**CELL DISRUPTION BY CHEMICAL METHOD**

1. **DETERGENTS**

A number of detergents will damage the lipoproteins of the microbial cell membrane and lead to release of intracellular components.

The compounds which can be used for this purpose include quaternary ammonium compounds, sodium lauryl sulphate, sodium dodecyl sulphate (SDS) and Triton X-lOO. Unfortunately, the detergents may cause some protein denaturation and may need to be removed before further purification stages can be undertaken.

Pullulanase is an enzyme which is bound to the outer membrane of Klebsiella pneumoniae. The cells were suspended in pH 7.8 buffer and 1% sodium cholate was added. The mixture was stirred for 1 hour to solubilize most of the enzyme. The use of Triton X-100 in combination with guanidine-HCl is widely and effectively used for the release of cellular protein.

1. **OSMOTIC SHOCK**

Osmotic shock caused by a sudden change in salt concentration will cause disruption of a number of cell types. However, the effect on microbial cells is normally minimal. It has proved to be a successful technique for the extraction of luciferase from *Photobacterium fischeri*.

A batch of 120 dm3 of broth was harvested and the cells collected as a cell paste in a Sharples centrifuge. Enzyme extraction was achieved by osmotic lysis using a ratio of 1 g of cell paste to 4 cm3 of cold distilled water with stirring for 15 to 30 minutes. A second extraction gave a small additional yield of enzyme. Only low levels of soluble protein were released using this technique.

1. **ALKALI TREATMENT**

Alkali treatment might be used for hydrolysis of microbial cell wall material provided that the desired enzyme will tolerate a pH of 11.5 to 12.5 for 20 to 30 minutes. Darbyshire (1981) has reported the use of this technique in the extraction of L-asparaginase.

1. **ENZYME TREATMENT**

There are a number of enzymes which hydrolyse specific bonds in cell walls of a limited number of micro-organisms. Enzymes shown to have this activity include lysozyme and enzyme extracts from leucocytes, Streptomyces spp., Micromonospora spp. Penicillium spp., Trichoderma spp., and snails. Although this is probably one of the most gentle methods available, unfortunately it is relatively expensive and the presence of the enzyme(s) may complicate further downstream purification processes.

 Chemical and enzymic methods for the release of intracellular products have not been used widely on a large scale, with the exception of lysozyme. However, their potential for the selective release of product and that they often yield a cleaner lysate mean that they are potentially invaluable tools in the recovery of fermentation products (Andrews and Asenjo, 1987; Andrews et at., 1990). Enzymes may also be used as a pretreatment to partially hydrolyse cell walls prior to cell disruption by mechanical methods.

References

1. Stanbury, The Recovery and Purification of Fermentation Products, Principles of Fermentation Technology, Second Edition.