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Fermentation Broth Clarification Techniques

PETER N. WHITTINGTON

Biotechnology and Separations Division, Warren Spring Laboratory, Gunnels Wood Road, Stevenage, Herts, SG1 2BX, UK

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ABSTRACT

This review describes the two main separation processes currently used to recover cultured cells from fermentation broth at process scale; centrifugation and microfiltration. The fundamental principles of each technique are presented and the significant parameters that affect process efficiency are highlighted. Strategies to optimize these parameters and hence process efficiency are suggested with reference to the properties of cells and fermentation broth.

The process enabling technology of flocculation is also reviewed, since it is possible to enhance centrifugal separation efficiency using this technique.

Index Entries: Centrifugation; microfiltration; fermentation; flocculation.

INTRODUCTION

Broth clarification is one of the most essential and ubiquitous initial operations in the sequence of separation processes used to purify the products of modern fermentation-based biotechnology (termed downstream processing). It is usually a solid/liquid separation process designed to recover the cultured cells from their suspending medium. Since the overall yield of product from a complex sequential process is a compound function of the efficiency of its individual unit operations, the importance of the preliminary stages is crucial.

This review will briefly introduce the range of separation processes currently available or under development before focusing on the two most commonly used: centrifugation and microfiltration. The process-enabling technology of flocculation is also reviewed, in its logical order, prior to these two unit operations.

In all sections, the author has drawn attention to some of the fundamental design theories that describe the principle of the process. This strategy is intended to help pinpoint the significant parameters that influence process efficiency (e.g., viscosity effects on sedimentation). This should aid process design, or at least experimental design, to enable process efficiency to be optimized. Clarification can involve either simple removal of all particulate material, or more selective enrichment of a particle fraction (e.g. cell organelles, such as mitochondria) that contains the product of interest.

For extracellular products, the purpose of cell harvesting is to simplify subsequent purification steps and prevent product degradation (e.g., continued production of proteases by Gram positive bacteria). Where the product is located periplasmically (e.g., insulin-like growth factor in recombinant *E. coli* cells (*1*)), a cell recovery operation is required to concentrate the cell suspension prior to enzymatic or osmotic shock treatment to liberate the product. After product release, a rapid particulate recovery step is required to recover surviving cells and the cell debris produced (particularly since further proteases and nucleases will also have been released). Where the product is intracellular, a similar preconcentration of the cell suspension will almost certainly be required to enable efficient cell disruption, which is necessary to release the free product, or the organelle with which it is associated.

A number of different techniques are currently available in various stages of development in order to perform these clarification processes. Certain techniques are more suited to certain applications and cell types (Table 1), this aspect of the technology will be referred to where relevant. An economic comparison of the commonly used harvesting techniques of centrifugation and microfiltration is given by Datar (2).

Techniques

The diversity of cultured cell types and fermentation processes has led to the design of a similarly diverse array of process schemes for product purification. Bacterial cells and cell debris are the most difficult to recover owing to their small size, low density, compressibility, and cohesivity. In contrast, mammalian cells are large and fragile (having no protective cell wall) and require specific, gentle systems to enable them to be recovered intact. The two most commonly used techniques for microbial cell harvesting in biotechnology are centrifugation (possibly following a flocculation step) and microfiltration. This review will concentrate on these two unit operations. Often, the two techniques are used in combination in a process scheme designed to remove all the cells in suspension. Thus, even if the centrifuge recovers 99% of the cells, 10^7 cells/mL will still re-

Table 1
Features of Biological Cells

Cell type	Shape	Size, μm	Density, kg/m^3	Relative shear resistance
Bacteria	rods	0.5–3	1050–1080	high
	cocci	5–10	1050–1090	f. high
Yeasts	spheres	5–10	1050–1090	
Fil Fungi Actinomycetes	long filaments (pellets of fibers)	1 × 100's	1050–1090	med.
Plant	irregular	20–100	1050–1090	low
Animal	nondefined	20–50		v. low
Cell flocs.		10–100's	1010–1080	variable
Cell debris		0.4	1010–1200	low

main from a fermentation broth originally containing 10^9 cells/mL. A microfiltration step should then recover the remaining cells.

A typical process scheme for broth or cell homogenate clarification could involve several techniques. The types of unit operations used in large scale clarification processes include those listed in Table 2; some of those listed are still at early stages of their development.

FLOCCULATION

Introduction

Flocculation can be defined as the process whereby particles are destabilized and induced to come together, make contact, and subsequently form large(r) agglomerates (3). Particles approaching 1 μm (10^{-6}m) or smaller in diameter are classified as colloids, particles smaller than 1 nm (10^{-9}m) as solutes. Bacteria, eukaryotic cell organelles, and cell debris are able to maintain a dispersed state in suspension (a colloid) owing to the overriding influence of interfacial forces and the diminishing influence of gravity. Therefore, a flocculation process is often required to enable clarification.

Before flocculation can occur, however, the colloidal dispersion must be destabilized, often termed coagulation, so that the particles cannot redisperse before separation. The coagulation process exploits the very interfacial or surface phenomena that stabilize the colloid.

Table 2
Separation Techniques

Physical property exploited	Density		Particle Size	Surface Activity Hydrophobicity	
Process	Sedimentation		Filtration	Flotation	2-phase aqueous extraction
	Gravity	Centrifugation			
Unit - Types	Settling tank -	Disc-stack -	Microfilter -	Dissolved gas -	Mixer -
		Multi chamber	Ultrafilters	Dispersed gas	Settlers
		Bucket/tube	Rotary Vacuum Drum Filter	Chemical	Counter-current Contactors
		Decanter	Plate and frame	Electrolytic	
		Cyclones		Biological	

Principal among these are the effects of the electrostatic surface charge, Brownian motion, steric effects, and the degree of hydration of the surface. Typically, coagulation is induced by adjusting the chemical environment of the particle suspension, e.g., addition of metal ions (as salts) to reduce/reverse charge repulsion, addition of small particles (''seeding'') to increase the frequency of Brownian collisions (termed perikinetic flocculation), or the use of surfactants to alter the hydrophobic/philic nature of the particle surface and reduce hydration stabilization. Some or all of these coagulation techniques may be employed in a 'flocculation process.' However, for appreciable floc formation, some degree of induced turbulence or fluid velocity gradients are required to promote particle collisions and subsequent attachment (termed orthokinetic flocculation).

