment [2].

High-pressure homogenizers operate by forcing a pressurized cell suspension through a narrow orifice between the valve and the valve seat (Fig. 1). The fluid leaves the gap in

the form of a radial jet that stagnates on an impact ring. Finally, it exits the homogenizer at low velocity and essentially atmospheric pressure [8, 9]. Cell disruption is caused by three different mechanisms: impingement on the valve, high liquid shear in the orifice, and sudden pressure drop upon discharge, causing finally an explosion of the cell. Universal acceptance for a single mechanism has not been reached. However, latest research has shown that fluid shear stress is the main cause of cell breakage [5, 10].

Many direct and indirect methods exist for measuring the degree of cell disruption. Indirect methods measure the release of specific enzymes or total protein from the cells during the disruption process [9]. According to Hetherington *et al*. [11], cell disruption, and consequently the rate of intracellular protein release, is a first-order kinetic process described by the relation:

$$\log[R_m / (R_m - R)] = k \times N \times P^a$$

where R is the amount of soluble protein, R_m is the maximum obtainable soluble protein, k is the temperature dependent rate constant, N is the number of passes through the homogenizer, P is the operating pressure, a is the pressure exponent.
The value of the superscript a is a measure of organism resistance to disruption and differs for diverse organisms. For a given organism, a has been found to be dependent on its growth history [12].

High-pressure homogenizers can be divided into three major classes: valve-type processors, fixed-geometry processors (microfluidizers) and constant pressure processors with fixed orifice. The valve may be a simple restricting needle as in the French Press or can have a more complex design with a combined valve seat and impact ring found in the APV Manton-Gaulin homogenizer [3, 5].

Fixed-geometry fluid processors (microfluidizers) differ from other high-pressure homogenizers. The instrument pressurizes and accelerates split streams of the cell suspension by the gas driven pumping of the liquid through fixed-geometry microchannels (obviously at 20–30 kpsi). The two high-velocity streams directly impinge on each other creating very high shear forces and pressure drop as the suspension exits the device. Microfluidizers range from the laboratory models processing small sample volumes (25 ml) to the biotechnological process models with up to 900 $l.h^{-1}$ throughput [5, 7].

Constant pressure processors are hydraulically operated disruption instruments where the sample is passed through a very small and fixed orifice. These cell disruptors function similarly to the original French Press; however, under contained and repeatable conditions (the desired pressure is maintained throughout the process). Useful features of these processors include the ability to achieve a maximum process pressure of 40 kpsi and a built-in cooling jacket. The Constant Systems instruments are available in a single shot bench (1–20 ml) to continuous process scale (405–565 $ml.min^{-1}$) models (Fig. 2) [5].

High-pressure homogenizers constitute an essential part of many industrial applications. In the pharmaceutical, cosmetic, chemical and food industries high-pressure homogenization is used for preparation or stabilization of emulsions and suspensions, for creating physical changes, such as viscosity changes, in products. Since high-pressure homogenization can be applied for cell disruption of dense microbial cultures, it can be anticipated that high-pressure homogenization will also cause a partial inactivation of the microbial population. This reduction of microorganisms, although not being the primary

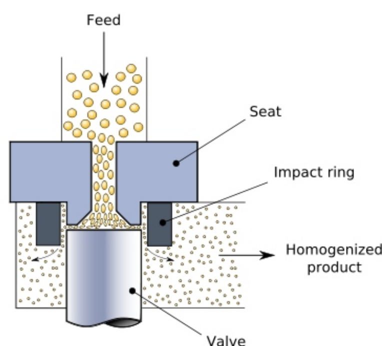

Fig. 1. The flow path through a simple homogenizing valve.

Fig. 2. High-pressure homogenizer (One Shot Model, Constant Systems Ltd.).

purpose of the process, is also potentially interesting since it may result in an extended shelf-life and improve the microbiological safety of the processed products [9, 13, 14].

In conclusion, the unique of proteins and differences in cellular envelope structures force a high degree of empiricism in selection and optimization of cell disruption and extract preparation techniques. However, a lot of tools and methods for successful isolation of biological extracts are available and have kept pace with the demands of modern structural and functional proteomics [5].

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