ISOLATION AND PURIFICATION OF PROTEINS

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ADDITIONAL VOLUMES IN PREPARATION

Preface

The whole area of protein purification has come a long way from being just a laboratory practice. Since earlier times, purification has remained an essential practice to enable one to characterize the proteins by their structural and functional properties, which may be necessary, for determining their role in cells and tissues or their potential for a certain application, etc. Today, it constitutes a key segment of protein production, which is a major biotechnology industry. Hence, increasing demands for process efficiency, predictability, and economy have encouraged constant improvements in the existing separation technology, and also development of some innovations.

Although the various separation techniques used in protein purification have been dealt with in great detail in a number of books, this new book can be seen as a supplement to already existing volumes, providing an overall approach to how to tackle the task of protein purification. The reader is given an idea of where the area of separation technology started from and where it stands today. The influence of other fields such as genetic technology, biochemical engineering, polymer chemistry, computer modeling, and automation on downstream processing is also presented.

This book provides a picture of the state of the art in separation technology as applied to protein purification. The conventional techniques used for protein separation, although still being used, are treated in brief as sufficient documentation about them already exists. With those techniques as the starting point, we focus on the developments that have taken place

around them to make them more efficient and cost-effective. An important issue is the trend toward designing compact downstream processing. The possibility of doing this by introduction of a separation technique that fulfils the functions of two or more separate techniques or by integrating the different techniques into one unit operation is shown. Recombinant DNA technology has had a great impact on bringing forward the limitations of existent downstream processing. The ways in which genetic techniques can be used to simplify the purification are discussed. Information is also given on techniques available for process monitoring and possibilities for process control, which becomes a necessity for large-scale processes and for integration of different stages of a purification scheme. Finally, essential information for commercial protein production, e.g., regulatory and economic considerations, and protein formulation, is included.

The underlying theme of the book is that it may not always be necessary to follow a long and difficult path to obtain a pure protein; proper planning and a smart choice and integration of separation techniques can be used to fulfil the need for an efficient, clean, and cost-effective process.

Leading researchers from both universities and industries have contributed to the chapters forming the book. They have provided first-hand knowledge of their area of expertise.

The book will arouse the interest of scientists in both universities and industries dealing with protein production. It can also be used as a reference or textbook for teachers and students in the areas of biochemistry, microbiology, and biotechnology.

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Introduction

Isolation and purification comprise the downstream processing (DSP) stage of the manufacture of bioproducts from raw materials such as fermentation media, cell homogenates, or other biological materials. Isolation involves primary recovery operations, such as broth conditioning, clarification, cell rupture, debris and nucleic acid removal, refolding, and concentration, with the objective of obtaining the product in solution from the production system. The resulting process stream contains the target protein mixed with other proteins and also other contaminants that differ in certain physicochemical properties. These differences are exploited in the purification process, in which a sequence of high-resolution operations, invariably based on chomatography, brings the product to a specified level of purity. The degree of purification required is dictated by the ultimate application of the product. The proteins meant for the therapetic use require extremely high levels (greater than 99%) of purity while industrial enzymes are relatively crude, concentrated solutions formulated to meet actual customer requirements for activity and stability. The product purity in turn determines the cost of purification, higher purity demanding higher costs due to the larger number of processing steps required and resultant lower yields.

Downstream processing of proteins started to attract attention toward the end of the 20th century when advances in recombinant DNA technology made it possible to produce almost any protein in a host of choice. Several protein-based therapeutics including a variety of monclonal antibodies have

already reached the market, while many more are in different stages of development in biotechnology companies around the world. With the increase in industrial production of high-value proteins, it was realized that the isolation and purification accounted for a significant fraction—in some cases up to 80%—of the total costs of the production process (Bonnerjea et al. 1986; Wheelwright 1987; Curling 2001). Thus, there has been a general consensus that reducing the number of separation steps and maximizing the yield at each step are necessary for an economical process. At the same time, with growing requirements of product purity and safety, increasing attention is being given to the importance of elimination from the process stream of contaminants arising from the production host such as residual host cell proteins, viruses, endotoxins and nucleic acids; culture media; leachates from the separation media, etc.; and also of the various isoforms of the product arising from posttranslational modification, denaturation, aggregation, etc. (Kalyanpur 2002). Downstream processing has thus been challenged with demands of high yields, resolving power, and cost efficiency. This has triggered remarkable developments in improvising process tools and innovative strategies for protein separation.

This book provides a comprehensive description of the various unit operations used for protein isolation and purification. Chapter 1 details the means for disruption of cells or tissue for making the target protein accessible to purification. Particular attention is given to chromatography as it forms the backbone for high-resolving protein separations (Chapters 2, 3, 10, 11, and 12). The genetic strategies used to modify recombinant proteins so as to facilitate selective capture are presented in Chapter 4. The techniques commonly employed for removal of solids (Chapters 5 and 6) and volume reduction (Chapters 6 and 7), as well as the techniques used for direct capture of the target protein from unclarified feedstock (Chapters 9 and 10), are described. A relatively recent use of crystallization as a powerful means of purification as an alternative to chromatography is discussed (Chapter 8.) Also described are the analytical tools available for process monitoring of downstream processing (Chapter 14). Finally, even aspects related to industrial production such as scale up (Chapter 13), product formulation (Chapter 15), and regulatory considerations (Chapter 16) are examined.

1. RATIONAL DESIGN OF MULTISTAGE DOWNSTREAM PROCESSING

The complexity of the raw materials and also that of the proteins, and the latter's limited stability under various environmental conditions, make the

design of downstream processing an art. Being a multistage operation, it is worthwhile to conceive a proper downstream scheme at an early stage if large-scale purification is to be developed. Among the various separation techniques, a larger variety is available for laboratory-scale operations in which aspects of cost or recyclability of reagents are less relevant while the options for process-scale operations can be limited. A process that has been designed for small quantities may not be optimal for manufacturing large quantities. Moreoever, the procedure is difficult to change once the purification procedure is set and regulatory approval of the product is in progress. Hence, one should use in the laboratory only such techniques that can realistically be used on a large scale.

There has been a great deal of interest in the development of systematic methods for synthesis of purification. According to Wheelwright (1987), downstream process design starts at the end (i.e., by defining the product: its desired purity, application, stability, etc.). The other prerequisite is the characterization of raw material. Information on the type of material (e.g., bacterial fermentation, mammalian cell culture, transgenics), major contaminants (e.g., host cell proteins, albumin, product variants), and the presence of solid bodies (e.g., cells, membrane debris, inclusion bodies) as well as on physical properties of the product (charge and titration curves, isoelectric point, molecular weight, surface hydrophobicity, specific binding properties, thermal stability, etc.) is helpful in exploring all the separation options available, including the constraints on conditions during processing such as range of pH and temperature.

A set of heuristics has been suggested as a guide to select the individual separation steps (Wheelwright 1987). Some of the recommendations are to choose: (1) separation processes based on different physical properties rather than multiple steps based on the same property, (2) processes that exploit the greatest differences in the physical properties of the product and the impurities, (3) the step reducing the process volume significantly as an early-stage operation, thus leading to a lower cost of processing, and (4) the most expensive step toward the end, when the amount of material to be treated in significantly reduced and most of the impurities have been removed.

Attempts have been made to apply mathematical models and computer-aided design for optimizing individual operations as well as to facilitate synthesis of a whole process (Vasquez-Alvarez et al. 2001). Another significant approach has been the use of artificial intelligence tools (e.g., expert systems that operate through the manipulation of heuristic rules, algebraic equations describing behavior of proteins, and databases that contain the characteristics of protein molecules) (Asenjo and Maugeri 1992). Some examples of expert systems such as Protein Purification Advisor,

Reactivate Planning P8, FPLC Assistant (Pharmacia), and Prot_Ex has been reported (Eriksson et al. 1991a; 1991b; Leser and Asenjo 1992). For implementing an expert system, recovery and purification sections of downstream processing have been treated separately because the information and available heuristic knowledge are different in each part (Leser and Asenjo 1992). For recovery operations, Prot_Ex could use heuristic rules from literature and human experts, while an important amount of quantitative data on physiochemical properties of both the protein and the main contaminating ones was needed to make a good selection of unit operations for purification. Selection of the purification sequence could be based on the separation selection coefficient (SSC) criterion that characterizes the ability of the purification step to separate two proteins or the purity criterion that compares the final purity level obtained after a particular chromatography step (Lienqueo et al. 1999; Lienqueo and Asenjo 2000).

The suitability of each separation step—both individually and in conjunction with the other steps—must be determined, so as to take into account the whole process rather than focusing on refining an isolated step. The possibility of process integration without affecting product purity and safety may also be evaluated. In this direction, one more recent approach has been to introduce certain selectivity (e.g. pseudoaffinity) early in the separation train, in order to reduce volume and remove a large fraction of impurities, while more high resolving techniques can then be applied to the smaller volumes left for processing (Kaul and Mattiasson 1992; Hatti-Kaul and Mattiasson 2001).

2. PRE-DOWNSTREAM FACTORS INFLUENCING PROTEIN ISOLATION

Choice of the raw material for production of a particular protein is determined by many factors, but is currently influenced greatly by recombinant DNA technology. Mammalian cells, *Escherichia coli*, and yeast are the main heterologous hosts for recombinant proteins, while transgenic animals and plants present attractive alternatives for reducing the upstream costs of protein production (Curling 2001; Mison and Curling 2000; Kalyanpur 2002).

Upstream processing and bioreactor conditions influence the DSP significantly and must be taken into consideration during process design. The host organism used often determines the choice of solid–liquid separation method (Chapter 5) and eventual cell lysis method (Chapter 1) to be used. Extra- /intracellular or periplasmic location of the target product, quantity of the product formed, and formation of inclusion bodies are

also considerations dependent on the host. Excretion of proteins into extracellular medium or even periplasm is often preferred so as to obviate the harsh cell disruption step, and also due to fewer contaminants that need to be dealt with during purification. On the other hand, expression of recombinant proteins as inclusion bodies in *E. coli* cells is rather commonplace, with the advantage that the protein is quite pure. The recovery of the product in active form, however, is often complicated, requiring solubilization of the aggregated protein under denaturing conditions followed by refolding of the solubilized protein that usually results in low yields of the active protein. Alternative solubilization methods that do not rely on high denaturant concentrations, and development of systems offering efficient and scalable refolding, would benefit industrial implementation of these processes (Hart et al. 1994; De Bernardez Clark 2001; Lee et al. 2001).

The nature of the medium used for fermentation has an effect on the growth form of many microorganisms and influences biomass removal, protein recovery, and even the effluent treatment. High viscosity of the medium will pose problems during biomass removal and so does a rich medium that promotes the formation of colloids.

In view of the above considerations, a strategy adopted by many industries is to express the protein in a standard production host for which cultivation conditions and the nature of contaminants are well characterized. Furthermore, a genetically fused purification tag is often used to distinguish the target protein from the contaminants and thus facilitate selective purification. A number of companies are in the business of providing affinity tag expression/purification systems (Constans 2002b).

3. FROM DISCRETE UNIT OPERATIONS TO INTEGRATED PROCESSING

Downstream processing of a protein has traditionally been planned with the aim of chromatography being the ultimate purification stage. Since chromatography in a packed bed has been the norm, a prerequisite is to process the crude feedstock during the isolation stage to yield a protein preparation in a clear and concentrated form. Developing the isolation stage of downstream processing normally involves choosing between alternative operations available (e.g., between high-pressure homogenization and bead milling for cell disruption, centrifugation and filtration for cell separation, and precipitation and ultrafiltration for concentration). On the other hand, design of the purification stage involves setting up an optimal chromatographic sequence with maximum yield. While size exclusion chromatography is used mainly as a polishing step for removal of aggregates, desalting,

and buffer exhange, process scale purification relies on adsorption chromatography in which the separation principles commonly used are ion exchange, hydrophobic interaction, and affinity. Screening of the chromatograhic media is often complicated by the numerous products commercially available (Rathore 2001). Besides selectivity for a particular separation, issues such as flow performance and physical and chemical stability of the matrix need to be considered during selection (Levison et al. 1997).

All the steps in a traditional downstream processing scheme are often performed as discrete packages. To avoid the losses accompanying each step and to achieve better process efficiency, the recommendation has been to introduce integrated and selective processes to fish out the target product from crude feedstock in a minimal number of downstream steps. The integrated processes could involve coupling of a capture step with the bioreactor and/or that of clarification, concentration, and preliminary purification into a single step. Selective approaches for product harvest are achieved by integrating affinity interactions with unit operations used for early stage processing. Several innovative approaches have been developed to meet the new needs (Kaul and Mattiasson 1992; Hatti-Kaul and Mattiasson 2001).

The techniques providing an integrated approach include extraction in aqueous two-phase systems (Hatti-Kaul 2000; Chapter 9) and expanded-bed adsorption chromatography (Mattiasson 1999; Chapter 10). They allow processing of whole-cell broth or homogenates to obtain a sufficiently/partially pure product without the need for individual clarification, concentration, and purification steps. Two-phase extraction has the advantage of having a capacity for high particulate loads and has also been found to be suitable for recovery of proteins from inclusion bodies and membranes (Hatti-Kaul 2000; 2001). However, expanded-bed adsorption seems to have gained favor in the biotechnology industry and its applications are facilitated by the availability of various matrices with different functionalities. The problem that still remains is the fouling of the matrix that can affect the performance for repeated use and is discouraging for process validation.

Extraction and expanded-bed adsorption are rendered more selective by further integration with affinity interactions. Similarily, selectivity has also be incorporated in other high-throughput processes, such as membrane filtration and precipitation to yield membrane affinity filtration (Chapter 6) and affinity precipitation (Chapter 7) processes, respectively. When using an affinity-based approach for "fishing" out the product from a crude material, one is of course limited to the use of affinity ligands that may lack absolute selectivity but are biologically and chemically stable.

Simultaneously, yet another development has been the application of crystallization (Chapter 8), a technique normally applied to ultrapure pro-

tein solutions to enable structural determination by X-ray crystallography, as a large-scale purification technique supported by ultrafiltration.

4. IMPROVEMENTS IN PROCESS TOOLS

Bioseparation advances have typically relied on the development of new techniques and materials. Although many of the separation principles have remained unchanged, developments have occurred in improving instrumentation for allowing rapid processing and contained operation, and also in the analytical tools with potential for on-line process monitoring.

Of significance are the developments in membrane processes that are currently used throughout downstream processing for broth clarification, product concentration, buffer exchange, desalting, and sterile filtration. New membrane materials, modules, and process designs have been developed with improvements in selectivity of membranes while maintaining their inherent high-throughput characteristics. High-performance tangential flow filtration and membrane chromatography are emerging new techniques for protein purification (van Reis and Zydney 2001; Kalyanpur 2002).

Developments have been seen in new commercial chromatography matrices based on different organic and inorganic materials. The matrix format is no longer limited to bead-shaped gels; adsorbents are being manufactured as monoliths, disks, and membranes for continous chromatography (Chapters 11 and 12). Matrices with pores in which the binding is not limited by slow process of diffusion have been made [e.g., the POROS and hybrid HyperD sorbents (Chapter 12; Schwartz et al. 2001)]. Some matrices with supermacropores capable of even separating cells have been presented (Arvidsson et al. 2002). Furthermore, different modes of operation have been worked out, such as displacement chromatography and radial flow chromatography. The latter has also led to new column designs, as increase in bed height is not necessary for scale-up.

Other chromatographic separation mechanisms have been investigated in recent years, especially with application for purification of monclonal antibodies that constitute the largest therapeutic products. Improvements have been made in hydroxyapatite chromatography, which can potentially be used for separation of two proteins with similar isoelectric point, molecular weight, and hydrophobicity (Constans 2002a). Another technique is hydrophobic charge induction chromatography, based on the use of dual-mode ligands that are designed to combine a molecular interaction supported by a mild hydrophobic association effect in the absence of salts, and when environmental pH is changed, the ligand becomes ionically

charged, resulting in desorption of the protein (Guerrier et al. 2000; 2001; Boschetti 2002).

Much attention has been given to ligand development for affinity chromatography with respect to robustness and selectivity, and also aspects related to validation. Much of the effort is being put into probing the combinatorial libraries based on a variety of chemical motifs for suitable affinity ligands (Amatschek et al. 1999; Romig et al. 1999; Teng et al. 1999; Lowe 2001; Sato et al. 2002). Combinatorial libraries containing random linear or constrained peptides, gene fragments, cDNA, and antibody libraries, presented on biological vehicles—especially bacteriophages—are widely used. From molecular models of the proteins, de novo synthesis of new mimetic chemical entities can be achieved from the molecules selected from chemical combinatorial libraries (Curling 2001). Synthetic chemical ligands (e.g., those based on triazinyl dyes) have been successfully developed for a number of proteins. Combinatorial chemical techniques are now beginning to have an impact on the discovery and design of ligands for glycoproteins, metal binding ligands for fusion peptides, etc. (Lowe 2001). A combination of the structural knowledge obtained by X-ray crystallography, NMR, or homology structures with defined or combinatorial chemical synthesis and advanced computational tools has provided a powerful route to the rational design of affinity ligands for simple protein purification.

5. PROTEIN PURIFICATION IN POST-GENOMIC ERA

As researchers attempt to understand the vast amount of genetic information, proteomics has become a major focus in the biotechnology industry since gene function is derived from the protein product it encodes. The challenge of studying proteins holistically is driving the development of analytical and preparative tools that allow the resolution and characterization of complex sets of protein mixtures in a high-throughput mode and the subsequent purification of target therapeutic protein. High-throughput processing typically involves automated instrumentation. Robotic systems for nucleic acid purification have recently been adapted for protein purification and are available from several companies, including Qiagen, Gilson, and Packard (Lesley 2001). Unlike DNA, however, protein complexity and diversity make the task of parallel processing rather difficult (Lesley 2001). On a small scale, parallel processing usually involves use of a 96-well plate format. Specialized 96-well plates clear cell debris via vacuum filtration and are also used to retain chromatography resin. Cell lysis is typically achieved using a combination of lysozyme and freeze-thaw cycles or non-ionic deter-

gents. Addition of nucleases helps to reduce viscosity and facilitate removal of cell debris at the low g forces commonly used with microtiter plates.