Advantages and Disadvantages

Increased particle size at a given density will aid centrifugal or gravity settling operations and improve filtration rates where the larger particles form a more porous filter cake, provided they are equally incompressible. Little work has yet been published on enhancing cross-flow microfiltration by flocculation though the increase in particle size may be expected to reduce membrane pore blockage.

Possible disadvantages arising from a flocculation step could include; incomplete flocculation producing no process advantage, denaturation of the protein product owing to chemical/shear action, toxicity of some synthetic polymeric flocculant monomers, and adverse effects on subsequent separation processes, such as ultrafiltration or chromatography, owing to the presence of free flocculant. Only process-specific trials, incorporating a cost-benefit analysis, will determine the need for a flocculation step.

The following series of elementary tests describe the procedures needed to optimize a coagulation of flocculation process for a particle suspension prior to its incorporation in a clarification process scheme. Optimization is necessary since many factors can affect flocculation efficiency. For biological systems, care must also be taken to protect product activity (e.g., enzyme), avoiding harsh chemical action or physical damage from excessive shear.

Optimizing Coagulation

Equipment

The apparatus normally used for coagulation tests is the standard jar test unit or jar flocculator. This consists of a rack of stirrers, driven by a single motor, under which glass beakers are placed containing the test suspension (e.g., fermentation broth). Beakers larger than 600 mL should be used to avoid errors in the accurate addition of coagulants or coagulant aids (flocculants) and reduce significant adsorption of polyelectrolytes to the glass surface (high surface area:volume ratio in small beakers). Tall beakers are preferred if a settling test is to be used as the index of flocculation efficiency.

A variable speed motor allows rapid initial dispersion of the coagulant, followed by slower speed conditioning during the maturation phase. Accurate speed control is essential since particle collision frequency and attachment efficiency is a function of the fluid velocity gradients. A constant speed motor with a gear arrangement for altering the stirrers' speed is sometimes recommended; alternatively accurate stirrer motors that monitor speed and torque are available. Ideally, the apparatus should be illuminated and a system for simultaneously adding coagulant/flocculant to all the beakers devised.

Coagulant Solutions

Stock solutions of each coagulant are prepared so that convenient volumes of solution correspond to the dosages to be used. Each coagulant (usually a metal salt) should be prepared as equal concentrations, expressed as the metal ion. Thus, 500 mg/L as the metal ion corresponds to 5.83 g/L alum ($Al_2(SO_4)_3 \cdot 16H_2O$), 2·51 g/L ferric sulphate ($Fe_2(SO_4)_3 \cdot 9H_2O$). Thus, adding 10 mL of 500 mg/L as Aluminum ions to a 1 L sample in beaker gives a final dosage of 5 mg/L (5 ppm) in the sample. Chemical stock solutions should be prepared regularly, preferably weekly, particularly for iron salts and polyelectrolytes. Reference to the manufacturers literature should indicate the shelf-life of particular concentrations of the reagent (e.g., 0.05% (w/v) polyacrylamide less than 1 week).

Assessing Coagulation Efficiency

Before embarking on a large series of tests, an indicator of flocculation efficiency should be decided upon by which the optimum type, its dosage, pH, and the conditioning regime can be determined. This criterion should ideally be process specific. Thus, some form of settling test is appropriate if centrifugation or gravity settling is to follow, a filtration test (e.g., Capillary Suction Time) if microfiltration is required. The test should be rapid and correlate with changes in full scale process efficiency. Typical tests/criteria include:

1. particle size analysis before and after flocculation (*see* "Equipment" section),
2. settling rate analysis—either quantitative by direct measurement of particle settling velocity or relative by measuring decrease in suspension turbidity with time,
3. measuring supernatant turbidity/absorbance after a standard settling period,
4. filtration though an appropriate filter and assessing filtrate clarity,
5. time required for floc formation,
6. extent of flocculation by monitoring the number of primary particles (e.g., single cells) (*see* "Equipment" section),

7. time taken for filtration of a set volume of suspension, e.g., 200 mL through a 0.45 μm membrane filter,
8. time required for removal of filtrate by capillary suction pressure (4), and
9. measurement of Dispersion Analyser response, sensitive to particle number and size increase (5).

Many other tests can be devised to suit the specific application though the examples listed above find wide application.

Jar Test Procedure

1. Prepare and assemble required coagulants and chemical solutions.
2. Place 500 mL of a representative suspension sample in each stirred beaker.
3. Agitate samples rapidly (e.g., 300 rpm).
4. Adjust pH of each sample, by adding acid/alkali, to appropriate value (e.g., for six samples: pH 4, 5, 6, 7, 8, and 9), ideally bracketing the isoelectric point of the particle.
5. Add coagulant solutions simultaneously to each sample and start stopwatch.
6. Maintain rapid stirring for a set time period (e.g., 1 min).
7. Reduce stirring speed to the minimum required to maintain flocs in suspension (same speed for each stirrer, 50 rpm). Alternatively, try a range of speeds and determine the optimum.
8. Maintain slow, 'conditioning' stirring for a set time period (e.g., 14 min).
9. Stop stirring.
10. Carry out floc analysis as appropriate (e.g., allow to settle, take sample for sizing, and flow through turbidometer).

