CHAPTER

10 The recovery and purification of fermentation products

INTRODUCTION

The extraction and purification of fermentation products may be difficult and costly. Ideally, one is trying to obtain a high-quality product as quickly as possible at an efficient recovery rate using minimum plant investment operated at minimal costs. Unfortunately, recovery costs of microbial products may vary from as low as 15% to as high as 70% of the total manufacturing costs (Aiba, Humphrey, & Millis, 1973; Swartz, 1979; Pace & Smith, 1981; Atkinson & Sainter, 1982; Datar, 1986). Obviously, the chosen process, and therefore its relative cost, will depend on the specific product. Atkinson and Mavituna (1991) indicate percentage of total costs being 15% for industrial ethanol, 20–30% for bulk penicillin G and up to 70% for enzymes. The extraction and purification of products such as recombinant proteins and monoclonal antibodies can account for 80–90% of the total processing costs (Doran, 2013). The high (and sometimes dominant) cost of downstream processing will affect the overall objective in some fermentations. A useful overview of relative downstream processing costs is given by Straathof (2011).

If a fermentation broth is analyzed at the time of harvesting, it will be discovered that the specific product may be present at a low concentration (typically 0.1–5 g dm^{-3}) in an aqueous solution that contains intact microorganisms, cell fragments, soluble and insoluble medium components, and other metabolic products. The product may also be intracellular, heat labile, and easily broken down by contaminating microorganisms. All these factors tend to increase the difficulties of product recovery. To ensure good recovery or purification, speed of operation may be the overriding factor because of the labile nature of a product. The processing equipment therefore must be of the correct type and also the correct size to ensure that the harvested broth can be processed within a satisfactory time limit. It should also be noted that each step or unit operation in downstream processing will involve the loss of some product as each operation will not be 100% efficient and product degradation may have occurred. Even if the percentage recovery for each step is very high, say for example, 90%, after five steps only around 60% of the initial product will be obtained. Hence, it is also important that the minimum number of unit operations possible are used to maximize product recovery.

The choice of recovery process is based on the following criteria:

1. The intracellular or extracellular location of the product.
2. The concentration of the product in the fermentation broth.
3. The physical and chemical properties of the desired product (as an aid to select separation procedures).
4. The intended use of the product.
5. The minimal acceptable standard of purity.
6. The magnitude of biohazard of the product or broth.
7. The impurities in the fermenter broth.
8. The marketable price for the product.

The main objective of the first stage for the recovery of an extracellular product is the removal of large solid particles and microbial cells usually by centrifugation or filtration (Fig. 10.1). In the next stage, the broth is fractionated or extracted into major fractions using ultrafiltration, reverse osmosis, adsorption/ion-exchange/gel filtration or affinity chromatography, liquid–liquid extraction, two phase aqueous extraction, supercritical fluid extraction, or precipitation. Afterward, the product-containing fraction is purified by fractional precipitation, further more precise chromatographic techniques and crystallization to obtain a product, which is highly concentrated and essentially free from impurities. Other products are isolated using modifications of this flow-stream. Finally, the finished product may require drying.

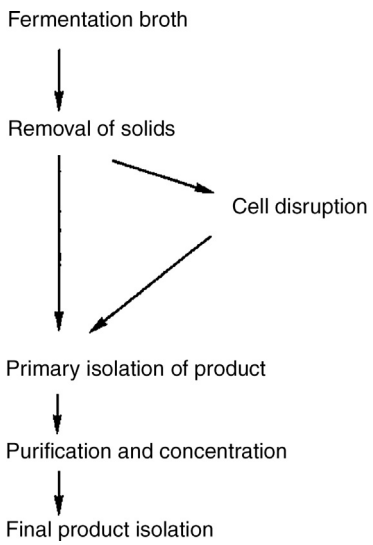

FIGURE 10.1 Stages in the Recovery of Product From a Harvested Fermentation Broth

It may be possible to modify the handling characteristics of the broth so that it can be handled faster with simpler equipment making use of a number of techniques:

1. Selection of a microorganism which does not produce pigments or undesirable metabolites.
2. Modification of the fermentation conditions to reduce the production of undesirable metabolites.
3. Precise timing of harvesting.
4. pH control after harvesting.
5. Temperature treatment after harvesting.
6. Addition of flocculating agents.
7. Use of enzymes to attack cell walls.

It must be remembered that the fermentation and product recovery are integral parts of an overall process. Because of the interactions between the two, neither stage should be developed independently, as this might result in problems and unnecessary expense. Darbyshire (1981) has considered this problem with reference to enzyme recovery. The parameters to consider include time of harvest, pigment production, ionic strength, and culture medium constituents. Large volumes of supernatants containing extracellular enzymes need immediate processing while harvesting times and enzyme yields might not be predictable. This can make recovery programs difficult to plan. Changes in fermentation conditions may reduce pigment formation. Corsano, Iribarren, Montagna, Aguirre, and Suarez (2006) discussed the integration and economic implications of downstream processing in batch and semicontinuous ethanol fermentations.

Certain antifoams remain in the supernatant and may affect centrifugation, ultrafiltration or ion-exchange resins used in recovery stages. Trials may be needed to find the most suitable antifoam (see also Chapter 4). The ionic strength of the production medium may be too high, resulting in the harvested supernatant needing dilution with demineralized water before it can be processed. Such a negative procedure should be avoided if possible by unified research and development programs. Media formulation is dominated by production requirements, but the protein content of complex media should be critically examined in view of subsequent enzyme recovery. This view is also shared by Topiwala and Khosrovi (1978), when considering water recycle in biomass production. They stated that the interaction between the different unit operations in a recycle process made it imperative that commercial plant design and operation should be viewed in an integrated fashion.

Flow sheets for recovery of penicillin, cephamycin C, citric acid, and micrococcal nuclease are given in Figs 10.2–10.5, to illustrate the range of techniques used in microbiological recovery processes. A series of comprehensive flow sheets for alcohols, organic acids, antibiotics, carotenoids, polysaccharides, intra- and extracellular enzymes, single-cell proteins, and vitamins have been produced by Atkinson and Mavituna (1991). Other reviews on separation and purification are available for penicillin (Swartz, 1979), amino acids (Samejima, 1972), enzymes (Aunstrup, 1979; Darbyshire, 1981), single-cell protein (Hamer, 1979) and polysaccharides (Pace &

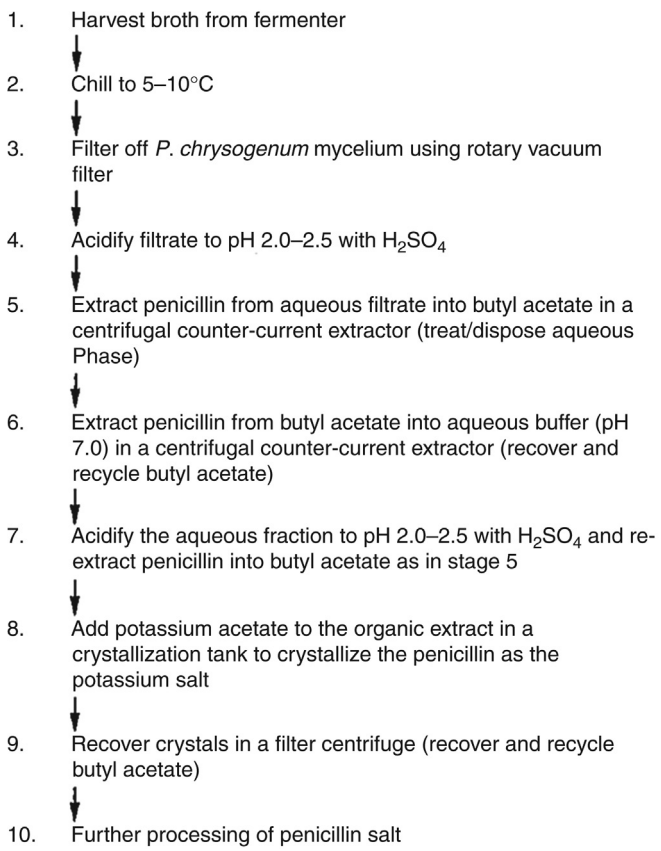

FIGURE 10.2 Recovery and Partial Purification of Penicillin G

Righelato, 1980; Smith & Pace, 1982). In the selection of processes for the recovery of biological products, it should always be understood that recovery and production are interlinked, and that good recovery starts in the fermentation by the selection of, among other factors, the correct media and time of harvesting.

The recovery and purification of many compounds may be achieved by a number of alternative routes. The decision to follow a particular route involves comparing the following factors to determine the most appropriate under a given set of circumstances:

Capital costs.
Processing costs.
Throughput requirements.
Yield potential.
Product quality.
Technical expertise available.
Conformance to regulatory requirements.

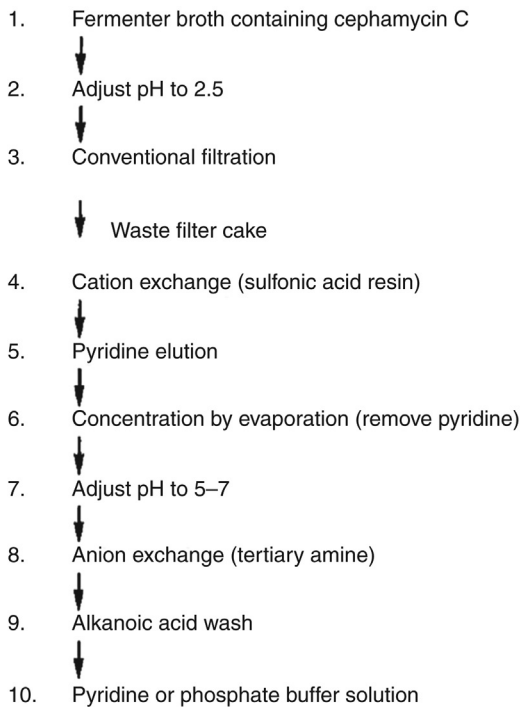

FIGURE 10.3 Purification of Cephamycin C: Sequential Ion Exchange Process (Omstead, Hunt, & Buckland, 1985)

Waste treatment needs.
Continuous or batch processing.
Automation.
Personnel health and safety (Wildfeuer, 1985).

The major problem currently faced in product recovery is the large-scale purification of biologically active molecules. For a process to be economically viable, large-scale production is required, and therefore large-scale separation, recovery, and purification. This then requires the transfer of small-scale preparative/analytical technologies (eg, chromatographic techniques) to the production scale while maintaining efficiency of the process, bioactivity of the product and purity of the product so that it conforms with the safety legislation and regulatory requirements. Developments in this field and remaining areas for development are documented by Pyle (1990).

REMOVAL OF MICROBIAL CELLS AND OTHER SOLID MATTER

Microbial cells and other insoluble materials are normally separated from the harvested broth by filtration or centrifugation. Because of the small size of many microbial cells, it will be necessary to consider the use of filter aids to improve

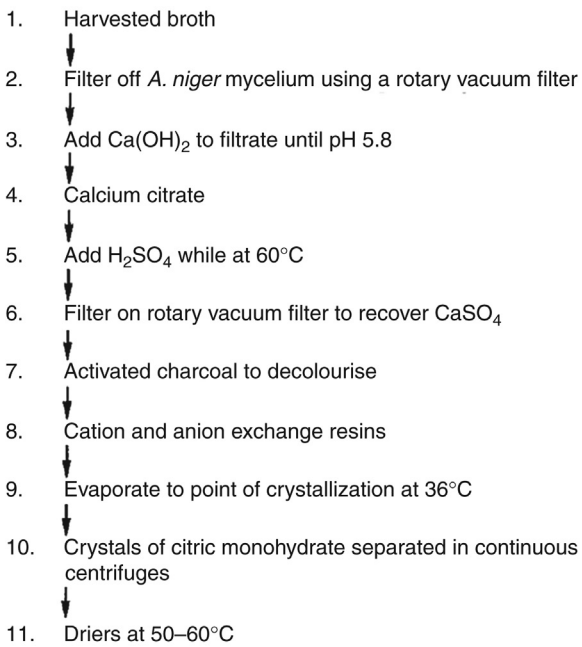

FIGURE 10.4 Recovery and Purification of Citric Acid (Sodeck et al., 1981)

filtration rates, while heat and flocculation treatments are employed as techniques for increasing the sedimentation rates in centrifugation. Flocculation can also be utilized in other downstream processing operations to aid product recovery. Hao, Xu, Liu, and Liu (2006) report the use of the flocculants chitosan and polyacrylamide on cell debris and soluble protein in the fermentation broth, to enhance the recovery of 1,3-propanediol by reactive extraction and distillation. The methods of cell and cell debris separation described in the following sections have been practiced for many years. Bowden, Leaver, Melling, Norton, and Whittington (1987) review some potential developments in cell recovery. These include the use of electrophoresis and dielectrophoresis to exploit the charged properties of microbial cells, ultrasonic treatment to improve flocculation characteristics and magnetic separations. Although not necessarily for the removal of cells, other downstream operations, which involve the application of an electrical field are also showing potential. One such process is electrodialysis that involves the transfer of ions from a dilute solution to a concentrated one through a semipermeable membrane by applying an electrical field (Moresi & Sappino, 2000). Lopez and Hestekin (2013) report the use of electrodialysis in the separation of organic acids in aqueous solution where the product, sodium butyrate, was successfully transferred from the aqueous phase into an ionic liquid phase through electrodialysis. A recovery rate of 99% was obtained with reduced energy input compared to traditional processing.

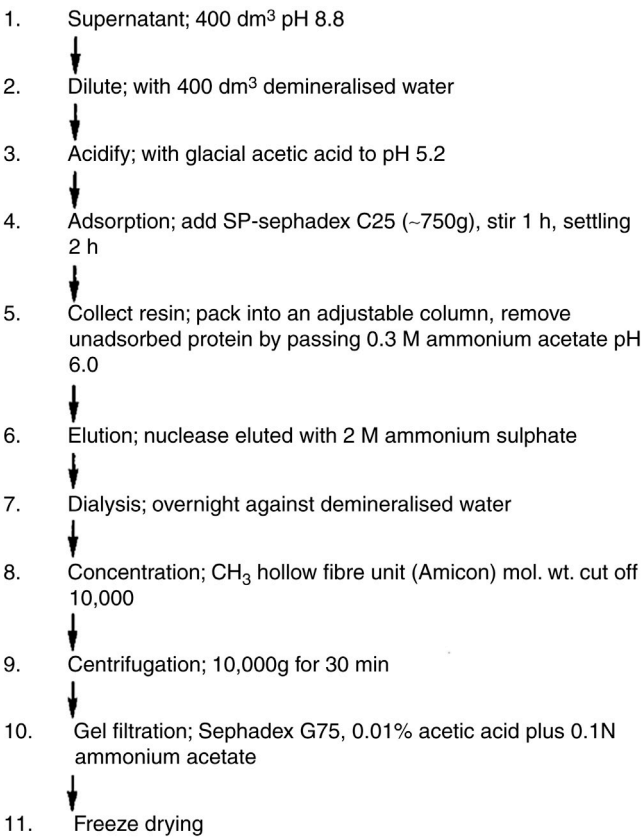

FIGURE 10.5 Purification of Micrococcal Nuclease (Darbyshire, 1981)

FOAM SEPARATION (FLOATATION)

Foam separation depends on using methods, which exploit differences in surface activity of materials. The material may be whole cells or molecular such as a protein or colloid, which is selectively adsorbed or attached to the surface of gas bubbles rising through a liquid, to be concentrated or separated and finally removed by skimming (Fig. 10.6). It may be possible to make some materials surface active by the application of surfactants such as long-chain fatty acids, amines, and quaternary ammonium compounds. Materials made surface active and collected are termed colligends whereas the surfactants are termed collectors. When developing this method of separation, the important variables, which may need experimental investigation are pH, air-flow rates, surfactants, and colligend-collector ratios. The recovery of surface active products is clearly an important potential application of this technique. Davis, Lynch, and Varley (2001) report the use of foam separation in the recovery

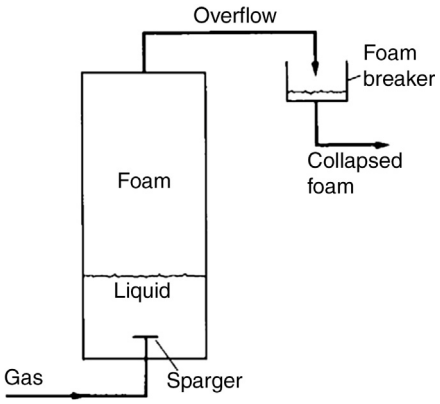

FIGURE 10.6 Schematic Flow Diagram for Foam Fractionation (Wang & Sinskey, 1970)

of the lipopeptide biosurfactant surfactin from *B. subtilis* cultures. They report that improved surfactin recovery can be achieved when foaming was simultaneous with the fermentation stage rather than as a nonintegrated semibatch process.

Rubin, Cassel, Henderson, Johnson, and Lamb (1966) investigated foam separation of *E. coli* starting with an initial cell concentration of 7.2×10^8 cells cm^{-3}. Using lauric acid, stearyl amine, or *t*-octyl amine as surfactants, it was shown that up to 90% of the cells were removed in 1 min and 99% in 10 min. The technique also proved successful with *Chlorella* sp. and *Chlamydomonas* sp. In other work with *E. coli,* Grieves and Wang (1966) were able to achieve cell enrichment ratios of between 10 and 1×10^6 using ethyl-hexadecyl-dimethyl ammonium bromide. DeSousa, Laluce, and Jafelicci (2006) examined the effects of a range of both organic and inorganic additives on floatation recovery of *Saccharomyces cerevisiae*. They report that compounds associated with cellular metabolism such as acetate and ethanol can improve floatation recovery of yeast cells.

PRECIPITATION

Precipitation may be conducted at various stages of the product recovery process. It is a particularly useful process as it allows enrichment and concentration in one step, thereby reducing the volume of material for further processing.

It is possible to obtain some products (or to remove certain impurities) directly from the broth by precipitation, or to use the technique after a crude cell lysate has been obtained.

Typical agents used in precipitation render the compound of interest insoluble, and these include:

1. Acids and bases to change the pH of a solution until the isoelectric point of the compound is reached and pH equals pI, when there is then no overall charge on the molecule and its solubility is decreased.

2. Salts such as ammonium and sodium sulfate are used for the recovery and fractionation of proteins. The salt removes water from the surface of the protein revealing hydrophobic patches, which come together causing the protein to precipitate. The most hydrophobic proteins will precipitate first, thus allowing fractionation to take place. This technique is also termed "salting out."
3. Organic solvents. Dextrans can be precipitated out of a broth by the addition of methanol. Chilled ethanol and acetone can be used in the precipitation of proteins mainly due to changes in the dielectric properties of the solution.
4. Nonionic polymers such as polyethylene glycol (PEG) can be used in the precipitation of proteins and are similar in behavior to organic solvents.
5. Polyelectrolytes can be used in the precipitation of a range of compounds, in addition to their use in cell aggregation.
6. Protein binding dyes (triazine dyes) bind to and precipitate certain classes of protein (Lowe & Stead, 1985).
7. Affinity precipitants are an area of much current interest in that they are able to bind to, and precipitate, compounds selectively (Niederauer & Glatz, 1992).
8. Heat treatment as a selective precipitation and purification step for various thermostable products and in the deactivation of cell proteases (Ng, Tan, Abdullah, Ling, & Tey, 2006).

FILTRATION

Filtration is one of the most common processes used at all scales of operation to separate suspended particles from a liquid or gas, using a porous medium which retains the particles but allows the liquid or gas to pass through. Gas filtration has been discussed in detail elsewhere (Chapters 5 and 7). It is possible to carry out filtration under a variety of conditions, but a number of factors will obviously influence the choice of the most suitable type of equipment to meet the specified requirements at minimum overall cost, including:

1. The properties of the filtrate, particularly its viscosity and density.
2. The nature of the solid particles, particularly their size and shape, the size distribution and packing characteristics.
3. The solids:liquid ratio.
4. The need for recovery of the solid or liquid fraction or both.
5. The scale of operation.
6. The need for batch or continuous operation.
7. The need for aseptic conditions.
8. The need for pressure or vacuum suction to ensure an adequate flow rate of the liquid.

THEORY OF FILTRATION

A simple filtration apparatus is illustrated in Fig. 10.7, which consists of a support covered with a porous filter cloth. A filter cake gradually builds up as filtrate passes

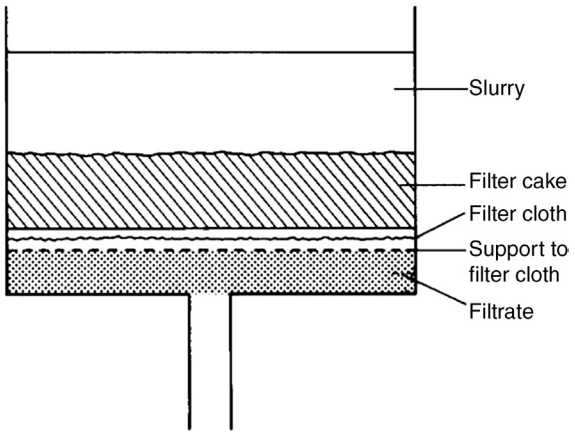

FIGURE 10.7 Diagram of a Simple Filtration Apparatus

through the filter cloth. As the filter cake increases in thickness, the resistance to flow will gradually increase.

Thus, if the pressure applied to the surface of the slurry is kept constant the rate of flow will gradually diminish. Alternatively, if the flow rate is to be kept constant the pressure will gradually have to be increased. The flow rate may also be reduced by blocking of holes in the filter cloth and closure of voids between particles, if the particles are soft and compressible. When particles are compressible, it may not be feasible to apply increased pressure.

Flow through a uniform and constant depth porous bed can be represented by the Darcy equation:

$$\text{Rate of flow} = \frac{dV}{dt} = \frac{KA\,\Delta P}{\mu L} \tag{10.1}$$

where μ, liquid viscosity; L, depth of the filter bed; ΔP, pressure differential across the filter bed; A, area of the filter exposed to the liquid; K, constant for the system.