Parallel expression and purification of the gene products are often simplified by utilization of purification tags. At the same time, however, instrumentation in the form of a minaturized high-throughput alternative for evaluation of chromatographic functionalities and binding and wash conditions has been developed for reducing chromatography development time and costs. This involves a family of protein biochips that carry functional groups typical of those used for chromatography adsorbents and that have been designed to bind proteins and peptides from complex mixtures (Santambien et al. 2002). After washing the individual spots under selective conditions, the retained target protein and/or impurity components bound to the array are analyzed by time-of-flight mass spectrometry. Besides allowing extremely rapid determination of the most effective combination of chromatographic modes in a protein purification scheme, the technology can be used for analysis of chromatography fractions to track target and impurity protein during process-scale purification.

It is apparent that advances in materials science will acompany the transformation of genetics and biology into protein products. Nanoscale materials are predicted to have a direct impact on genomics, proteomics, and high-throughput processes. Eventual production of an identified therapeutic product will, in all likelihood, depend on the sophistication of affinity purification.

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Solid–Liquid Separation

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1. INTRODUCTION

Separation and purification of biological products present special characteristics and difficulties when compared with separation and purification of other chemical products. The main difference is related to the fact that biological products are, in general, large and unstable molecules diluted in a complex solid-liquid mixture. The first step to be performed in a separation and purification protocol is usually a solid-liquid separation. This separation step can also be used at the end of a separation and purification protocol if the product has been concentrated, e.g., by precipitation or crystallization. In this chapter, some common unit operations used for solid-liquid separation in biotechnological processes are discussed, taking into account the peculiarities of biological particles. These unit operations are centrifugal separation and cake filtration.

Centrifugal separations are processes in which a centrifugal field is applied to promote the separation of particles, drops, or bubbles from a liquid, or particles and drops from a gas. This chapter will deal with the separation of biological particles from liquid media. The use of centrifuges to promote cell separations is widespread both in laboratory and in industry. A detailed description of the theory and types of centrifuges is seen in Sec. 2. This includes an item on separation efficiency that provides the theoretical basis for the understanding of centrifuge performance. In spite of the emergence of new separation methods, such as ultrasound-enhanced sedimentation (Hawkes et al. 1997) and dielectrophoresis separation (Pethig

and Markx 1997), it is expected that centrifuges will continue to play an important role in the separation of bioparticles in the near future.

Hydrocyclones also use centrifugal field to promote separation but have a great advantage over centrifuges. They have no moving parts. As such, they are extremely simple equipments. Recent works (Lübberstedt 2000a,b) have shown that they are able to separate mammalian cells. This opens the possibility of using them in perfusion systems or as a pretreatment in harvesting systems. A brief description of hydrocyclones is given in Sec. 2 of this chapter.

Conventional cake filtration is one of the oldest ways of carrying out solid-liquid separation. This is why it has been used since the beginning of the biotechnology industry. The cell high compressibility and the presence of submicrometer particles suspended in the liquid make the filtration of culture media a difficult task. The use of filter aids increases the cake permeability and porosity, facilitating the filtration of broths that would be otherwise difficult to filter. The theory of filtration, the use of filter aids and a description of the main types of filters are seen in Sec. 3 of this chapter.

The aggregation of bioparticles is an important step in many solid-liquid separation processes. Section 4 deals with flocculation, which is a common way of increasing the separability degree of suspensions with difficult settling properties or poor filterability.

Apart from two exceptions, all equations given in this chapter are dimensionally consistent. Therefore, they may be used with any coherent system of units. The two exceptions are Eqs. (46) and (52), which must be used only with the International System of Units (SI).

2. CENTRIFUGAL SEPARATION

Separation in a centrifugal field occurs only if there is a density difference between the particle and the liquid. Unfortunately, this difference is usually small when dealing with biological particles. Apart from that, their sizes are relatively small. These two facts explain why these particles attain very low terminal settling velocities when settling individually in the gravitational field, as shown in Table 1. Therefore, it is necessary to use high centrifugal accelerations and, usually, also high residence times in order to obtain high separation efficiencies of biological particles. Table 2 gives both the size and density ranges of some biological particles (Cann 1999; Datar 1984; Kildeso and Nielsen 1997; Linz et al. 1990; Lin et al. 1991; Ling et al. 1997; Pons and Vivier 1998; Bendixen and Rickwood 1994; Yuan et al. 1996b; Taylor et al. 1986; Werning and Voss, 1993), and Fig. 1 shows the typical size range of

Table 1 Typical Settling Velocities of Some Biological Particles Falling in Water at 20°C Under the Gravitational Field

Biological particle	Typical Settling Velocity (mm h^{-1})
Influenza virus	0.004
Cell debris of *Escherichia coli*	0.02
Interferon-γ as inclusion body	0.4
Escherichia coli	0.2
Saccharomyces cerevisiae	7
HeLa cell	30

some biological particles and the operational range of common separation processes.

Equation (1), known as Stokes' law, gives the settling velocity v of a spherical particle settling in the laminar region (Stokes region) under the influence of a gravitational or centrifugal field.

$$v = \frac{(\rho_s - \rho)bd^2}{18\mu} \tag{1}$$

where $b = g$ for gravitational field, $b = \omega^2 r$ for centrifugal fields, ρ_s and ρ are the particle and liquid densities, d is the particle diameter, μ is the liquid

Table 2 Ranges of Typical Sizes and Densities of Some Biological Particles

Biological particle	Size (μm)	Density (g cm^{-3})
Cells	0.5–400	1.005–1.14
Bacteria	0.5–10	1.05–1.11
Yeast	3–25	1.05–1.13
Mammalian cells	8–40	1.06–1.14
Insect cells	13–30	—
Plant cells	20–400	1.05–1.09
Nuclei from mammalian cells	2.5–5.0	—
Inclusion bodies	0.4–3.0	1.03–1.18
Cell debris	0.1–5	1.01–1.20
Virus	0.02–0.2	1.09–1.39

Data from Cann 1997; Datar 1984; Kildeso and Nielsen 1997; Linz et al. 1990; Lin et al. 1991; Ling et al. 1997; Pons and Vivier 1998; Bendixen and Rickwood 1994; Yuan et al. 1996b; Taylor et al. 1986; Werning and Voss 1993).

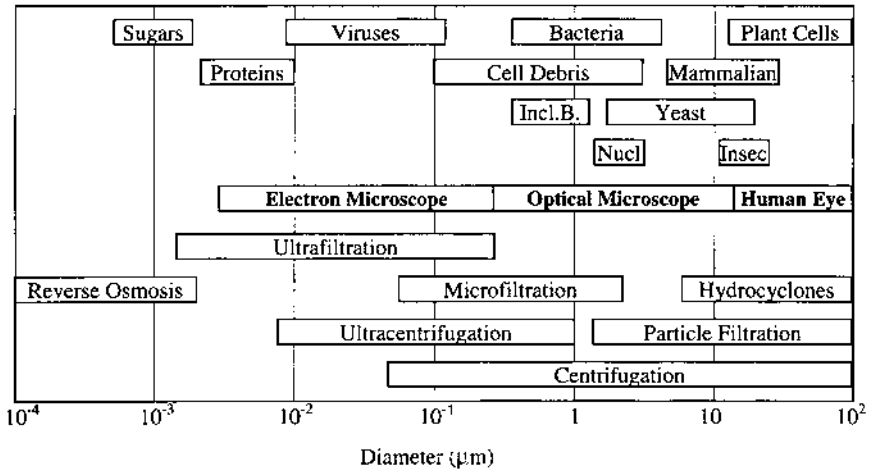

Figure 1 Typical sizes of some biological particles and the operational range of common separation processes. (Mammalian, mammalian cells; Inc.B., inclusion bodies; Nucl, mammalian cells nuclei; Insec, insect cells.)

viscosity, g is the gravity acceleration, ω is the angular velocity, and r the radial position of the particle.

Equation (1) is valid for a particle settling without the interference of other particles, i.e., for diluted systems. The particle settling velocity decreases as particle concentration increases. This phenomenon is known as *hindered settling*. Several equations can be found in the literature to account for this phenomenon. Nevertheless, a convenient method to calculate the hindered-settling velocity is by using Eq. (1) with the apparent density and viscosity of the suspension replacing the liquid density and viscosity, respectively (Heiskanen 1993).

The ratio between the settling velocity of a particle under a centrifugal field v_c and this velocity under the gravitational field v_g is known as g-factor ζ and is given by Eq. (2).

$$\zeta = \frac{v_c}{v_g} = \frac{\omega^2 r}{g} \tag{2}$$

The g factor is also known as g-number, centrifugation factor, acceleration factor, and, improperly, g force and relative centrifugal force. As Eq. (2) shows, ζ is also the ratio between the centrifugal and the gravitational accelerations. The angular velocity in Eq. (2) must be used in radians per second (units: s^{-1}). As a full revolution means 2π radians, the following

equation may be used to convert revolutions per minute (rpm) to radians per second:

$$\omega_{\text{rad/s}} = \frac{\pi \omega_{\text{rpm}}}{30} \tag{3}$$

For example, if a tubular centrifuge of 10.5 cm diameter is rotating at 15,000 rpm, from Eq. (3), its angular velocity is 1571 radians per second and, from Eq. (2), the g factor at the internal lateral wall of the bowl is 13,205. Therefore, the centrifugal acceleration at the wall is 13,205 times greater than the gravitational acceleration, i.e., $13{,}205 \times g$.

2.1 Separation Efficiency

When promoting a separation of particles from liquid, the degree of separation is an important parameter to be evaluated. This can be done through the use of some concepts as total efficiency, grade efficiency, and cut size. These concepts are applicable to any equipment whose performance remains constant if the operational conditions do not change. They are valid, therefore, for equipment such as sedimenting centrifuges, hydrocyclones, cyclones, gravitational classifiers, elutriators, and so forth.

Figure 2 shows a schematic diagram of a typical separator. Q, Q_o, and Q_u are volumetric flow rates, W_S, W_{So}, and W_{Su} are mass flow rates of solids, and y, y_o, and y_u are cumulative size distributions (undersize) of the feed, overflow and underflow, respectively.

The total efficiency E_T of a separator is defined by Eq. (4) and gives the mass fraction of solids recovered in the underflow. Authors who work with centrifuges usually refer to the fraction of unsedimented solids, which is equal to $1 - E_T$.

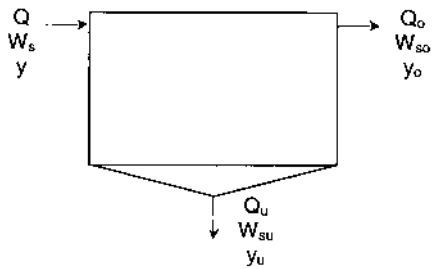

Figure 2 Schematic diagram of a typical separator.

$$E_T = \frac{W_{Su}}{W_S} \tag{4}$$

Assuming that there is no agglomeration or comminution inside the separator, a mass balance for the particles smaller than a given diameter d produces Eq. (5), which gives a relationship between the total efficiency and the particle size distributions.

$$E_T = \frac{y_o - y}{y_o - y_u} \tag{5}$$

The fraction of fluid that is discharged in the underflow is called flow ratio R_f, as given by Eq. (6).

$$R_f = \frac{Q_{Lu}}{Q_L} \tag{6}$$

where Q_{Lu} and Q_L are the volumetric flow rate of fluid in the underflow and in the feed, respectively.

Separators can operate with or without a fluid flow rate in the underflow. For instance, tubular centrifuges operate normally with $R_f = 0$ and scroll-type centrifuges with $R_f > 0$. A flow ratio equal to R_f means that this fraction of feed fluid is leaving the separator through the underflow. Since the fluid carries solid particles with it, some particles will be discharged into the underflow due not to the centrifugal action of the separator but to entrainment. In spite of some controversy regarding hydrocyclones (Frachon and Cilliers 1999; Roldán-Villsana et al. 1993; Del Villar and Finch 1992), this bypass is normally assumed to be equal to the flow ratio (Heiskanen 1993). Therefore, R_f is the minimal efficiency at which a separator will operate even if no centrifugal action takes place.

The reduced total efficiency E'_T, also called centrifugal efficiency, is used for separators with $R_f > 0$, and gives the separation efficiency taking into account only those particles that will be separated or not due to the centrifugal field intensity. Hence, E'_T does not consider the particles that are separated due to the flow ratio. The reduced total efficiency is defined by Eq. (7).

$$E'_T = \frac{E_T - R_f}{1 - R_f} \tag{7}$$

The total efficiency and the reduced total efficiency are useful when, for instance, analyzing the influence of operational conditions on a given solid-liquid separation. However, these figures only have meaning when accompanied by many other data, such as particle size distribution of the feed, densities of the solids and liquid, feed concentration of solids, feed flow rate, etc. If, however, the efficiency is calculated for every particle size pre-

sent in the feed, the resulting curve, known as grade efficiency curve, is usually independent of the solids size distribution and density and is constant for a particular set of operating conditions (Svarovsky 1990). The grade efficiency, G, gives the mass fraction of solids of a given diameter recovered in the underflow. Its definition is, thus, similar to the total efficiency but is applied to a given particle size, as shown in Eq. (8). For a better comprehension, the grade efficiency is the total efficiency that a separator would give if fed only with particles of a given size.

$$G = \frac{W_{\mathrm{Su}}\big|_{\mathrm{d}}}{W_{\mathrm{S}}\big|_{\mathrm{d}}} \tag{8}$$

As the particle size frequency x gives the fraction of particles of size d:

$$G = \frac{W_{\mathrm{Su}} x_{\mathrm{u}}}{W_{\mathrm{S}} x} \tag{9}$$

By definition, the frequency x is equal to the derivative of the cumulative size distribution y in relation to the diameter d. Therefore:

$$G = E_{\mathrm{T}} \frac{dy_{\mathrm{u}}}{dy} \tag{10}$$

Based on the mass balance given by Eq. (5), it is possible to obtain expressions similar to Eq. (10) based on y_{o} and y or on y_{o} and y_{u}.

$$G = 1 - (1 - E_{\mathrm{T}}) \frac{dy_{\mathrm{o}}}{dy} \tag{11}$$

$$\frac{1}{G} = 1 + \left(\frac{1}{E_{\mathrm{T}}} - 1 \right) \frac{dy_{\mathrm{o}}}{dy_{\mathrm{u}}} \tag{12}$$

Equations (10), (11), and (12) show that it is possible to obtain the grade efficiency curve based in the total efficiency and in two of the three size distributions.

Figure 3a shows a typical grade efficiency curve for a tubular centrifuge working without flow ratio. This curve is also known as partition or classification curve. The particle size, which corresponds to a grade efficiency of 50%, is known as cut size, d_{50}. A typical grade efficiency curve for a scroll-type centrifuge working with a 10% flow ratio is shown in Fig. 3b. As can be seen, the curve starts at the flow ratio value because the very fine particles present in the feed follow the flow and are therefore split in the same ratio as the fluid.

Similar to the total efficiency, the grade efficiency can also be reduced, producing the reduced grade efficiency G' as given by Eq. (13). Like the reduced total efficiency, G' does not consider the particles that are only

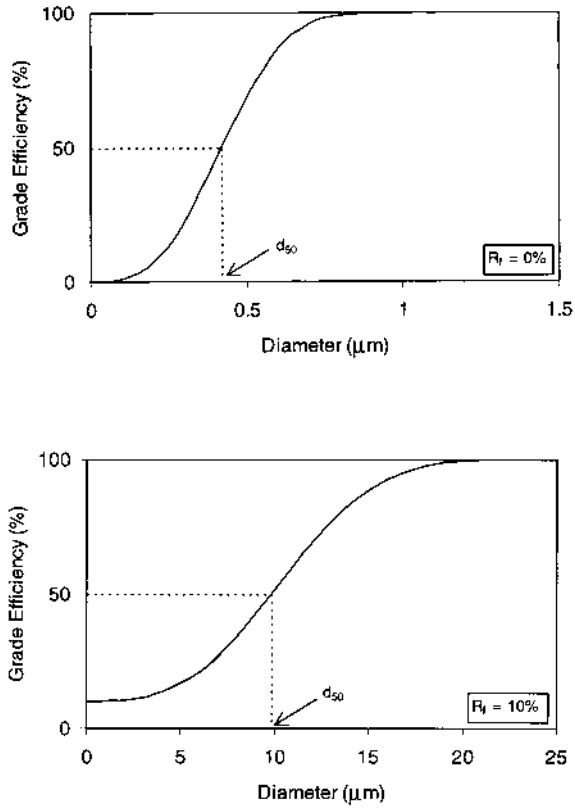

Figure 3 A typical grade efficiency curve for (a) a tubular centrifuge and (b) a scroll-type centrifuge, showing the cut size (d_{50}).

separated due to the flow ratio. A typical reduced grade efficiency curve is shown in Fig. 4. This curve was plotted based on the data from Fig. 3b. In analogy with the grade efficiency curve, the particle size that corresponds to $G' = 50\%$ is known as reduced cut size d'_{50}.

$$G' = \frac{G - R_f}{1 - R_f} \tag{13}$$

The grade efficiency curve (when $R_f = 0$) and the reduced grade efficiency curve (when $R_f > 0$) are usually presented as a function of d/d_{50} or d/d'_{50}, respectively (see an example in Fig. 5). Grade efficiency curves and reduced grade efficiency curves usually have an S shape for equipment that uses either screening (cake filters, microfilters, etc.) or particle dynamics in

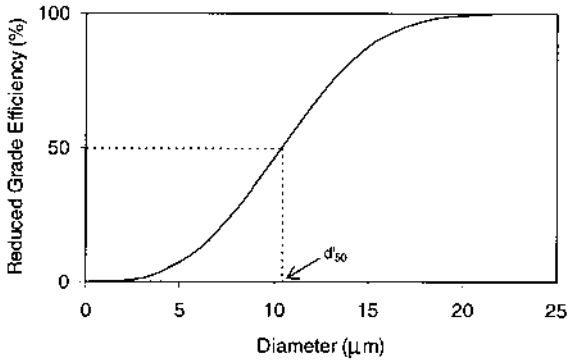

Figure 4 A typical reduced-grade efficiency curve and the reduced cut size (d'_{50}). This plot was based on data from Fig. 3b.

which body forces acting on the particles, such as gravity or centrifugal forces, are opposed by drag forces (elutriators, centrifuges, hydrocyclones, etc.) (Svarovsky 1990).