Results Analysis

After carrying out the jar test procedure for a range of coagulant types, dosages, and suspension pH values, a series of curves can be plotted graphically. Taking the pH value or coagulant dose as the fixed variable and the performance criterion (a) as the dependent variable, e.g., settling rate, the supernatant clarity is shown (Figs. 1 and 2). The two fixed variables, pH and dose, can form both axes, and the performance criterion values, plotted as contour lines, depict regions of optimal conditions (Fig. 3).

Assessing Flocculants

Flocculants are usually added to a particle suspension that already has been destabilized by adding a primary coagulant, i.e., a suspension that is coagulating. The intention here is to increase the rate of aggregation, the size of the flocs, and their strength.

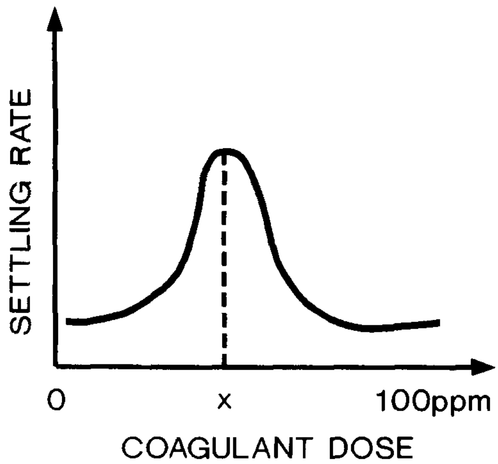

Fig. 1. Effect of coagulant dose on extent of flocculation, assessed as solids settling rate. Optimum dose indicated at dose "x" ppm.

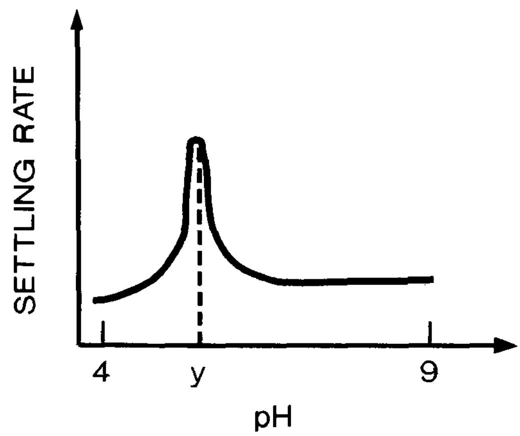

Fig. 2. Effect of suspension bulk pH on extent of flocculation, assessed as solids settling rate. Optimum pH indicated at pH "y."

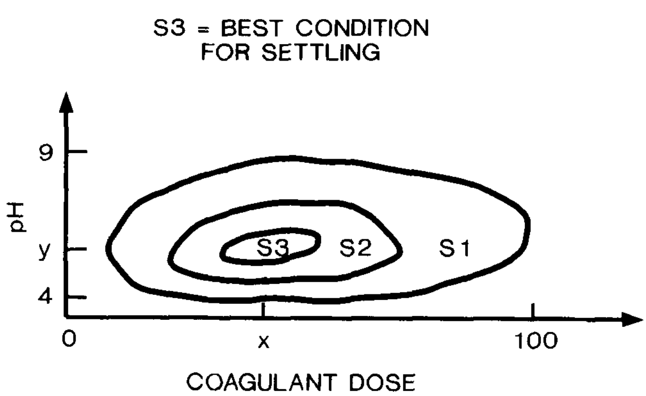

Fig. 3. Combined plot of effect of coagulant dose and suspension pH on extent of flocculation. Region S3 depicts optimal flocculation conditions, obtained by coagulant dose "x" ppm and pH "y." Regions S2 and S1 depict sub-optimal flocculation conditions.

Table 3
Flocculant Types

Naturals	Synthetics
Starch	Polyacrylamides
Gums	Polyamines/imines
Tannin	Cellulose derivatives (e.g., CMC)
Alginic acids	Polydiallyldimethyl ammonium chloride
Sugar/sugar acid polymers	
Chitosan (polyglucosamine)	

Flocculants, particularly-charged polyelectrolytes, can of course be used as the sole aggregating agent, in which case the flocculant is substituted for the coagulant in step 5 of the Jar Test Procedure.

Flocculant Choice

A bewildering array of flocculating materials are now available, all of which profess to be the ideal process aid. Some preliminary short listing of likely agents can be made on the basis of cost, availability, likely efficiency based on surface chemistry knowledge, and regulatory approval (e.g., FDA), which may preclude the use of toxic chemicals in a process producing a therapeutic agent. Flocculants can be initially classified into two broad groups—naturals and synthetics—according to their origins, though some could be termed semisynthetics (e.g., cellulose derivatives) (Table 3).

A particular class of polyelectrolyte flocculants can usually be subdivided on the basis of overall electrical charge character and molecular size. Thus, low and high molecular weight (10^5–15×10^6 d) polyacrylamides that are either cationic (positively charged), nonionic, or anionic (negatively charged) are available. Some polymers are amphoteric, i.e., their charge character is pH dependent since they contain both positive and negative charge groups. Initial trials with a range of doses of polymers of medium molecular weight and charge density, differing only in charge type, should identify the correct ionic type for the specific application. Further optimization tests, using polymers of varying weight and charge density (usually 0–30%), can then seek the optimal flocculant.

As a general rule, since most biological cells are negatively charged above pH 2–4 (surface groups principally $-PO_4^-$ and $-COO^-$), it is often found that cationic flocculants must be used at least as the primary flocculant. Further flocculation can sometimes be achieved by using an anionic or nonionic agent as a secondary flocculant.

Polymer Solutions

Researchers should always note the recommendations of the manufacturer/supplier with regard to preparation of solutions, particularly the method, strength of stock solutions, and shelf life. Maximum concentrations of stock polymer solutions are normally between 0.5 and 1% (w/v) (5–10 g/L solid polymer) and are limited by high solution viscosities that prevent homogeneous solution and ease of dispersion.