K itself is a term which depends on the specific surface area s (surface area/unit volume) of the particles making up the filter bed and the voidage Σ when they are packed together. The voidage is the amount of filter-bed area, which is free for the filtrate to pass through. It is normally 0.3–0.6 of the cross-sectional area of the filter bed. Thus K (Kozeny's constant) can be expressed as

$$K = \frac{\Sigma^2}{5(1-\Sigma)^2 s^2}$$

Unfortunately, s and Σ are not easily determined.

In most practical cases L is not readily measured but can be defined in terms of:

V = volume of filtrate passed in time t and
v = volume of cake deposited per unit volume of filtrate.

Then

$$L = \frac{vV}{A}$$

Substituting in Eq. (10.1):

$$\frac{dV}{dt} = \frac{KA^2\Delta P}{\mu v V} \tag{10.2}$$

This is a general equation relating the rate of filtration to pressure drop, cross-sectional area of the filter and filtrate retained. Eq. 10.2 can be integrated for filtration at constant pressure.

$$VdV = \frac{KA^2\Delta P\, dt}{\mu v V} \tag{10.3}$$

Integrating Eq. (10.3):

$$V^2 = \frac{2KA^2\Delta Pt}{\mu v} \tag{10.4}$$

Now in Eq. (10.4), ΔP is constant, μ is generally equal to 1, v can be determined by laboratory investigation and A^2 remains approximately constant. Thus, there is a linear relationship between V^2 and t. By carrying out small-scale filtration trials, it is therefore possible to obtain a value for K. It is then possible to reapply the equation for large-scale filtration calculations.

Although it is also possible to derive the equation for the pressure necessary to maintain a constant filtration rate, it has little practical application. The pressure is made up of two components. First, the pressure needed to pass the constant volume through the filter resistance and, second, an increasing pressure component, which is proportional to the resistance from the increasing cake depth. This filtration procedure would be complex to perform practically, and other methods of filtration are used to achieve constant flow rates, for example, vacuum drum filters.

USE OF FILTER AIDS

It is common practice to use filter aids when filtering bacteria or other fine or gelatinous suspensions which prove slow to filter or partially block a filter. Kieselguhr (diatomaceous earth) is the most widely used material. It has a voidage of approximately 0.85, and, when it is mixed with the initial cell suspension, improves the porosity of a resulting filter cake leading to a faster flow rate. Alternatively, it may be used as an initial bridging agent in the wider pores of a filter to prevent or reduce blinding. The term "blinding" means the wedging of particles which are not quite large enough to pass through the pores, so that an appreciable fraction of the filter surface becomes inactive.

The minimum quantity of filter aid to be used in filtration of a broth should be established experimentally. Kieselguhr is not cheap, and it will also absorb some of the filtrate, which will be lost when the filter cake is disposed. The main methods of using the filter aid are:

1. A thin layer of Kieselguhr is applied to the filter to form a precoat prior to broth filtration.
2. The appropriate quantity of filter aid is mixed with the harvested broth. Filtration is started, to build up a satisfactory filter bed. The initial raffinate is returned to the remaining broth prior to starting the true filtration.
3. When vacuum drum filters are to be used which are fitted with advancing knife blades, a thick precoat filter is initially built up on the drum (later section in this chapter).

In some processes such as microbial biomass production, filter aids cannot be used and cell pretreatment by flocculation or heating must be considered (see later sections in this chapter). In addition it is not normally practical to use filter aids when the product is intracellular and its removal would present a further stage of purification.

Plate and frame filters

A plate and frame filter is a pressure filter in which the simplest form consists of plates and frames arranged alternately. The plates are covered with filter cloths (Fig. 10.8) or filter pads. The plates and frames are assembled on a horizontal framework and held together by means of a hand screw or hydraulic ram so that there is no leakage between the plates and frames, which form a series of liquid-tight compartments. The slurry is fed to the filter frame through the continuous channel formed by the holes in the corners of the plates and frames. The filtrate passes through the filter cloth or pad, runs down grooves in the filter plates and is then discharged through outlet taps to a channel. Sometimes, if aseptic conditions are required, the outlets may lead directly into a pipe. The solids are retained within the frame and filtration is stopped when the frames are completely filled or when the flow of filtrate becomes uneconomically low.

On an industrial scale, the plate and frame filter is one of the cheapest filters per unit of filtering space and requires the least floor space, but it is intermittent in operation (a batch process) and there may be considerable wear of filter cloths as a result of frequent dismantling. This type of filter is most suitable for fermentation broths with a low solids content and low resistance to filtration. It is widely used as a "polishing" device in breweries to filter out residual yeast cells following initial clarification by centrifugation or rotary vacuum filtration. It may also be used for collecting high value solids that would not justify the use of a continuous filter. Because of high labor costs and the time involved in dismantling, cleaning, and reassembly, these filters should not be used when removing large quantities of worthless solids from a broth.

Pressure leaf filters

There are a number of intermittent batch filters usually called by their trade names. These filters incorporate a number of leaves, each consisting of a metal framework of

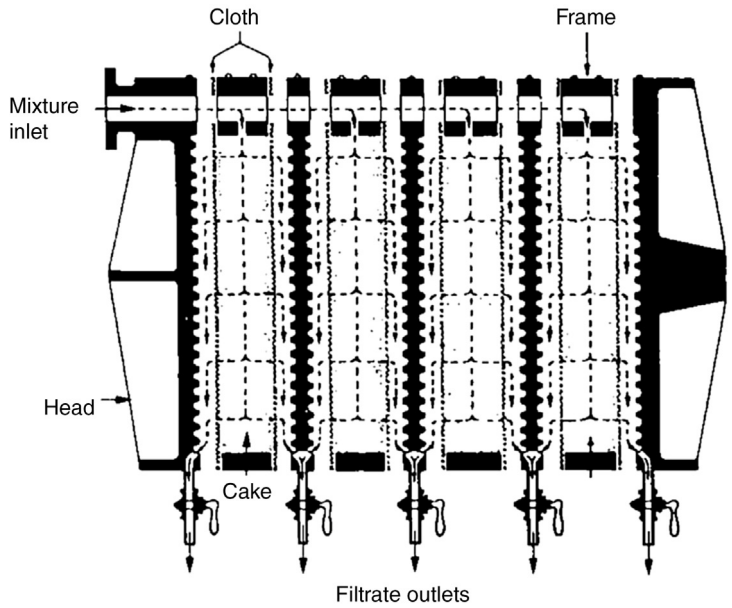

FIGURE 10.8 Flush Plate and Frame Filter Assembly

The cloth is shown away from the plates to indicate flow of filtrate in the grooves between pyramids (Purchas, 1971).

grooved plates, which is covered with a fine wire mesh, or occasionally a filter cloth and often precoated with a layer of cellulose fibers. The process slurry is fed into the filter, which is operated under pressure or by suction with a vacuum pump. Because the filters are totally enclosed it is possible to sterilize them with steam. This type of filter is particularly suitable for "polishing" large volumes of liquids with low solids content or small batch filtrations of valuable solids.

Vertical metal-leaf filter

This filter consists of a number of vertical porous metal leaves mounted on a hollow shaft in a cylindrical pressure vessel. The solids from the slurry gradually build up on the surface of the leaves and the filtrate is removed from the plates via the horizontal hollow shaft. In some designs the hollow shaft can be slowly rotated during filtration. Solids are normally removed at the end of a cycle by blowing air through the shaft and into the filter leaves.

Horizontal metal-leaf filter

In this filter, the metal leaves are mounted on a vertical hollow shaft within a pressure vessel. Often, only the upper surfaces of the leaves are porous. Filtration is continued until the cake fills the space between the disc-shaped leaves or when the operational pressure has become excessive. At the end of a process cycle, the solid cake can be discharged by releasing the pressure and spinning the shaft with a drive motor.

Stacked-disc filter

One kind of filter of this type is the Metafilter. This is a very robust device and because there is no filter cloth and the bed is easily replaced, labor costs are low. It consists of a number of precision-made rings, which are stacked on a fluted rod (Fig. 10.9). The rings (22 mm external diameter, 16 mm internal diameter, and 0.8 mm-thick) are normally made from stainless steel and precision stamped so that there are a number of shoulders on one side. This ensures that there will be clearances of 0.025–0.25 mm when the rings are assembled on the rods. The assembled stacks are placed in a pressure vessel, which can be sterilized if necessary. The packs are normally coated with a thin layer of Kieselguhr, which is used as a filter aid. During use, the filtrate passes between the discs and is removed through the grooves of the fluted rods, while solids are deposited on the filter coating. Operation is continued until the resistance becomes too high and the solids are removed from the rings by applying back pressure via the fluted rods. Metafilters are primarily used for "polishing" liquids such as beer.

CONTINUOUS FILTERS

Rotary vacuum filters

Large rotary vacuum filters are commonly used by industries, which produce large volumes of liquid which need continuous processing. The filter consists of a rotating, hollow, segmented drum covered with a fabric or metal filter, which is partially immersed in a trough containing the broth to be filtered (Fig. 10.10). The slurry is fed on to the outside of the revolving drum and vacuum pressure is applied internally so that the filtrate is drawn through the filter, into the drum and finally to a collecting vessel. The interior of the drum is divided into a series of compartments, to which the vacuum pressure is normally applied for most of each revolution as the drum slowly revolves (~1 rpm). However, just before discharge of the filter cake, air pressure may be applied internally to help ease the filter cake off the drum. A number of spray jets may be carefully positioned so that water can be applied to rinse the cake. This washing is carefully controlled so that dilution of the filtrate is minimal.

It should be noted that the driving force for filtration (pressure differential across the filter) is limited to 1 atmosphere (100 kN m^{-2}) and in practice it is significantly less than this. In contrast, pressure filters can be operated at many atmospheres pressure. A number of rotary vacuum drum filters are manufactured, which differ in the mechanism of cake discharge from the drum:

1. String discharge.
2. Scraper discharge.
3. Scraper discharge with precoating of the drum.

String discharge

Fungal mycelia produce a fibrous filter cake, which can easily be separated from the drum by string discharge (Fig. 10.11). Long lengths of string 1.5 cm apart are threaded over the drum and round two rollers. The cake is lifted free from the upper

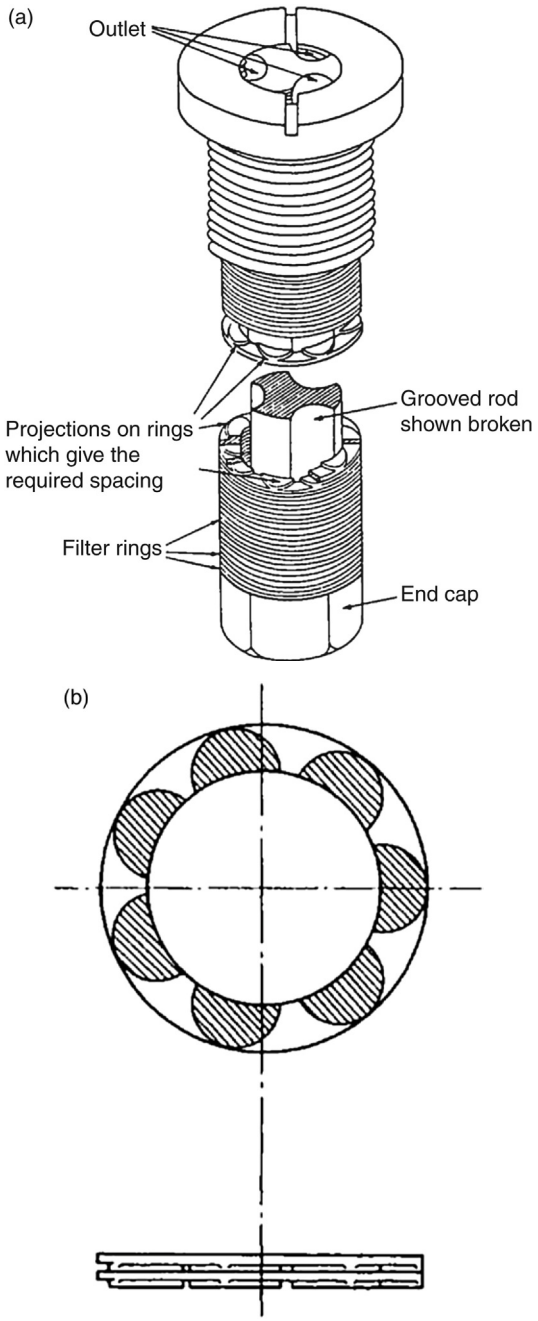

FIGURE 10.9

(a) Metafilter pack (Coulson & Richardson, 1991). (b) Rings for metafilter (Coulson & Richardson, 1991).

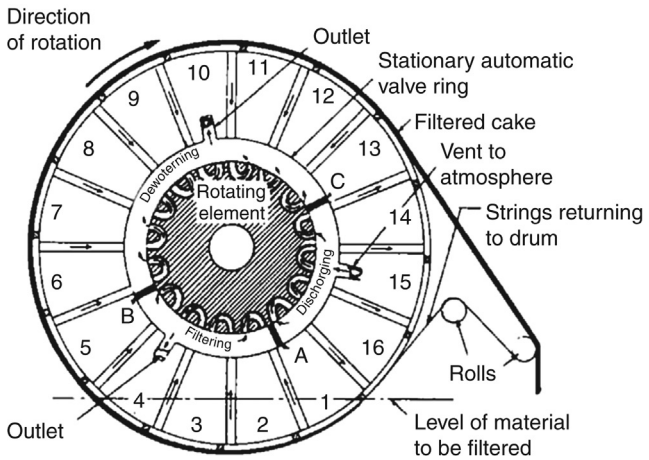

FIGURE 10.10 Diagram of String-Discharge Filter Operation

Sections 1 to 4 are filtering; sections 5 to 12 are dewatering; and section 13 is discharging the cake with the string discharge. Sections 14, 15 and 16 are ready to start a new cycle. A, B and C represent dividing members in the annular ring (Miller et al., 1973).

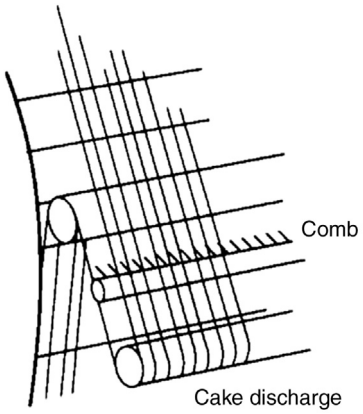

FIGURE 10.11 Cake Discharge on a Drum Filter Using Strings (Talcott, Willus, & Freeman, 1980)

part of the drum when the vacuum pressure is released and carried to the small rollers where it falls free.

Scraper discharge

Yeast cells can be collected on a filter drum with a knife blade for scraper discharge (Fig. 10.12). The filter cake which builds up on the drum is removed by an accurately positioned knife blade. Because the knife is close to the drum, there may be gradual wearing of the filter cloth on the drum.

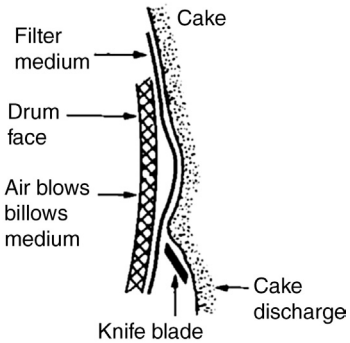

FIGURE 10.12 Cake Discharge on a Drum Using a Scraper (Talcott et al., 1980)

Scraper discharge with precoating of the drum

The filter cloth on the drum can be blocked by bacterial cells or mycelia of actinomycetes. This problem is overcome by precoating the drum with a layer of filter-aid 2–10 cm thick. The cake which builds up on the drum during operation is cut away by the knife blade (Fig. 10.13), which mechanically advances toward the drum at a controlled slow rate. Alternatively, the blade may be operated manually when there is an indication of "blinding" which may be apparent from a reduction in the filtration rate. In either case the cake is removed together with a very thin layer of precoat. A study of precoat drum filtration has been made by Bell and Hutto (1958). The operating variables studied include drum speed, extent of drum submergence, knife advance speed, and applied vacuum. The work indicated that optimization for a new process might require prolonged trials. Although primarily used for the separation of microorganisms from broth, studies have indicated (Gray, Dunnill, & Lilly, 1973) that rotary vacuum filters can be effective in the processing of disrupted cells.

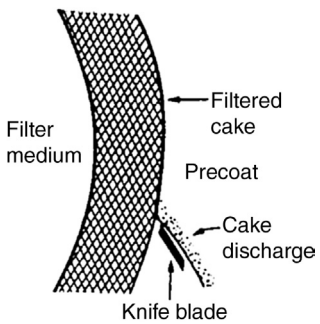

FIGURE 10.13 Cake Discharge on a Precoated Drum Filter (Talcott et al., 1980)

CROSS-FLOW FILTRATION (TANGENTIAL FILTRATION)

In the filtration processes previously described, the flow of broth was perpendicular to the filtration membrane. Consequently, blockage of the membrane led to the lower rates of productivity and/or the need for filter aids to be added, and these were serious disadvantages.

In contrast, an alternative which is rapidly gaining prominence both in the processing of whole fermentation broths (Tanny, Mirelman, & Pistole, 1980; Brown & Kavanagh, 1987; Warren, MacDonald, & Hill, 1991) and cell lysates (Gabler & Ryan, 1985; Le & Atkinson, 1985) is cross-flow filtration. Here, the flow of medium to be filtered is tangential to the membrane (Fig. 10.14a), and no filter cake builds up on the membrane.

The benefits of cross-flow filtration are:

1. Efficient separation, >99.9% cell retention.
2. Closed system; for the containment of organisms with no aerosol formation (see also Chapter 7).
3. Separation is independent of cell and media densities, in contrast to centrifugation.
4. No addition of filter aid (Zahka & Leahy, 1985).

The major components of a cross-flow filtration system are a media storage tank (or the fermenter), a pump, and a membrane pack (Fig. 10.14b). The membrane is usually in a cassette pack of hollow fibers or flat sheets in a plate and frame type

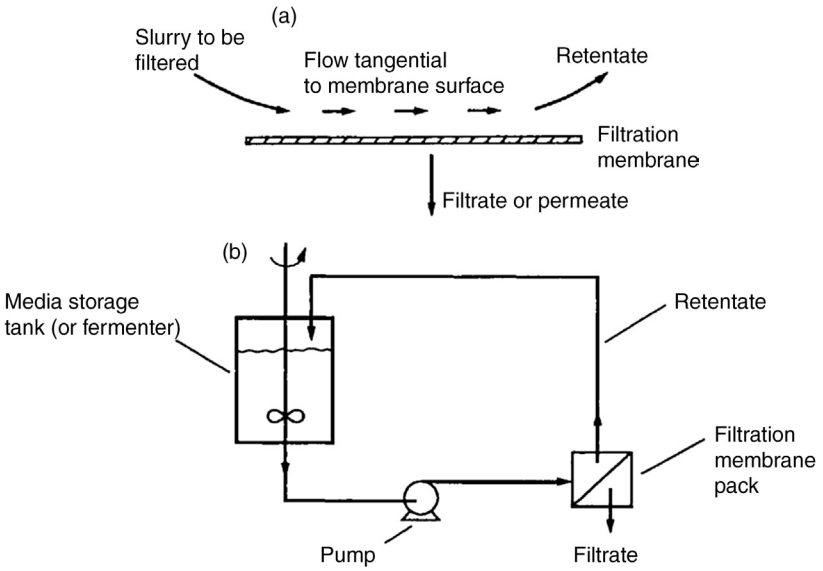

FIGURE 10.14

(a) Schematic diagram of cross-flow filtration. (b) Major components of a cross-flow filtration system.

stack or a spiral cartridge (Strathmann, 1985). In this way, and by the introduction of a much convoluted surface, large filtration areas can be attained in compact devices. Two types of membrane may be used; microporous membranes (microfiltration) with a specific pore size (0.45, 0.22 μm etc.) or an ultrafiltration membrane (see later section) with a specified molecular weight cut-off (MWCO). The type of membrane chosen is carefully matched to the product being harvested, with microporous and 100,000 MWCO membranes being used in cell separations.

The output from the pump is forced across the membrane surface; most of this flow sweeps the membrane, returning retained species back to the storage tank and generally less than 10% of the flow passes through the membrane (permeate). As this process is continued the cells, or other retained species are concentrated to between 5% and 10% of their initial volume. More complex variants of the process can allow in situ washing of the retentate and enclosed systems for containment and sterilization (Mourot, LaFrance, & Oliver, 1989). Russotti et al. (1995) report on a pilot scale system utilizing crossflow-microfiltration (pore sizes 0.22–0.65 μm) to harvest recombinant yeast cells to recover intracellular products.

Many factors influence filtration rate. Increased pressure drop will, up to a point increase flow across the membrane, but it should be remembered that the system is based on a swept clean membrane. Therefore, if the pressure drop is too great the membrane may become blocked. The filtration rate is therefore influenced by the rate of tangential flow across the membrane; by increasing the shear forces at the membrane's surface retained species are more effectively removed, thereby increasing the filtration rate. Adikane, Singh, and Nene (1999) report that a 2.9-fold increase in cross-flow velocity resulted in an average increase in flux across the membrane of 1.8-fold and a 41% reduction in processing time. Higher temperatures will increase filtration rate by lowering the viscosity of the media, though this is clearly of limited application in biological systems. Filtration rate is inversely proportional to concentration, and media constituents can influence filtration rate in three ways. Low molecular weight compounds increase media viscosity and high molecular weight compounds decrease shear at the membrane surface, both leading to a reduction in filtration rate. Finally, broth constituents can "foul" the membrane, primarily by adsorption onto the membrane's surface, causing a rapid loss in efficiency. This can be controlled by increasing the pore size in microporous membranes, modification of the membrane chemistry or media formulation in particular by reducing the use of antifoaming agents (Russotti et al., 1995). Lee, Chang, and Ju (1993) have shown that the pulses of air injected into the flow to a cross-flow filter increase the shear rate at the membrane surface reducing the effects of membrane fouling.

CENTRIFUGATION

Microorganisms and other similar sized particles can be removed from a broth by using a centrifuge when filtration is not a satisfactory separation method. Although a centrifuge may be expensive when compared with a filter it may be essential when:

1. Filtration is slow and difficult.
2. The cells or other suspended matter must be obtained free of filter aids.
3. Continuous separation to a high standard of hygiene is required.

Noncontinuous centrifuges are of extremely limited capacity and therefore not suitable for large-scale separation. The centrifuges used in harvesting fermentation broths are all operated on a continuous or semicontinuous basis. Some centrifuges can be used for separating two immiscible liquids yielding a heavy phase and light phase liquid, as well as a solids fraction. They may also be used for the breaking of emulsions.