Equation (14), which can be obtained from Eq. (10), shows that it is possible to estimate the total efficiency when the grade efficiency curve and the feed size distribution are known.

$$E_T = \int_0^1 G\, dy \tag{14}$$

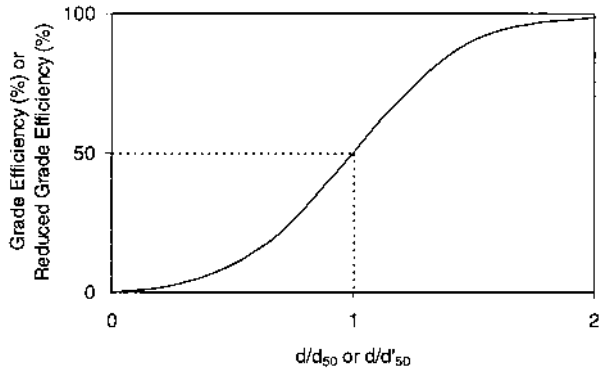

Figure 5 A typical grade efficiency curve as a function of the ratio between particle size and cut size (d/d_{50}). This S-shaped curve is usually found also for the reduced grade efficiency as a function of the ratio between particle size and reduced cut size (d/d'_{50}).

Based on Eqs. (7) and (14), a similar equation can be obtained for the reduced total efficiency:

$$E'_T = \int_0^1 G' dy \tag{15}$$

2.2 Centrifuges

Centrifugation is an important tool in the biotechnology industry. It is used for separation of whole cells, cell debris, protein precipitates, blood plasma fractionation, etc. In the laboratory, centrifuges are used in similar applications and also in some others, such as density gradient centrifugation (Patel et al. 1998) and centrifugal elutriation (Wilton and Strain 1998). An alternative application is the use of centrifuges in liquid-liquid extraction, where they may have several advantages over more classical methods (Ersson et al. 1998).

There are basically two families of centrifuges: sedimenting centrifuges and filtering centrifuges. The former uses centrifugal force to move the particles radially either outward or inward through a liquid, according to whether they are heavier or lighter than the suspending liquid. The principle of separation of these centrifuges is, therefore, sedimentation in a centrifugal field and, as such, they will be seen in this item. The latter, also known as centrifugal filter, uses the centrifugal field to promote the necessary pressure to force the mother liquor through both the filter media and the cake. The principles of filtration will be seen in item 3.

Types of Centrifuges

Some characteristics of the main industrial sedimenting centrifuges are shown in Table 3. For comparison, the data for ultracentrifuges are also included. Three different types of bowl can be seen in Fig. 6: the tubular bowl, the multichamber bowl, and the disk bowl.

Tubular Centrifuge. Figure 7 shows an industrial tubular centrifuge used for pharmaceutical and biotechnology applications. Tubular centrifuges use tubular bowls (see Fig. 6) with length-to-diameter ratios varying from 5 to 7 and diameters usually varying from 75 to 150 mm. They are the most efficient of all industrial sedimenting centrifuges due to their high angular velocities and thin settling zone. They can be used for separation of whole cells, cell debris, protein precipitates, and also for plasma fractionation, and polishing of solutions containing fines. In their laboratory version, speeds up to 50,000 rpm can be achieved leading to g

Table 3 Characteristics of the Main Industrial Centrifuges[a]

Type of centrifuge	*g* factor	Velocity (rpm)	Flow rate ($m^3\ h^{-1}$)	Conc. (% vol)	Solids removal[b]	Dewatering capacity[c]
Tubular	13000–20000	13000–18000	0.5–4	0–1	B	P
Multi-chamber	6000–11500	5000–10000	2–15	0–5	B	P
Disk	5000–13000	3000–15000	0.3–300	0–25	B, I, C	TS, TkS, P[d]
Scroll	1500–5000	1500–6000	0.5–120	3–60	C	TkS-P
Ultra-centrifuges	4×10^4–10^6	2×10^4–10^5	—	—	B	—

[a] For comparison, data for ultracentrifuges are also included.
[b] B = batch; I, intermittent; C, continuous.
[c] P, paste; TS, thin slurry; TkS, thick slurry.
[d] TS, nozzle type; TkS, solids-ejecting type; P, solid bowl type.

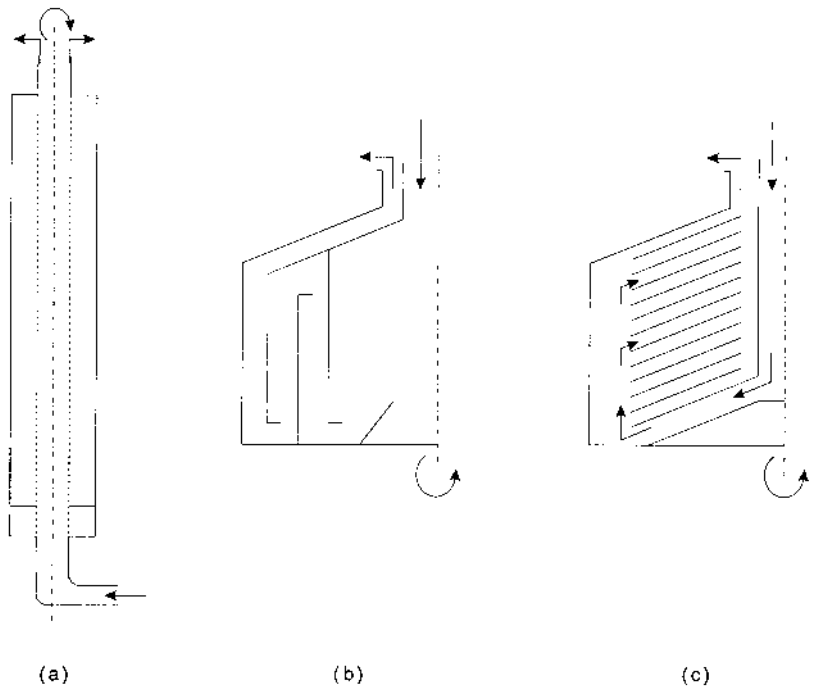

Figure 6 Three different types of bowls used in centrifuges: tubular bowl, multi-chamber bowl, and disk bowl.

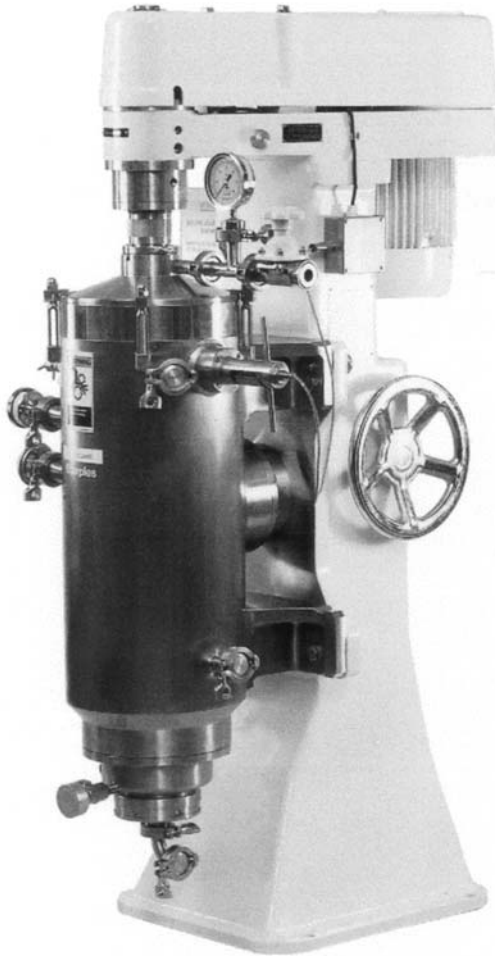

Figure 7 A Sharples supercentrifuge used for pharmaceutical and biotechnology applications. (Courtesy of Alfa Laval Separation AB, Sweden.)

factors as high as 62,000. Their main disadvantage is the small sludge holding space (2–10 L for the industrial version and maximum of 200 ml for the lab version), which makes their utilization viable only for dilute suspensions (concentrations lower than 1% by volume). Another disadvantage is that the separated solids must be manually removed.

Multichamber Centrifuge. As shown in Fig. 8, multichamber centrifuges have a certain number of concentric vertical compartments con-

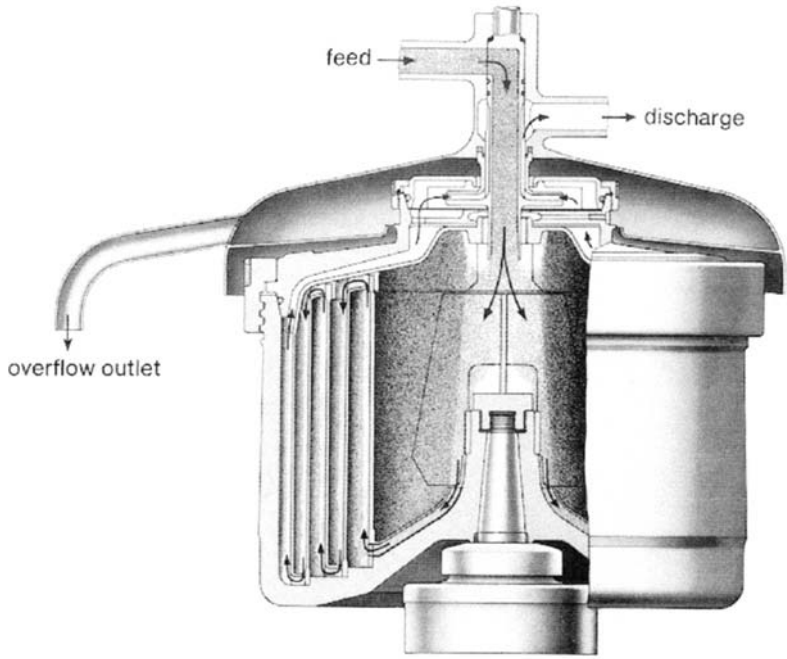

Figure 8 Multichamber centrifuge, vertical cut. (Courtesy of Westfalia Separator AG, Germany.)

nected in such a way that the suspension, which enters in the center, is forced to flow in series through the annuli between the cylinders. Thus, clarification occurs in the thin, axially flowing cylindrical layer of liquid. The thickness of the layers in the chambers decreases the further they are from the center, which implies a decreasing settling distance from the central chamber outwards. Since the centrifugal acceleration is directly proportional to the radius, the centrifugal force rises from the central chamber outward. Therefore, the coarser particles will be separated in the inner chambers and the finer particles in the outer (Hemfort 1984). These centrifuges use the same principle as the tubular ones but present the advantage of having a larger solids holding capacity of up to 65 L. They have usually two to six chambers, and their diameters range from 125 to 530 mm, with a length-to-diameter ratio of about 1. Their disadvantage is the relatively time-consuming process of solids removal. They are used for polishing liquids in the beverage, chemical, and pharmaceutical industries, and some machines can be refrigerated for special applications, such as human blood fractionating.

Disk-Stack Centrifuges. In a disk-stack centrifuge, the settling capacity is increased by means of a stack of conical disks, as shown in Fig. 6. A disk can be seen in Fig. 9. Both the bowl and the disk stack rotate at the same speed. The liquid is fed to the equipment through the center, flows underneath the disk stack, gaining angular velocity, and is split into many thin layers as it passes through the spaces between the disks, toward the overflow annulus at the top center. The centrifugal action makes the particles to move radially outward until they reach the upper conical surface of the individual separating space between two disks. Once settled, the particles slide down in a cohesive layer toward the disk periphery and then into the bowl sludge space. Typical diameters of these centrifuges are between 140 and 1000 mm, and two adjacent disks are usually 0.3–2 mm apart (Hemfort 1984). Disk-stack centrifuges are versatile devices and find application in the dairy, starch, food, and pharmaceutical industries.

There are basically three types of disk-stack centrifuges and they are related to the method of solids removal. These are the solid bowl type, the

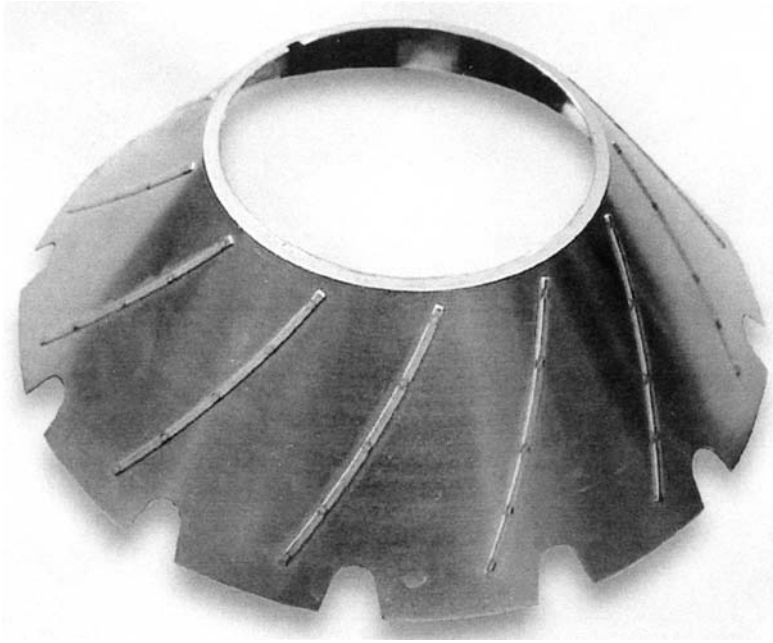

Figure 9 A disk with slanted caulks from a disk-stack centrifuge. (Courtesy of Alfa Laval Separation AB, Sweden.)

solids-ejecting type, and the nozzle type. In the solid bowl type, like the one shown in Fig. 6, the solids settle on the wall of the bowl and must be removed manually. Since the solids-holding capacity of these centrifuges is usually 5–20 L, their application is limited to suspensions with low concentrations (less than 1% by volume).

Figure 10 shows a solids-ejecting centrifuge. The intermittent solids ejection is achieved with an automated, periodic partial discharge of the sediment (Axelsson 1999). They can handle suspensions with concentrations up to 25% in volume. Solids-ejecting centrifuges are normally preferred for cell debris removal or for classifying centrifugation to separate inclusion bodies from cell debris. As inclusion bodies usually have higher settling velocities, they settle before the debris. These machines have a wide application, such as in breweries to separate yeast from the fermented broth, in the

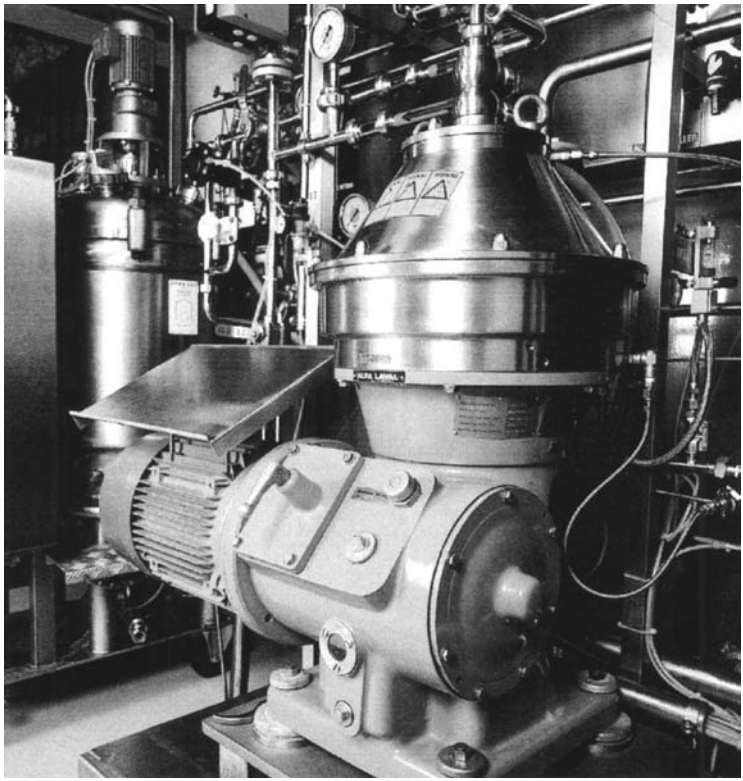

Figure 10 Solids-ejecting centrifuge. (Courtesy of Alfa Laval Separation AB, Sweden.)

wine industry, in antibiotic and vaccine production, and in the recovery of bioproducts obtained from manipulated cells.

In the nozzle type, a concentrated suspension is continuously discharged through nozzles, as shown in Fig. 11. They are, therefore, fully continuous machines. The nozzle diameters range from 0.5 to 3 mm, the number of nozzles ranges from 4 in small bowls to 20 in large ones, and the underflow-to-throughput ratio varies from 5% to 50%. As the feed liquid splits in overflow (clarified) and underflow (concentrated), the concepts of reduced total efficiency and reduced grade efficiency may be used here. Nozzle-type machines can process feed suspensions with high concentrations of up to 30% by volume. Nozzle centrifuges are used, for instance, in alcohol distilleries that employ the batchwise Melle-Boinot process, in baker's yeast production, in rDNA yeast processes, and in citric acid production.

Bacteria are normally harvested using disk-stack centrifuges. The dry solid contents by weight achieved by these centrifuges are usually in the 10–15% range for solids-ejecting types and 5–10% for nozzle types. If the bacteria can be flocculated, the fermented broth can be preconcentrated in a

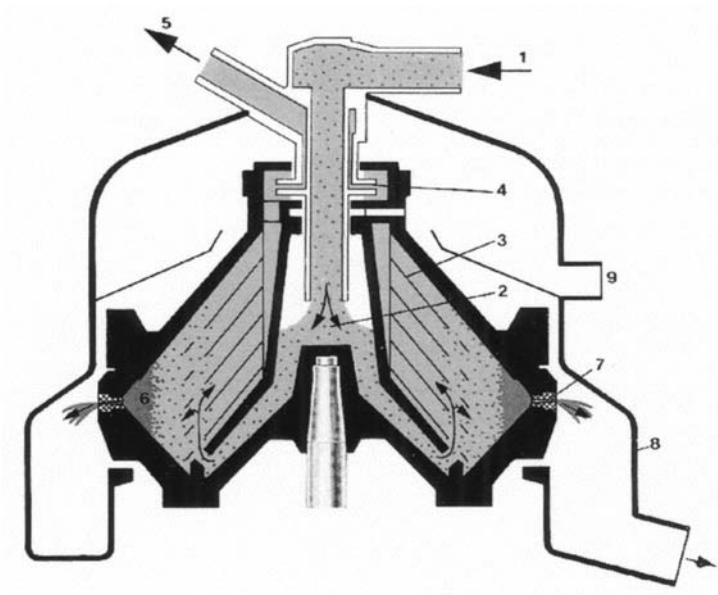

Figure 11 Nozzle type centrifuge. 1 feed, 2 inlet chamber, 3 disks, 4 centripetal pump, 5 clarified liquid discharge, 6 compaction zone, 7 nozzles, 8 concentrate catcher, 9 hood overflow. (Courtesy of Westfalia Separator AG, Germany.)

nozzle centrifuge followed by a further concentration in a scroll-type centrifuge. In this case, dry solids of 25–35% can be obtained.