More dilute solutions, as added to the cell suspension, can be prepared on an equal concentration or equal cost basis. Constant volume fractions of each concentration solution should be added to the sample, e.g. 20 mL to 1 L, to maintain a consistent cell concentration. There is likely to be a maximum practical concentration that can be added to a sample, often 0.1% (w/v) or 1000 ppm, imposed by the practicalities of dispensing viscous polymer solutions and ensuring complete dispersion.

Powdered polymer preparations require careful wetting and dissolution to ensure complete dispersion and the prevention of localized beads of polymer gel (termed 'fish-eyes'). A useful method is as follows:

1. Weigh the required amount of dry powder into a large dry beaker (minimum 600 mL).
2. Wet the powder with a small volume (3–5 mL) of methanol or ethanol and swirl the beaker.
3. Add 3-400 mL of distilled water rapidly, and simultaneously agitate the mixture vigorously (magnetic coupled stirring is ideal).
4. Slowly agitate the mixture until complete solution is apparent (30–60 min, perhaps more).
5. Dilute the solution to the correct stock concentration in a volumetric flask.

Liquid grade polymers can be diluted as normal, taking care to ensure complete dissolution. Final concentrations should be expressed as mg/g weight of cells or mg/m^2 cell surface area to enable direct comparison of the test results.

Flocculant Addition

As per Jar Test Procedure steps 1–5, then:

6. Maintain rapid stirring for 6 min.
7. Add flocculant solution into fluid vortex.
8. Continue rapid mixing for 1 min.
9. Reduce stirring speed (to 50 rpm) and maintain for 15 min (to provide low shear conditioning).
10. Analyze flocculated suspension according to a predetermined test (e.g. allow to settle, filter, sample for size analysis, and so on).

Optimizing Flocculation

Significant flocculation of particles in suspension is brought about by interparticle collisions caused by fluid or Brownian motion that result in particle attachment. Optimizing flocculation, therefore, relies on optimizing the hydrodynamics of the system causing these collisions. The principal influences are the fluid velocity gradient or shear rate (G) and the conditioning time (t). The rate of reduction of particle numbers, owing to collisions in a fluid, is given by the Smoluchowski equation

$$-\,dN / dt = 16/3\,\alpha\, G\, r^3 N^2 \tag{1}$$

where: G = shear rate, r = particle radius, N = particle number, and α = fraction of collisions resulting in attachment.

Integrating this expression with respect to time, gives

$$1/N = 1/N_o + 16/3\,\alpha\, G\, r^3 t \tag{2}$$

A series of tests, therefore, are required to apply different amounts of defined fluidic shear for various time periods to condition the flocculating suspension to different extents. An optimum level of conditioning should be found at some value of shear and time. Three main types of conditioning device are used to apply defined shear to a flocculating suspension.

AGITATED VESSEL. Either a simple stirred beaker or a variety of designs of baffled tank and stirrer paddle. Altering the speed of rotation of the stirrer will alter the shear rate applied, thus

$$\overline{G} = \sqrt{P / V\mu} \tag{3}$$

where $\overline{G}$ = mean shear rate (s^{-1}), P = power dissipated in fluid, V = fluid volume, and μ = fluid viscosity.

and $P = 2\,\pi\, nT$ (4)

where T = torque on stirrer (m^2 kg/s) and n = stirrer speed (revs/s).

Full descriptions of the use of these systems for flocculation trials are given by Bratby (3) and Ives (6).

COUETTE FLOCCULATOR. This consists of two coaxial cylinders 150 mm long having a small annular gap (3 mm) between them. The inner cylinder rotates, thereby imparting shear to the fluid present in the annular space.

Batch or continuous flow through tests can be performed on this unit, altering the shear rate applied by altering the rotational speed of the inner cylinder. The velocity gradient is linear across the gap and below 10/s (for the unit of dimensions given); the shear is uniform and laminar. This device and its use is also described by Ives (6).

LAMINAR TUBE FLOCCULATOR. The simplest means of applying uniform shear to a fluid is to flow it through a tube/pipe; the velocity of the fluid along the tube wall produces shear. The shear rate can be adjusted by changing the flow rate of the fluid or the tube diameter, conditioning time by altering the tube length. Tubes between 1 and 5 mm in internal diameter can produce a wide range of shear rates in laminar flow (30 mL/min), 30–3400/s.

$$G = 8Q / 3\pi r^3 \tag{5}$$

where: Q = volumetric flowrate (m^3/s) and r = pipe diameter (m).

The residence time of the fluid in the pipe is given by

$$t = \pi r^2 L / Q \tag{6}$$

Combining eqs. (5) and (6), we can see that the product of shear and time, the Camp number, is determined by tube dimensions alone (*7*).

$$Gt = 8L / 3r \tag{7}$$

Some form of rapid mix device is required to ensure dispersion of the coagulant and/or flocculant in the particle suspension; a small (5–10 mL residence volume) flow-through stirred vessel of constant dimensions and stirring rate is ideal.

Fluid flow through a pipe will only be laminar for a Reynolds number <2000; above this value the flow becomes turbulent (5000 for coiled tubes) (*8*). The dimensionless Reynolds number (Re), which describes the state of flow, can be calculated from:

$$Re = \rho Dv / \mu \tag{8}$$

where ρ = fluid density (kg m^{-3}), D = pipe diameter (m), v = fluid velocity (ms^{-7}), and v = fluid viscosity (kg/m/s).

A fuller description of the technique is given by Gregory (*8*).

Some workers have attempted to apply the same overall amount of conditioning to larger scales of operation by keeping the product of shear and conditioning time, *Gt*, constant. Also known as the Camp number, after its pioneer, Gt values of 10^4–10^5 are quoted as being optimal for flocculation purposes (*7*). However, in practice, very high shear rates for short time periods reduce the resultant floc size owing to floc breakup. Thus, testwork is still required to optimize both G and t for the specific application where flocculation is required.