According to Stoke's law, the rate of sedimentation of spherical particles suspended in a fluid of Newtonian viscosity characteristics is proportional to the square of the diameter of the particles, thus the rate of sedimentation of a particle under gravitational force is:

$$V_g = \frac{d^2 g(\rho_P - \rho_L)}{18\mu} \tag{10.5}$$

where V_g, rate of sedimentation (m s^{-1}); d, particle diameter (m); g, gravitational constant (m s^{-2}); ρ_p, particle density (kg m^{-3}); ρ_L, liquid density (kg m^{-3}); μ, viscosity (kg m^{-1} s^{-1}).

This equation can then be modified for sedimentation in a centrifuge:

$$V_c = \frac{\mathrm{d}\omega^2 r(\rho_P - \rho_L)}{18\mu} \tag{10.6}$$

where V_c, rate of sedimentation in the centrifuge (ms^{-1}); ω, angular velocity of the rotor (s^{-1}); r, radial position of the particle (m).

Dividing Eq. (10.6) by Eq. (10.5) yields

$$\frac{\omega^2 r}{g}$$

This is a measure of the separating power of a centrifuge compared with gravity settling. It is often referred to as the relative centrifugal force and given the symbol "Z."

It is evident from this formula that factors influencing the rate of sedimentation over which one has little or no control are the difference in density between the cells and the liquid (increased temperature would lower media density but is of little practical use with fermentation broths), the diameter of the cells (could be increased by coagulation/flocculation), and the viscosity of the liquid. Ideally, the cells should have a large diameter, there should be a large density difference between cell and liquid and the liquid should have a low viscosity. In practice, the cells are usually very small, of low density and are often suspended in viscous media. Thus it can be seen that the angular velocity and diameter of the centrifuge are the major factors to

be considered when attempting to maximize the rate of sedimentation (and therefore throughput) of fermentation broths.

CELL AGGREGATION AND FLOCCULATION

Following an industrial fermentation, it is quite common to add flocculating agents to the broth to aid dewatering (Wang, 1987). The use of flocculating agents is widely practiced in the effluent-treatment industries for the removal of microbial cells and suspended colloidal matter (Delaine, 1983).

It is well known that aggregates of microbial cells, although they have the same density as the individual cells, will sediment faster because of the increased diameter of the particles (Stokes law). This sedimentation process may be achieved naturally with selected strains of brewing yeasts, particularly if the wort is chilled at the end of fermentation, and leads to a natural clearing of the beer.

Microorganisms in solution are usually held as discrete units in three ways. First, their surfaces are negatively charged and therefore repulse each other. Second, because of their generally hydrophilic cell walls a shell of bound water is associated with the cell which acts as a thermodynamic barrier to aggregation. Finally, due to the irregular shapes of cell walls (at the macromolecular level) steric hindrance will also play a part.

During flocculation, one or more mechanisms besides temperature can induce cell flocculation:

1. Neutralization of anionic charges, primarily carboxyl and phosphate groups, on the surfaces of the microbial cells, thus allowing the cells to aggregate. These include changes in the pH and the presence of a range of compounds, which alter the ionic environment.
2. Reduction in surface hydrophilicity.
3. The use of high molecular weight polymer bridges. Anionic, nonionic, and cationic polymers can be used, though the former two also require the addition of a multivalent cation.

Flocculation usually involves the mixing of a process fluid with the flocculating agent under conditions of high shear in a stirred tank, although more compact and efficient devices have been proposed (Ashley, 1990). This stage is known as coagulation, and is usually followed by a period of gentle agitation when floes developed initially are allowed to grow in size. The underlying theoretical principles of cell flocculation have been discussed by Atkinson and Daoud (1976).

Nakamura (1961) described the use of various compounds for flocculating bacteria, yeasts and algae, including alum, calcium salts, and ferric salts. Other agents which are now used include tannic acid, titanium tetrachloride and cationic agents such as quaternary ammonium compounds, alkyl amines, and alkyl pyridinium salts. Gasner and Wang (1970) reported a many 100-fold increase in the sedimentation rate of *Candida intermedia* when recoveries of over 99% were readily obtained. They found that flocculation was very dependent on the choice of additive, dosage, and

conditions of floe formation, with the most effective agents being mineral colloids and polyelectrolytes. Nucleic acids, polysaccharides, and proteins released from partly lysed cells may also bring about agglomeration. In SCP processes, phosphoric acid has been used as a flocculating agent since it can be used as a nutrient in medium recycle with considerable savings in water usage (Hamer, 1979).

The majority of flocculating agents currently in use are polyelectrolytes, which act by charge neutralization and hydrophobic interactions to link cells to each other. In processes where the addition of some toxic chemicals is to be avoided, alternative techniques have been adopted. One method is to coagulate microbial protein, which has been released from the cells by heating for short periods. Kurane (1990) reports the use of bioflocculants obtained from *Rhodococcus erythropolis.* They are suggested as being safer alternatives to conventional flocculants. Warne and Bowden (1987) suggest the use of genetic manipulation to alter cell surface properties to aid aggregation. Flocculating agents such as crosslinked cationic polymers may also be used in the processing of cell lysates and extracts prior to further downstream processing (Fletcher et al., 1990). Bentham, Bonnerjea, Orsborn, Ward, and Hoare (1990) utilized borax as a flocculating agent for yeast cell debris prior to decanter centrifugation.

RANGE OF CENTRIFUGES

A number of centrifuges will be described which vary in their manner of liquid and solid discharge, their unloading speed and their relative maximum capacities. When choosing a centrifuge for a specific process, it is important to ensure that the centrifuge will be able to perform the separation at the planned production rate, and operate reliably with minimum manpower. Largescale tests may therefore be necessary with fermentation broths or other materials to check that the correct centrifuge is chosen.

Basket centrifuge (perforated-bowl basket centrifuge)

Basket centrifuges are useful for separating mould mycelia or crystalline compounds. The centrifuge is most commonly used with a perforated bowl lined with a filter bag of nylon, cotton, etc. (Fig. 10.15). A continuous feed is used, and when the basket is filled with the filter cake, it is possible to wash the cake before removing it. The bowl may suffer from blinding with soft biological materials so that high centrifugal forces cannot be used. These centrifuges are normally operated at speeds of up to 4000 rpm for feed rates of 50–300 dm^3 min^{-1} and have a solids holding capacity of 30–500 dm^3. The basket centrifuge may be considered to be a centrifugal filter.

Tubular-bowl centrifuge

This is a centrifuge to consider using for particle size ranges of 0.1–200 μm and up to 10% solids in the in-going slurry. Fig. 10.16a shows an arrangement used in a Sharples Super-Centrifuge. The main component of the centrifuge is a cylindrical bowl (or rotor) (A in Fig. 10.16), which may be of a variable design depending on application, suspended by a flexible shaft (B), driven by an overhead motor or air

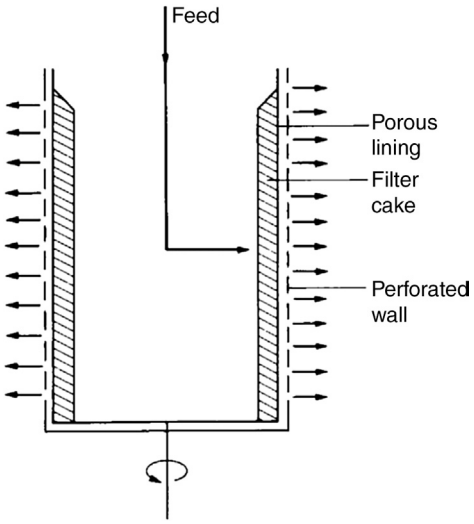

FIGURE 10.15 Diagram of Basket Centrifuge

turbine (C). The inlet to the bowl is via a nozzle attached to the bottom bearing (D). The feed which may consist of solids and light and heavy liquid phases is introduced by the nozzle (E). During operation solids sediment on the bowl wall while the liquids separate into the heavy phase in zone (G) and the light phase in the central zone (H). The two liquid phases are kept separate in their exit from the bowl by an adjustable ring, with the heavy phase flowing over the lip of the ring. Rings of various sizes may be fitted for the separation of liquids of various relative densities. Thus the centrifuge may be altered to use for:

1. Light-phase/heavy-phase liquid separation.
2. Solids/light-liquid phase/heavy-liquid phase separation.
3. Solids/liquid separation (using a different rotor, Fig. 10.16b).

The Sharpies laboratory centrifuge with a bowl radius of approximately 2.25 cm can be operated with an air turbine at 50,000 rpm to produce a centrifugal force of approximately 62,000g, but has a bowl capacity of only 200 cm^3 with a throughput of 6–25 $dm^3\ h^{-1}$. The largest size rotor is the Sharpies AS 26, which has a bowl radius of 5.5 cm and a capacity of 9 dm^3, a solids capacity of 5 dm^3 and a throughput of 390–2400 $dm^3\ h^{-1}$.

The advantages of this design of centrifuge are the high centrifugal force, good dewatering, and ease of cleaning. The disadvantages are limited solids capacity, difficulties in the recovery of collected solids, gradual loss in efficiency as the bowl fills, solids being dislodged from the walls as the bowl is slowing down, and foaming. Plastic liners can be used in the bowls to help improve batch cycle time. Alternatively a spare bowl can be changed over in about 5 min.

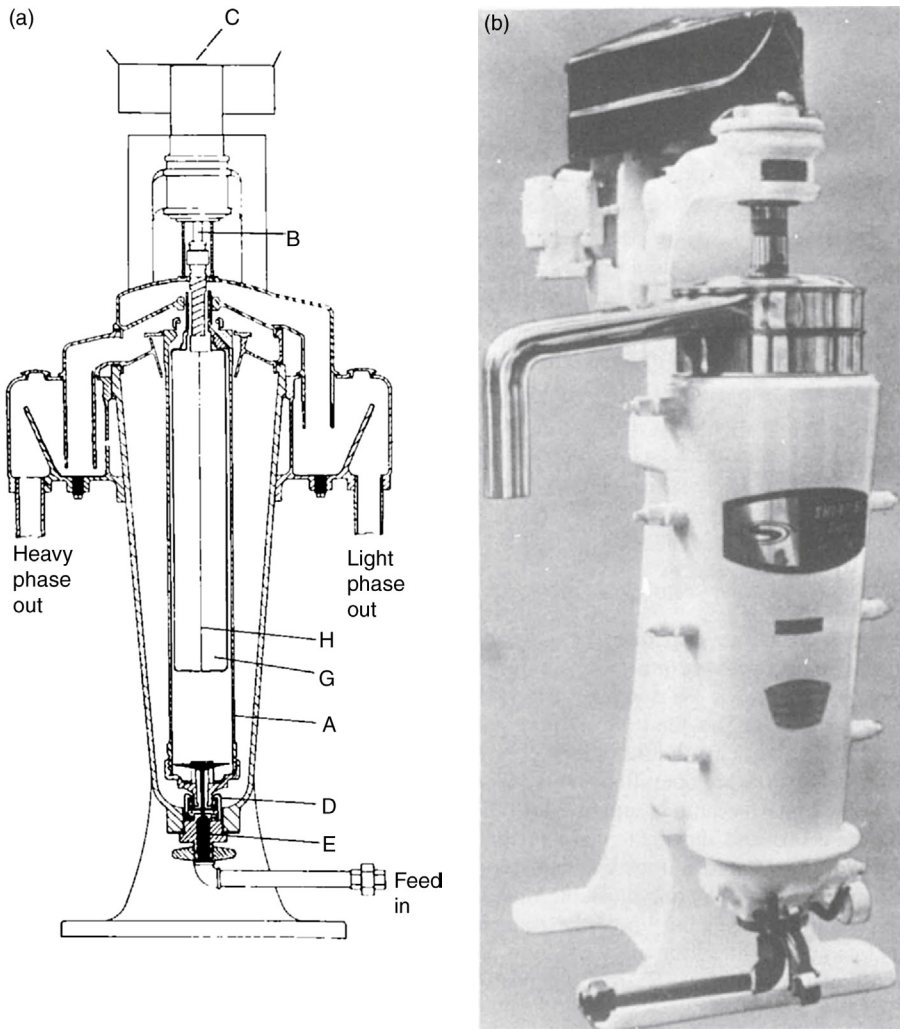

FIGURE 10.16

(a) Section of a Sharples Stiper-Centrifuge (Alfa Laval Sharpies, Camberley, UK).
(b) A Sharples Super-Centrifuge assembled for discharge of one liquid phase (Alfa Laval Sharpies, Camberley, UK)

The solid-bowl scroll centrifuge (decanter centrifuge)

This type of centrifuge is used for continuous handling of fermentation broths, cell lysates and coarse materials such as sewage sludge (Fig. 10.17). The slurry is fed through the spindle of an archimedean screw within the horizontal rotating solids bowl. Typically the speed differential between the bowl and the screw is in the range 0.5–100 rpm (Coulson & Richardson, 1991). The solids settling on the walls of

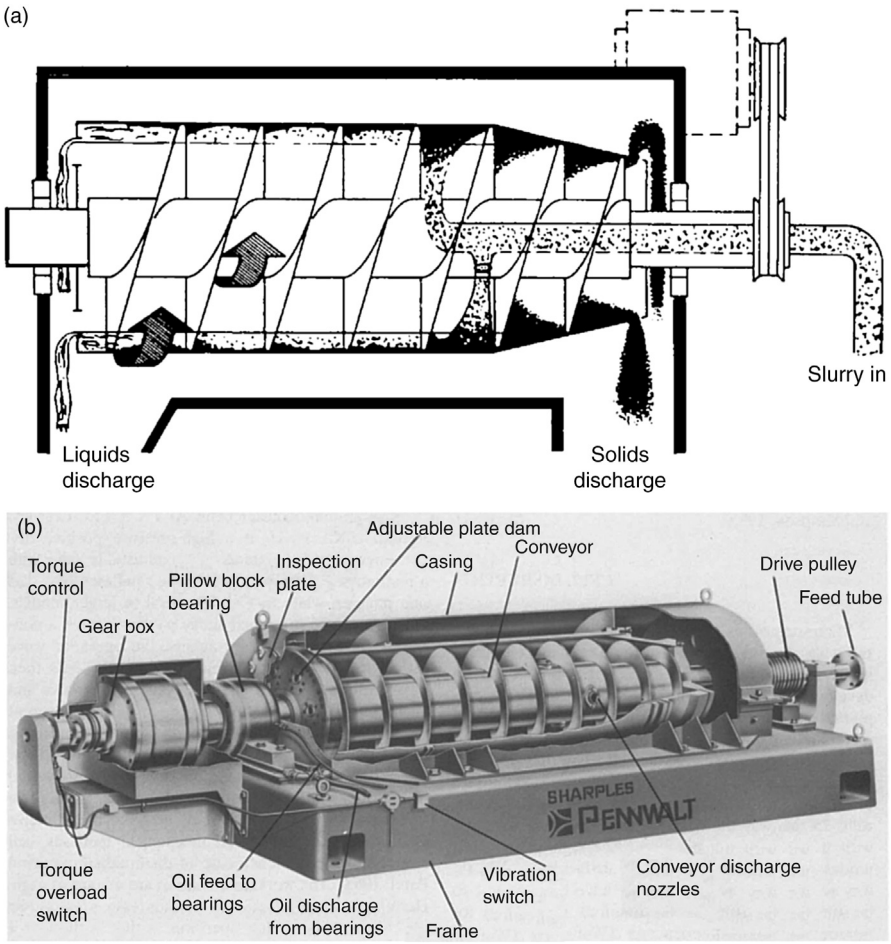

FIGURE 10.17

(a) Diagram of a solid-bowl scroll centrifuge (Alfa Laval Sharpies Ltd, Camberley, UK). (b) Cutaway view of a Sharpies Super-D-Canter continuous solid-bowl centrifuge, Model P-5400 (Alfa Laval Sharpies Ltd, Camberley, UK).

the bowl are scraped to the conical end of the bowl. The slope of the cone helps to remove excess liquid from the solids before discharge. The liquid phase is discharged from the opposite end of the bowl. The speed of this type of centrifuge is limited to around 5000 rpm in larger models because of the lack of balance within the bowl, with smaller models having bowl speeds of up to 10000 rpm. Bowl diameters are normally between 0.2 and 1.5 m, with the length being up to 5 times the diameter. Feed rates range from around 200 $dm^3\ h^{-1}$ to 200 $m^3\ h^{-1}$ depending on scale of operation and material being processed. A number of variants on the basic design are available:

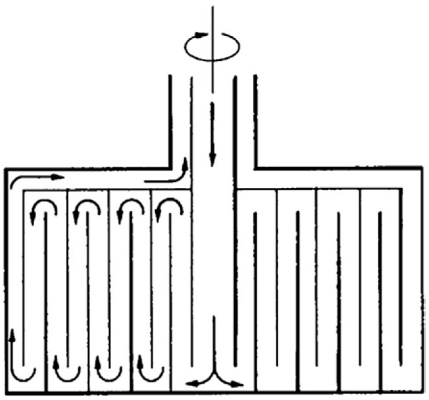

FIGURE 10.18 L.S. of a Multichamber Centrifuge

1. Cake washing facilities (screen bowl decanters).
2. Vertical bowl decanters.
3. Facility for in-place cleaning.
4. Biohazard containment features; steam sterilization in situ, two or three stage mechanical seals, control of aerosols, containment casings, and the use of high pressure sterile gas in seals to prevent the release of microorganisms.

Multichamber centrifuge

Ideally, this is a centrifuge for a slurry of up to 5% solids of particle size 0.1–200 μm diameter. In the multichamber centrifuge (Fig. 10.18), a series of concentric chambers are mounted within the rotor chamber. The broth enters via the central spindle and then takes a circuitous route through the chambers. Solids collect on the outer faces of each chamber. The smaller particles collect in the outer chambers where they are subjected to greater centrifugal forces (the greater the radial position of a particle, the greater the rate of sedimentation).

Although these vessels can have a greater solids capacity than tubular bowls and there is no loss of efficiency as the chamber fills with solids, their mechanical strength and design limits their speed to a maximum of 6500 rpm for a rotor 46-cm diameter with a holding capacity of up to 76 dm^3. Because of the time needed to dismantle and recover the solids fraction, the size and number of vessels must be of the correct volume for the solids of a batch run.

Disc-bowl centrifuge

This centrifuge relies for its efficiency on the presence of discs in the rotor or bowl (Fig. 10.19). A central inlet pipe is surrounded by a stack of stainless-steel conical discs. Each disc has spacers so that a stack can be built up. The broth to be separated flows outward from the central feed pipe, then upward and inward between the discs at an angle of 45 degrees to the axis of rotation. The close packing of the discs assists

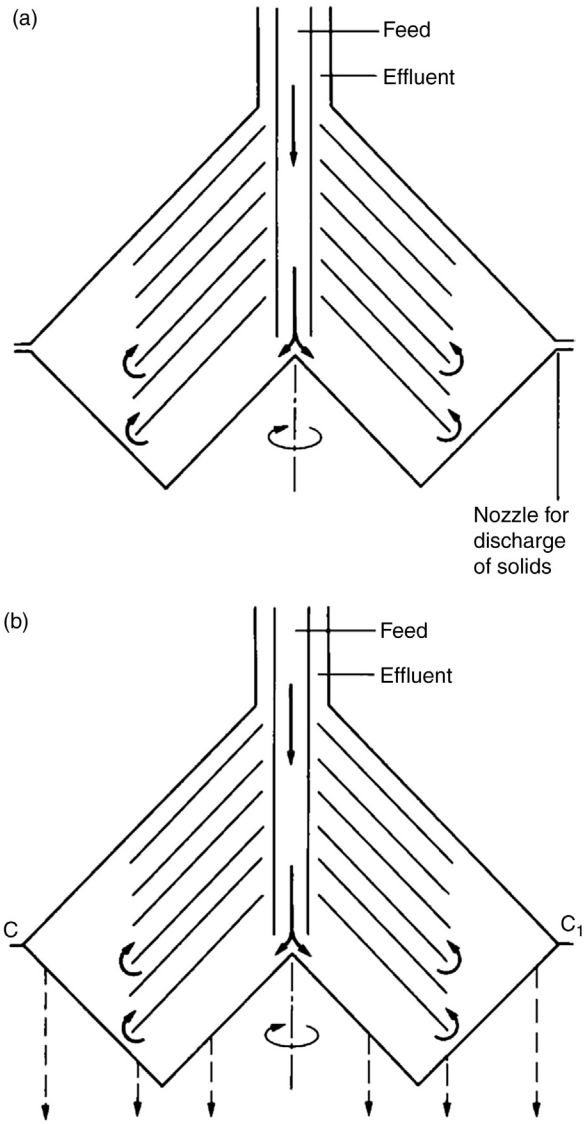

FIGURE 10.19

(a) L.S. of disc-bowl centrifuge with nozzle discharge. (b) L.S. of disc-bowl centrifuge with intermittent discharge. (Solids discharged when rotor opens intermittently along the section C–Cj.)