Scroll-Type Centrifuges. Figure 12 shows a scroll-type machine, also known as a decanter centrifuge. It is equipped with a screw conveyor that continuously discharges the deposited solids. The bowl and the screw conveyor rotate in the same direction, but the latter rotates with a velocity slightly lower or higher than the former. This speed difference is usually 5–100 rpm (Svarovsky 1990). The bowl diameters range from 150 to 1200 mm, and the length/diameter ratios are usually 4–5. The scroll-type machine was developed to deal with large quantities of solids; therefore, they can handle suspensions with concentrations in the 3–60% range. Their conventional mechanical design limits the *g* factor to a maximum of 5000. Thus, their application in biotechnology is limited to the dewatering of biological sludges. Alfa Laval, a well-known centrifuges producer, has claimed that due to design innovations they were able to achieve *g* factors as high as 10,000. These innovations include suspended main bearings, a gear box dynamically decoupled from the bowl, and a swimming conveyor.

New Types of Centrifuges. Shear stress is always present in centrifugal separations in a relatively high degree. This poses a problem when dealing for instance with mammalian cells, which are shear sensitive. The Centritech centrifuge was developed aiming at shear stress minimization. It uses a sterilized and flexible plastic bag that is placed in a rotor and a "principle of the inverted comma" that requires no seals between rotating and no rotating parts. The operational *g* factor is only 100, which is enough to separate mammalian cells in these machines. The feed enters at one top end of the plastic bag and the overflow exits at the other top end. The cell-concentrated suspension is withdrawn intermittently through the

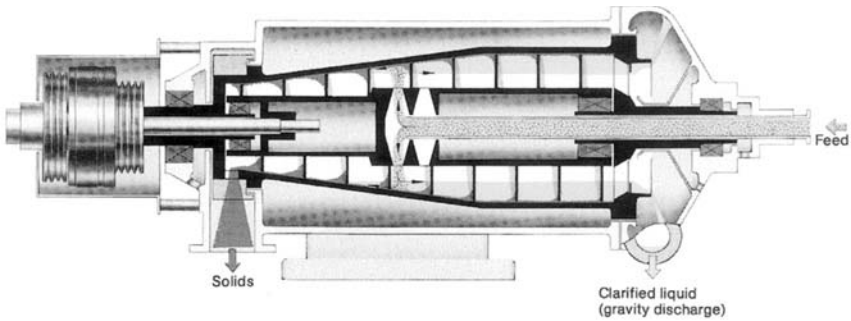

Figure 12 Decanter centrifuge. (Courtesy of Westfalia Separator AG, Germany.)

underflow pipe situated at the bottom end of the plastic bag. It has been reported that a high viable cell concentration of around 1.4×10^7 cells ml^{-1} could be achieved in a 50-L bioreactor performing perfusion culture of CHO cells (Apelman and Björling 1991). In another work, the growth rates and monoclonal antibody production were comparable to those obtained with a filtration-perfusion system when operating the centrifuge in an intermittent fashion (Johnson et al. 1996).

Another new design is the inverted chamber bowl used in the Powerfuge. These centrifuges operate with an intermittent solids discharge and are available in four different sizes, with bowl diameters varying from 150 to 600 mm. They can process from 0.01 to 6 $m^3\ h^{-1}$ of feed suspension and have a solids holding capacity from 0.9 to 60 L. The suspension is fed in the top of the machine and the clarified liquid is discharged through a centrate port. The separated solids are periodically removed during a fully automated scraping cycle. These centrifuges can operate with g factors of up to 20,000, except the largest size that can achieve $15{,}000 \times g$. The main applications of the Powerfuge are cell separation, blood plasma fractionation, and recovery of vaccines.

Grade Efficiency and Cut Size for Tubular Centrifuges

In order to derive an expression for the grade efficiency of tubular centrifuges, the following assumptions are made:

- The particles are homogeneously distributed at the entrance, i.e., at the bottom ($z = 0$) of the annulus formed by the R_1 and R_2 radii (see Fig. 13).
- The particle is a sphere with a smooth surface and the interaction between particles is neglected, i.e., there is no hindered settling.
- The particles settle within the Stokes region, and the end effects can be neglected.
- The particle is separated when it reaches the lateral wall of the centrifuge.
- There is no slip between the particles and the liquid flow in the axial direction.
- The liquid rotates at the same speed as the bowl.
- The liquid velocity profile in the liquid shell is uniform, i.e., the liquid flows as plug flow.

Figure 13 shows a schematic diagram of a tubular centrifuge. If a particle entering at $r = R_1$ and $z = 0$ reaches the wall at $z = L$, it will be separated with 100% efficiency. This particle is the smallest size to be separated with 100% grade efficiency and is known as critical size d_{100}. This

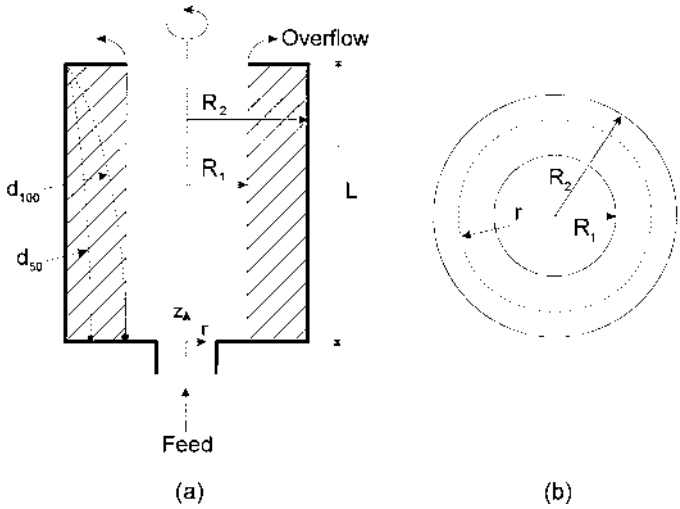

Figure 13 Schematic diagram of a tubular centrifuge showing the cut size d_{50} and the critical size d_{100}.

means that if this particle enters in any other position it will be more easily separated or that larger particles entering in $r = R_1$ and $z = 0$ will be separated before reaching $z = L$. Another interesting situation is the case of a particle that enters at the radius $r = R_{50}$ that divides in two equal parts the area of the annulus formed by R_1 and R_2. If this particle is collected in $z = L$, it will have 50% grade efficiency, since the particles of the same size that enter in $R_{50} < r \leq R_2$ will be collected and those entering in $R_1 \leq r < R_{50}$ will escape. This particle is known as cut size d_{50}. The trajectories of these two particles have been drawn in Fig. 13a. For the same reason, the particle that is collected with 20% efficiency is the one that will be collected in $z = L$ when entering the centrifuge in a position r that divides the area of the annulus in 20% between r and R_2 and 80% between R_1 and r. Therefore, the grade efficiency G of a particle of size d that is collected in $z = L$ when entering in a radius r is the fraction of the total annulus area lying between r and R_2, as given by Eq. (16).

$$G = \frac{R_2^2 - r^2}{R_2^2 - R_1^2} \tag{16}$$

The settling velocity of this particle is given by Eq. (1). As this velocity is a function of radial position, it is better written in a differential form. Therefore, replacing v by dr/dt and b by $\omega^2 r$ in Eq. (1):

$$\frac{dr}{dt} = \frac{(\rho_s - \rho)\omega^2 r d^2}{18\mu} \tag{1b}$$

The residence time t_r of this particle in the bowl is given by:

$$t_r = \frac{V_s}{Q} \tag{17}$$

where Q is the feed flow rate and V_s is the effective volume of the centrifuge, i.e., the volume of the liquid shell, as given by Eq. (18).

$$V_s = \pi\left(R_2^2 - R_1^2\right)L \tag{18}$$

Thus, this particle will have to travel the distance between r and R_2 in the available residence time. So, from Eq. (1b):

$$\int_r^{R_2} \frac{dr}{r} = \frac{(\rho_s - \rho)\omega^2 d^2}{18\mu} \int_0^{t_r} dt \tag{19}$$

Integrating Eq. (19), and replacing r and t_r as given by eqs. (16) and (17), respectively:

$$G = \frac{R_2^2}{R_2^2 - R_1^2}\left\{1 - \exp\left[-\frac{(\rho_s - \rho)\omega^2 V_s d^2}{9\mu Q}\right]\right\} \qquad \text{for } d \le d_{100} \tag{20a}$$

$$G = 1 \qquad \text{for } d > d_{100} \tag{20b}$$

The critical size d_{100} can be obtained from Eq. (20a), when $G = 1$:

$$d_{100} = \left[\frac{18\mu Q}{(\rho_s - \rho)\omega^2 V_s}\ln\left(\frac{R_2}{R_1}\right)\right]^{1/2} \tag{21}$$

And the cut size d_{50} can also be obtained from Eq. (20a), when $G = 0.5$:

$$d_{50} = \left[\frac{9\mu Q}{(\rho_s - \rho)\omega^2 V_s}\ln\left(\frac{2R_2^2}{R_2^2 + R_1^2}\right)\right]^{1/2} \tag{22}$$

From Equations (20) and (22) it is possible to obtain an expression for the curve $G(d/d_{50})$:

$$G = \frac{1}{1 - (R_1/R_2)^2}\left\{1 - \left[\frac{2}{1 + (R_1/R_2)^2}\right]^{-(d/d_{50})^2}\right\} \qquad \text{for } d \le d_{100} \tag{23a}$$

$$G = 1 \qquad \text{for d} > d_{100} \tag{23b}$$

If the size distribution of the biological particle in a given suspension and the desired total efficiency are known, the needed cut size can be obtained from Eqs. (14) and (23). Thus, the flow rate that will give this total efficiency can be obtained from Eq. (22). Unfortunately, this theoretical flow rate is always too high in comparison with the experimental value that truly gives the desired total efficiency. There are several reasons for such a deviation, and many of them are related to the initial assumptions made at the beginning of this item. Possibly, the main reason is the hypothesis of plug flow. According to Hemfort (1984), most of the liquid filling the shell seen in Fig. 13 is stationary with respect to the bowl. Only a thin cylindrical layer flows upward from the bottom and is discharged over the bowl overflow weir. This means that Eq. (17) overestimates the residence time and, consequently, the grade efficiency. A correction factor for the theoretical flow rate will be seen in the next item.

The Sigma Factor and the Scale-up of Centrifuges

The terms in Eq. (22) can be rearranged to give:

$$Q = 2\left[\frac{(\rho_s - \rho)g d_{50}^2}{18\mu}\right] \frac{\omega^2 V_s}{g \ln\left(\dfrac{2R_2^2}{R_2^2 + R_1^2}\right)} \tag{24}$$

The term inside the square brackets is the cut size settling velocity under the gravitational field, v_{g50} [see Eq. (1)], and its neighbor term is constant for a given centrifuge operating at a constant speed. Equation (24) can then be rewritten as:

$$Q = 2v_{g50}\Sigma \tag{25}$$

where Σ is known as sigma factor, machine parameter, or theoretical capacity factor. As Eq. (24) shows, Σ is a function of the centrifuge geometry and its speed. Thus, the sigma factor for tubular centrifuges is given by:

$$\Sigma_{\text{tubular}} = \frac{\pi\left(R_2^2 - R_1^2\right) L\omega^2}{g \ln\left(\dfrac{2R_2^2}{R_2^2 + R_1^2}\right)} \tag{26}$$

The sigma factor has the dimension of an area and represents the area of a settling tank capable of giving the same total efficiency under the gravitational field. That explains why it is also known as equivalent clarification area. According to Svarovsky (1990), this interpretation of Σ is false since,

due to Brownian diffusion and convection currents among others, the settling tank would hardly perform as well as a centrifuge.

Through a similar procedure, the sigma factor can also be derived for multichamber, disk-stack, and scroll-type centrifuges.

$$\Sigma_{\text{multichamber}} = \frac{\pi\omega^2 L}{3g} \sum_{\text{i=0}}^{\text{i=n}} \frac{R_{2i+2}^3 - R_{2i+1}^3}{R_{2i+2}^3 - R_{2i+1}^3} \tag{27}$$

where L is the height of the chambers, $n + 1$ is the number of chambers, and indexes of R (radius) with even and odd numbers are related to inner and outer radii of the chamber, respectively.

$$\Sigma_{\text{disc}} = \frac{2\pi\omega^2 n\left(R_2^3 - R_1^3\right)}{3g\ tg\theta} \tag{28}$$

where θ is half cone angle of the disks, n is the number of disks, and R_1 and R_2 are the minimal and maximal radii of the disk, respectively.

$$\Sigma_{\text{scroll}} = \frac{\pi\omega^2}{4g}\left[2L_1\left(3R_2^2 + R_1^2\right) + L_2\left(R_2^2 + 3R_2R_1 + 4R_1^2\right)\right] \tag{29}$$

where L_1 and L_2 are the length of the cylindrical and the conical parts of the bowl, respectively, R_1 is the inner radius of liquid and R_2 is the inner radius of the cylindrical part.

An analysis of Eq. (25) shows that, when working with the same centrifuge at the same speed (constant Σ):

$$\frac{Q_2}{Q_1} = \frac{\left(v_{g_{50}}\right)_2}{\left(v_{g_{50}}\right)_1} = \left[\frac{(d_{50})_2}{(d_{50})_1}\right]^2 \qquad \text{for constant } \Sigma \tag{30}$$

Equation (30) shows that, when working with the same suspension at the same temperature, a reduction in flow rate leads to a decrease in cut size. This generates a higher grade efficiency for each diameter present in the feed size distribution [see, for instance, Eq. (23)]. According to Eq. (14), higher grade efficiencies produce higher total efficiencies. In other words, changes in feed flow rate have a great influence in performance. For instance, if $Q_2/Q_1 = 1/4$, $(d_{50})_2/(d_{50})_1 = 1/2$. Such a reduction in cut size will produce most probably a great increase in the collected solids (or a great decrease in the fraction of unsedimented solids). How large the increase is will be a function of the feed size distribution and the grade efficiency curve. As cells usually have narrow size distributions, small reductions in cut size can produce relatively high increments in efficiency. For instance, Higgins et al. (1978) obtained an increase in separation efficiency of *Escherichia coli* from 58% to 99.5% when decreasing the flow rate to 25% of its initial value. In resume, reductions in flow rate lead to higher efficiencies. This effect can also be

understood through the following way: according to Eq. (17), a lower flow rate increases the residence time of the particles in the bowl, i.e., the particles have more time to settle.

Another way of increasing efficiency is to increase the particle settling velocity. According to equation (1), it is possible through increases in density difference, angular velocity and particle diameter (through, for instance, flocculation) or by viscosity reduction (achievable, for instance, through higher temperatures)

As mentioned at the end of last section, the theoretical flow rate calculated with Eq. (25) is always overestimated. Axelsson (1999) suggested the introduction of an efficiency factor to correct the theoretical flow rate given by Eq. (25). In his equation, instead of using v_{g50}, he uses v_{g100}, i.e., the settling velocity of the critical size d_{100}. Axelsson's equations are:

$$Q_{\text{theor}} = v_{g_{100}} \Sigma \tag{31}$$

and

$$Q_{\text{actual}} = \eta v_{g_{100}} \Sigma \tag{32}$$

The efficiency factors η suggested by Axelsson (1999) are within the ranges 0.90–0.98, 0.45–0.73, and 0.54–0.67 for tubular, disk-stack, and scroll-type centrifuges, respectively. As $(d_{50}/d_{100})^2 = 0.5$ for disk centrifuges (Svarovsky 1990), the theoretical flow rates calculated with Eqs. (25) and (31) are identical, for the same centrifuge and speed (constant Σ). That is not the case, for instance, for tubular centrifuges where, according to Eqs. (21) and (22), the ratio between the flow rates given by Eqs. (25) and (31) is a function of R_1 and R_2 and, for the same centrifuge, is usually in the 0.6–0.9 range.

Equation (25) or (31) can be used for scale-up purposes. For instance, data from a pilot centrifuge can be used to predict the flow rate of an industrial centrifuge in order to get the same performance. Equation (25) when applied to both centrifuges becomes:

Pilot centrifuge: $$Q_1 = 2\left(v_{g_{50}}\right)_1 \Sigma_1 \tag{33}$$

Industrial centrifuge: $$Q_2 = 2\left(v_{g_{50}}\right)_2 \Sigma_2 \tag{34}$$

To get the same performance, the cut size d_{50} in both centrifuges should be the same. Therefore, $(v_{g50})_1 = (v_{g50})_2$, hence:

$$\frac{Q_1}{\Sigma_1} = \frac{Q_2}{\Sigma_2} \tag{35}$$

The flow rate in the pilot machine should be adjusted to produce the desired total efficiency; then it is possible to calculate the flow rate for the industrial centrifuge, if both sigma factors are known. The same Eq. (35) can be obtained if Eq. (31) is used with the condition of both centrifuges producing the same critical size d_{100}.

Scale-up of centrifuges of the same type using Eq. (35) is fairly reliable (Svarovsky 1990), and the results are better when geometrically similar machines are compared (Axelsson 1999). These limitations are mainly due to the assumption that to produce the same performance both centrifuges should give the same cut size. This is a necessary condition, but not sufficient. As Eq. (14) shows, a way to guarantee the same total efficiency is by having the same size distribution y and the same grade efficiency G. The first condition is attended when working with the same suspension. To satisfy the second condition, not only must the cut size be the same, but the centrifuges must be geometrically similar [see Eq. (23a)]. In resume, Eq. (35) can be used to compare the performances of geometrically similar centrifuges. If this is not the case, but the centrifuges are of the same type, it can be used with caution. However, it should not be used to predict performance of machines of different types.

Since Svarovsky (1990) stated that the only way to fully describe the performance of a sedimenting centrifuge is by the grade efficiency curve, only a few groups have been using this concept (Wong et al. 1997; Maybury et al. 1998). It would also be good to see the manufacturers of centrifuges adopting this concept.

