CHAPTER III

DISINTEGRATION OF CELLS

3.1 Methods of disintegration

Various possible methods for disintegrating microorganism may be summarized as shown in Figure 3-1.

3.1.1 Non mechanical methods

3.1.1.1 Drying

Various drying techniques have been used. Acetone powders of microorganisms can be prepared by pouring an aqueous suspension of cells into not less than 10 volumes of acetone at -20° C or lower, followed by filtration. Certain enzyme activities are retained, and these enzymes can be extracted with aqueous buffer solutions. Sieved yeast, and some bacteria, can be air dried at room temperature for two or three days. Partial autolysis occurs and some soluble enzymes may be extracted by stirring with buffer solution for a couple of hours (Wiseman, 1969).

3.1.1.2 Lysis

Lysis can be caused by physical, chemical or enzymic treatments.

3.1.1.2.1 Physical

3.1.1.2.1.1 Freezing-thawing

Repeated freezing and thawing are used early for disintegration of microbes. When cells of yeast Saccharomyces cerevisiae are cooled rapidly to -30° C or below, fewer than 0.01% survive. In contrast; when they are cooled slowly, up to 50% survive. The effect

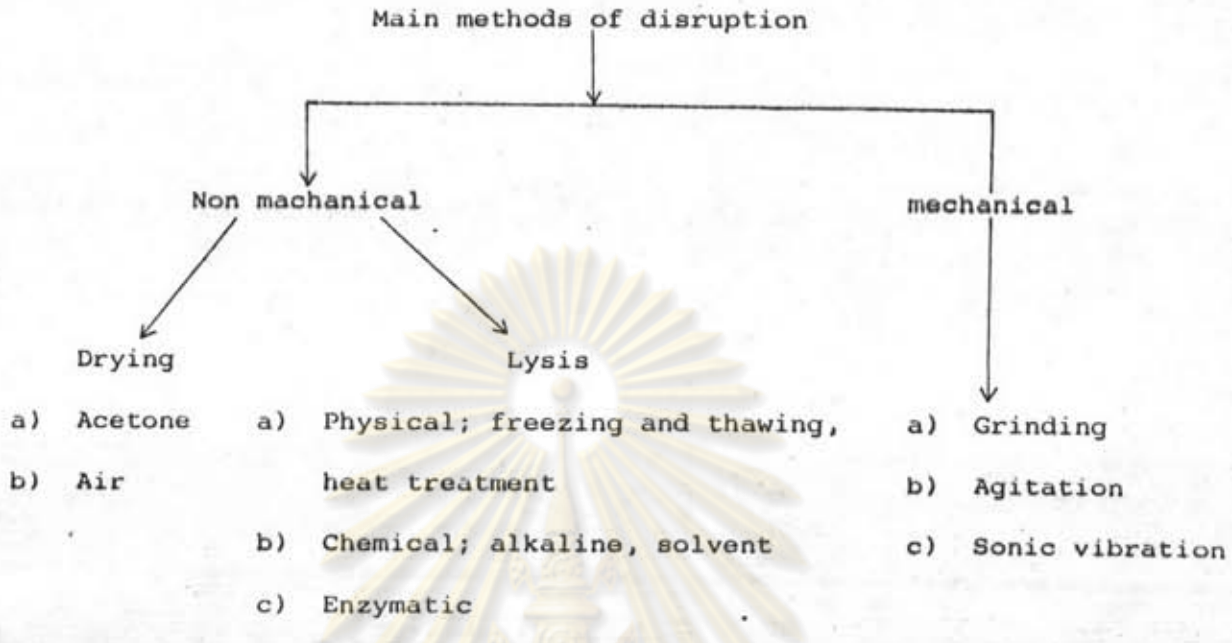

Figure 3-1 Types of non mechanical and mechanical cell disruption

of cooling rate on survival is reflected in the morphological apperance of cells both before and after thawing (Mazur, 1961).

3.1.1.2.1.2 Heat treatment rupture

With the use of difference temperatures rupture of the cell walls are obtained in several microbial species. The temperature used for rupture also "coagulate" the protoplasm. Therefore heat treatment is generally followed by a procedure which solubilized the now accessible protoplasm. Proteolytic enzymes with broad activity have often accomplished this.

3.1.1.2.1.3 Chemical

3.1.1.2.1.3.1 Alkaline or detergent breakdown

When microorganisms are subjected to alkaline, most components except the cell wall are dissolved. Detergents often lyse cells by disorganizing the cell membrane. However, the concentrations needed destroy many biological activities.