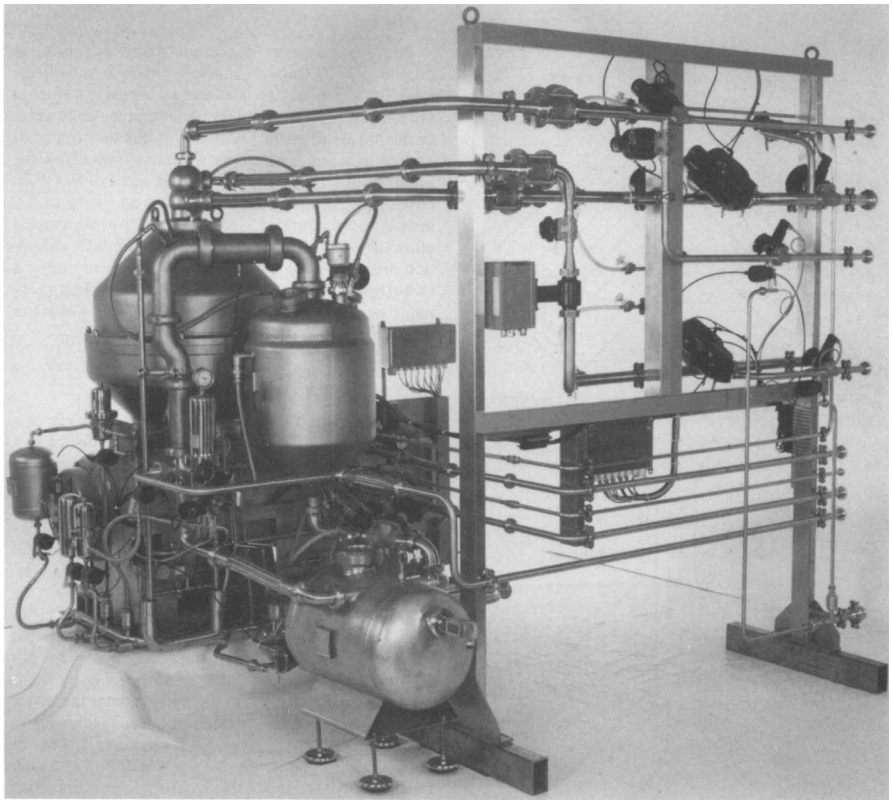

FIGURE 10.20 Alfa Laval BTUX 510 Disc Stack Centrifuge (Alfa Laval Sharples Ltd, Camberley, UK)

rapid sedimentation and the solids then slide to the edge of the bowl, provided that there are no gums or fats in the slurry, and eventually accumulate on the inner wall of the bowl. Ideally, the sediment should form a sludge which flows, rather than a hard particulate or lumpy sediment. The main advantages of these centrifuges are their small size compared with a bowl without discs for a given throughput. Some designs also have the facility for continuous solids removal through a series of nozzles in the circumference of the bowl or intermittent solids removal by automatic opening of the solids collection bowl. The arrangement of the discs makes this type of centrifuge laborious to clean. However, recent models such as the Alfa Laval BTUX 510 (Alfa Laval Sharpies Ltd, Camberley, Surrey, U.K.) system (Fig. 10.20) are designed to allow for cleaning in situ. In addition, this and similar plant have the facility for in situ steam sterilization and total containment, incorporating double seals to comply with containment regulations (see also Chapter 7). Feed rates range from 45 to 1800 dm^3 min^{-1}, with rotational speeds typically between 5000 and 10,000 rpm. The Westfalia CSA 19–47–476 is also steam sterilizable and has been used for the

sterile collection of organisms (Walker, Narendranathan, Brown, Woolhouse, & Vranch, 1987). Similarly, the Westfalia CSA 8 can be modified for contained operation and steam sterilization (Frude & Simpson, 1993).

CELL DISRUPTION

Microorganisms are protected by extremely tough cell walls. In order to release their cellular contents a number of methods for cell disintegration have been developed (Wimpenny, 1967; Hughes, Wimpenny, & Lloyd, 1971; Harrison, 2011). Any potential method of disruption must ensure that labile materials are not denatured by the process or hydrolyzed by enzymes present in the cell. Huang, Andrews, and Asenjo (1991) report the use of a combination of different techniques to release products from specific locations within yeast cells. In this way the desired product can be obtained with minimum contamination. Although many techniques are available which are satisfactory at laboratory scale, only a limited number have been proved to be suitable for large-scale applications, particularly for intracellular enzyme extraction (Wang et al., 1979; Darbyshire, 1981). Containment of cells can be difficult or costly to achieve in many of the methods described later and thus containment requirements will strongly influence process choice. Methods available fall into two major categories:

Physicomechanical methods

1. Liquid shear.
2. Solid shear.
3. Agitation with abrasives.
4. Freeze–thawing.
5. Ultrasonication.
6. Hydrodynamic cavitation.

Chemical and biological methods

1. Detergents.
2. Osmotic shock.
3. Alkali treatment.
4. Enzyme treatment.
5. Solvents.

PHYSICOMECHANICAL METHODS

Liquid shear (high-pressure homogenizers)

Liquid shear is the method which has been most widely used in large scale enzyme purification procedures (Scawen, Atkinson, & Darbyshire, 1980). High-pressure homogenizers used in the processing of milk and other products in the food industry have proved to be very effective for microbial cell disruption. One machine, the

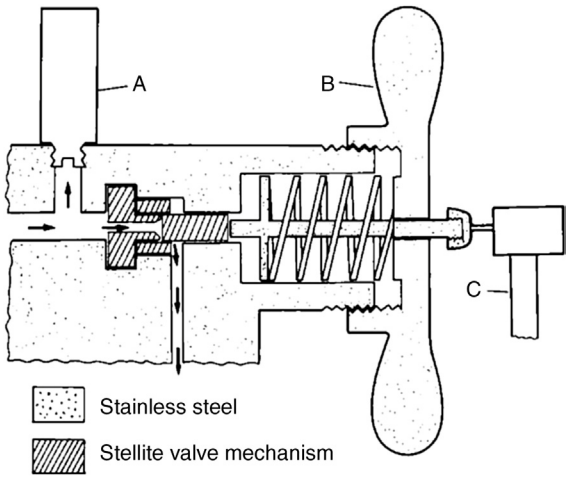

FIGURE 10.21 Details of Homogonizer Valve Assembly (Brookman, 1974)

A, 0–50,000 psi pressure transducer; *B,* pressure-control handwheel; *C,* linear variable displacement transformer; *(→)* direction of flow.

APV-Manton Gaulin-homogenizer (The APV Co. Ltd, Crawley, Surrey, UK), which is a high-pressure positive displacement pump, incorporates an adjustable valve with a restricted orifice (Fig. 10.21). The smallest model has one plunger, while there are several in larger models. During use, the microbial slurry passes through a nonreturn valve and impinges against the operative valve set at the selected operating pressure. The cells then pass through a narrow channel between the valve and an impact ring followed by a sudden pressure drop at the exit to the narrow orifice. There are various discharge valve designs (Fig. 10.22) with sharp edged orifices being preferred for cell disruption. The large pressure drop across the valve is believed to cause cavitation in the slurry and the shock waves so produced disrupt the cells. Brookman (1974) considered the size of the pressure drop to be very important in achieving effective disruption, and as with all mechanical methods, cell size and shape influence ease of disruption (Wase & Patel, 1985). The working pressures are extremely high. Hetherington, Follows, Dunhill, and Lilly (1971) used a pressure of 550 kg cm^{-2} for a 60% yeast suspension. A throughput of 6.4 kg soluble protein h^{-1} with 90% disruption could be achieved with a small industrial machine. In larger models, flow rates of up to 600 dm^3 h^{-1} are now possible and operating pressures of 1200 bar are utilized in some processes (Asenjo, 1990). Darbyshire (1981) has stressed the need for cooling the slurry to between 0 and 4°C to minimize loss in enzyme activity because of heat generation during the process. The increase in slurry temperature is approximately proportional to the pressure drop across the valve. Because of problems caused by heat generation and because cell suspensions can be surprisingly abrasive, it is common practice to operate such homogenizers in a multipass mode but at a lower pressure. The degree of disruption and consequently the amount of protein released will

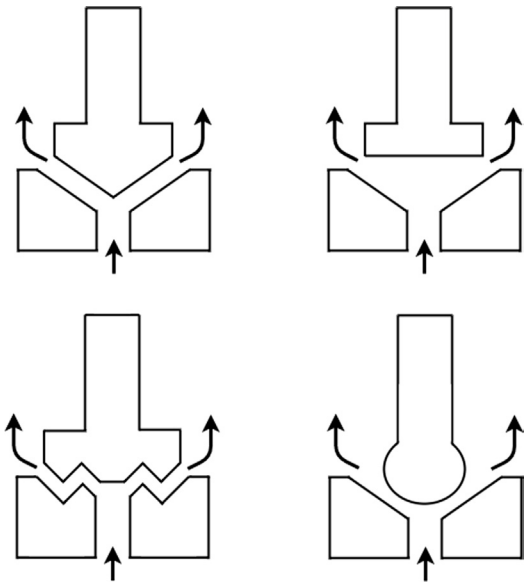

FIGURE 10.22 Discharge Valve Designs in High-Pressure Homogenizers

influence the ease of subsequent separation of the product from the cell debris in high-pressure homogenizers and bead mills (Agerkvist & Enfors, 1990). A careful balance must therefore be made between percentage release of product and the difficulty and cost of further product purification.

Solid shear

Pressure extrusion of frozen microorganisms at around −25°C through a small orifice is a well established technique at a laboratory scale using a Hughes press or an X-press to obtain small samples of enzymes or microbial cell walls. Disruption is due to a combination of liquid shear through a narrow orifice and the presence of ice crystals. Magnusson and Edebo (1976) developed a semicontinuous X-press operating with a sample temperature of −35°C and an X-press temperature of −20°C. It was possible to obtain 90% disruption with a single passage of *S. cereuisiae* using a throughput of 10 kg yeast cell paste h^{-1}. This technique might be ideal for microbial products which are very temperature labile.

Agitation with abrasives (high speed bead mills)

Mechanical cell disruption can also be achieved in a disintegrator containing a series of rotating discs/impellers on a central drive shaft and a charge of small beads. Beads are typically 0.1−3 mm diameter depending on the type of microorganism and impeller tip speeds are in the order of 15 m s^{-1}. The beads are made of mechanically resistant materials such as glass, alumina ceramics and some titanium compounds

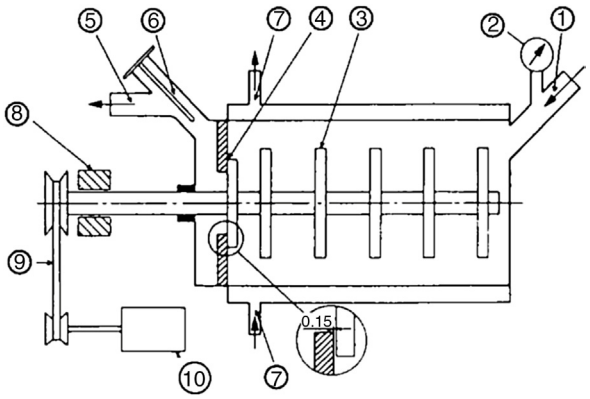

FIGURE 10.23 Simplified Drawing of the Dyno-Muhle KD5 (Mogren et al., 1974)

1, Inlet of suspension; *2,* manometer; *3,* rotating disc; *4,* slit for separation of glass beads from the suspension; *5,* outlet of suspension; *6,* thermometer; *7,* cooling water, inlet and outlet; *8,* bearings; *9,* variable V-belt drive; *10,* drive motor. Cylinder dimensions: inside length 33 cm; inside diameter 14 cm.

(Fig. 10.23). Disruption is achieved through interparticle collision and solid shear (Harrison, 2011). In a small disintegrator, the Dyno-Muhle KD5 (Wiley A. Bachofen, Basle, Switzerland), using a flow rate of 180 dm^3 h^{-1}, 85% disintegration of an 11% w/v suspension of *S. cereuisiae* was achieved with a single pass (Mogren, Lindblom, & Hedenskog, 1974). Although temperatures of up to 35°C were recorded in the disintegrator, the specific enzyme activities were not considered to be very different from values obtained by other techniques. Dissipation of heat generated in the mill is one of the major problems in scale up, though this can generally be overcome with the provision of a cooling jacket. In another disintegrator, the Netzsch LM20 mill (Netzsch GmbH, Selb, Germany), the agitator blades were alternately mounted vertically and obliquely on the horizontal shaft (Fig. 10.24). A flow rate of up to 400 dm^3 h^{-1} was claimed for a vessel with a nominal capacity of 20 dm^3 (Rehacek & Schaefer, 1977).

Freezing–thawing

Freezing and thawing of a microbial cell paste will inevitably cause ice crystals to form and their expansion followed by thawing will lead to some subsequent disruption of cells. It is slow, with limited release of cellular materials, and has not often been used as a technique on its own, although it is often used in combination with other techniques. β-Glucosidase has been obtained from *S. cerevisiae* by this method (Honig & Kula, 1976). A sample of 360 g of frozen yeast paste was thawed at 5°C for 10 h. This cycle was repeated twice before further processing.

Ultrasonication (ultrasonic cavitation)

High frequency vibration (~20 kHz) at the tip of an ultrasonication probe leads to cavitation (the formation of vapor cavities in low pressure regions), and shock waves

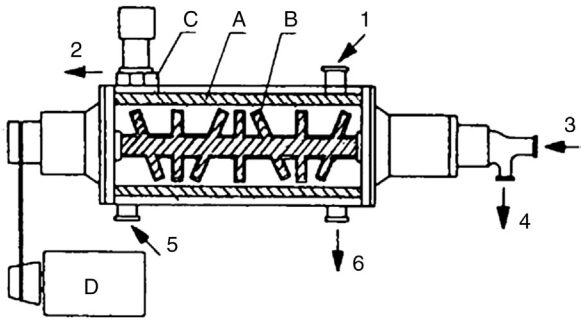

FIGURE 10.24 Simplified Drawing of the Netzsch Model LM-20 Mill (Rehacek & Schaefer, 1977)

A, cylindrical grinding vessel with cooling jacket; *B,* agitator with cooled shaft and discs; *C,* annular vibrating slot operator; *D,* variable-speed-drive motor; *1 and 2,* product inlet and outlet; *3 and 4,* agitator cooling inlet and outlet; *5 and 6,* vessel-cooling inlet and outlet.

generated when the cavities collapse cause cell disruption. The method can be very effective on a small scale (5–500 mL), but a number of serious drawbacks make it unsuitable for large-scale operations. Power requirements are high, there is a large heating effect so cooling is needed, the probes have a short working life and are only effective over a short range. Continuous laboratory sonicators with hold-up volumes of around 10 cm^3 have been shown to be effective (James, Coakley, & Hughes, 1972).

Hydrodynamic cavitation

Cavitation similar to that generated by ultrasonication probes can also be generated by fluid flow. When fluid flows through an orifice an increase in velocity is accompanied by a decrease in pressure of the fluid. When the pressure falls to the vapor pressure of the fluid cavitation occurs resulting in cell damage/disruption (Harrison, 2011).

CHEMICAL AND BIOLOGICAL METHODS

Detergents

A number of detergents will damage the lipoproteins of the microbial cell membrane and lead to the release of intracellular components. The compounds which can be used for this purpose include quaternary ammonium compounds, sodium lauryl sulfate, sodium dodecyl sulfate (SDS) and Triton X-100. Anionic detergents such as SDS disorganize the cell membrane while cationic detergents are believed to act on lipopolysaccharides and phospholipids of the membrane. Nonionic detergents such as Triton X-100 cause partial solubilization of membrane proteins (Harrison, 2011). Unfortunately, the detergents may cause some protein denaturation and may need to be removed before further purification stages can be undertaken. The stability of the desired product must be determined when using any detergent system. 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 h to solubilize most of the enzyme (Kroner, Hustedt, Granda, & Kula, 1978). The use of Triton X-100 in combination with guanidine-HCl is widely and effectively used for the release of cellular protein (Naglak & Wang, 1992; Hettwer & Wang, 1989), Hettwer and Wang (1989) obtaining greater than 75% protein release in less than 1 h from *Escherichia coli* under fermentation conditions.

Osmotic shock

Osmotic shock caused by a sudden change in salt concentration will cause disruption of a number of cell types. Cells are equilibrated to high osmotic pressure (typically 1 M salt solutions). Rapid exposure to low osmotic pressure causes water to quickly enter the cell. This increases the internal pressure of the cell resulting in cell lysis. Osmotic shock is of limited application except where the cell wall is weakened or absent. Application on a large scale is limited by the cost of chemicals, increased water use, and possible product dilution (Harrison, 2011).

Alkali treatment

Alkali treatment might be used for hydrolysis of microbial cell wall material provided that the desired product will tolerate a pH of 10.5–12.5 for up to 30 min. Chemical costs can be high both in terms of alkali required and neutralization of the resulting lysate. Darbyshire (1981) has reported the use of this technique in the extraction of L-asparaginase.

Enzyme treatment

There are a number of enzymes which hydrolyze specific bonds in cell walls of a limited number of microorganisms. Enzymes shown to have this activity include lysozyme, produced from hen egg whites and other natural sources, and other enzyme extracts from leucocytes, *Streptomyces* spp., *Staphylococcus* spp., *Micromonospora* spp. *Penicillium* spp., *Trichoderma* spp., and snails. Lysozyme hydrolyses β-1-4 glucosidic bonds in the polysaccharide chains of peptidoglycan causing cell lysis. 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. Enzyme lysis in large scale operations is limited by the availability and cost of appropriate enzymes. The use of immobilized lysozyme has been investigated by a number of workers and may provide the solution to such problems (Crapisi, Lante, Pasini, & Spettoli, 1993).

Solvents

Solvents extract the lipid components of the cell membrane causing the release of intracellular components and are applicable across a wide range of microorganisms. Solvents used include alcohols, dimethyl sulfoxide, methyl ethyl ketone, and toluene. However, their toxicity, flammability, and ability to cause protein denaturation requires careful consideration (Harrison, 2011).

Chemical and enzymatic 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 & Asenjo, 1987; Andrews, Huang, & Asenjo, 1990; Harrison, 2011). Enzymes may also be used as a pretreatment to partially hydrolyze cell walls prior to cell disruption by mechanical methods.

LIQUID–LIQUID EXTRACTION

The separation of a component from a liquid mixture by treatment with a solvent in which the desired component is preferentially soluble is known as liquid–liquid extraction. The specific requirement is that a high percentage extraction of product must be obtained but concentrated in a smaller volume of solvent.

Prior to starting a large-scale extraction, it is important to find out on a small scale the solubility characteristics of the product using a wide range of solvents. A simple rule to remember is that "like dissolves like." The important "likeness" as far as solubility relations are concerned is in the polarities of molecules. Polar liquids mix with each other and dissolve salts and other polar solids. The solvents for nonpolar compounds are liquids of low or nil polarity.

The dielectric constant is a measure of the degree of molar polarization of a compound. If this value is known it is then possible to predict whether a compound will be polar or nonpolar, with a high value indicating a highly polar compound. The dielectric constant D of a substance can be measured by determining the electrostatic capacity C of a condenser containing the substance between the plates. If C_0 is the value for the same condenser when completely evacuated then

$$D = \frac{C}{C_0}$$

Experimentally, dielectric constants are obtained by comparing the capacity of the condenser when filled with a given liquid with the capacity of the same condenser containing a standard liquid whose dielectric constant is known very accurately. If D_1 and D_2 are the dielectric constants of the experimental and standard liquids and C_1 and C_2 are the electrostatic capacities of a condenser when filled with each of the liquids, then

$$\frac{D_1}{D_2} = \frac{C_1}{C_2}$$

The value of D_1 can be calculated since C_1 and C_2 can be measured and D_2 is known. The dielectric constants for a number of solvents are given in Table 10.1.

The final choice of solvent will be influenced by the distribution or partition coefficient K where

$$K = \frac{\text{Concentration of solute in extract}}{\text{Concentration of solute in raffinate}}$$

Table 10.1 Dielectric Constants of Solvents at 25°C (Arranged in Order of Increasing Polarity)

Solvent	Dielectric Constant
Hexane	1.90 (least polar)
Cyclohexane	2.02
Carbon tetrachloride	2.24
Benzene	2.28
Di-ethyl ether	4.34
Chloroform	4.87
Ethyl acetate	6.02
Butan-2-ol	15.8
Butan-l-ol	17.8
Propan-l-ol	20.1
Acetone	20.7
Ethanol	24.3
Methanol	32.6
Water	78.5 (most polar)

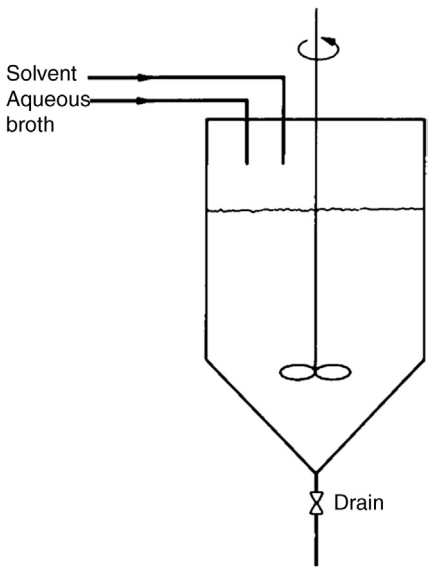

FIGURE 10.25 Diagram of a Single-Stage Extraction Unit

The value of K defines the ease of extraction. When there is a relatively high K value, good stability of product and good separation of the aqueous and solvent phases, then it may be possible to use a single-stage extraction system (Fig. 10.25). A value of 50 indicates that the extraction should be straightforward whereas a value of 0.1 shows that the extraction will be difficult and that a multistage process will be

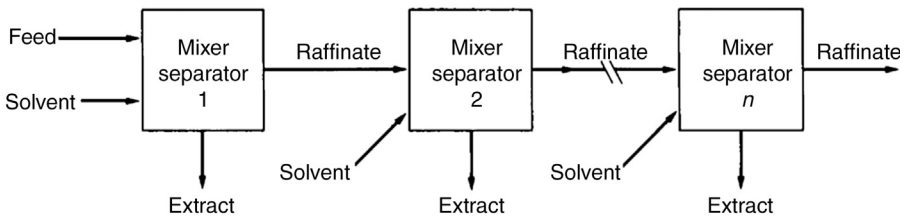

FIGURE 10.26 Diagram of a Cocurrent Flow Extraction System

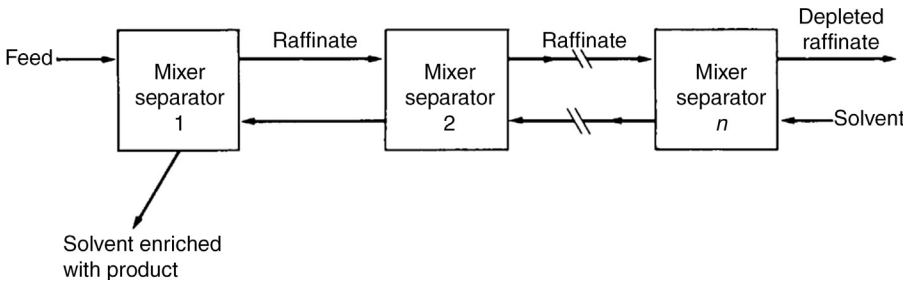

FIGURE 10.27 Diagram of a Countercurrent Extraction System

necessary. Unfortunately, in a number of systems the value of K is low and cocurrent or countercurrent multistage systems have to be utilized. The cocurrent system is illustrated in Fig. 10.26. There are n mixer/separator vessels in line and the raffinate goes from vessel 1 to vessel n. Fresh solvent is added to each stage, the feed and extracting solvent pass through the cascade in the *same* direction. Extract is recovered from each stage. Although a relatively large amount of solvent is used, a high degree of extraction is achieved.

