subjected to a high pressure. The cylinder has a needle valve at the base, and the cells disrupt as they are extruded through the valve to atmospheric pressure. The Ribi fractionator is similar to a French press but is capable of continuous operation. It requires a preconcentrated cell paste or slurry. The Rannie high-pressure homogenizer is advertised to operate up to 1000 bar and to have a capacity of up to 40,000 l/h.

Other mechanical homogenizers make use of small beads (20 to 50 mesh). Some of these can be effectively used at rather large scales. The Dyno-Mill is available in sizes up to 275 l and can process 2000 kg/h of a cell suspension or about 340 kg dw/h of yeast. The Dyno-Mill can work with algae, bacteria, and fungi. The principle of operation is to pump the cell suspension through a horizontal grinding chamber filled with about 80% beads. Within the grinding chamber is a shaft with specially designed discs. When rotated at high speeds, high shearing and impact forces from millions of beads break cell walls. The broken cells are then discharged. A primary advantage of bead over pressure devices is better temperature control. Also this device is fully enclosed, preventing release of organisms in an aerosol, which is a critical consideration with pathogenic or genetically modified organisms.

For a solid medium, such as frozen cell paste or cells attached to or within a solid matrix, different processing options are available. Ball mills can be operated on large-scale processes. More specialized pieces of equipment include the Hughes and X-presses. The Hughes press consists of a split block with a half-cylinder hollowed in each face. The frozen cell paste is placed in the hollow, and pressure is applied to it from the cylinder into channels cut in the block. In the X-press, frozen cells are forced continuously through a small hole in a disc between two cylinders at low temperature and pressure. Cell disruption takes place because of deformation of organisms embedded in the ice. Pressure levels of several hundred bars can be obtained with the X-press.

With any of these approaches there is potential for damage to the product. Clearly heat denaturation is a major potential problem. The release of proteases from cellular compartments can lead to enzymatic degradation of the product. Some techniques, such as bead mills, have comparatively long residence times, so that if 100% cell breakage is desired, some fragile compounds released early in the process may be damaged. Once cell breakage occurs, products are released and encounter an oxidizing environment (due to air) that can cause, for protein products, rapid denaturation and aggregation. Typically this effect can be mitigated by addition of a reducing agent (e.g., β-mercaptoethanol) and a chelating agent (e.g., EDTA) to complex metal ions that may accelerate the oxidation process. Product quality considerations are essential to selection of a method for cell breakage.

11.3.2 Nonmechanical Methods

Osmotic shock and rupture with ice crystals are commonly used methods. By slowly freezing and then thawing a cell paste, the cell wall and membrane may be broken, releasing enzymes into the media. Changes in the osmotic pressure of the medium may result in the release of certain enzymes, particularly periplasmic proteins in gram-negative cells.[†] Cell-wall preparation of certain organisms can be made by heat-shocking. Freeze-drying after treatment with acetone, butanol, and buffers results in cell-wall disruption.

[†]The periplasmic space in a gram-negative cell is the space between the inner and outer membranes.