3.1.1.2.1.3.2 Solvent extraction

A great number of organic solvents have been used both in the laboratory and in the industry for the preparation of particular protoplasmic or cell wall components. Also this treatment inactivates several biological activities. The solvents are chloroform, toluol, amylacetate, ethylacetate.

3.1.1.2.1.4 Enzymatic

Enzymatic digestion of the microorganisms can be used for disintegration with enzymes that attack accessible cell supporting structures. Lysosomes cleave the β -(1→4) linkage between the N-acetyl-muramic acid peptide and N-acetyl-glucosamine in the rigid murein layer of the bacterial cell wall.

The added exogenous enzymes such as protease, nuclease, lipase work in a synergistic fashion with the yeast

endogenous enzymes to achieve the autolytic degradation of cellular material (Chao et al, 1980).

3.1.2 Mechanical methods

3.1.2.1 Grinding

Grinding with abrasive particles is supposed to cut up the microbial cell wall so that the cell content is released. A simple arrangement is to mix cell paste and abrasive (e. g. glass, quartz sand, alumina) in a mortar and grind the mixture with a pestle. No abrasive has to be added, if the grinding is performed at very low temperatures, e. g. after cooling with dry ice or liquid nitrogen. In these cases the water crystals, which at temperatures below -100° C are very hard, are supposed to act as abrasives. Grinding with mortar and pestle is laborious and the yield is small. Baker's yeast (27% dry weight) treated together with dry ice in an electric homogenizer gave approximately 20% extract after 2 minutes of treatment and centrifugation. Prolonged treatment of the cells increased the yield little. This method, which has a relatively low efficiency, is useful when the starting cell material is inexpensive (Perlman, 1969).

3.1.2.2 Agitation with abrasive particles

Agitation of suspensions of microorganisms mixed with small glass beads has, in many cases, been a convenient method for cell disruption. Usually cell concentrations around 10-100 mg/ml, alkali-free glass beads with diameter 0.1-0.5 mm. at approximately the same weight as the microbial suspension, and frequencies around 50-100 cycles/sec have been employed. The efficiency of disintegration is greater, when the treatment vessel is not filled up. After the treatment the beads are usually separated from the cell homogenate by filtration and may be used again. For large scale waring blendors, colloid mills

and a continuous flow shaker with plastic beads have been used to provide the agitation. The disintegration is thought to result from shear forces. These methods have been widely and successfully employed for the preparation of large cell-wall fragments. Presence of glass beads in a suspension of microorganisms under sonic treatment reduces further cell wall comminution.

3.1.2.3 Sonic vibration

Sonic and ultrasonic waves have been widely employed for disintegration of cells. Since there is no basic difference between them, they will be considered together as sonic waves. These waves, which are alternations of the pressure, cause streaming in the liquid. Under certain conditions bubbles form, grow and coalesce until they reach their resonant size, when they vibrate violently and eventually collapse. This physical phenomenon, known as cavitation, causes inter alia free radical formation. Oxidative free radicals are capable of inactivating unstable compounds like enzymes. In order to evade damage by the free radicals, addition of cysteine or other SH compounds have been used and found to protect several enzymes. Disruption of cells occurs also in the absence of observable bubble formation or cavitation, probably as a consequence of shearing due to the eddying motions which are induced by the vibration. When cavitation occurred, the eddying was more violent and the disintegration more efficient. Also in the presence of cavitation disintegration is caused by shearing action associated with bubble induced eddying and related motions.

3.2 Comparison between different methods

The chemical methods will probably have fairly special use, mainly because alkali, detergents and solvents destroy many biological

compounds, and the enzymes require rather high temperatures and have a narrow range of susceptible microorganisms. Freezing-thawing has low efficiency and uses long periods at risk temperatures. The heat treatment "coagulates" the protoplasm but makes it accessible to added enzymes. Drying technique has low efficiency. These methods might therefore mainly be used for preparation of low molecular weight compounds and stable macromolecules.

Mycelial filaments seem to be relatively resistant to sonic vibration. Little quantitative information is available on the disintegration by agitation with abrasive particles. Rapidly growing cells are generally more susceptible to the physical and mechanical methods than stationary cells. Sonic treatment seems to be particularly effective at "solubilizing" the cell envelopes which might make separation more difficult.

The breakage technique should generate high efficiency and cost saving. The efficiency of each techniques depends on the microorganism used. The efficiency of physical treatment such as freezing and thawing is low. Mechanical disintegration such as grinding requires high power consumption and the efficiency is low. Therefore, the general uses for preparation of commercial yeast extract are chemical and enzymatic treatments such as autolysis, plasmolysis and hydrolysis (Peppler, 1982).