A countercurrent system is illustrated in Fig. 10.27. There are a number of mixer/separators connected in series. The extracted raffinate passes from vessel 1 to vessel n while the product-enriched solvent is flowing from vessel n to vessel 1. The feed and extracting solvent pass through the cascade in *opposite* directions. The most efficient system for solvent utilization is countercurrent operation, showing a considerable advantage over batch and cocurrent systems. Unless there are special reasons the counter-current system should be used. In practice, the series of countercurrent extractions are conducted in a single continuous extractor using centrifugal forces to separate the two liquid phases. The two liquid streams are forced to flow countercurrent to each other through a long spiral of channels within the rotor.

The Podbielniak centrifugal extractor (Fig. 10.28) consists of a horizontal cylindrical drum revolving at up to 5000 rpm about a shaft passing through its axis. The liquids to be run countercurrent are introduced into the shaft, with the heavy liquid entering the

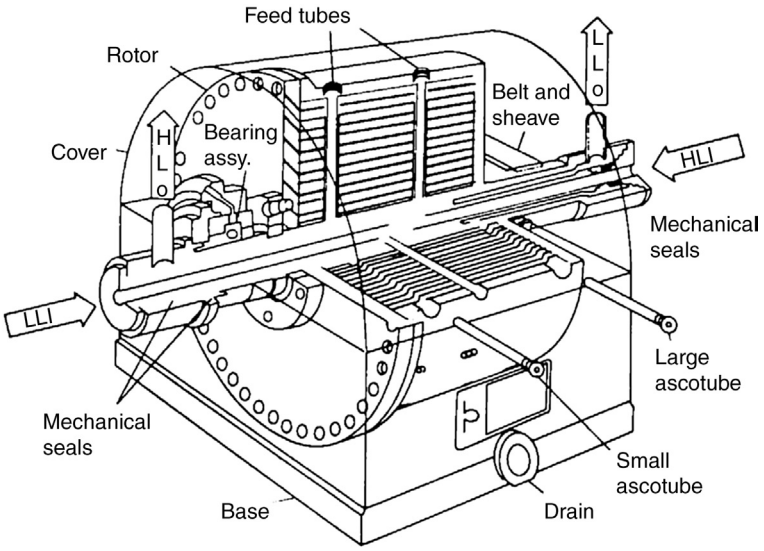

FIGURE 10.28 Diagram of the Podbielniak Extractor (Queener & Swartz, 1979)

HLI, LLI, HLO, and LLO indicate heavy and light liquid in and out.

drum at the shaft while the light liquid is led by an internal route to the periphery of the drum. As the drum rotates, the heavy liquid is forced to the periphery of the drum by centrifugal action where it contacts the light liquid. The solute is transferred between the liquids and the light liquid is displaced back toward the axis of the drum. The heavy liquid is returned to the drum's axis via internal channels. The two liquid streams are then discharged via the shaft. Flow rates in excess of 100,000 $dm^3\ h^{-1}$ are possible in the largest models. Probably the most useful property of this type of extractor is the low hold-up volume of liquid in the machine compared with the throughput.

Penicillin G is an antibiotic which is recovered from fermentation broths by centrifugal countercurrent solvent extraction. At neutral pHs in water penicillin is ionized, while in acid conditions this ionization is suppressed and the penicillin is more soluble in organic solvents. At pH 2 to 3, the distribution ratio of total acid will be

$$K = \frac{(\mathrm{RCOOH})\mathrm{org}}{(\mathrm{RCOOH})\mathrm{aq} + (\mathrm{RCOO^-})\mathrm{aq}}$$

For penicillin this value may be as high as 40 in a suitable solvent (Podbielniak, Kaiser, & Ziegenhorn, 1970). The penicillin extraction process may involve the four following stages:

1. Extraction of the penicillin G from the filtered broth into an organic solvent (amyl or butyl acetate or methyl iso-butyl ketone).
2. Extraction from the organic solvent into an aqueous buffer.
3. Extraction from aqueous buffer into organic solvent.
4. Extraction of the solvent to obtain the penicillin salt.

At each extraction stage progressively smaller volumes of extradant are used to achieve concentration of the penicillin (Fig. 10.2). Unfortunately, penicillin G has a half-life of 15 min at pH 2.0 at 20°C. The harvested broth is therefore initially cooled to 0–3°C. The cooled broth is then acidified to pH 2–3 with sulfuric or phosphoric acid immediately before extraction. This acidified broth is quickly passed through a Podbielniak centrifugal countercurrent extractor using about 20% by volume of the solvent in the counter flow. Ideally, the hold-up time should be about 60–90 s. The penicillin-rich solvent then passes through a second Podbielniak extractor countercurrent to an aqueous NaOH or KOH solution (again about 20% by volume) so that the penicillin is removed to the aqueous phase (pH 7.0 to 8.0) as the salt.

$$\mathrm{RCOOH(org) + NaOH(aq) \rightarrow RCOO^{-}Na^{+} + H_2O}$$

These two stages may be sufficient to concentrate the penicillin adequately from a broth with a high titer. Penicillin will crystallize out of aqueous solution at a concentration of approximately 1.5×10^6 units cm^{-3}. If the broth harvested initially contains 60,000 units cm^{-3}, and two fivefold concentrations are achieved in the two extraction stages, then the penicillin liquor should crystallize. If the initial broth titer is lower than 60,000 units cm^{-3} or the extractions are not so effective, the solvent and buffer extractions will have to be repeated. At each stage the spent liquids should be checked for residual penicillin and solvent usage carefully monitored. Since the solvents are expensive and their disposal is environmentally sensitive they are recovered for recirculation through the extraction process. The success of a process may depend on efficient solvent recovery and reuse.

SOLVENT RECOVERY

A major item of equipment in an extraction process is the solvent-recovery plant which is usually a distillation unit. It is not normally essential to remove all the raffinate from the solvent as this will be recycled through the system. In some processes the more difficult problem will be to remove all the solvent from the raffinate because of the value of the solvent and problems which might arise from contamination of the product.

Distillation may be achieved in three stages:

1. Evaporation, the removal of solvent as a vapor from a solution.
2. Vapor–liquid separation in a column, to separate the lower boiling more volatile component from other less volatile components.
3. Condensation of the vapor, to recover the more volatile solvent fraction.

Evaporation is the removal of solvent from a solution by the application of heat to the solution. A wide range of evaporators is available. Some are operated on a batch basis and others continuously. Most industrial evaporators employ tubular heating surfaces. Circulation of the liquid past the heating surfaces may be induced by boiling or by mechanical agitation. In batch distillation (Fig. 10.29), the vapor from the

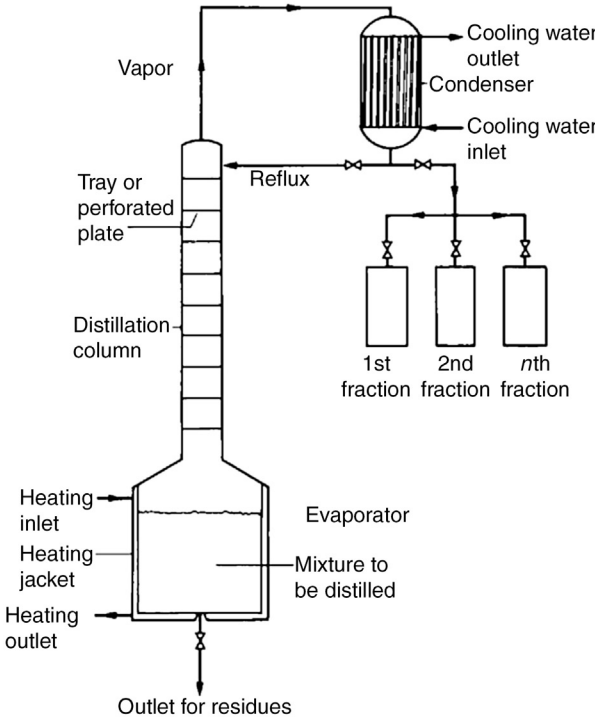

FIGURE 10.29 Diagram of a Batch Distillation Plant With a Tray or Perforated-Plate Column

boiler passes up the column and is condensed. Part of the condensate will be returned as the reflux for countercurrent contact with the rising vapor in the column. The distillation is continued until a satisfactory recovery of the lower-boiling (more volatile) component(s) has been accomplished. The ratio of condensate returned to the column as reflux to that withdrawn as product is, along with the number of plates or stages in the column, the major method of controlling the product purity. A continuous distillation (Fig. 10.30) is initially begun in a similar way as with a batch distillation, but no condensate is withdrawn initially. There is total reflux of the condensate until ideal operating conditions have been established throughout the column. At this stage the liquid feed is fed into the column at an intermediate level. The more volatile components move upward as vapor and are condensed, followed by partial reflux of the condensate. Meanwhile, the less volatile fractions move down the column to the evaporator (reboiler). At this stage part of the bottoms fraction is continuously withdrawn and part is reboiled and returned to the column.

Countercurrent contacting of the vapor and liquid streams is achieved by causing:

1. Vapor to be dispersed in the liquid phase (plate or tray column),
2. Liquid to be dispersed in a continuous vapor phase (packed column).

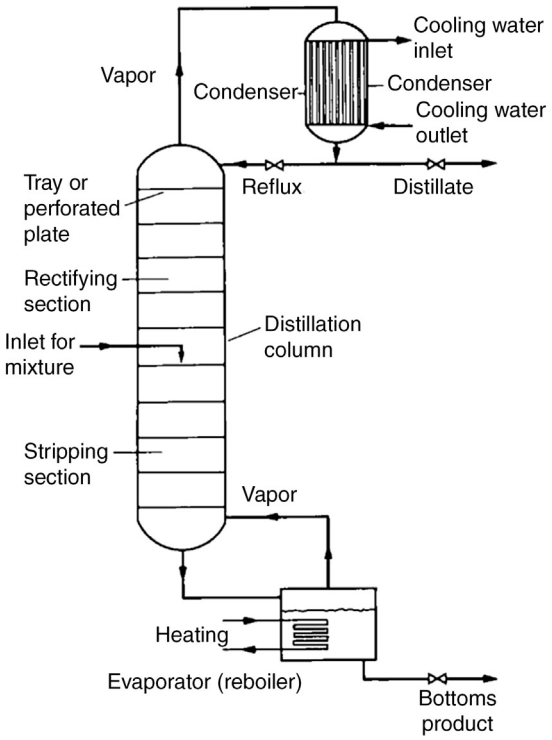

FIGURE 10.30 Diagram of a Continuous Distillation Plant With a Tray or Perforated-Plate Column

The plate or tray column consists of a number of distinct chambers separated by perforated plates or trays. The rising vapor bubbles through the liquid which is flowing across each plate, and is dispersed into the liquid from perforations (sieve plates) or bubble caps. The liquid flows across the plates and reaches the reboiler by a series of overflow wires and down pipes.

A packed tower is filled with a randomly packed material such as rings, saddles, helices, spheres or beads. Their dimensions are approximately one-tenth to one-fiftieth of the diameter of the column and are designed to provide a large surface area for liquid-vapor contacting and high voidage to allow high throughput of liquid and vapor.

The heat input to a distillation column can be considerable. The simplest ways of conserving heat are to preheat the initial feed by a heat exchanger using heat from:

1. The hot vapors at the top of the column,
2. Heat from the bottoms fraction when it is being removed in a continuous process,
3. A combination of both.

Since it is beyond the scope of this text to consider the distillation process more fully the reader is therefore directed to Coulson and Richardson (1991).

TWO-PHASE AQUEOUS EXTRACTION

Liquid–liquid extraction is a well established technology in chemical processing and in certain sectors of biochemical processing. However, the use of organic solvents has limited application in the processing of sensitive biologicals. Aqueous two-phase systems, on the other hand, have a high water content and low interfacial surface tension and are regarded as being biocompatible (Mattiasson & Ling, 1987).

Two-phase aqueous systems have been known since the late 19th century, and a large variety of natural and synthetic hydrophilic polymers are used today to create two (or more) aqueous phases. Phase separation occurs when hydrophilic polymers are added to an aqueous solution, and when the concentrations exceed a certain value two immiscible aqueous phases are formed. Settling time for the two phases can be prolonged, depending on the components used and vessel geometry. Phase separation can be improved by using centrifugal separators (Huddlestone et al., 1991), or novel techniques such as magnetic separators (Wikstrom, Flygare, Grondalen, & Larsson, 1987).

Many systems are available:

1. Nonionic polymer/nonionic polymer/water, for example, polyethylene glycol/dextran.
2. Polyelectrolyte/nonionic polymer/water, for example, sodium carboxymethyl cellulose/polyethylene glycol.
3. Polyelectrolyte/polyelectrolyte/water, for example, sodium dextran sulfate/sodium carboxymethyl cellulose.
4. Polymer/low molecular weight component/water, for example, dextran/propyl alcohol.

The distribution of a solute species between the phases is characterized by the partition coefficient, and is influenced by a number of factors such as temperature, polymer (type and molecular weight), salt concentration, ionic strength, pH, and properties (eg, molecular weight) of the solute. As the goal of any extraction process is to selectively recover and concentrate a solute, affinity techniques such as those applied in chromatographic processes can be used to improve selectivity. Examples include the use of PEG–NADH derivatives in the extraction of dehydrogenases, *p*-aminobenzamidine in the extraction of trypsin and cibacron blue in the extraction of phosphofructokinase. It is possible to use different ligands in the two phases leading to an increase in selectivity or the simultaneous recovery and separation of several species (Cabral & Aires-Barros, 1993). Wohlgemuth (2011) reports that a comparison of aqueous two-phase separation and ion-exchange chromatography shows that the process yield and costs are lower for the aqueous two-phase process. Two phase aqueous systems have found application in the purification of many solutes; proteins, enzymes (Gonzalez, Pencs, & Casas, 1990; Guan, Wu, Treffry, & Lilley, 1992), recombinant proteins using a PEG/salt system (Gu & Glatz, 2006), β-carotenene and lutein from cyanobacterial fermentations utilizing a PEG/salt process, (Chavez-Santoscoy, 2010), antibiotics (Guan, Zhu, & Mei, 1996), cells and subcellular particles, and in extractive bioconversions. The cost of phase forming

polymers and chemicals have limited the use of aqueous two-phase processes in industrial applications (Wohlgemuth, 2011), however some aqueous two-phase systems for handling large-scale protein separation have emerged, the majority of which use PEG as the upper phase forming polymer with either dextran, concentrated salt solution or hydroxypropyl starch as the lower phase forming material (Mattiasson & Kaul, 1986). Hustedt, Kroner, and Papamichael (1988) demonstrated the application of continuous cross-current extraction of enzymes (fumarase and penicillin acylase) by aqueous two-phase systems at production scale.

REVERSED MICELLE EXTRACTION

Reversed micelle extraction is potentially an attractive alternative to conventional solvent extraction for the recovery of bioproducts as the solute of interest remains in an aqueous environment at all times and hence can be considered "biocompatible." A reversed micelle is a nanometer scale droplet of aqueous solution stabilized in a nonpolar environment by a surfactant at the interface between the two liquids (Fig. 10.31). The minimum concentration of surfactant required for micelles is known as the critical micelle concentration (CMC) and is highly system specific. There are a number of limitations to reversed micelle extraction including costs and low rates of mass transfer (Krijgsman, 1992).

SUPERCRITICAL FLUID EXTRACTION

The technique of supercritical fluid extraction utilizes the dissolution power of supercritical fluids, that is, fluids above their critical temperature and pressure. Its advantages include the use of moderate temperatures, good solvent, and transport properties (high diffusivity and low viscosity), and that cheap and nontoxic fluids are available.

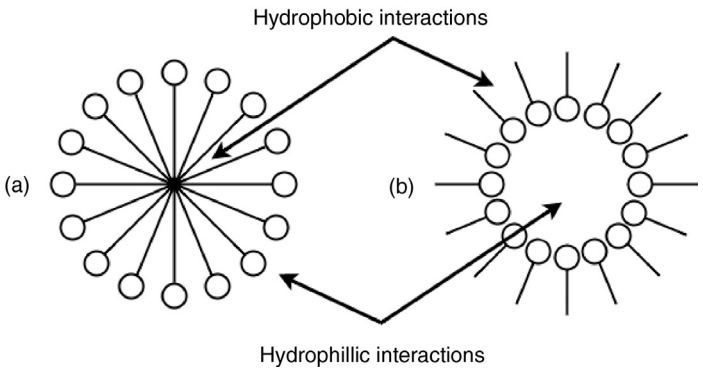

FIGURE 10.31 Micelles

(a) Normal micelle. (b) Reversed micelle.

Supercritical fluids are used in the extraction of hop oils, caffeine, vanilla, vegetable oils, and β-carotene. It has also been shown experimentally that the extraction of certain steroids and chemotherapeutic drugs can be achieved using supercritical fluids. Other current and potential uses include the removal of undesirable substances such as pesticide residues, removal of bacteriostatic agents from fermentation broths, the recovery of organic solvents from aqueous solutions, cell disintegration, destruction and treatment of industrial wastes, and liposome preparation. There are, however, a number of significant disadvantages in the utilization of this technology:

1. Phase equilibria of the solvent/solute system is complex, making design of extraction conditions difficult.
2. The most popular solvent (carbon dioxide) is nonpolar and is therefore most useful in the extraction of nonpolar solutes. Though cosolvents can be added for the extraction of polar compounds, they will complicate further downstream processing.
3. The use of high pressures leads to high capital costs for plant, and operating costs may also be high.

Thus, the number of commercial processes utilizing supercritical fluid extraction is relatively small, due mainly to the existence of more economical processes. However, its use is increasing, for example, the recovery of high value biologicals, when conventional extractions are inappropriate, and in the treatment of toxic wastes (Bruno, Nieto De Castro, Hamel, & Palavra, 1993). Super critical CO_2 extraction has been described by Fabre, Condoret, and Marty (1999) for the extraction of 2-phenylethyl alcohol (rose aroma) from cell free extracts of *Kluyveromyces marxianus* fermentations at pressures of 200 bar and temperatures between 35 and 45°C. The distribution coefficient was found to be twice that of conventional solvent extraction using *n*-hexane with greater than 90% product extraction.

ADSORPTION

Adsorption can be a useful technique for the separation of a product from a dilute aqueous phase and the use of polymer absorbers for the recovery of small molecules is well established. A range of polymers (eg, ion-exchangers) are available on a large scale. After extraction of the product onto the absorber, the product can then be recovered by solvent elution/extraction and the absorber can then be recycled (Wohlgemuth, 2011). Kujawska, Kujawski, Bryjak, and Kujawski (2015) report the application of absorptive polymers and zeolites for product recovery in acetone-butanol-ethanol fermentations. The use of ion-exchange resins for the recovery of lactic acid have been described by Dave, Patil, and Suresh (1997), Rincon, Fuertes, Rodriguez, Rodriguez, and Monteagudo (1997), and Moldes, Alonso, and Parajo (2003), and recombinant hepatitis B antigen by Ng et al. (2006). Further details of adsorption, ionexchange etc. are further covered in the later section on chromatography.

REMOVAL OF VOLATILE PRODUCTS

Distillation (evaporation) is a well established process, which can be used for the separation of volatile products from less volatile materials. Examples of products include ethanol (both alcoholic beverages and biofuel), flavors, and fragrances. Batch and continuous fractional distillation has been addressed earlier in this chapter (solvent recovery).

A relatively new emerging membrane based alternative to distillation for the recovery of volatile products is pervaporation (Vane, 2005). The term is derived from *Per*meation and E*vaporation*. In the process a liquid stream containing two or more miscible components is in contact with one side of a polymeric or inorganic membrane. Components from the liquid stream permeate through the membrane and the "permeate" evaporates into the vapor phase. This vapor can then be condensed. Different chemical entities will have differing affinity for the membrane and different permeation rates and thus with the correct choice of membrane, the desired product can be concentrated into the vapor phase. For example, if the membrane is hydrophobic in nature it will concentrate hydrophobic molecules. A schematic representation of a pervaporation process is shown in Fig. 10.32. There are numerous reported examples of pervaporation being used for the recovery of ethanol (Levandowicz et al., 2011; Staniszewski, Kujawski, & Lewandowska, 2007; Vane, 2005; Ikegami et al., 2003) and acetone-butanol-ethanol (Liu, Liu, & Feng, 2005; Cai et al., 2016; Kujawska et al., 2015).

Other techniques for the recovery of volatile products include flash extraction in acetone-butanol fermentations (Shi, Zhang, Chen, & Mao, 2005) and gas stripping in acetone-butanol-ethanol fermentations (Qureshi & Blaschek, 2001; Kujawska et al., 2015).

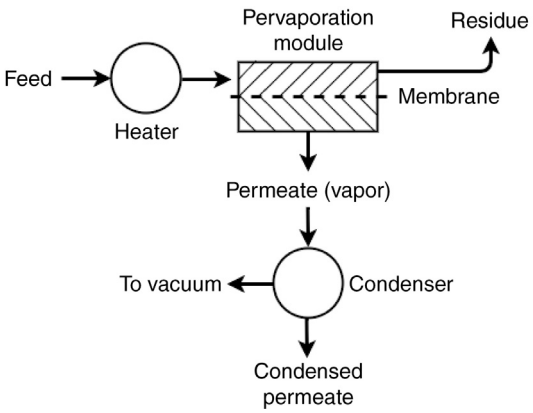

FIGURE 10.32 Schematic Representation of a Pervaporation Process

CHROMATOGRAPHY

In many fermentation processes, chromatographic techniques are used to isolate and purify relatively low concentrations of metabolic products. Chromatographic methods separate solutes based on charge, polarity, size, and affinity. In this context, chromatography will be concerned with the passage and separation of different solutes as liquid (the mobile phase) is passed through a column, that is, *liquid chromatography*. Gas chromatography, when the mobile phase is a gas, is a widely used analytical technique but has little application in the recovery of fermentation products. Depending on the mechanism by which the solutes may be differentially held in a column, the techniques can be grouped as follows:

1. Adsorption chromatography.
2. Ion-exchange chromatography.
3. Gel permeation chromatography.
4. Affinity chromatography.
5. Reverse phase chromatography.
6. High performance liquid chromatography.