CENTRIFUGATION

Theory

Centrifugal sedimentation is essentially the application of high radial acceleration to a particle suspension by rotational motion. Particles denser than their suspending medium will move outward to the perimeter of the centrifuge bowl. Conversely, particles or liquid lighter than the bulk fluid will move inward. The Brownian diffusion forces, which hinder or prevent the settling of very fine particles under gravity, are overcome in a centrifugal field.

The sedimentation velocity of a particle in a less dense fluid under the influence of gravity is given by Stokes' law

$$Vg = D^2 (\rho_p - \rho_1) / 18\mu\, g \tag{9}$$

where Vg = sedimentation velocity, D = particle size, ρ_p = density of particle, ρ_1 = density of liquid, μ = fluid viscosity, and g = gravitational acceleration.

In centrifugation, the acceleration term 'g' is replaced by $\omega^2 r$, where 'r' is the distance of the particle from the center of rotation and 'ω' is the angular velocity of the centrifuge (radians/s). The rotor rotates at 'n' revs s^{-1}.

$$\omega = \pi\, n / 30 \tag{10}$$

The centrifugal force on a particle of mass 'm,' rotating with angular velocity 'ω,' at a radius 'r' from the center of rotation is given by

$$F = mr\omega^2 \tag{11}$$

The relative centrifugal force (RCF), compared to gravity, is given by

$$RCF = r\omega^2 / g \tag{12}$$

It can be seen from eq. (9) that a particle's sedimentation velocity can be increased by: increasing the speed of the centrifuge, and hence the centrifugal acceleration and decreasing the viscosity of the suspending fluid.

Decreasing the volumetric throughout will increase the efficiency of separation since the particles have longer to settle. Furthermore, modern multichamber and disk-stack centrifuges use walls or conical disk inserts to reduce the particles' settling distance (and hence settling time) and increase the settling area in the centrifuge.

Where batch or semicontinuous processing is practiced in an opening bowl disk-stack unit, the interval between solids ejections is important. This can be calculated from the solids content of the feed, its flowrate, and the volume of the solids collection space. Complete recovery is assumed in the following equation

$$T_I = V_1 \times 60 \times 100 / Q \times V_2 \tag{13}$$

where T_I = shooting interval (min), V_1 = volume of solids collecting space (L), Q = feed volumetric flowrate (1/h), and V_2 = % solids in feed suspension. V_2 is found experimentally by tube centrifugation tests (broth volume less supernatant volume, expressed as percent).

Optimizing Centrifuge Performance

To some extent, the basis of this process has been addressed in the preceding section; however, this section highlights those process parameters of the most importance to separation performance. Certainly for optimum protein purification, the feed stream to the product purification steps must be as clear as possible and free from particulates that can block filters and blind chromatography columns. It is unlikely in practice that a form of continuous or semicontinuous centrifugation will recover 100% of the particulates, cells, and cell debris, from suspension. Stokes' law, used to calculate the particle settling velocity, assumes no particle interactions (which occur in the highly-populated broths of interest), that particles are spherical and does not account for hydrodynamic effects in the centrifuge owing to the fluid's motion. Furthermore, the liquid flow velocity is nonuniform; there is likely to be a distribution of residence times, and reentrainment of settled particles by the adjacently moving supernatant (centrate) can occur.

Particle Size

Sedimentation velocity increases as the square of particle size, underlining the significance of this factor. Coagulation and/or flocculation of the individual particles will increase their resultant particle size (*see* Flocculation section). Briefly, particle agglomeration can be induced by:

1. chilling to temperatures of $<20°C$ (particularly with yeast cells),
2. pH adjustment—as an indication, the optimum is pH 3–6, rapid uneven adjustment can have an additional effect,
3. altering the ionic strength of the suspending fluid in conjunction with pH adjustment. Higher ionic strengths will compress the electrical double layer, which helps to stabilize cells and, hence, destabilize the colloid,
4. increasing the number of particles in suspension increases the collision frequency and, hence, the rate of perikinetic flocculation,
5. addition of multivalent metal ions (notably Al^{3+} and Fe^{3+}) to inhibit charge stabilization of the colloid. Polyaluminum chloride is a unique reagent combining this mechanism of flocculation with polymer bridging, and
6. use of polymeric flocculants, singly or in addition to metal ion coagulation, to physically attach to cells and bridge between or destabilize them (charge-patch model) to form flocs.

Density Differences

A density difference between the particles and their suspending medium is necessary for any form of sedimentation, irrespective of the particle/floc size. Cells are only slightly denser than their suspending fluid; thus, any means of increasing their density contrast is always worthwhile. Possibilities include: reducing suspending fluid density by dilution or altering its solute composition, or increasing cell/particle density—overproduction of product protein to form dense inclusion bodies, seeded settling tests with inorganic particle addition (e.g., resin beads and metal powders) (*9*).

Liquid Viscosity

Settling velocity (Vs) is reduced by an increase in the viscosity of the suspending fluid, $Vs/18\mu$; therefore, this factor is very significant, particularly for cell debris separation where intracellular macromolecules have been released into the medium (e.g., DNA, proteins, and RNA). Viscosity reduction techniques include

1. increasing the temperature (opposite to particle size methods, *see* "Particle Size" section) at the risk of causing protein denaturation,
2. dilution of the process stream,
3. pH, salt, or alcohol treatment to precipitate viscous macromolecules (beware of precipitating the product), and
4. use of DNase and RNase and proteases (e.g., papain and trypsin) to cleave viscous macromolecules into smaller, less viscous molecules.