Test Tube Centrifugation

At the early stage of a bioprocess development, the amount available of biological material is usually not enough to permit tests in a pilot centrifuge. At this stage, test tube centrifugation can be carried out as a preliminary test to determine the degree of separability of a given biological particle suspension. This spin test is usually done at different settling times and speeds, and the result is one or more sets of values that give an acceptable supernatant. It also gives an indication of solids compressibility and rheology, and dry solids content after centrifugation (Axelsson 1999).

Figure 14 shows a schematic view of a swing-out test tube (bottle) centrifuge. As the settling velocity is a function of radial position, from Eq. (1b):

$$\frac{dr}{dt} = \frac{(\rho_s - \rho)\omega^2 r d^2}{18\mu} = \frac{\omega^2 r}{g} v_g \tag{1b}$$

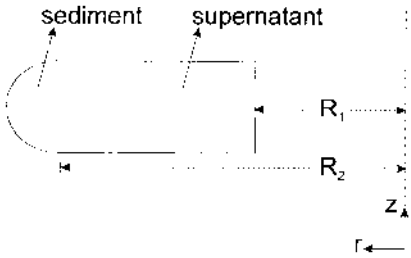

Figure 14 Schematic view of a test tube of a swing-out test tube (bottle) centrifuge.

Supposing R_2 is the radius at the sediment surface and the particles are well distributed in the test tube, a particle with cut size d_{50} that is located in the test tube surface ($r = R_1$) at the beginning of the centrifugation ($t = 0$) will have to travel half the distance between R_1 and R_2 in the centrifugation time (t_c). Thus:

$$\int_{R_1}^{(R_1+R_2)/2} \frac{dr}{r} = \frac{\omega^2}{g} v_{g_{50}} \int_0^{t_c} dt \tag{36}$$

Then:

$$v_{g_{50}} = \frac{g}{\omega^2 t_c} \ln\left(\frac{R_1 + R_2}{2R_1}\right) \tag{37}$$

From Eq. (25):

$$\frac{Q}{\Sigma} = \frac{2g}{\omega^2 t_c} \ln\left(\frac{R_1 + R_2}{2R_1}\right) \tag{38}$$

An equation based on the critical size d_{100} can also be formulated. This particle will have to travel from R_1 until R_2 in the centrifugation time; therefore, from Eq. (36):

$$v_{g_{100}} = \frac{g}{\omega^2 t_c} \ln\left(\frac{R_2}{R_1}\right) \tag{39}$$

From equation (31):

$$\frac{Q}{\Sigma} = \frac{g}{\omega^2 t_c} \ln\left(\frac{R_2}{R_1}\right) \tag{40}$$

Q can be understood here as the ratio V/t_c, where V is the volume of material in the centrifuge tube.

For the same centrifuge and speed, i.e., for the same Σ, Eq. (38) usually gives flow rates from 5% to 15% higher than Eq. (40).

A similar procedure applied to test tube centrifuge with angle head (see Fig. 15) yields equations (41) and (42) based on d_{50} and (43) and (44) based on d_{100}.

$$v_{g_{50}} = \frac{g}{\omega^2 t_c} \ln\left(1 + \frac{D}{2R_1 \cos\gamma}\right) \tag{41}$$

$$\frac{Q}{\Sigma} = \frac{2g}{\omega^2 t_c} \ln\left(1 + \frac{D}{2R_1 \cos\gamma}\right) \tag{42}$$

$$v_{g_{100}} = \frac{g}{\omega^2 t_c} \ln\left(1 + \frac{D}{R_1 \cos\gamma}\right) \tag{43}$$

$$\frac{Q}{\Sigma} = \frac{g}{\omega^2 t_c} \ln\left(1 + \frac{D}{R_1 \cos\gamma}\right) \tag{44}$$

where D is the test tube diameter and γ is angle of the bottle relative to the vertical axis.

For the same centrifuge and speed, i.e., for the same Σ, Eq. (42) usually gives flow rates from 5% to 15% higher than Eq. (44).

Maybury et al. (1998) adapted the sigma theory aiming at predicting the performance of a disk-stack centrifuge based on tests with a bench-top centrifuge. Taking into account the acceleration and deceleration stages present in the test tube centrifuge, they found a "corrected" equation similar to Eq. (38). Based on their equation, they could predict well the performance of the industrial centrifuge when treating suspensions of polyvinyl acetate.

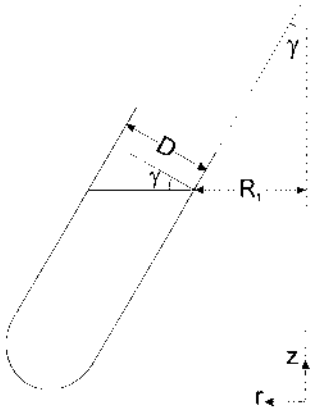

Figure 15 Schematic view of a test tube centrifuge with angle head.

For yeast cell debris, the predictions were good in the high-capacity zone of the industrial centrifuge. However, there was some evidence of overprediction for small capacities. For protein precipitates, the test tube centrifuge always overpredicted the efficiency given by the disk-stack centrifuge. This difference was attributed to the most likely shear-related damage of sensitive precipitate particles in the high shear stress regions of the disk-stack centrifuge.

2.3 Hydrocyclones

A hydrocyclone is a very simple equipment, as shown in Fig. 16. It consists of a conical section joined to a cylindrical portion, which is fitted with a tangential inlet and closed by an end plate with an axially mounted overflow pipe, also called vortex finder. The end of the cone terminates in a circular

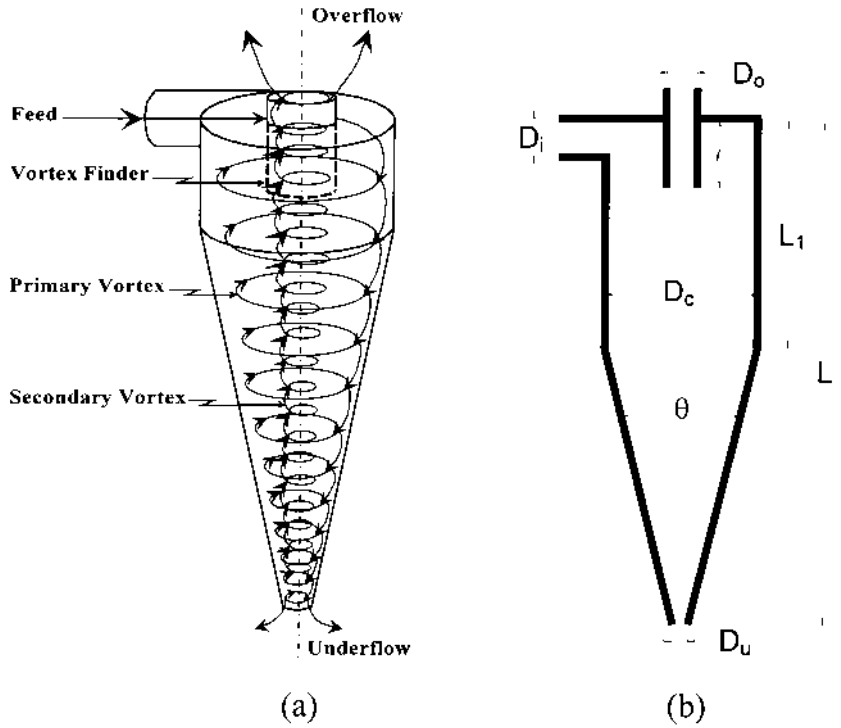

Figure 16 Perspective view of a hydrocyclone showing the internal flow (a), and a schematic view showing the main dimensions (b).

apex opening, called underflow orifice. Despite its simplicity, hydrocyclones are very efficient when promoting solid-liquid separations.

Unlike centrifuges, which use the same separation principle (sedimentation in a centrifugal field), hydrocyclones have no moving parts and the vortex motion is performed by the fluid itself. As Fig. 16a shows, the feed is introduced tangentially through the inlet duct into the upper part of the cylindrical section, acquiring a strong swirling downward movement. In hydrocyclones designed for solid-liquid separations, the underflow orifice is smaller than the overflow diameter; therefore, only a fraction of the feed liquid escapes through the underflow, carrying the coarser (or denser) particles. Most of the flow reverses its vertical direction and goes up in an even stronger vortex motion and out through the overflow pipe, carrying the smaller (or lighter) particles.

Based on correlations published in the literature and reviewed in books (Bradley 1965; Svarovsky 1984; Heiskanen 1993), it is possible to establish the effect of geometrical (see Fig. 16b) and operational variables on capacity and cut size of hydrocyclones, as shown in Table 4 (Matta and Medronho 2000). For instance, an increase in underflow diameter promotes a small increase in capacity and a relatively high reduction in cut size. As a reduction in cut size leads to an increase in total efficiency, it is possible to change the hydrocyclone efficiency by simply changing the underflow diameter, without great changes in flow rate.

Hydrocyclones were originally designed to promote solid-liquid separations, but nowadays they are also used for solid-solid, liquid-liquid, and gas-liquid separations. Potential new applications, such as cell separation, are being developed, and some works can be found in the literature.

Table 4 Effect of Increases in the Values of Geometrical and Operational Variables on Capacity and Cut Size of Conventional Hydrocyclones[a]

	D_c	D_i	D_o	D_u	L	ℓ	θ	ΔP	C_v
Capacity	+ + +	+ +	+ +	+	+	—	—	+ +	—[c]
Cut size[b]	+ +	+ +	+ +	– –	–	+	+	– –	+ +

[a] +, increase; –, reduction. D_c, hydrocyclone diameter; D_i, feed inlet diameter; D_o, overflow diameter; D_u, underflow diameter; L, hydrocyclone length; ℓ, vortex finder length; θ, angle of the hydrocyclone cone; ΔP, pressure drop; C_v, feed volumetric concentration.

[b] Reductions in cut size mean higher efficiencies and vice versa.

[c] Flow rate increases slightly with feed concentration from 0% to 3–5% and then decreases continuously.

These works refer to separation of mammalian cells (Lübberstedt et al. 2000a,b), separation of yeast either from fermented broths or from water suspensions (Rickwood et al. 1992; Yuan et al. 1996a,b; Harrison et al. 1994; Cilliers and Harrison 1996, 1997), separation of yeast from filter aids (Rickwood et al. 1996; Matta and Medronho 2000), and separation of biological sludge in wastewater treatments (Thorwest and Bohnet 1992; Ortega-Rivas and Medina-Caballero 1996; Bednarski 1996; Marschall 1997; Müller 2000).

The use of hydrocyclones for separating mammalian cells from the culture medium opens the possibility of using them to perform perfusion in bioreactors. In contrast to most solid particles, which are insensitive to shear, mammalian cells can be damaged by the relatively high levels of shear stress existing in hydrocyclones. Figure 17 shows the influence of pressure drop on the viability of HeLa cells in the underflow and overflow of three different hydrocyclones (Lübberstedt et al. 2000a). It can be seen that in the underflow of the three hydrocyclones the viability did not decrease with pressure drops up to 4 bar, and in the overflow, it started to decrease for pressure drops higher than 3.5 bar. Therefore, in spite of the relatively high values of shear stresses generated inside hydrocyclones, the cells appear to resist up to a certain limit of pressure drop. This is probably due to the extremely low average residence time of the cells inside the equipment, which lies in the range between 0.03 and 0.1 s. Since hydrocyclones are low-cost equipment and, for this application, their maintenance costs would be virtually nonexistent, their use in perfusion would decrease both capital and operational costs (Lübberstedt et al. 2000b). They could be also used, in some applications, as a pretreatment for a harvesting process.

The performance of hydrocyclones can be estimated with the following equations (Coelho and Medronho 2001):

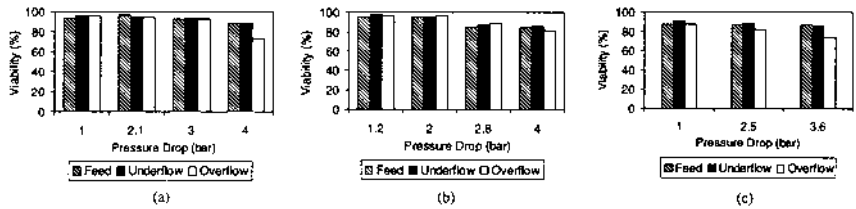

Figure 17 Influence of pressure drop on cell viability in the underflow and overflow of the following hydrocyclones: Mozley (a), Dorr-Oliver (b), and Bradley (c) with diameters of 10 mm, 10 mm, and 7 mm, respectively (Lübberstedt et al. 2000a).

$$\mathrm{Stk}_{50}\mathrm{Eu} = 0.12\left(\frac{D_c}{D_o}\right)^{0.95}\left(\frac{D_c}{L-\ell}\right)^{1.33}\left[\ell n\left(1/R_f\right)\right]^{0.79}\exp(12.0\,C_v) \tag{45}$$

$$\mathrm{Eu} = 43.5D_c^{0.57}\left(\frac{D_c}{D_i}\right)^{2.61}\left(\frac{D_c}{D_o^2+D_u^2}\right)^{0.42}\left(\frac{D_c}{L-\ell}\right)^{0.98}\mathrm{Re}^{0,12}\exp(-0.51C_v) \tag{46}$$

$$R_f = 1.18\left(\frac{D_c}{D_o}\right)^{5.97}\left(\frac{D_u}{D_c}\right)^{3.10}\mathrm{Eu}^{-0.54} \tag{47}$$

$$G' = 1-\exp\left[-0.693\left(\frac{d}{d_{50}'}\right)^3\right] \tag{48}$$

where the symbol definitions can be found in Table 4. $\mathrm{Stk}_{50}\mathrm{Eu}$ is the product between the Stokes number Stk_{50} and the Euler number Eu, Re is the Reynolds number, and R_f is the flow ratio given by Eq. (6). $\mathrm{Stk}_{50}\mathrm{Eu}$, Eu, and Re are given by Eqs. (49) to (51), respectively.

$$\mathrm{Stk}_{50}\mathrm{Eu} = \frac{\pi(\rho_s-\rho)\Delta P\,D_c\left(d_{50}'\right)^2}{36\mu\rho Q} \tag{49}$$

$$\mathrm{Eu} = \frac{\pi^2\,\Delta P D_c^4}{8\rho Q^2} \tag{50}$$

$$\mathrm{Re} = \frac{4\rho Q}{\pi\mu D_c} \tag{51}$$

Equations (45), (47), and (48) are totally based on dimensionless groups and dimensionless variables, and Eqs. (49) to (51) are the definitions of well-known dimensionless groups. Therefore, these equations can be used with any coherent system of units as, for instance, the International System of Units (SI). This is not the case for Eq. (46), where only the SI units may be employed.

Equations (45) to (48) can be used for performance prediction of hydrocyclones. For instance, the reduced cut size and the flow rate can be calculated based on Eqs. (45) and (49); and (46), (50), and (51), respectively, leading to Eqs. (52) and (53).

$$d_{50}' = \frac{1.17D_c^{0.64}}{D_o^{0.48}(L-\ell)^{0.67}}\left[\frac{\mu\rho Q}{(\rho_s-\rho)\Delta P}\right]^{0.50}\left[\ell n\left(\frac{1}{R_f}\right)\right]^{0.40}\exp(6.0C_v) \tag{52}$$

$$Q = 0.18_D c^{-0.22} D_i^{1.23} (D_o^2 + D_u^2)^{0.20} (L - \ell)^{0.46} \mu^{0.057} \rho^{-0.53} \Delta P^{0.47} \exp(0.24 C_v) \quad (53)$$

Equation (52) can be used with any coherent system of units as, for instance, the SI units. However, Eq. (53) must be used only in SI units.

If the hydrocyclone dimensions and the suspension data (liquid and particle densities, liquid viscosity, concentration of solids, and particle size distribution) are known, the hydrocyclone performance can be predicted according to the following steps:

1. Select the pressure drop to be used.
2. Calculate the flow rate using Eq. (53).
3. Calculate Eu and R_f using Eqs. (50) and (47), respectively.
4. Calculate d'_{50} using Eq. (52).
5. Calculate the predicted reduced total efficiency E'_T using the particle size distribution of the feed suspension and Eqs. (15) and (48).
6. Calculate the total efficiency E_T using Eq. (7).

Manufacturers of hydrocyclones produce only a limited range of cyclone diameters and, in order to be able to cover a wide range of cut sizes and flow rates, each cyclone of a given size can be operated with different openings sizes (inlet, overflow and underflow) through the use of interchangeable parts. This approach requires accurate knowledge of how geometrical variables affect the equipment performance. An alternative approach is to use a custom-made hydrocyclone based on a geometrically similar family. Although a hydrocyclone is a very simple apparatus to build, this approach is not widely used. A simple procedure to design two well-known families of geometrically similar hydrocyclones (Castilho and Medronho 2000) has been recently proposed.

3. FILTRATION

Filtration is the separation of most of the solids present in a solid-fluid mixture by forcing the fluid through a porous barrier known as filter medium or septum, which can be a cloth, membrane, paper, screen, or a bed of solids. The conventional filtration of biological particles suspended in a liquid will be treated in this chapter. The use of membranes for protein isolation will be seen in Chap. 7. The conventional filtration can be divided into two main types—deep-bed filtration and cake filtration—as shown in Fig. 18. In the former, the particles are smaller than the porous diameters of the filter medium. Therefore, they penetrate into the pores of the bed and are

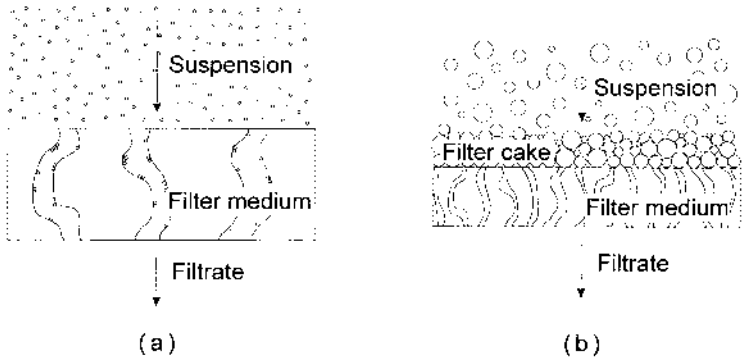

Figure 18 Schematic view of a deep-bed filtration (a) and of a cake filtration (b).

collected due to a series of mechanisms, like gravity, diffusion, and inertia (Svarovsky 1990). In cake filtration, the particles larger than the porous diameters are collected over the surface of the filter medium forming a cake, which has pores even smaller than those of the medium. Therefore, the cake acts as a porous medium for the subsequent incoming suspension, capturing even smaller particles. Cake filtration has a wider application in biotechnology than deep-bed filtration. Hence, only the former will be seen here. More detailed information on filtration can be found in more specialized books (Meltzer and Jornitz 1998; Dickenson 1997; Rushton et al. 1996; Svarovsky 1990).