Chromatographic techniques are also used in the final stages of purification of a number of products. The scale-up of chromatographic processes can prove difficult, mainly as a result of the pressures used causing compaction of the column packing materials, and there is much current interest in the use of mathematical models and computer programs to translate data obtained from small-scale processes into operating conditions for larger scale applications (Cowan, Gosling, & Laws, 1986; Cowan, Gosling, & Sweetenham, 1987).

ADSORPTION CHROMATOGRAPHY

Adsorption chromatography involves binding of the solute to the solid phase primarily by weak Van de Waals forces. The materials used for this purpose to pack columns include inorganic adsorbents (active carbon, aluminum oxide, aluminum hydroxide, magnesium oxide, silica gel) and organic macroporous resins. Adsorption and affinity chromatography are mechanistically identical, but are strategically different. In affinity systems selectivity is designed rationally while in adsorption selectivity must be determined empirically.

Dihydrostreptomycin can be extracted from filtrates using activated charcoal columns. It is then eluted with methanolic hydrochloric acid and purified in further stages (Nakazawa, Shibata, Tanabe, & Yamamoto, 1960). Some other applications for small-scale antibiotic purification are quoted by Weinstein and Wagman (1978). Active carbon may be used to remove pigments to clarify broths. Penicillin-containing solvents may be treated with 0.25–0.5% active carbon to remove pigments and other impurities (Sylvester & Coghill, 1954).

Macro-porous adsorbents have also been tested. The first synthetic organic macro-porous adsorbents, the Amberlite XAD resins, were produced by Rohm and

Haas in 1965. These resins have surface polarities, which vary from nonpolar to highly polar and do not possess any ionic functional groups. Voser (1982) considers their most interesting application to be in the isolation of hydrophilic fermentation products. He stated that these resins would be used at Ciba-Geigy in recovery of cephalosporin C (acidic amino acid), cefotiam (basic amino acid), desferrioxamine B (basic hydroxamic acid) and paramethasone (neutral steroid).

ION EXCHANGE

Ion exchange can be defined as the reversible exchange of ions between a liquid phase and a solid phase (ion-exchange resin) which is not accompanied by any radical change in the solid structure. Cationic ion-exchange resins normally contain a sulfonic acid, carboxylic acid, or phosphonic acid active group. Carboxy-methyl cellulose is a common cation exchange resin. Positively charged solutes (eg, certain proteins) will bind to the resin, the strength of attachment depending on the net charge of the solute at the pH of the column feed. After deposition solutes are sequentially washed off by the passage of buffers of increasing ionic strength or pH. Anionic ion-exchange resins normally contain a secondary amine, quaternary amine, or quaternary ammonium active group. A common anion exchange resin, DEAE (diethylaminoethyl) cellulose is used in a similar manner to that described earlier for the separation of negatively charged solutes. Other functional groups may also be attached to the resin skeleton to provide more selective behavior similar to that of affinity chromatography. The appropriate resin for a particular purpose will depend on various factors such as bead size, pore size, diffusion rate, resin capacity, range of reactive groups, and the life of the resin before replacement is necessary. Weak-acid cation ion-exchange resins can be used in the isolation and purification of streptomycin, neomycin, and similar antibiotics.

In the recovery of streptomycin, the harvested filtrate is fed on to a column of a weak-acid cationic resin such as Amberlite IRC 50, which is in the sodium form. The streptomycin is adsorbed on to the column and the sodium ions are displaced.

$$\mathrm{RCOO^-Na^+_{(resin)}} + \text{streptomycin} \rightarrow \mathrm{RCOO^-}\ \text{streptomycin}^+_{(\text{resin})} + \mathrm{NaOH}$$

Flow rates of between 10 and 30 bed volumes per hour have been used. The resin bed is now rinsed with water and eluted with dilute hydrochloric acid to release the bound streptomycin.

$$\mathrm{RCOO^-}\ \text{streptomycin}^+_{(\text{resin})} + \mathrm{HCl} \rightarrow \mathrm{RCOOH_{(resin)}} + \text{streptomycin}^+\ \mathrm{Cl^-}$$

A slow flow is used to ensure the highest recovery of streptomycin using the smallest volume of eluent. In one step, the antibiotic has been both purified and concentrated, may be more than 100-fold. The resin column is regenerated to the sodium form by passing an adequate volume of NaOH slowly through the column and rinsing with distilled water to remove excess sodium ions.

$$RCOOH_{(resin)} + NaOH \rightarrow RCOO^{-}Na^{+}_{(resin)} + H_2O$$

The resin can have a capacity of 1 g of streptomycin g^{-1} resin. Commercially, it is not economic to regenerate the resin completely, therefore the capacity will be reduced. In practice, the filtered broth is taken through two columns in series while a third is being eluted and regenerated. When the first column is saturated, it is isolated for elution and regeneration while the third column is brought into operation.

Details for isolation of some other antibiotics are given in Weinstein and Wagman (1978). Ion-exchange chromatography may be combined with HPLC in, for example, the purification of somatotropin using DEAE cellulose columns and β-urogastrone in multigram quantities using a cation exchange column (Brewer & Larsen, 1987).

GEL PERMEATION

This technique is also known as gel exclusion and gel filtration. Gel permeation separates molecules on the basis of their size. The smaller molecules diffuse into the gel more rapidly than the larger ones, and penetrate the pores of the gel to a greater degree. This means that once elution is started, the larger molecules which are still in the voids in the gel will be eluted first. A wide range of gels are available, including crosslinked dextrans (Sephadex and Sephacryl) and crosslinked agarose (Sepharose) with various pore sizes depending on the fractionation range required.

One early industrial application, although on a relatively small scale, was the purification of vaccines (Latham, Michelsen, & Edsall, 1967). Tetanus and diphtheria broths for batches of up to 100,000 human doses are passed through a 13 dm^3 column of G 100 followed by a 13 dm^3 column of G 200. This technique yields a fairly pure fraction which is then concentrated 10-fold by pressure dialysis to remove the eluent buffer (Na_2HPO_4).

AFFINITY CHROMATOGRAPHY

Affinity chromatography is a separation technique with many applications since it is possible to use it for separation and purification of most biological molecules on the basis of their function or chemical structure. This technique depends on the highly specific interactions between pairs of biological materials such as enzyme–substrate, enzyme–inhibitor, antigen–antibody, etc. The molecule to be purified is specifically adsorbed from, for example, a cell lysate applied to the affinity column by a binding substance (ligand) which is immobilized on an insoluble support (matrix). Eluent is then passed through the column to release the highly purified and concentrated molecule. The ligand is attached to the matrix by physical absorption or chemically by a covalent bond. The pore size and ligand location must be carefully matched to the size of the product for effective separation. The latter method is preferred whenever possible. Porath (1974) and Yang and Tsao (1982) have reviewed methods and coupling procedures.

Coupling procedures have been developed using cyanogen bromide, bisoxiranes, disaziridines, and perio-dates, for matrixes of gels and beads. Four polymers, which are often used for matrix materials are agarose, cellulose, dextrose, and polyacrylamide. Agarose activated with cyanogen bromide is one of the most commonly used supports for the coupling of amino ligands. Silica based solid phases have been shown to be an effective alternative to gel supports in affinity chromatography (Mohan & Lyddiatt, 1992).

Purification may be several 1000-fold with good recovery of active material. The method can however be quite costly and time consuming, and alternative affinity methods such as affinity cross-flow filtration, affinity precipitation, and affinity partitioning may offer some advantages (Janson, 1984; Luong, Nguyen, & Male, 1987). Affinity chromatography was used initially in protein isolation and purification, particularly enzymes. Since then many other large-scale applications have been developed for enzyme inhibitors, antibodies, interferon, and recombinant proteins (Janson & Hedman, 1982; Ostlund, 1986; Folena-Wasserman, Inacker, & Rosenbloom, 1987; Nachman, Azad, & Bailón, 1992), and on a smaller scale for nucleic acids, cell organelles, and whole cells (Yang & Tsao, 1982). In the scale-up of affinity chromatographic processes (Katoh, 1987) bed height limits the superficial velocity of the liquid, thus scale-up requires an increase in bed diameter or adsorption capacity.

REVERSE PHASE CHROMATOGRAPHY (RPC)

When the stationary phase has greater polarity than the mobile phase it is termed "normal phase chromatography." When the opposite is the case, it is termed "reverse phase chromatography." RPC utilizes a solid phase (eg, silica) which is modified so as to replace hydrophilic groups with hydrophobic alkyl chains. This allows the separation of proteins according to their hydrophobicity. More-hydrophobic proteins bind most strongly to the stationary phase and are therefore eluted later than less-hydrophobic proteins. The alkyl groupings are normally eight or eighteen carbons in length (C_8 and C_{18}). RPC can also be combined with affinity techniques in the separation of, for example, proteins and peptides (Davankov, Kurganov, & Unger, 1990).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a high resolution column chromatographic technique. Improvements in the nature of column packing materials for a range of chromatographic techniques (eg, gel permeation and ion-exchange) yield smaller, more rigid, and more uniform beads. This allows packing in columns with minimum spaces between the beads, thus minimizing peak broadening of eluted species. It was originally known as high *pressure* liquid chromatography because of the high pressures required to drive solvents through silica based packed beds. Improvements in the performance led to the name change and its widespread use in the separation and purification of a wide

range of solute species, including biomolecules. HPLC is distinguished from liquid chromatography by the use of improved media (in terms of their selectivity and physical properties) for the solid (stationary) phase through which the mobile (fluid) phase passes.

The stationary phase must have high surface area/unit volume, even size and shape and be resistant to mechanical and chemical damage. However, it is factors such as these which lead to high pressure requirements and cost. This may be acceptable for analytical work, but not for preparative separations. Thus, in preparative HPLC some resolution is often sacrificed (by the use of larger stationary-phase particles) to reduce operating and capital costs. For very high value products, large-scale HPLC columns containing analytical media have been used. Fast protein liquid chromatography (FPLC) is a variant of HPLC which is more suited to large scale purification processes (Doran, 2013).

Affinity techniques can be merged with HPLC to combine the selectivity of the former with the speed and resolving power of the latter (Forstecher, Hammadi, Bouzerna, & Dautre-vaux, 1986; Shojaosadaty & Lyddiatt, 1987).

CONTINUOUS CHROMATOGRAPHY

Although the concept of continuous enzyme isolation is well established (Dunnill & Lilly, 1972), the stage of least development is continuous chromatography. Jungbauer (2013) described the possible continuous chromatography alternatives to batch chromatography. These include annular chromatography, carrousel chromatography, and various configurations of moving bed chromatography. However as far back as 1969, Fox, Calhoun, and Eglinton (1969) developed a continuous-fed annular column for this purpose (Fig. 10.33). It consisted of two concentric cylindrical sections clamped

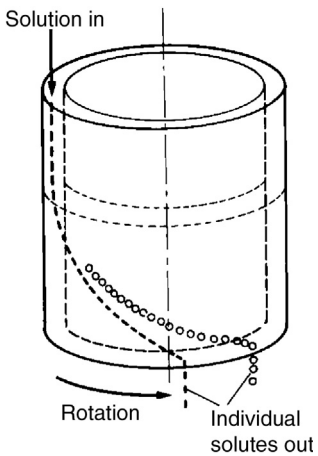

FIGURE 10.33 The Principle of Continuous-Partition Chromatography

---, faster-moving component; O O, slower-moving component (Fox et al., 1969).

to a base plate. The space (1 cm wide) between the two sections was packed with the appropriate resin or gel giving a total column capacity of 2.58 dm^3. A series of orifices in the circumference of the base plate below the column space led to collecting vessels. The column assembly was rotated in a slow-moving turntable (0.4–2.0 rpm). The mixture for separation was fed to the apparatus by an applicator rotating at the same speed as the column, thus allowing application at a fixed point, while the eluent was fed evenly to the whole circumference of the column. The components of a mixture separated as a series of helical pathways, which varied with the retention properties of the constituent components. This method gave a satisfactory separation and recovery but the consumption of eluent and the unreliable throughput rate were not considered to be satisfactory for a large-scale method (Nicholas & Fox, 1969; Dunnill & Lilly, 1972).

MEMBRANE PROCESSES

ULTRAFILTRATION AND REVERSE OSMOSIS

Both processes utilize semipermeable membranes to separate molecules of different sizes and therefore act in a similar manner to conventional filters.

ULTRAFILTRATION

Ultrafiltration can be described as a process in which solutes of high molecular weight are retained when the solvent and low molecular weight solutes are forced under hydraulic pressure (between 2 and 10 atmospheres) through a membrane of a very fine pore size, typically between 0.001 and 0.1 μm. It is therefore used for product concentration and purification. A range of membranes made from a variety of polymeric materials, with different molecular weight cut-offs (500–500,000), are available which makes possible the separation of macromolecules such as proteins, enzymes, hormones, and viruses. It is practical only to separate molecules whose molecular weights are a factor of ten different due to variability in pore size (Heath & Belfort, 1992). Because the flux through such a membrane is inversely proportional to its thickness, asymmetric membranes are used where the membrane (~0.3 μm thick) is supported by a mesh around 0.3 mm thick.

When considering the feasibility of ultrafiltration, it is important to remember that factors other than the molecular weight of the solute affect the passage of molecules through the membranes (Melling & Westmacott, 1972). There may be concentration polarization caused by accumulation of solute at the membrane surface, which can be reduced by increasing the shear forces at the membrane surface either by conventional agitation or by the use of a cross-flow system (see previous section). Second, a slurry of protein may accumulate on the membrane surface forming a gel layer which is not easily removed by agitation. Formation of the gel layer may be partially controlled by careful choice of conditions such as pH (Bailey & Ollis, 1986). Finally,

equipment and energy costs may be considerable because of the high pressures necessary; this also limits the life of ultrafiltration membranes.

There are numerous examples of the use of ultrafiltration for the concentration and recovery of biomolecules: viruses (Weiss, 1980), enzymes (Atkinson & Mavituna, 1991), antibiotics (Pandey et al., 1985), xanthan gum (Lo, Yang, & Min, 1997), and surfactin (Chen, Chen, & Juang, 2007). Tessier, Bouchard, and Rahni (2005) described the application of ultrafiltration coupled to nanofiltration in the purification of benzylpenicillin. The nanofiltration membrane was used to concentrate the permeates produced by ultrafiltration with recoveries of ~90% being obtained. Details of large scale applications are given by Lacey and Loeb (1972) and by Ricketts, Lebherz, Klein, Gustafson, and Flickinger (1985). Affinity ultrafiltration (Luong et al., 1987; Luong & Nguyen, 1992) is a novel separation process developed to circumvent difficulties in affinity chromatography. It offers high selectivity, yield, and concentration, but it is an expensive batch process and scale up is difficult.

REVERSE OSMOSIS

Reverse osmosis (also described as hyperfiltration) is a separation process where the solvent molecules are forced by an applied pressure to flow through a semipermeable membrane in the opposite direction to that dictated by osmotic forces, and hence is termed reverse osmosis. It is used for the concentration of smaller molecules than is possible by ultrafiltration as the pores are 1–10 angstroms diameter. Concentration polarization is again a problem and must be controlled by increased turbulence at the membrane surface. Nanofiltration is a modified form of reverse osmosis, which utilizes charged membranes to separate small solutes and charged species based on both charge and size effects (Doran, 2013).

LIQUID MEMBRANES

Liquid membranes are insoluble liquids (eg, an organic solvent) which are selective for a given solute and separate two other liquid phases. Extraction takes place by the transport of solute from one liquid to the other. They are of great interest in the extraction and purification of biologicals for the following reasons:

1. Large area for extraction.
2. Separation and concentration are achieved in one step.
3. Scale-up is relatively easy.

Their use has been reported in the extraction of lactic acid (Chaudhuri & Pyle, 1990) and citric acid using a supported liquid membrane (Sirman, Pyle, & Grandison, 1990). The utilization of selective carriers to transport specific components across the liquid membrane at relatively high rates has increased interest in recent years (Strathmann, 1991). Liquid membranes may also be used in cell and enzyme immobilization, and thus provide the opportunity for combined production

and isolation/extraction in a single unit (Mohan & Li, 1974, 1975). The potential use of liquid membranes has also been described for the production of alcohol reduced beer as having little effect on flavor or the physicochemical properties of the product (Etuk & Murray, 1990).

DRYING

The drying of any product (including biological products) is often the last stage of a manufacturing process (McCabe, Smith, & Harriot, 1984; Coulson & Richardson, 1991). It involves the final removal of water or other solvents from a product, while ensuring that there is minimum loss in viability, activity, or nutritional value. Drying is undertaken because:

1. The cost of transport can be reduced.
2. The material is easier to handle and package.
3. The material can be stored more conveniently in the dry state.

A detailed review of the theory and practice of drying can be found in Perry and Green (1984). It is important that as much water as possible is removed initially by centrifugation or in a filter press to minimize heating costs in the drying process. Driers can be classified by the method of heat transfer to the product and the degree of agitation of the product. For some products simple tray driers, where the product is placed on trays over which air is passed in a heated oven may be sufficient. A vacuum may be applied to aid evaporation at lower temperatures. In contact driers, the product is contacted with a heated surface. An example of this type is the drum drier (Fig. 10.34), which may be used for more temperature stable bioproducts. A slurry is run onto a slowly rotating steam heated drum, evaporation takes place and the dry product is removed by a scraper blade in a similar manner as for rotary vacuum

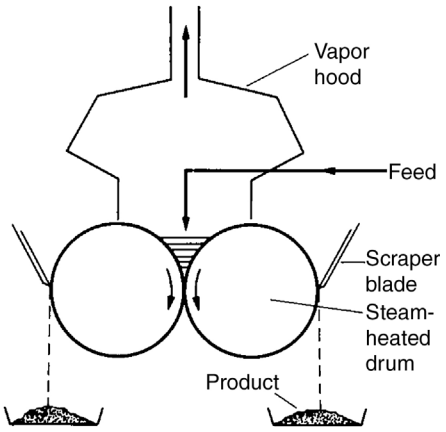

FIGURE 10.34 Cross-Section of a Drum Drier

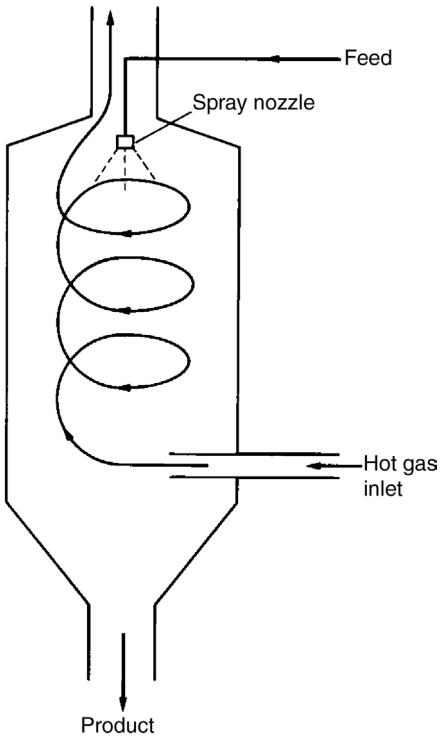

FIGURE 10.35 Counter-Current Spray Drier

filtration. The solid is in contact with the heating surface for 6–15 s and heat transfer coefficients are generally between 1 and 2 kW m^{-2} K^{-1}. Vacuum drum driers can be used to lower the temperature of drying.

A spray drier (Fig. 10.35) is most widely used for drying of biological materials when the starting material is in the form of a liquid or paste. The material to be dried does not come into contact with the heating surfaces, instead, it is atomized into small droplets through, for example, a nozzle or by contact with a rotating disc. The wide range of atomizers available is described in Coulson and Richardson (1991). The droplets then fall into a spiral stream of hot gas at 150–250°C. The high surface area:volume ratio of the droplets results in a rapid rate of evaporation and complete drying in a few seconds, with drying rate and product size being directly related to droplet size produced by the atomizer. The evaporative cooling effect prevents the material from becoming overheated and damaged. The gas-flow rate must be carefully regulated so that the gas has the capacity to contain the required moisture content at the cool-air exhaust temperature (75–100°C). In most processes, the recovery of very small particles from the exit gas must be conducted using cyclones or filters. This is especially important for containment of biologically active compounds. The jet spray drier is particularly suited to handling heat sensitive materials. Operating at

a temperature of around 350°C, residence times are approximately 0.01 s because of the very fine droplets produced in the atomizing nozzle.

Spray driers are the most economical available for handling large volumes, and it is only at feed rates below 6 kg min^{-1} that drum driers become more economic.

Freeze drying (also known as lyophilization or cryodesiccation) is an important operation in the production of many biologicals and pharmaceuticals. The material is first frozen and then dried by sublimation in a high vacuum followed by secondary drying to remove any residual moisture. The great benefit of this technique is that it does not harm heat sensitive materials. Freeze drying is generally more energy intensive than other forms of drying.

Fluidized bed driers are used increasingly in the pharmaceutical industry. Heated air is fed into a chamber of fluidized solids, to which wet material is continuously added and dry material continuously removed. Very high heat and mass-transfer rates are achieved, giving rapid evaporation and allowing the whole bed to be maintained in a dry condition.

CRYSTALLIZATION

Crystallization is an established method used in the initial recovery of organic acids and amino acids, and more widely used for final purification of a diverse range of compounds. Crystallization is a two stage process, the formation of nuclei in a supersaturated solution and crystal growth, which proceed simultaneously and can be independently controlled to some extent. Industrial crystallizers may be batch or continuous processes with supersaturation being achieved by cooling or by removal of solvent (evaporative crystallization).