Grade Efficiency and Sigma Concept

These are important factors when considering the continuous or semicontinuous processing of cell suspensions in process scale centrifuges. In a given size distribution of particles fed to a centrifuge, some will be too small to be recovered in the time they are exposed to the centrifugal field (their residence time in the centrifuge). Thus, in a given particle size distribution, there will be a corresponding distribution of recovery efficiencies, termed the grade efficiency.

This can be assessed by size analysis of the feed suspension and the centrate or clarified stream. The grade recovery can be calculated from

$$R = 1 - Fc(d)\,Cc \,/\, Ff(d)\,Cf \tag{14}$$

where $Fc(d)$ = fraction of particles of size (d) in centrate, $Ff(d)$ = fraction of particles of size (d) in feed, and Cf, Cc = solids concentration in feed and centrate streams, respectively.

Particles above and below a certain critical size (the 'cut' size) will be recovered at higher and lower efficiencies, respectively, under the given operating conditions of the centrifuge. The grade efficiency function, G(d), has a value of 100% if all particles of that size are recovered and 0% if none are recovered (Fig. 4).

The cut size or limit particle diameter (d lim) can be calculated from the following equation

$$Q = [\Delta\rho d\,\mathrm{lim}^2 \,/\, 18\,\mu\,g]\,[2\pi \,/\, 3 \cdot \omega^2 g\, N \cot\sigma\, (r_1^3 - r_2^3)] \tag{15}$$

where Q = feed flowrate, N = no. of disks in centrifuge bowl, σ = conical half angle—smaller angle between disks and centrifuge axis, r_1 = outer disk radius, r_2 = inner disk radius, and $\Delta\rho$ = density

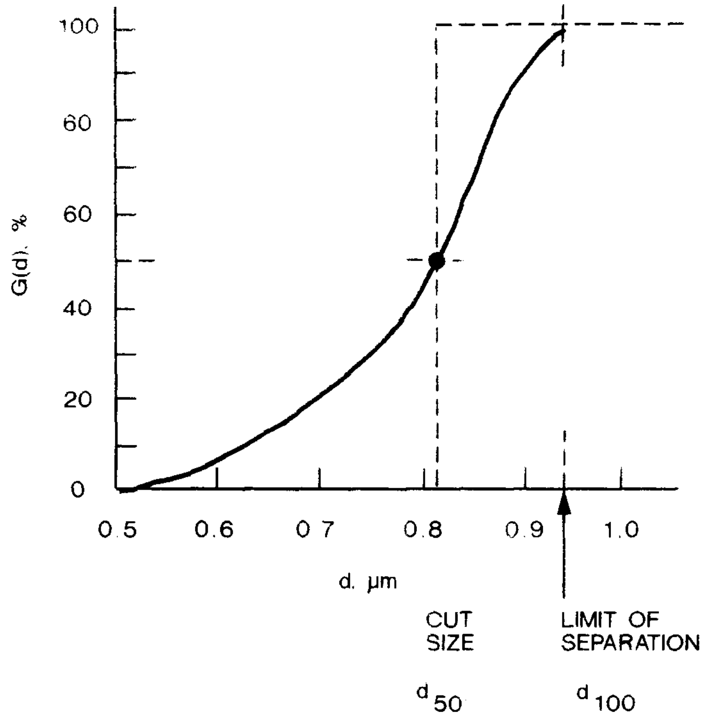

Fig. 4. Typical grade efficiency curve for a sedimenting centrifuge.

difference between particles and fluid. (Similarly, the operating conditions required to recover particles of a given size can be calculated from the same equation.)

The first expression in parentheses in the equation refers to the properties of the feed suspension (and calculates its settling velocity), the second expression determines the sedimentation area in the centrifuge (termed 'Σ' or sigma).

Hence:

$$Q = Vg\,\lim.\,\Sigma \qquad (16)$$

where Q=volumetric flowrate of particle feed suspension, *Vg lim.* = gravitational settling velocity of 'limit' particle, and Σ=equivalent settling area of a sedimentation tank that performs the same degree of separation as the centrifuge. The sigma factor ('Σ') enables scale-up of the separation with centrifuges of the same type and design. From

$$Q_1 / \Sigma_1 = Q_2 / \Sigma_2 \qquad (17)$$

Prediction and Assessment of Process Performance

Settling Tests

Simple laboratory scale settling tests, under gravity or in a centrifugal field, can be used to calculate the sedimentation rates of the particles in their suspending fluid. Furthermore, the extent of compaction of the sediment in a known centrifugal field can help predict separation performance at larger scales and importantly measure the volume occupied by the sediment.

These tests can also be used to assess the increase in particle size owing to coagulation or flocculation, as evidenced by an increase in the sedimentation rate. This is the most important, dynamic measure of the extent of flocculation relevant to centrifugation.

Gravity Settling

It is important to measure the maximal free settling velocity of the particle suspension in its suspending fluid. To minimize irregularities owing to wall effects, a reasonable sized measuring cylinder should be used (200–1000 mL) if at all possible. A particle suspension settles under gravity in three phases: first, the induction phase, when the particles begin to settle and are accelerated by gravity; second, the free settling phase, when the particles settle at their maximum velocities; and third, the compression phase, where high particle numbers cause particle interactions and the settling rate slows (hindered settling). Monitoring the particle horizon height in the measuring cylinder determines these phases. The usual procedure is

1. fill graduated cylinder with particle suspension,
2. monitor height of falling particle horizon with time, and
3. calculate settling velocity of horizon, and, hence, particles in 'free settling' phase.

Settling velocity = distance settled by horizon/ time

Most microbial suspensions settle very slowly, entailing a test duration of several hours, and may be too dilute for a distinct horizon to be apparent. Flocculation will greatly increase the particle size and settling velocity. Centrifugal settling tests will reduce the test duration; stroboscopic centrifuges are available that allow real-time monitoring of particle sedimentation in the centrifuge. Monitoring the decrease in optical absorbance as particles settle in a spectrophotometer cuvet gives a measure of the relative settling rates of particles. This is useful for evaluating flocculant and floc conditioning performance.