Cake filtration of fermented broths is not an easy task. Biological particles are normally highly compressible. The broth usually also contains colloidal and submicrometer particles, which usually play an important role in determining the filtration properties. Therefore, the main problems of filtering fermented broths are the blinding of the filter medium due the presence of submicrometer particles and the blinding of the cake due to the microorganism's high compressibility. To overcome these problems, some powders with special properties are added to the system, improving the filtration characteristics of the original suspension. These powders are called filter aids and will be described in detail in Sec. 3.5.

If necessary, after filtration, the cake may be washed to remove liquid containing dissolved materials in order either to clean the cake from soluble contaminants or to recover product retained in the cake pores.

3.1 Theory of Filtration

In spite of filtration being a straightforward procedure, the theory underlying it is very complex. The great majority of biological solids produce

compressible cakes that deform themselves continuously under pressure. Therefore, cake porosity ε and permeability k are a function of time and position, i.e., in a given point, ε and k decrease with time, having lower values near the filter medium than away from it. Apart from that, the cake is a porous medium that grows as filtration proceeds. Hence, filtration is an unsteady problem with moving boundaries. Regarding the filter medium, its resistance can increase with time due to some penetration of fine solids into the medium. This resistance may also change with changes in pressure drop, due to the compression of the fibers that compose the medium. However, the medium resistance can always be considered approximately constant, since these small variations in resistance are negligible in comparison with the total resistance offered by the cake and medium together. Use of the conservation equations (continuity and motion equations) to solve such a problem is extremely difficult not only due to the resulting nonlinear equations but also due to the unreliability of the constitutive equations. A way to overcome these difficulties is through the simplified theory of filtration, which incorporates the following assumptions:

Plane deformation of the compressible cake.
One-directional flow of incompressible Newtonian fluid.
"Darcyan" flow, i.e., low fluid velocities.
Fluid velocities inside the cake are much larger than solid velocities; consequently, they are independent of position.
In the motion equation, the acceleration, the viscous force, and the gravitational force are negligible in comparison with the pressure force and the force the fluid exerts on the solids.
Cake porosity and permeability in a given point are a function of the pressure on the solids P_s, which is defined as the difference between the pressures in the cake face and in that point ($P_s = P_\ell - P$).

Figure 19 gives a detailed schematic view of a cake filtration. In this figure, ΔP_c and ΔP_m are the pressure drops across the cake and the filter medium, respectively, ℓ_c and ℓ_m are the cake and medium thickness, A is the filtration area, and dx is a thin slice of the cake.

The equation of motion for the fluid is given by:

$$\rho\left\{\varepsilon\frac{\partial}{\partial t}\left(\frac{\vec{q}}{\varepsilon}\right)+\left[\nabla\left(\frac{\vec{q}}{\varepsilon}\right)\right]\vec{q}\right\}=-\nabla P+\nabla\cdot\vec{\tau}+\frac{\mu}{k}\vec{q}+\rho\vec{q} \tag{54}$$

where ρ and μ are the fluid density and viscosity, respectively, ε and k are the cake porosity and permeability, respectively, t is time, q is the average fluid velocity ($q = Q/A$), Q is the volumetric flow rate, τ is the stress tensor, and g is the gravity acceleration.

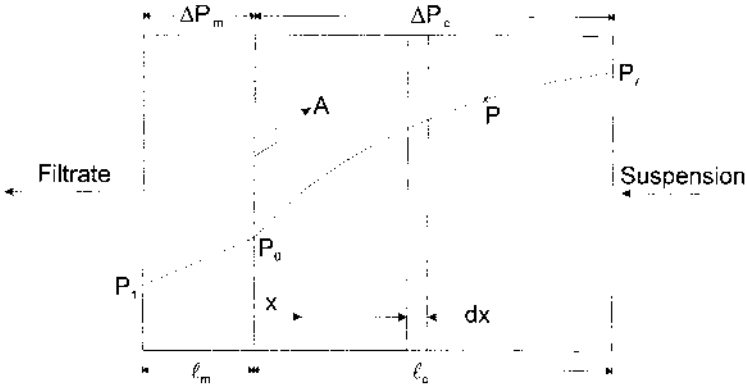

Figure 19 Detailed schematic view of a cake filtration. (Symbol definitions can be found in the text.)

Equation (55) is obtained if the assumptions listed above are applied to equation (54). This equation is known as Darcy's law.

$$\frac{dP}{dx} = \frac{\mu q}{k} \tag{55}$$

The mass of solids dm in a cake slice of thickness dx is given by equation (56), where ρ_s is the density of the solids.

$$dm = \rho_s(1-\varepsilon)\mathrm{Adx} \tag{56}$$

Replacing dx in equation (55) by equation (56), assuming that P_ℓ is constant, replacing dP by $-d(P_\ell - P)$, and integrating the resulting equation from $P = P_0$ to $P = P_\ell$:

$$\Delta P_c = \frac{\langle\alpha\rangle \mu q m}{A} \tag{57}$$

where m is the total mass of solids in the cake and $<\alpha>$ is the average specific cake resistance given by Eq. (58).

$$\langle\alpha\rangle = \frac{\Delta P_c}{\displaystyle\int_0^{\Delta P_c} \frac{dP_s}{\alpha}} \tag{58}$$

and $\alpha = [k\rho_s(1-\varepsilon)]^{-1}$ is the specific cake resistance.

Assuming that the volume of liquid entrapped in the cake is negligible in comparison with the volume of filtrate:

$$m \cong \rho c V \tag{59}$$

where V is the volume of filtrate and c is the solid concentration in the feed expressed as mass of solids by mass of liquid.

Substitution of Eq. (59) into Eq. (57) gives:

$$\Delta P_c = \frac{\langle \alpha \rangle \mu q \rho c V}{A} \tag{60}$$

Equation (61) can be obtained if Eq. (55) is applied to the filter medium and integrated from $P = P_0$ to $P = P_1$ for $x = 0$ to $x = -\ell_m$.

$$\Delta P_m = R_m \mu q \tag{61}$$

where $R_m = \ell_m / k_m$ and k_m are the resistance and permeability of the filter medium, respectively.

The total pressure drop ΔP can be obtained adding the cake pressure drop with the pressure drop in the filter medium, both expressed by Eqs. (60) and (61), respectively.

$$\Delta P = \mu q \left(\frac{\langle \alpha \rangle \rho c V}{A} + R_m \right) \tag{62}$$

but:

$$q = \frac{Q}{A} = \frac{1}{A} \frac{dV}{dt} \tag{63}$$

then:

$$\frac{dt}{dV} = \frac{\mu}{A \Delta P} \left(\frac{\langle \alpha \rangle \rho c V}{A} + R_m \right) \tag{64}$$

Equation (64) is the general filtration equation for compressible cakes. The same result is obtained if the cake is incompressible. In this case, as the specific cake resistance is constant, $< \alpha >= \alpha$.

For compressible cakes, the specific cake resistance increases with pressure drop according to the following empirical equation:

$$\langle \alpha \rangle = \alpha_o (\Delta P)^n \tag{65}$$

where α_o is the average specific cake resistance at unit applied pressure drop and n is a compressibility index, which is zero for incompressible cakes and varies between 0.3 and 1.0 for most cakes of biological particles. Table 5 gives the values of α_o and n for some microorganisms. Nakanishi et al. (1987) found that the $\langle \alpha \rangle$ value is not only a function of the microorganism size, but also more strongly of its shape. They explain this dependency as being related to the difference in porosity obtained as a function of shape.

Table 5 Values of the Average Specific Cake Resistance at Unit Applied Pressure Drop α_o and Compressibility Index n for Some Microorganisms (Nakanishi et al. 1987)

Microorganisms	Morphology	α_o at 1 bar (10^{12} m kg^{-1})	n
Baker's yeast	Elliptical	0.4	0.45
Micrococcus glutamicus	Elliptical	3.8	0.31
Bacillus circulans	Rod shaped	30.0	1.00
Rhodopseudomonas spheroides	Rod shaped	54.0	0.88
Escherichia coli	Rod shaped	670.0	0.79

3.2 Constant Pressure Filtration

Filtration is normally carried out at constant pressure, as is the case in vacuum filtration, or at constant flow rate (to be seen in the next item). If the filtration is conducted at constant pressure drop, Eq. (64) can be integrated to give:

$$\frac{t}{V} = \frac{\mu}{A\Delta P}\left(\frac{\langle\alpha\rangle \rho c V}{2A} + R_m\right) \tag{66}$$

Equation (66) is a straight line, if the experimental points of t/V are plotted against V, with the following gradient and intercept:

$$\text{Gradient} = \frac{\mu\langle\alpha\rangle\rho c}{2A^2\Delta P} \tag{67}$$

$$\text{Intercept} = \frac{\mu R_m}{A\Delta P} \tag{68}$$

The average specific cake resistance $\langle\alpha\rangle$ and the filter medium resistance R_m can, therefore, be easily calculated. Filtration at the desired pressure drop should be carried out and the volumes of filtrate at different times measured. With the experimental values of t and V, the values of t/V should be calculated; then a linear regression should be done using t/V as the dependent variable and V as the independent variable. The values of $\langle\alpha\rangle$ and R_m could be then calculated using Eqs. (67) and (68). Figure 20 shows a plot of t/V against V for the filtration of a pectinase extract from fermented solids (solid-state fermentation of wheat bran by *Aspergillus niger*). The filtration was conducted (Ferreira et al. 1995) in a vacuum filter leaf with an area of 78.5 cm^2, under a pressure drop of 0.6 bar, and using perlite as filter aid (precoat-bodyfeed method, as described in Sec. 3.5). The solids concentra-

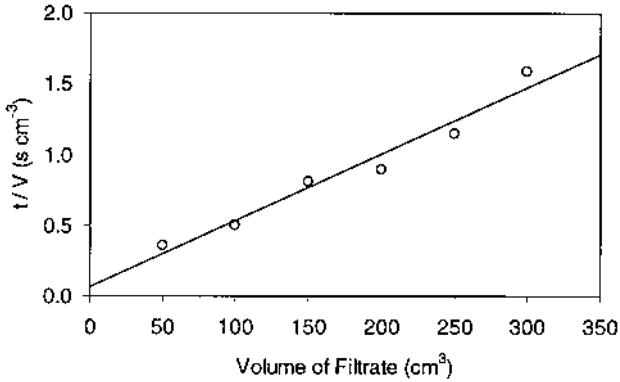

Figure 20 Filtration of pectinase extract from fermented solids (solid-state fermentation of wheat bran by *Aspergillus niger*) using diatomaceous earth as filter aid (precoat-bodyfeed method) in a vacuum filter leaf, under a constant pressure drop of 0.6 bar (Ferreira et al., 1995).

tion was 0.084 g of solids per gram of extract. The calculated resistances were $\langle\alpha\rangle = 4.2 \times 10^{10}$ cm g^{-1} and $R_m = 3.1 \times 10^8$ cm^{-1}.

It should be pointed out that at the very beginning of a constant pressure filtration, since there is no cake formation yet, the resistance to the flow is minimal. Therefore, at this point ($t = 0$), the filtrate flow rate is maximal. As soon as the flow starts, cake begins to be formed on the filter medium, continuously increasing the flow resistance. The net effect is, thus, a continuously decreasing flow rate.

3.3 Constant Rate Filtration

If a positive displacement pump is used to promote filtration, the flow rate through the filter will be constant. In this case, dt/dV in Eq. (64) is constant, so that

$$\frac{dt}{dV} = \frac{t}{V} = \frac{1}{Q} \tag{69}$$

Under this condition, Eq. (64) can be rearranged as follows:

$$\Delta P = \frac{\mu Q}{A}\left(\frac{\langle\alpha\rangle \rho c Q t}{A} + R_m\right) \tag{70}$$

Equation (70) is a straight line, if the experimental points of pressure drop ΔP are plotted against time, with the following gradient and intercept:

$$\text{Gradient} = \frac{\mu \langle \alpha \rangle \rho c Q^2}{A^2} \tag{71}$$

$$\text{Intercept} = \frac{\mu Q R_m}{A} \tag{72}$$

The resistances $\langle \alpha \rangle$ and R_m can then be calculated through a filtration experiment, in a way similar to that in the constant-pressure filtration case.

In a constant rate filtration, the pump delivers a constant flow rate against an increasing cake resistance due to the continuous growth of the cake thickness. In order to give a constant flow rate, the pressure delivered by the pump must also rise with time. Equation (70) shows that this growth in ΔP is linear with time. Since this pressure can rise considerably, most filters that use constant-rate filtration have a pressure relief valve in their pumping system. Figure 21 shows a plot of ΔP against t for yeast filtration at constant flow rate using perlite as filter aid (precoat-bodyfeed method). The filter used was a plate-and-frame press. The calculated resistances were $\langle \alpha \rangle = 6.6 \times 10^{10}$ cm g^{-1} and $R_m = 8.6 \times 10^9$ cm^{-1}.

3.4 Filtration Using a Centrifugal Pump

Most pressure filtration systems use a centrifugal pump for delivering the slurry to the filter. In this situation, the filtration follows neither a constant-pressure filtration nor a constant-rate filtration. This occurs because the flow rate delivered by the pump is a function of the resistance offered by the system, as shown schematically in Fig. 22. At the beginning of the filtration,

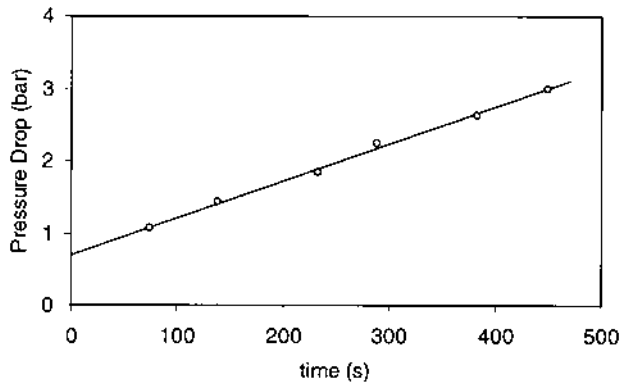

Figure 21 Filtration of yeast using perlite as filter aid (precoat-bodyfeed method) in a plate-and-frame press at constant flow rate.

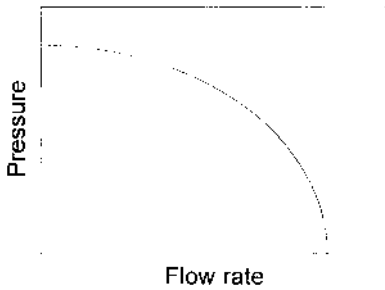

Figure 22 Typical centrifugal pump characteristic.

there is no cake, so the system resistance is at its minimum and the flow rate is at its maximum. As cake starts to build up, the resistance grows and, thus, the flow rate starts to decrease continuously as the pressure drop increases, following the pump characteristic. In other words, the time to filter a given volume of filtrate is a function of the flow rate, which is a function of the pressure drop.

Assuming that the cake volume is negligible compared to the volume of filtrate V, it is possible to write the instantaneous flow rate as:

$$Q = \frac{dV}{dt} \tag{73}$$

Therefore:

$$t = \int_0^V \frac{dV}{Q} \tag{74}$$

The relationship between V and Q given by equation (64) can be rewritten as:

$$\frac{\Delta P}{Q} = \frac{\mu \langle \alpha \rangle \rho c}{A^2} V + \frac{\mu R_m}{A} \tag{75}$$

where the relationship between ΔP and Q is given by the pump characteristics. Thus, through the use of equations (74) and (75) and the pump characteristics, it is possible to calculate the time needed for filtering a desired volume V of filtrate. This is not a straightforward procedure, since the integral expressed by equation (74) must be, in general, numerically solved. However, if a good centrifugal pump is being used (one that possesses a relatively flat pump characteristic curve in the low Q region) and if the pressure drop rapidly attains a constant value, the assumption of pressure constant filtration can be used, simplifying the problem. Figure 23

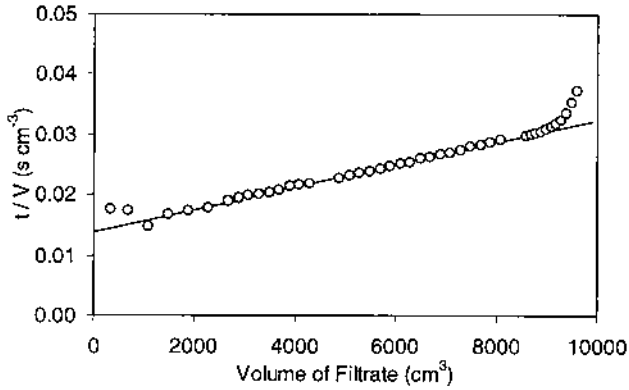

Figure 23 Filtration of yeast using a centrifugal pump and diatomaceous earth as filter aid (precoat-bodyfeed method) in a plate-and-frame press.

shows a plot of t/V against V for yeast filtration using diatomaceous earth as filter aid (precoat-bodyfeed method). A plate-and-frame filter press with a filtration area of 894 cm^2 was employed (pilot filter with square plates and frames of 15.24 cm), at a pressure drop of 3.0 bar. The solids concentration was 0.15 g L^{-1}. As can be seen in this figure, the initial and the final points do not lie in a straight line and must be discarded from the linear regression. The initial points do not fit to a straight line because at the beginning the pressure drop is not constant, so Eq. (66) does not describe this initial stage. At the end, the cake fills all the frames and, consequently, it becomes more and more difficult to filter a given ΔV. In other words, the time to filter a given ΔV increases exponentially. The calculated resistances, discarding the initial and final points, were $\langle\alpha\rangle = 6.6 \times 10^{10}$ cm g^{-1} and $R_m = 8.6 \times 10^9$ cm^{-1}.