In citric acid production, the filtered broth is treated with $Ca(OH)_2$ so that the relatively insoluble calcium citrate crystals will be precipitated from solution. Checks are made to ensure that the $Ca(OH)_2$ has a low magnesium content, since magnesium citrate is more soluble and would remain in solution. The calcium citrate is filtered off and treated with sulfuric acid to precipitate the calcium as the insoluble sulfate and release the citric acid. After clarification with active carbon, the aqueous citric acid is evaporated to the point of crystallization (Lockwood & Irwin, 1964; Sodeck, Modl, Kominek, & Salzbrunn, 1981; Atkinson & Mavituna, 1991). Crystallization is also used in the recovery of amino acids; Samejima (1972) has reviewed methods for glutamic acid, lysine, and other amino acids. The recovery of cephalosporin C as its sodium or potassium salt by crystallization has been described by Wildfeuer (1985). In 1,3-propanediol fermentations salt by-products (sodium succinate and sodium sulfate) of the fermentation need to be removed before recovery of 1,3-propanediol. Wu, Ren, Xu, and Liu (2010) describe recovery, rather than simply removal, of these salts at high yield and purity by batch crystallization. Buque-Taboada, Straathof, Heijnen, and van der Wielen (2006) review the application of in situ crystallization in by-product recover from fermentation broths as soon as the product is formed when such products have an inhibitory or degrading effect on further product formation.

WHOLE BROTH PROCESSING

The concept of recovering a metabolite directly from an unfiltered fermentation broth is of considerable interest because of its simplicity, the reduction in process stages, and the potential cost savings. It may also be possible to remove the desired fermentation product continuously from a broth during fermentation so that inhibitory effects due to product formation and product degradation can be minimized throughout the production phase (Roffler, Blanch, & Wilke, 1984; Diaz, 1988). It can also be used to continuously remove undesirable byproducts from a fermentation broth which might otherwise inhibit cell growth or degrade a desired extracellular product (Agrawal & Burns, 1997; Demirci & Pongtharangku, 2007).

The continuous downstream processing of biopharmaceuticals via centrifugation, filtration, extraction, precipitation, crystallization, and chromatography has been reviewed by Jungbauer (2013). Ó Meadhra (2005) describes potential systems for the continuous crystallization of products from fermentation.

Bartels, Kleiman, Korzun, and Irish (1958) developed a process for adsorption of streptomycin on to a series of cationic ion-exchange resin columns directly from the fermentation broth, which had only been screened to remove large particles so that the columns would not become blocked. This procedure could only be used as a batch process. Belter, Cunningham, and Chen (1973) developed a similar process for the recovery of novobiocin. The harvested broth was first filtered through a vibrating screen to remove large particles. The broth was then fed into a continuous series of well-mixed resin columns fitted with screens to retain the resin particles, plus the absorbed novobiocin, but allow the streptomycete filaments plus other small particulate matter to pass through. The first resin column was removed from the extraction line after a predetermined time and eluted with methanolic ammonium chloride to recover the novobiocin.

Karr, Gebert, and Wang (1980) developed a reciprocating plate extraction column (Fig. 10.36) to use for whole broth processing of a broth containing 1.4 g dm^{-3} of a slightly soluble organic compound and 4% undissolved solids provided that chloroform or methylene chloride were used for extraction. Methyl-iso-butyl ketone, diethyl ketone, and iso-propyl acetate were shown to be more efficient solvents than chloroform for extracting the active compound, but they presented problems since they also extracted impurities from the mycelia, making it necessary to filter the broth before beginning the solvent extraction. Considerable economies were claimed in a comparison with a process using a Podbielniak extractor, in investment, maintenance costs, solvent usage, and power costs but there was no significant difference in operating labor costs.

An alternative approach is to remove the metabolite continuously from the broth during the fermentation. Cycloheximide production by *Streptomyces griseus* has been shown to be affected by its own feedback regulation (Kominek, 1975). Wang, Kominek, and Jost (1981) have tested two techniques at laboratory scale

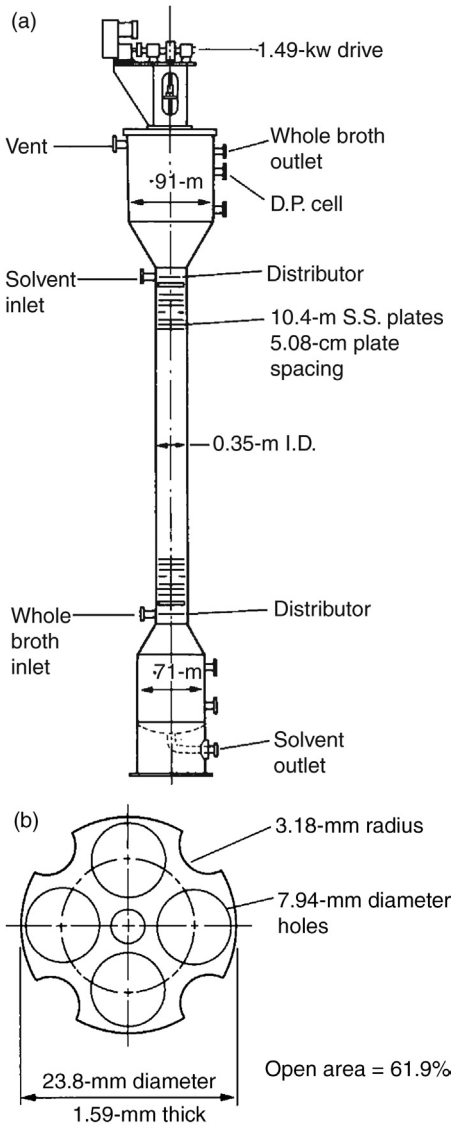

FIGURE 10.36

(a) Diagram of a 0.35-m internal diameter reciprocating plate column (Karr et al., 1980). (b) Plan of a 23.8-m stainless-steel plate for a 25-mm diameter reciprocating plate test column (Karr et al., 1980).

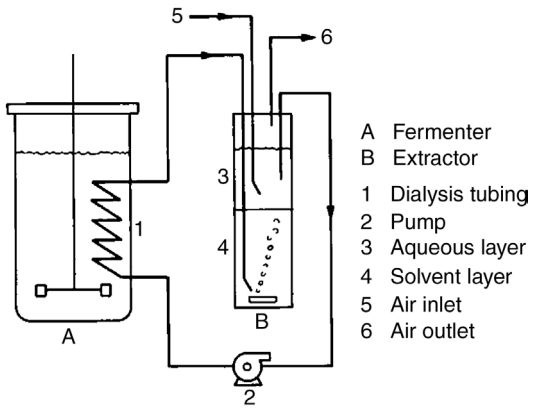

FIGURE 10.37 Dialysis-Extraction Fermentation System (Wang et al., 1981)

for improving the production of cycloheximide. In a dialysis method (Fig. 10.37), methylene chloride was circulated in a dialysis tubing loop which passed through a 10 dm^{-3} fermenter. Cycloheximide in the fermentation broth was extracted into the methylene chloride. It was shown that the product yield could be almost doubled by this dialysis-solvent extraction method to over 1200 µg cm^{-3} as compared with a control yield of approximately 700 pg cm^{-3}. In a resin method, sterile beads of XAD-7, an acrylic resin, as dispersed beads or beads wrapped in an ultrafiltration membrane, were put in fermenters 48 h after inoculation. Some of the cycloheximide formed in the broth is absorbed by the resin. Recovery of the antibiotic from the resin is achieved by solvents or by changing the temperature or pH. When assayed after harvesting, the control (without resin) had a bioactivity of 750 µg cm^{-3}. Readings of total bioactivity (from beads and broth) for the bead treatment and the membrane-wrapped bead treatments were 1420 µg cm^{-3} and 1790 µg cm^{-3} respectively. Agrawal and Burns (1997) described the use of a membrane based system for whole broth processing to separate lysozyme from feed mixture containing lysozyme, myoglobin, and yeast cells for in situ product removal.

Roffler et al. (1984) reviewed the use of a number of techniques for the in situ recovery of fermentation products:

1. Vacuum and flash fermentations for the direct recovery of ethanol from fermentation broths.
2. Extractive fermentation (liquid–liquid and two-phase aqueous) for the recovery of ethanol, organic acids, and toxin produced by *Clostridium tetani.*
3. Adsorption for the recovery of ethanol and cycloheximide.
4. Ion-exchange in the extraction of salicylic acid and antibiotics.
5. Dialysis fermentation in the selective recovery of lactic acid, salicylic acid, and cycloheximide.

Hansson, Stahl, Hjorth, Uhlen, and Moks (1994) have used an expanded adsorption bed for the recovery of a recombinant protein produced by *E. coli* directly from the fermentation broth. The protein was produced in high yields (550 mg dm^{-3}) and >90% recovery together with concentration (volume reduction) and removal of cells was achieved on the expanded bed. Affinity chromatography was used for further purification, and again an overall yield of >90% obtained.

REFERENCES

Adikane, H. V., Singh, R. K., & Nene, S. N. (1999). Recovery of penicillin G from fermentation broth by microfiltration. *Journal of Membrane Science*, *162*, 119–123.

Agerkvist, I., & Enfors, S. -O. (1990). Characterisation of *E. coli* cell disintegrates from a bead mill and high pressure homogenizers. *Biotechnology and Bioengineering*, *36*(11), 1083–1089.

Agrawal, A., & Burns, M. A. (1997). Application of membrane based preferential transport to whole broth processing. *Biotechnology and Bioengineering*, *55*(4), 581–591.

Aiba, S., Humphrey, A. E., & Millis, N. F. (1973). Recovery of fermentation products. In *Biochemical engineering* (2nd ed., pp. 346–392). New York: Academic Press.

Andrews, B. A., & Asenjo, J. A. (1987). Enzymatic lysis and disruption of microbial cells. *Trends in Biotechnology*, *5*, 273–277.

Andrews, B. A., Huang, R. B., & Asenjo, J. A. (1990). Differential product release from yeast cells by selective enzymatic lysis. In D. L. Pyle (Ed.), *Separations for Biotechnology 2* (pp. 21–28). London: Elsevier.

Asenjo, J. A. (1990). Cell disruption and removal of insolubles. In D. L. Pyle (Ed.), *Separations for Biotechnology 2* (pp. 11–20). London: Elsevier.

Ashley, M. H. J. (1990). Conceptual design of a novel flocculation device. In D. L. Pyle (Ed.), *Separations for Biotechnology 2* (pp. 29–37). London: Elsevier.

Atkinson, B., & Daoud, I. S. (1976). Microbial floes and flocculation in fermentation process engineering. *Advances in Biochemical Engineering*, *4*, 41–124.

Atkinson, B., & Mavituna, F. (1991). *Biochemical Engineering and Biotechnology Handbook* (2nd ed.). London: Macmillan.

Atkinson, B., & Sainter, P. (1982). Development of downstream processing. *Journal of Chemical Technology and Biotechnology*, *32*, 100–108.

Aunstrup, K. (1979). Production, isolation and economics of extracellular enzymes. In L. B. Wingard, & E. Katzir-Katchalski (Eds.), *Applied Biochemistry and Bioengineering* (pp. 27–69). (Vol. 2). New York: Academic Press.

Bailey, J. E., & Ollis, D. F. (1986). *Biochemical Engineering Fundamentals* (2nd ed.). New York: McGraw-Hill, pp. 768–769.

Bartels, C. R., Kleiman, G., Korzun, J. N., & Irish, D. B. (1958). A novel ion-exchange method for the isolation of streptomycin. *Chemical Engineering Progress*, *54*, 49–51.

Bell, G. B., & Hutto, F. B. (1958). Analysis of rotary pre-coat filter operation. 1. New concepts. *Chemical Engineering Progress*, *54*, 69–76.

Belter, P. A., Cunningham, F. L., & Chen, J. W. (1973). Development of a recovery process for novobiocin. *Biotechnology and Bioengineering*, *15*, 533–549.

Bentham, C. C., Bonnerjea, J., Orsborn, G. B., Ward, P. N., & Hoare, M. (1990). The separation of affinity flocculated yeast cell debris using a pilot plant scroll decanter centrifuge. *Biotechnology and Bioengineering*, *36*(4), 397–401.

Bowden, C. P., Leaver, G., Melling, J., Norton, M. G., & Whittington, P. N. (1987). Recent and novel developments in the recovery of cells from fermentation broths. In M. S. Verrall, & M. J. Hudson (Eds.), *Separations for Biotechnology* (pp. 49–61). Chichester: Ellis Horwood.

Brewer, S. J., & Larsen, B. R. (1987). Isolation and purification of proteins using preparative HPLC. In M. S. Verrall, & M. J. Hudson (Eds.), *Separations for Biotechnology* (pp. 113–126). Chichester: Ellis Horwood.

Brookman, J. S. G. (1974). Mechanism of cell disintegration in a high pressure homogenizer. *Biotechnology and Bioengineering*, *16*, 371–383.

Brown, D. E., & Kavanagh, P. R. (1987). Cross-flow separation of cells. *Process Biochemistry*, *22*(4), 96–101.

Bruno, T. J., Nieto De Castro, C. A., Hamel, J. -F. P., & Palavra, A. M. F. (1993). Supercritical fluid extraction of biological products. In J. F. Kennedy, & J. M. S. Cabrai (Eds.), *Recovery Processes for Biological Materials* (pp. 303–354). Chichester: Wiley.

Buque-Taboada, E. M., Straathof, A. J. J., Heijnen, J. J., & van der Wielen, L. A. M. (2006). In situ product recovery (ISPR) by crystallization: basic principles, design, and potential applications in whole-cell biocatalysis. *Applied Microbiology and Biotechnology*, *71*, 1–12.

Cabral, J. M. S., & Aires-Barros, M. R. (1993). Liquid-liquid extraction of biomolecules using aqueous two-phase systems. In J. F. Kennedy, & J. M. S. Cabral (Eds.), *Recovery Processes for Biological Materials* (pp. 273–302). Chichester: Wiley.

Cai, D., Chen, H., Chen, C., Hu, S., Wang, Y., Chang, Z., Miao, Q., Qin, P., Wang, Z., Wang, J., & Tan, T. (2016). Gas stripping-pervaporation process for energy saving product recovery from acetone-butanol-ethanol (ABE) fermentation broth. *Chemical Engineering Journal*, *287*, 1–10.

Chaudhuri, J. B., & Pyle, D. L. (1990). A model for emulsion liquid membrane extraction of organic acids. In D. L. Pyle (Ed.), *Separations for Biotechnology 2* (pp. 112–121). London: Elsevier Applied Science.

Chavez-Santoscoy, A. (2010). Application of aqueous two-phase systems for potential extractive fermentation of cyanobacterial products. *Chemical Engineering and Technology*, *33*(1), 177–182.

Chen, H. -L., Chen, Y. -S., & Juang, R. -S. (2007). Separation of surfactin from fermentation broths by acid precipitation and two stage dead-end ultrafiltration processes. *Journal of Membrane Science*, *299*, 114–121.

Corsano, G., Iribarren, O., Montagna, J. M., Aguirre, P. A., & Suarez, E. G. (2006). Economic tradeoffs involved in the design of fermentation processes with environmental constraints. *Chemical Engineering Research and Design*, *84*(A10), 932–942.

Coulson, J. M., & Richardson, J. F. (1991) *Chemical Engineering* (Vol. 2). (4th ed.). Oxford: Pergamon Press.

Cowan, G. H., Gosling, I. S., & Laws, J. F. (1986). Physical and mathematical modelling to aid scale-up of liquid chromatography. *Journal of Chromatography A*, *363*, 31–36.

Cowan, G. H., Gosling, I. S., & Sweetenham, W. P. (1987). Modelling for scale-up and optimisation of packed-bed columns in adsorption and chromatograpy. In M. S. Verrall, & M. J. Hudson (Eds.), *Separations for Biotechnology* (pp. 152–175). Chichester: Ellis Horwood.

Crapisi, A., Lante, A., Pasini, G., & Spettoli, P. (1993). Enhanced microbial cell lysis by the use of lysozyme immobilised on different carriers. *Process Biochemistry*, *28*(1), 17–21.

Darbyshire, J. (1981). Large scale enzyme extraction and recovery. In A. Wiseman (Ed.), *Topics in Enzyme and Fermentation Biotechnology* (pp. 147–186). (Vol. 5). Chichester: Ellis Horwood.

Datar, R. (1986). Economics of primary separation steps in relation to fermentation and genetic engineering. *Process Biochemistry*, *21*(1), 19–26.

Davankov, V. A., Kurganov, A. A., & Unger, K. (1990). Reversed phase high performance liquid chromatography of proteins and peptides on polystyrene coated silica supports. *Journal of Chromatography*, *500*, 519–530.

Dave, S. M., Patil, S. S., & Suresh, A. K. (1997). Ion exchange for product recovery in lactic acid fermentation. *Separation Science and Technology*, *32*(7), 1273–1294.

Davis, D. A., Lynch, H. C., & Varley, J. (2001). The application of foaming for the recovery of Surfactin from *B. subtilis* ATCC21332 cultures. *Enzyme and Microbial Technology*, *28*, 346–354.

Delaine, J. (1983). Physico-chemical pretreatment of process effluents. *Institution of Chemical Engineers Symposium Series*, *77*, 183–203.

Demirci, A., & Pongtharangku, T. (2007). Online recovery of nisin during fermentation and its effect on nisin production in a biofilm reactor. *Applied Microbiology and. Biotechnology*, *74*, 555–562.

DeSousa, S. R., Laluce, C., & Jafelicci, A., Jr. (2006). Effects of organic and inorganic additives on floatation recovery of washed cells of *Saccaromyces cerevisiae* resuspended in water. *Colloids and Surfaces B: Biointerfaces*, *48*, 77–83.

Diaz, M. (1988). Three-phase extractive fermentation. *Trends in Biotechnology*, *6*(6), 126–130.

Doran, P. M. (2013). *Bioprocess Engineering Principles* (pp. 445–578) (2nd ed.). Oxford: Academic Press.

Dunnill, P., & Lilly, M. D. (1972). Continuous enzyme isolation. *Biotechnology and Bioengineering Symposium*, *3*, 97–113.

Etuk, B. R., & Murray, K. R. (1990). Potential use of liquid membranes for alcohol reduced beer production. *Process Biochemistry*, *25*(1), 24–32.

Fabre, C. E., Condoret, J. -S., & Marty, A. (1999). Extractive fermentation or aroma with supercritical CO_2. *Biotechnology and Bioengineering*, *64*(4), 392–400.

Fletcher, K., Deley, S., Fleischaker, R. J., Forrester, I. T., Grabski, A. C., & Strickland, W. N. (1990). Clarification of tissue culture fluid and cell lysates using Biocryl bioprocessing aids. In D. L. Pyle (Ed.), *Separations for Biotechnology 2* (pp. 142–151). London: Elsevier.

Folena-Wasserman, G., Inacker, R., & Rosenbloom, J. (1987). Assay, purification and characterisation of a recombinant malaria circumsporozoite fusion protein by high performance liquid chromatography. *Journal of Chromatography*, *411*, 345–354.

Forstecher, P., Hammadi, H., Bouzerna, N., & Dautre-vaux, M. (1986). Rapid purification of antisteriod anti-bodies by high performance liquid affinity chromatography. *Journal of Chromatography A*, *369*, 379–390.

Fox, J. B., Calhoun, R. C., & Eglinton, W. J. (1969). Continuous chromatography apparatus. 1. Construction. *Journal of Chromatography A*, *43*, 48–54.

Frude, M. J., & Simpson, M. T. (1993). Steam sterilisation of a Westfalia CSA 8 centrifuge. *Process Biochemistry*, *28*, 297–303.

Gabler, R., & Ryan, M. (1985). Processing cell lysate with tangential flow filtration. In D. LeRoith, J. Shiloach, & T. J. Leahy (Eds.), *Purification of Fermentation Products*, (pp. 1–20), ACS, Symposium Series, 271, A.C.S, Washington, D.C.

Gasner, C. C., & Wang, D. I. C. (1970). Microbial recovery enhancement through flocculation. *Biotechnology and Bioengineering*, *12*, 873–887.

Gonzalez, M., Pencs, C., & Casas, L. T. (1990). Partial purification of β-galactosidase from yeast by an aqueous two phase system method. *Process Biochemistry*, *25*(5), 157–161.

Gray, P. P., Dunnill, P., & Lilly, M. D. (1973). The clarification of mechanically disrupted yeast suspensions by rotary vacuum precoat filtration. *Biotechnology and Bioengineering*, *15*, 309–320.

Grieves, R. B., & Wang, S. L. (1966). Foam separation of *Escherichia coli* with a cationic surfactant. *Biotechnology and Bioengineering*, *8*, 323–336.

Gu, Z., & Glatz, C. E. (2006). Aqueous two-phase extraction for protein recovery from corn extracts. *Journal of Chromatography B*, *845*, 38–50.

Guan, Y., Wu, X. -Y., Treffry, T. E., & Lilley, T. H. (1992). Studies on the isolation of penicillin acylase from *Escherichia coli* by aqueous two-phase partitioning. *Biotechnology and Bioengineering*, *40*(4), 517–524.

Guan, Y., Zhu, Z., & Mei, L. (1996). Technical aspects of extractive purification of penicillin fermentation broth by aqueous two-phase partitioning. *Separation Science and Technology*, *31*(18), 2589–2597.

Hamer, G. (1979). Biomass from natural gas. In A. H. Rose (Ed.), *Economic Microbiology* (pp. 315–360). (Vol. 4). London: Academic Press.

Hansson, M., Stahl, S., Hjorth, R., Uhlen, M., & Moks, T. (1994). Single step recovery of a secreted recombinant protein by expanded bed adsorption. *Biotechnology*, *12*(3), 285–288.

Hao, J., Xu, F., Liu, H., & Liu, D. (2006). Downstream processing of 1,3-propanediol fermentation broth. *Journal of Chemical Technology and Biotechnology*, *81*, 102–108.

Harrison, S.T. L. (2011) Cell distruption. In: M. Moo-Young (Ed. in chief), *Comprehensive Biotechnology, Volume 2,* (2nd ed.) (pp. 619–639). Oxford: Elsevier.

Heath, C. A., & Belfort, G. (1992). Synthetic membranes in biotechnology. *Advances in Biochemical Engineering/Biotechnology*, *47*, 45–88.

Hetherington, P. J., Follows, M., Dunhill, P., & Lilly, M. D. (1971). Release of protein from baker's yeast (*Saccharomyces cerevisiae*) by disruption in an industrial homogenizer. *Transactions of the Institution of Chemical Engineers*, *49*, 142–148.

Hettwer, D. J., & Wang, H. Y. (1989). Protein release from *Escherichia coli* cells permeabilized with guanidine-HCl and Triton X-100. *Biotechnology and Bioengineering*, *33*, 886–895.