Viscosity

The importance of fluid viscosity, where the fluid is that in which the particles are suspended, is demonstrated by Stokes' law (Eq. (9)), where it can be seen that increasing viscosity will rapidly reduce the settling velocity of the particle suspension. This is a particular problem when centrifugation to remove cell debris is required since intracellular macromolecules, such as DNA, RNA, proteins, and polysaccharides, are released during cell disruption, drastically increasing the liquid viscosity. This adverse increase in viscosity can be limited or alleviated by the use of enzymes (DNase, proteases, or amylases) or by changing the solution pH (usually increasing). The risk of breaking down the desired product by this treatment should be assessed.

Particle Size Measurement

Particle size is one of the most critical factors affecting the separation performance of a particle suspension. The sedimentation velocity of a particle is a function of the square of the particle size. Thus, a 2 μm cell will have very nearly 4x the settling velocity of a 1 μm cell of equal density. The size of the particles in a cell suspension is one of the few factors over which some control can be exerted (by flocculation, coagulation, and so on); thus, it is important to be able to measure this parameter.

Great care should always be taken to minimize the influence of sample handling on the subsequent analysis (e.g., pipetting small volumes through narrow pipet tips) if a real and representative answer is sought.

MICROSCOPIC ANALYSIS. A number of techniques are available for measuring particle sizes, both simple and sophisticated. Perhaps the simplest, and least susceptible to sample handling, is direct measurement by observation of the sample with a microscope fitted with a graticle eyepiece. Particles in the sample can be directly measured and their size calculated, allowing for the magnification as necessary. This technique can be combined with photography to give a permanent record of the observed particle size; photographs of particles of known size (latex spheres) at the same magnification can be used as calibration.

COULTER COUNTER SIZING. A Coulter Counter (Coulter Instruments Ltd, Luton, UK) measures particle size electrically as the change in resistance when particles pass through an orifice of known size situated between two electrodes connected conductively via the orifice. The instrument calculates the volume of the particles in suspension and produces cumulative distributions of the number and volume of particles. This information is useful when predicting and later assessing the separation performance of the centrifuge (particularly the grade separation efficiency) and any increase in particle size owing to flocculation.

Although the Coulter Counter can give highly resolved results, care must be taken when interpreting the data since significant shear forces occur as the particles near and pass through the orifice. Thus, fragile particles, such as mammalian cells or loose flocs of microbial cells, will be distorted or even disrupted. Measurement of the particle size is said to take place before the shear forces are able to distort the particle; however, care should still be exercised and the experimentor should be aware of this effect. Problems can occur with blockages of the fine orifice if broad particle size distributions are being measured.

Finally, it should be noted that since the Coulter instrument only measures the size as the resistance change owing to the presence of the cells in the orifice region, large diffuse flocs that contain appreciable amounts of solvent can appear smaller than the reality since the entrained solvent is not measured, although it contributes to the floc's volume. Comparing data between this instrument and the laser gives a qualitative indication of floc density, a factor affecting floc strength. Dense flocs will produce broadly similar size results; looser, weaker flocs will be undersized by the Coulter, compared to the laser method.

LASER LIGHT SCATTERING. A third system for particle size analysis is based on laser light scattering (Malvern Instruments Ltd, Malvern, UK). The instrument detects the extent of scattering (diffraction) of light owing to the presence of the particles in the path of a collimated laser light beam. The system's computer calculates the size of the particles causing the measured extent of light diffraction. This is an expensive and sophisticated system that enables rapid and accurate analysis of numerous samples in batch or continuous fashion. Extraneous background light can interfere with the measuring system, and some shear forces are present in the sample cell by virtue of the magnetic stirrer used to keep the sample dispersed. A flow-through sample cell can be used for online monitoring of dilute particle suspensions ($<0.3\%$ w/v at 1 μm). The volume distribution data produced is reliable although a few large particles can mask the presence of a larger number of smaller particles owing to the data weighting system employed by the computer.

Equipment

Although there are a wide variety of sedimenting centrifuge types, fewer are suited to biotechnological applications where high speeds, low solids volumes, low shear, and often contained operation are ideal. Few machines can demonstrate all these attributes, in particular low shear operation has yet to be fully mastered; however, the more commonly used types are reviewed here.

Disk-Stack Centrifuge

The most appropriate type of pilot scale centrifuge for biotechnological applications, given the nature of the solids and liquids involved, is the disk-stack centrifuge. Basket, multichamber, and scroll-type centrifuges are slower, and small solids removal and contained operation is less efficient. Slower speed centrifugation is necessary for shear sensitive mammalian cell recovery, perhaps combined with flocculation (*10*); imperforate bowl and multichamber type machines have also been used for this application (*11*). Machines are also available for much larger scales of operation; the interested reader is referred to (*12*) for a comprehensive review of these centrifuge types. Biotechnological applications have been reviewed by Wiesmann (*13*) and Atkinson and Mavituna (*14*).