The procedure described above is only valid for filtrations where the pressure drop reaches a constant level in a reasonably low time, which should be not superior to 10% of the filtration time. If that is not the case, Eq. (64) may be integrated from a point t_1, V_1 at the beginning of the truly constant period:

$$\int_{t_1}^{t} dt = \frac{\mu\langle\alpha\rangle\rho c}{A^2\Delta P}\int_{V_1}^{V} V dV + \frac{\mu R_m}{A\Delta P}\int_{V_1}^{V} dV \tag{76}$$

Then:

$$\frac{t - t_1}{V - V_1} = \frac{\mu\langle\alpha\rangle\rho c}{2A^2\Delta P}(V + V_1) + \frac{\mu R_m}{A\Delta P} \tag{77}$$

Equation (77) is a straight line when $(t - t_1)/(V - V_1)$ is plotted against $V + V_1$. The resistances $\langle \alpha \rangle$ and R_m may, then, be evaluated through a linear regression.

3.5 Filter Aids

Most fermented broths tend to blind the filter medium due to the presence of submicrometer particles and/or to blind the cake due to the high compressibility of microorganisms. The best way to solve these problems is to use filter aids, which are particles that can protect the filter medium and/or increase the permeability and porosity and decrease the compressibility of the cake. They are used extensively in the chemical industry to aid the filtration of solids that tend to form compressible cakes. In biotechnology, filter aids find application in the antibiotic, beer, and wine industries; in serum purification; in *in vitro* preparations; in enzyme isolation; in plasmid DNA recovery; and so on.

Filter aids are used in two ways: precoat and/or body feed. The precoat method is used to protect the filter medium against fouling due to colloidal particles, since such particles would penetrate into the filter medium, increasing its resistance and eventually blinding it. This can be avoided by initially filtering a diluted suspension (e.g., 0.5% by weight) containing only filter aid, which will deposit on the filter medium. Recirculating the filter aid slurry is recommended since the coarser particles will be first retained on the septum followed by the small ones. Average precoating velocities should be less than 7×10^{-4} m s^{-1} and the pressure drop should be at least 7 kPa. For higher viscosity liquids, much lower rates should be used. This precoat will act as the filtering medium rather than the filter cloth itself, and this filter action involves both sieve retention and adsorptive removal. An additional advantage of precoating the septum is that it facilitates the removal of the cake at the end of the filtration period. When used in pressure filtrations, precoat employs usually only a thin layer of about 1.5–3 cm thickness, equivalent to 0.5–1.0 kg/m^2 of filter area. However, when filtering slimy or gelatinous fermentation broth, a rotary-drum vacuum filter, using a thick layer of filter aid as precoat, is recommended. In this case, a thin layer of filter aid should be removed on every rotation together with the deposited solids. The usual range of precoat thickness is from 5 to 15 cm, and the blade advance varies from 25 to 250 μm per drum revolution. A new precoat is usually needed when the thickness of the old one reaches 0.5–1.5 cm. The recommended concentration for the precoat formation in vacuum filtration is from 2% to 5% by weight. A 5- to 10-cm precoat thickness may be achieved in 1 h, except for very fine filter aids where the time for forming a 5-cm precoat can be as high as 2 h.

The second way of using filter aids is as body feed. In this case, the filter aid is added to the fermented broth at the end of the fermentation. Actually, the body-feed method is usually employed together with the precoat method. The use of filter aid as body feed increases the porosity and permeability of the cake and decreases its compressibility, consequently reducing the cake resistance. The amount of filter aid to be used as body feed should be at least equal to the weight of biomass present. Nevertheless, up to 10 times more filter aid would be necessary if the bioparticles form highly compressible cakes. Filter aids are normally used in a one-time basis, although it is possible to recover them through resuspension of the cake followed by separation of the filter aid from the biological particles using a hydrocyclone (Matta and Medronho 2000).

Within a variety of filter aids, practically only three types have found wide application in the fermentation industry: (1) diatomaceous earth, also known as kieselguhr; (2) perlites; and (3) to a much smaller extent, cellulose. Diatomaceous earths are the most commonly used type of filter aid. They are porous and friable sedimentary rock composed by skeletal remains of single-celled aquatic plants called diatoms. These microscopic algae can extract silica from water to form their skeletal structure (see Fig. 24). They have approximately 90% silica and offer an enormous surface for the adsorption of colloidal particles (Fig. 25). Perlite is a glassy volcanic rock chemically composed primarily of aluminum silicates. It also has in its composition from 2% to 6% combined water. When quickly heated to above 870°C, the crude rock pops as the combined water vaporizes and creates countless tiny glass bubbles. This light-weight material is then milled, sized, and packed. Cellulose is not as frequently used in the biotechnology industry as diatomaceous earth or even perlites. Their greater application is, perhaps, in mixtures with diatomaceous earth or perlite to improve precoat

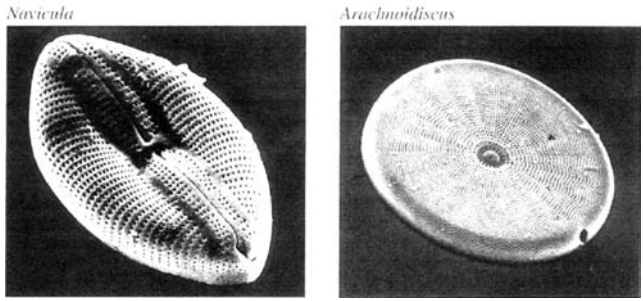

Figure 24 Skeletal structure of two different diatoms. (Courtesy of Celite Corporation.)

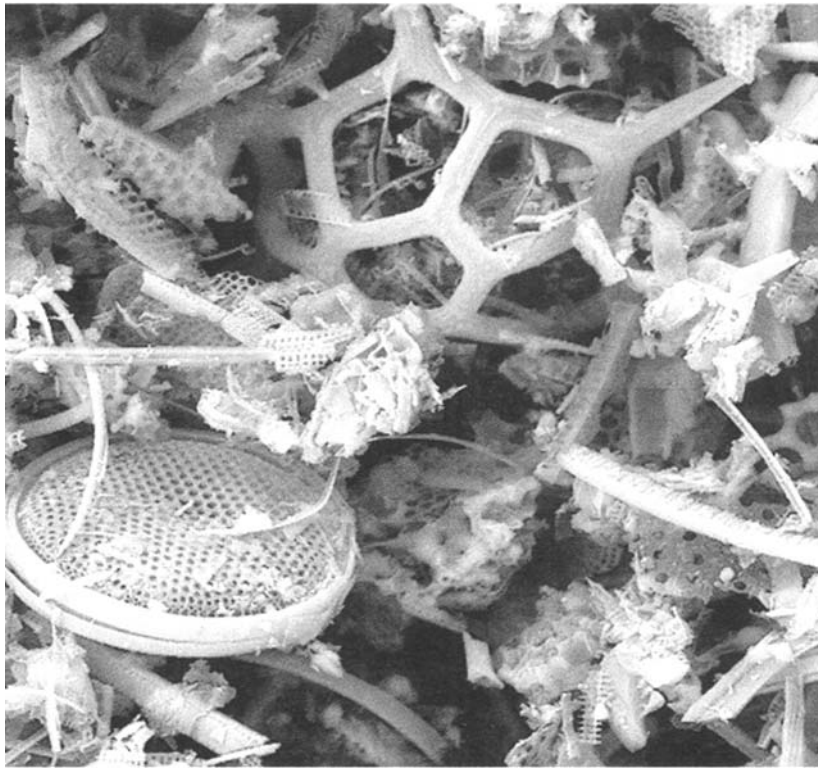

Figure 25 Scanning electron micrographs (1000×) of a commercial grade (Standard Super-Cell) of diatomaceous earth. (Courtesy of Celite Corporation.)

stability. Cellulose also finds application where the presence of trace amounts of silica is undesirable, in the filtration of hot caustic solutions, or when incineration of the cake is desirable. Table 6 gives a comparison between these three filter aid types, which are commercialized by different producers with a variety of brand names such as Celite 500, Filter Cell, Standard Super-Cell, Hyflo Super-Cell, Hyflo HV, Dicalite 215, Superaid, Speedflow, Speedplus, Speedex, Clarcel (all diatomaceous earths), Harbolite, Europerl, Dicalite 428, Grefco 436, Sil-Kleer, Clarcel-Flo (all perlites), and Fibra-Cell, Dicacel, Solka-Floc, and Clar-O-Cel (all celluloses). Figure 26 shows a scanning electron micrograph of 10-μm latex beads in a cake of Hyflo Super-Cell.

The use of filter aids usually improves the filtration of fermentation broths. However, a disadvantage may be the possible adsorption of product

Table 6 A Comparison Between the Main Properties of the Three Main Types of Filter Aids Used in the Biotechnology Industry

Filter aid	Wet density ($g\ cm^{-3}$)	Permeability ($10^{-8}\ cm^2$)	Median pore size (μm)	Compressibility
Diatomite	0.27–0.44	0.02–25	0.5–22	Low
Perlite	0.08–0.32	0.4–8.0	6–18	Medium
Cellulose	0.10–0.38	0.4–8.3	—	High

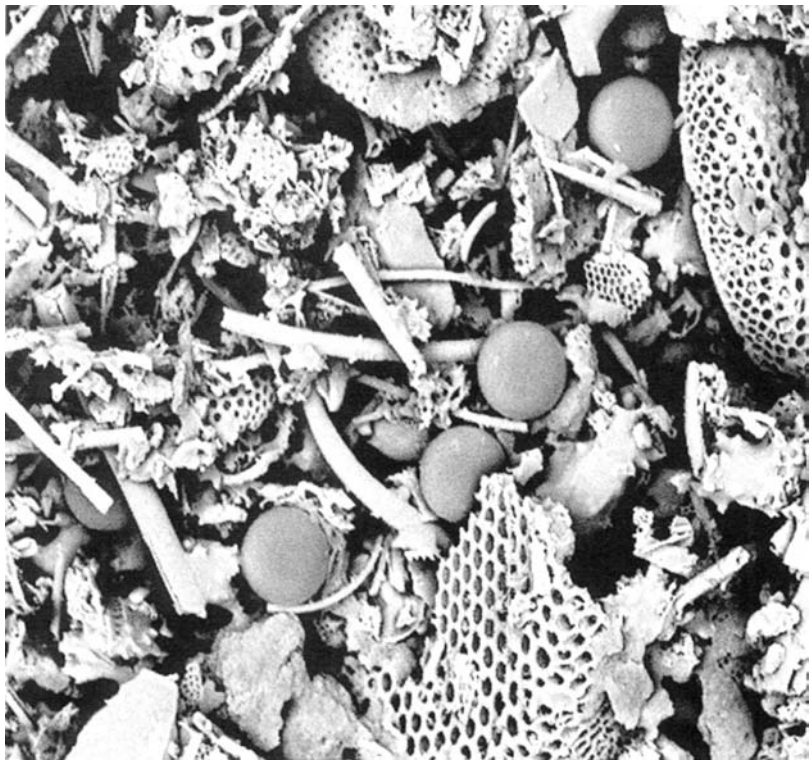

Figure 26 Scanning electron micrograph (1000×) of 10-μm latex beads in a cake of Hyflo Super-Cell. (Courtesy of Celite Corporation.)

to the filter aid. For instance, plasmid genes (Theodossiou et al. 1999), as well as aminoglycoside antibiotics and proteins (Wagman et al. 1975) can bind to diatomite and cellulose.

3.6 Types of Filters

There is an enormous variety of filter types. For instance, Nutsche filters, filter presses, liquid bag filters, external-cake tubular filters, and pressure-leaf filters are batch cake filters. Rotary drum filters (pressure or vacuum), disk filters, and horizontal vacuum filters are continuous filters. Each of these filter types has a great number of variations. For example, there are more than 100 design variations of filter presses. Filters have filtration areas varying from 1000 m^2 in some large units used in the chemical industry, down to 0.1 mm^2 in some microfilters used in conjunction with microreactors (Ehrfeld et al. 2000). Obviously, it is not possible to cover all of these filters in this chapter. Therefore, only the two most important filters will be summarized here, i.e., the filter press and the rotary vacuum filter. The readers can find more detailed descriptions of filters in other sources (Perry and Green 1997; Smith 1998; Svarovsky 1990).

Filter Presses

Filter presses have been used since the early days of the biotechnology industry, but nowadays they are increasingly being replaced by membrane filters. In spite of that, they still find applications ranging from fine pharmaceuticals and beverages to large volumes of waste products (Smith 1998). They are very simple and low-capital-cost equipments. They are available in a wide range of materials, such as metal, plastics, and wood. They are flexible, since their capacity can be easily adjusted by adding or removing plates and frames. When properly operated, a drier cake is obtained than that obtained with most other filters. However, they also have disadvantages. The main disadvantage is the high labor requirement, although a reasonable degree of automation can be obtained in some types of filter presses. In addition, they often present problems of leaking that render them unsuitable for the processing of hazardous liquids.

There are two main types of filter presses: recessed-plate and plate-and-frame. The former is composed only of plates, and the latter of plates and frames. In the recessed-plate press, both faces of each plate have a recess that acts as a chamber where the cake is formed between adjacent plates. These filters can be automated but inspection must be done after the end of each filtration cycle to ensure cake release. Some designs have a rubber diaphragm between plates that, at the end of filtration, compresses the

cake to squeeze out more liquid. Compression pressures of up to 16 bar can be achieved, and the bioparticle cake compressibility helps in deliquoring of the cake (Mackay 1996).

The plate-and-frame press (Fig. 27) is an alternate assembly of hollow frames, where the cake is formed, and plates, which are covered on both sides with a filter medium (usually a cloth). When compared with a recessed-plate press, it has the advantage of forming more uniform cakes. But the recessed-plate press design has fewer parts and, therefore, about half as many joints, resulting in tighter closure. Therefore, leaking is a larger problem in plate-and-frame than in recessed-plate presses. In plate-and-frame presses, the feed is pumped into the frames, and the filtrate is driven from the filter through special orifices in the plates. Frames and plates are usually rectangular, but they can also be, for instance, circular. There are basically two different procedures for washing the cake: simple washing and thorough washing. In the former, the wash liquid is pumped at the end of filtration through the cake using the same entrance in the frames as was used for the suspension. This kind of washing is only recommended for very uniform and highly permeable cakes (Perry 1997), which is usually not the case for cakes formed by bioparticles. The second type of washing uses special plates, sometimes called washing plates or three-button plates. The filter is

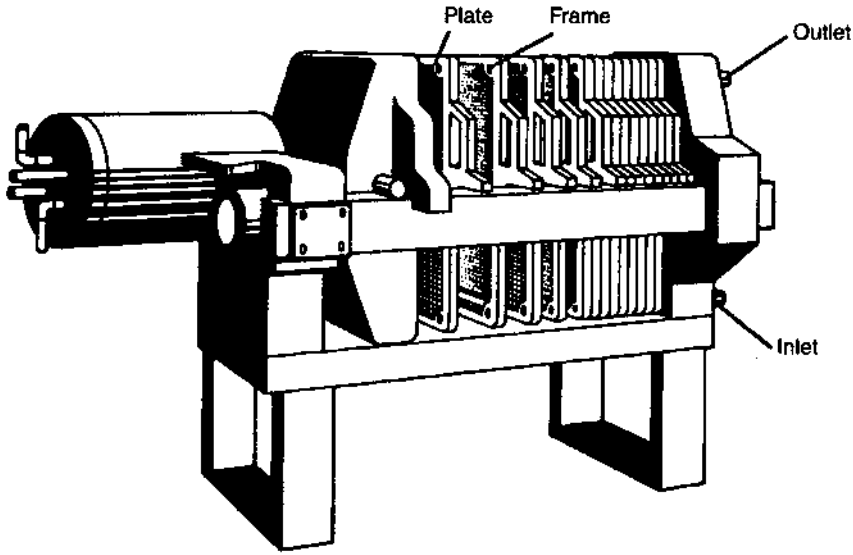

Figure 27 A perspective view of a plate-and-frame press. (Courtesy of Celite Corporation.)

assembled using the normal plates (one button), the frames (two buttons), and the washing plates (three buttons) in the following order: 1-2-3-2-1-2-3-2-1. In other words, the washing plate is always placed between two frames containing the cake. The wash liquid is then injected through the washing plates, passes through the entire cake, and exits through the one-button plates.

If the washing is done at the same pressure drop as at the end of filtration, the washing flow rate will be constant and equal to the flow rate at the end of filtration. This is true because during the washing the cake resistance does not increase because only liquid is passing through it. Therefore, Eq. (56) can be used to calculate the washing flow rate. As $d\mathrm{t}/d\mathrm{V} = 1/Q$, where Q is the flow rate for simple washing:

$$Q_\ell = \frac{V_\ell}{t_\ell} = \frac{A\,\Delta P}{\mu\left(\dfrac{\langle\alpha\rangle \rho c V}{A} + R_\mathrm{m}\right)} \tag{78}$$

If the filter has washing plates, the flow rate for thorough washing can be proved to be one-fourth of the simple washing flow rate. Therefore, the time to wash the cake with a volume V_ℓ of washing liquid will be four times greater in the thorough washing.

The scale-up of plate-and-frame press is possible if the following information is obtained with a pilot plate-and-frame press, operating under the same conditions and with the same suspension as the industrial filter (index p is used here for the pilot filter variables and i for the industrial filter variables):

- The average specific cake resistance $\langle\alpha\rangle$, the filter medium resistance R_m, and the average cake porosity $\langle\varepsilon\rangle$
- The filtration time t_p needed to occupy with cake all volume within the frames of thickness e_p, and the volume of filtrate V_p obtained after t_p
- The required ratio between the volume of washing liquid and cake volume ($\beta' = V_\ell/V_\mathrm{t}$)

A complete filtration cycle involves three different times: filtration time t, washing time t_ℓ (if required), and time t_d for cake removal, cleaning, and filter reassembling. Therefore, the filtrate production p will be given by:

$$p = \frac{V}{t + t_\ell + t_\mathrm{d}} \tag{79}$$

Applying Eqs. (64) and (78) to both pilot and industrial filter, it is possible to deduce the scale-up Eqs. (80) to (82), for filtration and washing conducted at the same constant pressure.

$$A_i = A_p \frac{V_i}{V_p} \frac{e_p}{e_i} \tag{80}$$

$$t_i = t_p \left(\frac{e_i}{e_p} \right)^2 \tag{81}$$

$$t_{\ell i} = k \frac{\beta'}{\beta} t_i \tag{82}$$

where $k = 2$ for simple washing and $k = 8$ for thorough washing, e is the frame thickness, and β is the ratio between the filtrate volume V and the cake volume V_t. β can be obtained through Eq. (83).

$$\beta = \frac{V}{V_t} = \frac{(1 - \langle \varepsilon \rangle)\rho_s}{\rho c} \tag{83}$$

where

$$V_t = \left(\frac{A}{2} \right) e \tag{84}$$

In Eq. (84), the filtration area is divided by 2 because the filtration area of each frame is twice the frame face area.