Honig, W., & Kula, M. R. (1976). Selectivity of protein precipitation with polyethylene glycol fractions of various molecular weights. *Analytical Biochemistry*, *72*, 502–512.

Huang, R. -B., Andrews, B. A., & Asenjo, J. A. (1991). Differential product release (DPR) of proteins from yeast: a new technique for selective product recovery from microbial cells. *Biotechnology and Bioengineering*, *38*(9), 977–985.

Huddlestone, J., Veide, A., Kohler, K., Flanagan, J., En-fors, S. -O., & Lyddiatt, A. (1991). The molecular basis of partitioning in aqueous two-phase systems. *Trends in Biotechnology*, *9*, 381–388.

Hughes, D. E., Wimpenny, J. W. T., & Lloyd, D. (1971). The disintegration of microorganisms. In J. R. Norris, & D. W. Ribbons (Eds.), *Methods in Microbiology* (pp. 1–54). (Vol. 5B). London: Academic Press.

Hustedt, H., Kroner, K. -H., & Papamichael, N. (1988). Continuous cross-current aqueous two-phase extraction of enzymes from biomass. Automated recovery in production scale. *Process Biochemistry*, *23*(5), 129–137.

Ikegami, T., Kitamoto, D., Negishi, H., Haraya, K., Matsuda, H., Nitanai, Y., Koura, N., Sano, T., & Yanagishita, H. (2003). Drastic improvement of bioethanol recovery using pervaporation separation technique employing a silicone rubber coated silicate membrane. *Journal of Chemical Technology and Biotechnology*, *78*, 1006–1010.

James, C. J., Coakley, W. T., & Hughes, D. E. (1972). Kinetics of protein release from yeast sonicated in batch and flow systems at 20 kHz. *Biotechnology and Bioengineering*, *14*(1), 33–42.

Janson, J. -C. (1984). Large scale purification—state of the art and future prospects. *Trends in Biotechnology*, *2*(2), 31–38.

Janson, J. -C., & Hedman, P. (1982). Large-scale chromatography of proteins. *Advances in Biochemical Engineering*, *25*, 43–99.

Jungbauer, A. (2013). Continuous downstream processing of biopharmaceuticals. *Trends in Biotechnology*, *31*(8), 479–492.

Karr, A. E., Gebert, W., & Wang, M. (1980). Extraction of whole fermentation broth with a Karr reciprocating plate extraction column. *Canadian Journal of Chemical Engineering*, *58*, 249–252.

Katoh, S. (1987). Scaling-up affinity chromatography. *Trends in Biotechnology*, *5*, 328–331.

Kominek, L. A. (1975). Cycloheximide production by *Streptomyces griseus;* alleviation of end-product inhibition by dialysis extraction fermentation. *Antimicrobial Agents and Chemotherapy*, *7*, 856–860.

Krijgsman, J. (1992). Product recovery in bioprocess technology. In R. O. Jenkins (Ed.), *BIOTOL Series* (pp. 247–250). Oxford: Butterworth-Heinemann.

Kroner, K. H., Hustedt, S., Granda, S., & Kula, M. R. (1978). Technical aspects of separation using aqueous two-phase systems in enzyme isolation processes. *Biotechnology and Bioengineering*, *20*, 1967–1988.

Kujawska, A., Kujawski, J., Bryjak, M., & Kujawski, W. (2015). ABE fermentation products recovery methods—a review. *Renewable and Sustainable Energy Reviews*, *48*, 648–661.

Kurane, R. (1990). Separation of biopolymers: separation of suspended solids by microbial flocculant. In D. L. Pyle (Ed.), *Separations for Biotechnology 2* (pp. 48–54). London: Elsevier.

Lacey, R. E., & Loeb, S. (1972). *Industrial Processing with Membranes*. New York: Wiley-Interscience.

Latham, W. C., Michelsen, C. B., & Edsall, G. (1967). Preparative procedure for the purification of toxoids by gel filtration. *Applied Microbiology*, *15*, 616–621.

Le, M. S., & Atkinson, T. (1985). Crossflow microfiltration for recovery of intracellular products. *Process Biochemistry*, *20*(1), 26–31.

Lee, C. -K., Chang, W. -G., & Ju, Y. -H. (1993). Air slugs entrapped cross-flow filtration of bacterial suspensions. *Biotechnology and Bioengineering*, *41*(5), 525–530.

Liu, F., Liu, L., & Feng, X. (2005). Separation of acetone-butanol-ethanol (ABE) from dilute aqueous solutions by pervaporation. *Separation Purification Technology*, *42*, 273–282.

Lo, Y. -M., Yang, S. -T., & Min, D. B. (1997). Ultrafiltration of xanthan gum fermentation broth: process and economic analyses. *Journal of Food Engineering*, *31*, 219–236.

Lockwood, L. B., & Irwin, W. E. (1964). Citric acid. In *Kirk-Othmer Encyclopedia of Chemical Technology* (pp. 524–541). (Vol. 5). New York: Wiley.

Lopez, A. M., & Hestekin, J. A. (2013). Separation of organic acids from water using ionic liquid assisted electrodialysis. *Separation and Purification Technology*, *116*, 162–169.

Lowe, C. R., & Stead, C. V. (1985). The use of reactive dyestuffs in the isolation of proteins. In M. S. Verrall (Ed.), *Discovery and Isolation of Microbial Products* (pp. 148–158). Chichester: Ellis Horwood.

Luong, J. H. T., & Nguyen, A. -L. (1992). Novel separations based on affinity interactions. *Advances in Biochemical Engineering and Biotechnology*, *47*, 137–158.

Luong, J. H. T., Nguyen, A. L., & Male, K. B. (1987). Recent developments in downstream processing based on affinity interactions. *Trends in Biotechnology*, *5*, 281–286.

Magnusson, K. E., & Edebo, L. (1976). Large-scale disintegration of micro-organisms by freeze-pressing. *Biotechnology and Bioengineering*, *18*, 975–986.

Mattiasson, B., & Kaul, R. (1986). Use of aqueous two-phase systems for recovery and purification in biotechnology. In J.A. Asenjo & J. Hong (Eds.), *Separation, Recovery, Purification in Biotechnology* (pp. 78–92). ACS., Symposium Series 314, A.C.S., Washington, D.C.

Mattiasson, B., & Ling, T. G. I. (1987). Extraction in aqueous two-phase systems for biotechnology. In M. S. Verrall, & M. J. Hudson (Eds.), *Separations for Biotechnology* (pp. 270–292). Chichester: Ellis Horwood.

McCabe, W. L., Smith, J. C., & Harriot, P. (1984). *Unit Operations in Chemical Engineering* (4th ed.). New York: McGraw-Hill.

Melling, J., & Westmacott, D. (1972). The influence of pH value and ionic strength on the ultrafiltration characteristics of a penicillinase produced by *Escherichia coli* strain W3310. *Journal of Applied Chemistry*, *22*, 951–958.

Miller, S. A. (1973). Filtration. In R. H. Perry, & C. H. Chilton (Eds.), *Chemical Engineers' Handbook*. New York: McGraw-Hill.

Mogren, H., Lindblom, M., & Hedenskog, G. (1974). Mechanical disintegration of micro-organisms in an industrial homogenizer. *Biotechnology and Bioengineering*, *16*, 261–274.

Mohan, R. R., & Li, N. N. (1974). Reduction and separation of nitrate and nitrite by liquid membrane encapsulated enzymes. *Biotechnology and Bioengineering*, *16*(4), 513–523.

Mohan, R. R., & Li, N. N. (1975). Nitrate and nitrite reduction by liquid membrane-encapsulated whole cells. *Biotechnology and Bioengineering*, *17*(8), 1137–1156.

Mohan, S. B., & Lyddiatt, A. (1992). Silica based solid phases for affinity chromatography. Effect of pore size and ligand location upon biochemical productivity. *Biotechnology and Bioengineering*, *40*(5), 549–563.

Moldes, A. B., Alonso, J. L., & Parajo, J. C. (2003). Recovery of lactic acid from simultaneous saccharification and fermentation using anion exchange resins. *Bioprocess and Biosystems Engineering*, *25*, 357–363.

Moresi, M., & Sappino, F. (2000). Electrodialytic recovery of some fermentation products from model solutions: techno-economic feasibility study. *Journal of Membrane Science*, *164*, 129–140.

Mourot, P., LaFrance, M., & Oliver, M. (1989). Aseptic concentration of microbial cells by cross-flow filtration. *Process Biochemistry*, *24*(1), 3–8.

Nachman, M., Azad, A. R. M., & Bailón, P. (1992). Efficient recovery of recombinant proteins using membrane based immunoaffinity chromatography (MIC). *Biotechnology and Bioengineering*, *40*(5), 564–571.

Naglak, T. J., & Wang, H. Y. (1992). Rapid protein release from *Escherichia coli* by chemical permeabilization under fermentation conditions. *Biotechnology and Bioengineering*, *39*(7), 733–740.

Nakamura, H. (1961). Chemical separation methods for common microbes. *Journal of Biochemical and Microbiological Technology and Engineering*, *3*, 395–403.

Nakazawa, K., Shibata, M., Tanabe, K., & Yamamoto, H. (1960). Di-hydrostreptomycin. US patent 2,931,756.

Ng, M. Y. T., Tan, W. S., Abdullah, N., Ling, T. C., & Tey, B. T. (2006). Heat treatment of unclarified *Escherichia coli* homogenate improved the recovery efficiency of recombinant hepatitis B core antigen. *Journal of Virological Methods*, *137*, 134–139.

Nicholas, R. A., & Fox, J. B. (1969). Continuous chromatography apparatus. II Application. *Journal of Chromatography*, *43*, 61–65.

Niederauer, M. Q., & Glatz, G. E. (1992). Selective precipitation. *Advances in Biochemical Engineering and Biotechnology*, *47*, 159–188.

Ó Meadhra, R. (2005). Continuous crystallization strategy for the recovery of fermentation products. *Transactions of the Institute of Chemical Engineers, Part A*, *83*(A8), 1000–1008.

Omstead, D. R., Hunt, G. R., & Buckland, B. G. (1985). Commercial production of Cephamycin antibiotics. In H. W. Blanch, S. Drew, & D. I. G. Wang (Eds.), *Comprehensive Biotechnology* (pp. 187–210). (Vol. 3). New York: Pergamon.

Ostlund, C. (1986). Large-scale purification of monoclonal antibodies. *Trends in Biotechnology*, *4*(11), 288–293.

Pace, G. W., & Righelato, R. H. (1980). Production of extracellular polysaccharides. *Advances in Biochemical Engineering*, *15*, 41–70.

Pace, G. W., & Smith, I. H. (1981). Recovery of microbial polysaccharides. In *Abstracts of Communications. Second European Congress of Biotechnology, Eastbourne* (pp. 36). London: Society of Chemical Industry.

Pandey, R. C., Kalita, C. C., Gustafson, M. E., Kline, M. C., Leidhecker, M. E., & Ross, J. T. (1985). Process developments in the isolation of Largomycin F-II, a chemoprotein antitumor antibiotic. In D. LeRoith, J. Shiloach, & T. J. Leahy (Eds.), *Purification of Fermentation Products* (pp 133–153). ACS Symposium Series 271. ACS, Washington, DC.

Perry, R. H., & Green, D. W. (1984). *Perry's Chemical Engineers' Handbook* (6th ed.). New York: McGraw-Hill.

Podbielniak, W. J., Kaiser, H. R., & Ziegenhorn, G. J. (1970). Centrifugal solvent extraction. In *The History of Penicillin Production. Chemical Engineering Progress Symposium*, *66*(100), 44–50.

Porath, j (1974). Affinity techniques—general methods and coupling procedures. In W. B. Jakoby, & M. Wilchek (Eds.), *Methods in Enzymology* (pp. 13–30). (Vol. 34). New York: Academic Press.

Purchas, D. B. (1971). *Industrial Filtration of Liquids* (2nd ed.). London: Leonard Hill, pp. 209.

Pyle, D.L. (Ed.), (1990). *Separations for Biotechnology 2*, SCI/Elsevier Applied Science, London.

Queener, S., & Swartz, R. W. (1979). Penicillins: biosynthetic and semisynthetic. In A. H. Rose (Ed.), *Secondary Products of Metabolism, Economic Microbiology* (pp. 35–122). (Vol. 3). London: Academic Press.

Qureshi, N., & Blaschek, H. P. (2001). Recovery of butanol from fermentation broth by gas stripping. *Renewable Energy*, *22*, 557–564.

Rehacek, J., & Schaefer, J. (1977). Disintegration of microorganisms in an industrial horizontal mill of novel design. *Biotechnology and Bioengineering*, *19*, 1523–1534.

Ricketts, R. T., Lebherz, W. B., Klein, F., Gustafson, M. E., & Flickinger, M. C. (1985). Application, sterilisation, and decontamination of ultrafiltration systems for large scale production of biologicals. In D. LeRoith, J. Shiloach, T. J. Leahy (Eds.), *Purification of Fermentation Products* (pp. 21–49). ACS., Symposium Series, 271, ACS, Washington, D.C.

Rincon, J., Fuertes, J., Rodriguez, J. F., Rodriguez, L., & Monteagudo, J. M. (1997). Selection of a cation exchange resin to produce lactic acid solutions from whey fermentation broths. *Solvent Extraction and Ion Exchange*, *15*(2), 329–345.

Roffler, S. R., Blanch, H. W., & Wilke, C. R. (1984). *In situ* recovery of fermentation products. *Trends in Biotechnology*, *2*(5), 129–136.

Rubin, A. J., Cassel, E. A., Henderson, O., Johnson, J. D., & Lamb, J. C. (1966). Microflotation: new low gas-flow rate foam separation technique for bacteria and algae. *Biotechnology and Bioengineering*, *8*, 135–151.

Samejima, H. (1972). Methods for extraction and purification. In K. Yamada, S. Kinoshita, T. Tsunoda, & K. Aida (Eds.), *The Microbial Production of Amino Acids* (pp. 227–259). Tokyo: Kodanska.

Scawen, M. D., Atkinson, A., & Darbyshire, J. (1980). Large scale enzyme purification. In R. A. Grant (Ed.), *Applied Protein Chemistry* (pp. 281–324). London: Applied Science Publishers.

Shi, Z., Zhang, C., Chen, J., & Mao, Z. (2005). Performance evaluation of acetone-butanol continuous flash extractive fermentation process. *Bioprocess and Biosystems Engineering*, *27*, 175–183.

Shojaosadaty, S. A., & Lyddiatt, A. (1987). Application of affinity HPLC to the recovery and monitoring operations in biotechnology. In M. S. Verrall, & M. J. Hudson (Eds.), *Separations for Biotechnology* (pp. 436–444). Chichester: Ellis Horwood.

Sirman, T., Pyle, D. L., & Grandison, A. S. (1990). Extraction of citric acid using a supported liquid membrane. In D. L. Pyle (Ed.), *Separations for Biotechnology 2* (pp. 245–254). London: Elsevier Applied Science.

Smith, I. H., & Pace, G. W. (1982). Recovery of microbial polysaccharides. *Journal of Chemical Technology and Biotechnology*, *32*, 119–129.

Sodeck, G., Modl, J., Kominek, J., & Salzbrunn, G. (1981). Production of citric acid according to the submerged fermentation. *Process Biochemistry*, *16*(6), 9–11.

Staniszewski, M., Kujawski, W., & Lewandowska, M. (2007). Ethanol production from whey in bioreactor with co-immobilized enzyme and yeast cells followed by pervaporative recovery of product—kinetic model predictions. *Journal of Food Engineering*, *82*, 618–625.

Straathof, A.J. J. (2011). The proportion of downstream costs in fermentative production processes. In M. Moo-Young (Ed. in chief), *Comprehensive Biotechnology, Vol. 2,* (2nd ed.) (pp. 811–814). Oxford: Elsevier.

Strathmann, H. (1985). Membranes and membrane processes in biotechnology. *Trends in Biotechnology*, *3*(5), 112–118.

Strathmann, H. (1991). Fundamentals of membrane separation processes. In C.A. Costa, & J.S. Cabrai (Eds.), *Chromatographic and Membrane Processes in Biotechnology* (pp. 153–175). NATO ASI Series, Dordrecht, The Netherlands: Kluwer Academic Publishers.

Swartz, R. R. (1979). The use of economic analyses of penicillin G manufacturing costs in establishing priorities for fermentation process improvement. *Annual Report on Fermentation Processes*, *3*, 75–110.

Sylvester, J. C., & Coghill, R. D. (1954). The penicillin fermentation. In L. A. Underkofler, & R. J. Hickey (Eds.), *Industrial Fermentations* (pp. 219–263). (Vol. 2). New York: Chemical Publishing Co.

Talcott, R. M., Willus, C., & Freeman, M. P. (1980). Filtration. In *Kirk-Othmer Encyclopedia of Chemical Technology* (pp. 284–337). (Vol. 10). New York: Wiley.

Tanny, G. B., Mirelman, D., & Pistole, T. (1980). Improved filtration technique for concentrating and harvesting bacteria. *Applied and Environmental Microbiology*, *40*(2), 269–273.

Tessier, L., Bouchard, P., & Rahni, M. (2005). Separation and purification of benzylpenicillin produced by fermentation using coupled ultrafiltration and nanofiltration technologies. *Journal of Biotechnology*, *116*, 79–89.

Topiwala, H. H., & Khosrovi, B. (1978). Water recycle in biomass production processes. *Biotechnology and Bioengineering*, *20*, 73–85.

Vane, L. M. (2005). A review of pervaporation for product recovery from biomass fermentation processes. *Journal of Chemical Technology and Biotechnology*, *80*, 603–629.

Voser, W. (1982). Isolation of hydrophylic fermentation products by adsorption chromatography. *Journal of Chemical Technology and Biotechnology*, *32*, 109–118.

Walker, P. D., Narendranathan, T. J., Brown, D. C., Woolhouse, F., & Vranch, S. P. (1987). Containment of micro-organisms during fermentation and downstream processing. In M. S. Verall, & M. J. Hudson (Eds.), *Separations for Biotechnology* (pp. 467–482). Chichester: Ellis Horwood.

Wang, D. I. C. (1987). Separation for biotechnology. In M. S. Verall, & M. J. Hudson (Eds.), *Separations for Biotechnology* (pp. 30–48). Chichester: Ellis Horwood.

Wang, D. I. C., & Sinskey, A. J. (1970). Collection of microbial cells. *Advances in Applied Microbiology*, *12*, 121–152.

Wang, D. I. C., Cooney, C. L., Demain, A. L., Dunnill, P., Humphrey, A. E., & Lilly, M. D. (1979). Enzyme isolation. In *Fermentation and Enzyme Technology* (pp. 238–310). New York: Wiley.

Wang, H. Y., Kominek, L. A., & Jost, J. L. (1981). On-line extraction fermentation processes. In M. Moo-Young, C. W. Robinson, & C. Vezina (Eds.), *Advances in Biotechnology* (pp. 601–607). (Vol. 1). Toronto: Pergamon Press.

Warne, S. R., & Bowden, C. P. (1987). Biosurface properties and their significance to primary separation: a genetic engineering approach to the utilisation of bacterial auto-aggregation. In M. S. Verrall, & M. J. Hudson (Eds.), *Separations for Biotechnology* (pp. 90–104). Chichester: Ellis Horwood.

Warren, R. K., MacDonald, D. G., & Hill, G. A. (1991). Cross-flow filtration of *Saccharomyces cerevisae*. *Process Biochemistry*, *26*(6), 337–342.

Wase, D. A. J., & Patel, Y. R. (1985). Effect of cell volume on disintegration by ultrasonics. *Journal of Chemical Technology and Biotechnology*, *35*, 165–173.

Weinstein, M. J., & Wagman, G. H. (1978). *Antibiotics, isolation, separation and purification*. Amsterdam: Elsevier.

Weiss, S. A. (1980). Concentration of baboon endogenous virus in large scale production by use of hollow fibre ultrafiltration technology. *Biotechnology and Bioengineering*, *22*(1), 19–31.

Wikstrom, P., Flygare, S., Grondalen, A., & Larsson, P. O. (1987). Magnetic aqueous two phase separation: A new technique to increase rate of phase separation using dextran-ferrofluid or larger iron particles. *Analytical Biochemistry*, *167*, 331–339.

Wildfeuer, M. E. (1985). Approaches to cephalosporin C purification from fermentation broth. In D. LeRoith, J. Shiloach, & T.J. Leahy (Eds.), *Purification of fermentation products* (pp. 155–174). ACS Symposium Series 271. ACS, Washington, DC.

Wimpenny, J. W. T. (1967). Breakage of micro-organisms. *Process Biochemistry*, *2*(7), 41–44.

Wohlgemuth, R. (2011). Product recovery. In M. Moo-Young (Ed. in chief), *Comprehensive biotechnology, Vol. 2,* 2nd ed. (pp. 591–601). Oxford: Elsevier.

Wu, R. C., Ren, H. J., Xu, Y. Z., & Liu, D. (2010). The final recovery of salt from 1,3-propanadiol fermentation broth. *Separation and Purification Technology*, *73*, 122–125.

Yang, C. M., & Tsao, G. T. (1982). Affinity chromatography. *Advances in Biochemical Engineering*, *15*, 19–42.

Zahka, J., & Leahy, T.J. (1985). Practical aspects of tangential flow filtration in cell separations. In D. LeRoith, J. Shiloach, & T.J. Leahy (Eds.), Purification of fermentation products (pp. 51–69). ACS Symposium Series 271. ACS, Washington, DC.

FURTHER READING

Levandowicz, G., Bialas, W., Marcewski, B., & Szymanowska, D. (2011). Application of membrane distillation for ethanol recovery during fuel ethanol production. *Journal of Membrane Science*, *375*, 212–219.

Perry, R. H., & Chilton, G. H. (1974). *Chemical engineers' handbook* (5th ed.). New York: McGraw-Hill.

Pritchard, M., Scott, J. A., & Howell, J. A. (1990). The concentration of yeast suspensions by crossflow filtration. In D. L. Pyle (Ed.), *Separations for biotechnology 2* (pp. 65–73). London: Elsevier.

Russotti, G., Osawa, A. E., Sitrin, R. D., Buckland, B. C., Adams, W. R., & Lee, S. S. (1995). Pilot-scale harvest of recombinant yeast employing microfiltration: a case study. *Journal of Biotechnology*, *42*, 235–246.