With the above equations it is possible to select a plate-and-frame press for an industrial operation, based on the results obtained in the laboratory using a pilot filter. The scaled-up filtration area should be increased by 25% as a factor of uncertainty (Perry 1997). It should be pointed out that the ratios given by β and β' hold for both filters and that the volume of filtrate V_p for the pilot filter is the one at which the cake fills all frames. This volume can be estimated through Eq. (84) or through a plot of t/V against V. For instance, for the filtration shown in Fig. 23, the volume of filtrate and the time of filtration at the point where the frames are full are 9000 cm^3 and 280 s, respectively.

The Rotary Vacuum Filter

The rotary vacuum filters are widely used for continuous large-scale filtrations. They are not truly continuous filters but can be operated for long batch times ranging from 1 to 10 days. They are especially useful for highly compressible particles, such as those found in fermented broths, which blind the cake after forming just a very thin layer. In such a case, a thick precoat of filter aid, as described in Sec. 3.5, is normally employed. Rotary vacuum filters are used for the filtration of waste sludges and have been used for yeast separations for more than a century. They find also wide application in

the pharmaceutical industry, although here they are being replaced by other separation methods, such as membrane filtration.

Despite the existence of many design variations, the rotary vacuum filter consists basically of a horizontal-axis rotating drum, as shown schematically in Fig. 28. The cylindrical area of the drum is a grid structure covered by a filter medium with filtration areas varying from 0.4 to 190 m^2. The drum rotates at low speeds, usually between 0.1 and 10 rpm, and is partially submerged in the suspension. A typical submergence of 30–35% of the drum diameter is normally applied. Vacuum is applied inside the drum, so liquid is sucked through the filter medium as the drum rotates. The solids are thus deposited over the filter cloth, forming a cake. At each full rotation of the drum, a cycle of filtration is completed, which includes filtration, cake washing (when needed), cake drying, and cake removal. Vacuum from 15 to 60 cm of mercury is usually employed, with the lower range (15–30 cm Hg) being used for coarser particles and porous cakes, such as those formed by filter aids, and the higher range being used for thicker cakes and when a reduced moisture is important. There are two main types of drum: single compartment and multicompartment. The single compartment is usually a hollow cylinder whereas the multicompartment has a more complicated mechanical design, with internal pipes and valves. Different methods of cake discharge have been developed over the years: knife discharge, precision knife discharge (used in precoat filtration), roller discharge (for sticky cakes), string discharge (for fibrous cakes), belt discharge (for sticky cakes), and so forth. The proper cake-discharge method is mainly a function of the cake characteristics.

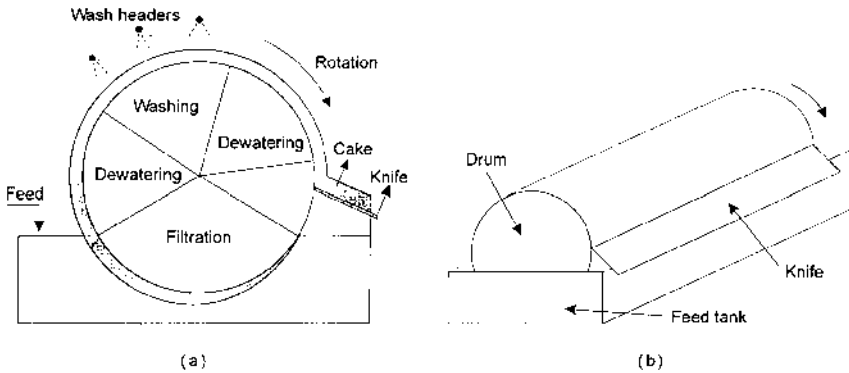

Figure 28 Rotary vacuum filter: schematic front view (a) and simplified perspective view (b).

Selection or performance predictions of rotary vacuum filters can be carried out using the so-called leaf tests. With these tests, it is possible to determine the flux of filtrate, expressed as filtrate flow rate per filtration area, and therefore the area of the filter. Detailed explanation about leaf tests as well as other laboratory tests used for vacuum or pressure filters can be found elsewhere (Rushton et al. 1996). It should be emphasized that the Buchner funnel is not an adequate substitute for a leaf test because of the significant resistance to flow produced by the drainage surface. It may be used for comparative flocculation evaluations, although the capillary suction time test is more appropriate for such application (Rushton et al. 1996).

4. FLOCCULATION

Small particles, such as cell debris or whole cells, may be aggregated to form flocs large enough to be removed by an appropriate process, such as gravity settling, centrifugation, or filtration. The scientific basis of aggregation is complex. It is based on the interfacial electrochemistry of small particles and its relation with colloid stability. Detailed information on flocculation can be found in more specialized books (Dobias 1993; Ives 1978).

An aggregation process may be distinguished as coagulation or flocculation, and the definition of both may vary with the area of application. For workers in the water treatment area, coagulation is the step of mixing an appropriate chemical to destabilize a colloid, and flocculation is the posterior stage of soft mixing that will promote aggregation through particle-particle collisions. For many colloid researchers, coagulation is the process whereby aggregation is caused by the addition of ions and flocculation by the addition of polymers. Since neither of these definitions describes properly the aggregation process, only the term ''flocculation'' will be adopted here.

Many flocculants found in the market are not approved for food and pharmaceutical use, and most of those approved are expensive. However, some alternative and cheap biocompatible flocculants, such as borax, may be used (Tsoka et al. 2000).

4.1 Colloidal Interactions

The principles that apply to colloidal solutions are also valid for larger particles, but in this case other forces, such as gravitational forces, may become more important. In any case, aggregation may be applied to particles from colloidal size up to the visible size range. The main types of interactions occurring when two particles approach each other are van der

Waals attraction, electrical interaction, solvation forces, steric interaction, and polymer bridging (Gregory 1986). Specific forces may also act in special cases, such as magnetic forces (Ives 1978). The universal van der Waals attractive forces are inversely proportional to the distance between the colloidal particles, are directly proportional to the size of the particle, and are almost independent of ionic strength for most particles (not always valid for bioparticles). Electrical interactions appear as a consequence of positive or negative charges that particles normally present at their surface when dispersed in water. These charges occur due either to preferential adsorption of certain ions from solution or to ionization of groups at the particle surface. As the system can have no net charge, the solution near a charged surface will develop a countercharge. The particle charge and the countercharge together form an electrical double layer. For biological surfaces, the most important groups to suffer ionization are carboxylic acid and amine groups, characteristic of amino acids and proteins. There is usually a characteristic pH value at which these charges at the particle surface are neutralized. Many bioparticles have this point of zero charge in the acid region of pH; consequently, their surfaces hold a negative charge at neutral pH (Gregory 1986). The van der Waals and electrostatic forces are "long range" forces, i.e., they can act at several nanometers of distance. Solvation (hydration) forces are short range forces that play a role in particle-particle interaction, usually adding an extra repulsion. They appear due to a different structure that water assumes close to solid surfaces. Steric interaction can occur if the particles are covered by an adsorbed layer, particularly of polymeric material. In a collision, compression or interpenetration of the adsorbed layers may occur. Compression always generates repulsion and interpenetration can produce either repulsion (more frequent) or attraction, depending on the existing interaction between the polymer and the solvent. In biological systems, polymers produced by microorganisms play an important role in controlling stability (Gregory 1986). Polymer bridging occurs when a polymer promotes an opposite effect that occurs in a steric stabilization. Thus, instead of stabilizing the particles, the polymer promotes flocculation. This difference in behavior can be understood if one looks at the particle surface. Polymer bridging occurs only at very diluted polymer concentrations. Therefore, the same polymer molecule, usually with a very high molecular weight, can have more than one particle attached to it, i.e., one or more polymer molecules bridge a floc. In the steric stabilization, which uses much higher polymer concentrations, the polymer covers all or most of the particle surface, thus, there is no surface left to bind to a polymer molecule already bound to another particle. Therefore, if polymer bridging is desired, tests must be carried out to determine the point of optimal concentration. As shown in Fig. 29, flocculation usually increases with polymer concentra-

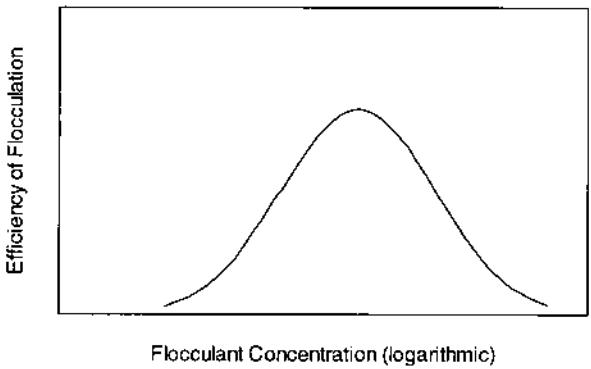

Figure 29 Efficiency of flocculation as a function of flocculant concentration.

tion, passes through a maximum, and then decreases due to the continuous adsorption of more polymer molecules by a given particle. The optimal surface coverage for bridging flocculation has been estimated to be around 50% (Cumming et al. 1994). It is important to point out that bridging is not the only way of action of polymeric flocculants. When using polyelectrolytes (charged polymers), charge neutralization may also be responsible for flocculation (Gregory 1986).

4.2 Aggregation Mechanisms

The presence of several forces, some attractive and some repulsive, that show different behavior according to the medium conditions (pH, temperature, salt concentration, etc.) makes aggregation a very complex theoretical matter. Nevertheless, there are several ways of promoting it, such as addition of chemicals to reduce the stability of the original suspension, neutralization of the electrical forces, addition of polymers to link particles (bridging), and so on (Ives 1978). In any situation, aggregation will only occur if the particles collide with each other. Collisions can occur due to Brownian motion, velocity gradients within the liquid, or differential settling of the particles. Brownian motion of the particles happens due to the thermal energy of the liquid. Velocity gradients can be obtained through stirring the liquid, and differential settling occurs when the particles have different sedimentation velocities. The mechanism of flocculation due to Brownian motion is called *perikinetic flocculation*, and the one due to velocity gradients is known as *orthokinetic flocculation*. Differential settling is usually treated as a special case of orthokinetic flocculation, since the particle motion rela-

tive to the liquid also creates velocity gradients. It has been shown (Ives 1978) that, under most significant cases of stirring, the perikinetic rates dominate for particle sizes under 1 μm and the orthokinetic rates dominate for larger particles.

4.3 Flocculation Agents

The surface charge of most bioparticles, such as whole cells and cells debris, depends on the pH of the medium and is usually negative at neutral pH. Therefore, a continuous reduction in pH will lead to a continuous reduction in these negative charges, until zero charge is reached. At this point, if the particles collide, the van der Waals forces may be strong enough to flocculate the particles. Actually, this flocculation can occur not only at the point of zero charge but also in a narrow range around it. It is possible to estimate the point of zero charge by measuring the ζ potential as a function of pH using microelectrophoresis and, for the majority of bioparticles, it is located in the 4.5–6.5 pH range (Mackay 1996). Many fermentation broths have buffering properties. If this is the case, the amount of acid required to reach the proper pH range needed for flocculation may be high (Mackay 1996).

The addition of an electrolyte to a culture medium containing charge-stabilized bioparticles may lead to flocculation. This happens because electrolytes increase the ionic strength of the medium, which decreases the electrical double-layer thickness and, consequently, reduces particle-particle repulsion. When this electrostatic repulsion is reduced, van der Waals forces may predominate and, thus, the particles can flocculate. This effect is more prominent for dissolved salts containing high-valence ions (usually the cation) of opposite charge to the bioparticle. Aluminum and iron salts, usually sulfates or chlorides, are the most common inorganic flocculant agents. In some cases, the addition of flocculation aids, which are insoluble particles that act as nucleating sites, improves the quality and size of the flocs. Common flocculation aids are activated silica, activated carbon, clay, metal oxides, and paper pulp (Daniels 1993).

Polymeric flocculants are organic polymers with a linear or branched chain. They can both decrease the electrical double-layer thickness and hence the strength of repulsion and/or adsorb on different particles bridging them. They can be natural or synthetic and, regarding electrical charge, anionic, cationic, or nonionic. Natural flocculants, such as alginates, starches, gelatines, celluloses (e.g., carboxymethylcellulose), and chitins (e.g., chitosan), have been used since ancient times and still find applications, for instance, in the beer and wine industries. However, the great majority of polyelectrolytes are synthetic, and of these polyacrylamide is the most widely used. It is obtained through polymerization of acrylamide

monomer, and polymers with high molecular weight of up to around 2×10^7 can be obtained. In spite of being theoretically nonionic, polyacrylamide usually has a small anionic character due to unintentional hydrolysis of some amide groups to form carboxyl groups (Gregory 1986). Cationic polyelectrolytes can be obtained through copolymerization of acrylamine with a positively charged monomer (e.g., amine, imine, or quaternary ammonium group). Anionic polyelectrolytes are obtained by deliberate hydrolysis of polyacrylamide or through copolymerization of acrylamine with acrylic acid. Other examples of polymeric flocculants are polyethylene oxide (nonionic), polyvinyl alcohol (nonionic), polyethylenimine (cationic), polydiallyldimethylammonium chloride (cationic), and sodium polystyrene sulfonate (anionic).

5. MAIN MANUFACTURERS OF EQUIPMENT

5.1 Centrifuges

Alfa Laval: http://www.alfalaval.com/
Baker Hughes: http://www.bakerhughes.com
Beckman Coulter: http://www.beckman.com/
CARR Separations: http://www.carrsep.com
Eppendorf: http://www.eppendorf.com/
Flottweg: http://www.flottweg.com/
Hettich-Zentrifugen: http://www.hettich-zentrifugen.de/
Kendro: http://www.kendro.com
Rousselet Robatel: http://robatel.com
Sigma-Aldrich: http://www.sigma-aldrich.com
The Western States Machine: http://www.westernstates.com
Westfalia Separators: http://www.westfalia-separator.com

5.2 Hydrocyclones

AKW: http://www.akwauv.com
Dorr-Oliver: http://www.dorr-oliver.com
Krebs Engineers: http://www.krebsengineers.com
Richard Mozley: http://www.mozley.co.uk

5.3 Filters

Alsop Engineering: http://www.alsopengineering.com
Baker Hughes: http://www.bakerhughes.com

BHS-Sonthofen: http://www.bhs-sonthofen.de
D. R. Sperry: http://www.drsperry.com
Dorr-Oliver: http://www.dorr-oliver.com
Giovanola Freres: http://www.giovanola.ch/
Larox: http://www.larox.com
Netzsch Filtrationstechnik: http://www.netzsch.com/

5.4 Filter Aids

Ceca: http://www.ceca.fr
Dicalite: http://www.dicalite-europe.com
Grefco Minerals: http://www.grefco.com
Solka-Floc: http://www.solkafloc.com
World Minerals: http://www.worldminerals.com/

NOMENCLATURE

A	filtration area (m^2)
b	intensity of the field of forces ($m\ s^{-2}$)
c	concentration of solids as mass of solids by mass of liquid
C_v	feed volumetric concentration
C_{vu}	underflow volumetric concentration
d	particle diameter (m)
D	test tube diameter (m)
D_c	hydrocyclone diameter (m)
D_i	feed inlet diameter (m)
D_o	overflow diameter (m)
D_u	underflow diameter (m)
d_{50}	cut size (m)
d'_{50}	reduced cut size (m)
e	thickness of the frame (m)
E_T	total efficiency
E'_T	reduced total efficiency
Eu	Euler number
g	gravity acceleration ($m\ s^{-2}$)
G	grade efficiency
G'	reduced grade efficiency
k	cake permeability (m^2)
k_m	filter medium permeability (m^2)
ℓ	vortex finder length (m)
L	length (m)

ℓ_c	cake thickness (m)
ℓ_m	filter medium thickness (m)
m	mass of solids in the cake (kg)
p	filtrate production ($m^3\ s^{-1}$)
P	pressure (Pa)
q	average fluid velocity ($m\ s^{-1}$)
Q	volumetric flow rate of the feed suspension ($m^3\ s^{-1}$)
Q_ℓ	volumetric flow rate of cake washing ($m^3\ s^{-1}$)
Q_L	volumetric flow rate of water in the feed ($m^3\ s^{-1}$)
Q_{Lu}	volumetric flow rate of water in the underflow ($m^3\ s^{-1}$)
Q_u	volumetric flow rate of the underflow ($m^3\ s^{-1}$)
r	radius (m)
R	radius (m)
Re	Reynolds number
R_f	flow ratio
R_m	filter medium resistance (m^{-1})
Stk_{50}	Stokes number
t	time (s)
t_c	centrifugation time (s)
t_d	time for cake removal, cleaning, and filter reassembling (s)
t_f	filtration time (s)
t_ℓ	washing time (s)
t_r	residence time (s)
v	particle settling velocity ($m\ s^{-1}$)
V	volume of filtrate (m^3)
v_g	particle settling velocity under the gravitational field ($m\ s^{-1}$)
V_ℓ	volume of washing liquid (m^3)
V_s	volume of the liquid shell in the centrifuge (m^3)
V_t	cake volume (m^3)
x	particle size frequency in the feed
x_u	particle size frequency in the underflow
W_S	mass flow rate of the feed ($kg\ s^{-1}$)
W_{Su}	mass flow rate of the underflow ($kg\ s^{-1}$)
y	cumulative particle size distribution (undersize) of the feed suspension
y_o	cumulative particle size distribution (undersize) of the overflow
y_u	cumulative particle size distribution (undersize) of the underflow
α	specific cake resistance ($m\ kg^{-1}$)
$\langle\alpha\rangle$	average specific cake resistance ($m\ kg^{-1}$)
α_o	average specific cake resistance at unit-applied pressure drop ($m\ kg^{-1}$)
β	ratio between the filtrate volume and the cake volume
β'	ratio between the volume of washing liquid and the cake volume

γ angle of the bottle
ΔP pressure drop (Pa)
ε cake porosity
ζ g factor
η efficiency factor
θ angle of the hydrocyclone cone
μ viscosity of liquid (Pa s)
ρ density of liquid (kg m^{-3})
ρ_s density of solids (kg m^{-3})
Σ sigma factor (m^2)
τ stress tensor (Pa)
ω angular velocity (s^{-1})

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