Disk-stack centrifuges contain a stack of conical discs in the bowl (Fig. 5) that rotate with the bowl and increase its settling area. The feed suspension is dispersed and accelerated radially from the center of the bowl to the area between the disk-stack and the bowl wall. The suspension

Operating principles and constructional features

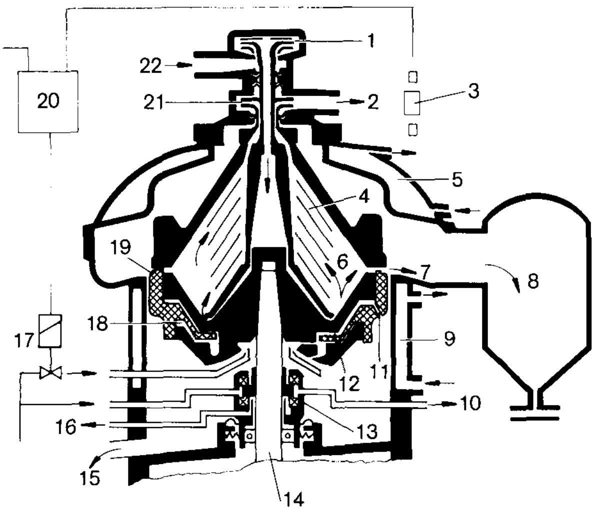

Fig. 5. Continuous disk-stack separator with opening bowl discharge mechanism (reprinted by permission of Westfalia Separator Ltd, Milton Keynes, UK).

then flows radially inward through the channels between the disks toward the bowl outlet at the top. The denser particles in this suspension are forced radially outwards by the centrifugal force against the inner surface of the discs. Having 'settled' against the disk, the particle is forced to slide down along the disk surface and out to the bowl perimeter (the solids collecting space). After a period of time (T_I, *see* Eq. (13)), the solids collecting space fills with collected cells that must be removed before they interfere with the separation on the disks and are reentrained in the feed. There are several methods for performing this operation, including: manual removal from a solid bowl, continuous discharge via peripheral nozzles, and intermittent solids ejection via peripheral ports or an opening bowl (*see* Fig. 5).

Centrifuge speeds up to around 12,000 rpm are attained by the newer machines, enabling high flowrates (several hundred liters per hour, e.g., 200 L/h *E. coli* (*1*) and good separation. The latest machines (e.g. Alfa-Laval BTPX, Westfalia CSA series) are completely contained to eliminate aerosols, have reduced shear inlet zones, essential for some cell and floc types, and can be cleaned, steam sterilized, and cooled. Feed solids concentrations up to 15% w/v can be handled.

MICROFILTRATION

Introduction

The preceding sections demonstrate the problematic process of broth clarification using conventional separation technologies. The small size, low density, and glutinous nature of biological cells and their components means that these technologies, particularly at large process scales, are at or approaching the limit of their efficient operating range. A recent development is the technology of microfiltration, usually applied as cross-flow microfiltration. This technique operates by the application of hydrostatic pressure across a semipermeable membrane filter, while causing the feed-stream to flow across the filter surface to minimize fouling. Microfiltration membranes are usually classified in terms of the size of particles they can retain with high probability (>99%), size ratings between 0.02–10 μm in size are common, though significant undesirable retention of smaller macromolecules, such as proteins, can occur during processing. The filters are usually isotropic (symmetric) though recently anisotropic (asymmetric) or 'skinned' microfilters have become available. These have smaller more defined pores in the filter's separating surface, supported by a more rigid porous backing material. The pressure difference across the filter forces the smaller solutes and water (or solvent) through the filter pores as permeate, while retaining and concentrating the cell/particle suspension, the retentate.

Since the species that cannot pass through the membrane are rejected at the filter surface and are continually removed from the filter unit, they do not accumulate in the filter or its housing. This allows high permeate flux rates to be maintained for longer periods and high concentration factors to be achieved (up to 60% wet wt/vol in retentate).

Microfiltration is principally used for:

1. cell and cell debris removal (i.e., clarification) to enable product recovery from the permeate,
2. concentration of a cell suspension prior to disruption,
3. recycling biomass and continuous product removal from continuous/batch fermentations, and
4. sterile filtration of a variety of fluids (e.g., heat-labile fermentation media)

The main advantages of microfiltration include:

1. operation at ambient temperature,
2. high separation efficiency,
3. rapid processing possible,
4. performance is not strongly dependent on cell size or density,
5. process aids (e.g., flocculants) are not required,
6. good containment, and
7. compact, simple, and reliable equipment.

Theory

The theory describing the mechanisms operating during microfiltration of a particle suspension is not well developed, and an accurate quantitative model that would enable process optimization does not exist. Theoretical models derived for the flow of fluids through tubes (Hagen-Poiseuille) or porous particulate beds (Carman-Kozeny) have been applied to microfiltration and quantitatively do allow some prediction of likely operating efficiency (interested readers should refer to Hanisch (*15*), Porter (*16*), or Gutman (*17*) for a fuller description).

The Hagen-Poiseuille equation predicts that the solvent (water) flux through a filter, consisting of cylindrical capillary-like pores, is given by

$$J = \epsilon d^2 \Delta P / 32 \mu L \tag{18}$$

where ϵ = membrane porosity, d = pore diameter, L = membrane thickness, ΔP = pressure drop across filter, and μ = solvent viscosity. The Carman-Kozeny equation is more realistic and assumes that the filter pores may be tortuous, vary in size, and blind routes may exist, here

$$J = \epsilon^2 \Delta P / k^1 (1 - \epsilon)^2 S_o^2 \mu L \tag{19}$$

where k = constant dependent on pore structure and S_o = surface area/unit particle volume.

Both models predict that the permeate flux will increase in direct proportion with transmembrane pressure and inversely with fluid viscosity. The relation between flux and viscosity is usually demonstrated when an increase in flux is obtained if the operating temperature is raised. Care should be taken not to cause heat denaturation of the product, however. Similarly, reducing fluid viscosity using nucleases will increase the flux rate when filtering cell debris suspensions. Increasing pore size will also increase flux rates, as predicted by the models; this is shown in the dramatic increase in flux rates for microfiltration membranes, compared with ultrafiltration membranes.

The flux rates observed for microfiltration of particulate suspensions are not as dependent on concentration as in the ultrafiltration of macromolecules. The permeability of the concentrated layer above the filter is a strong function of the size of its composite material, thus

$$P = d_p^2 \epsilon^3 / 108 (1 - \epsilon)^2 \tag{20}$$

where P = permeability, d_p = particle size, and ϵ = porosity.

Increasing particle size from 10^{-8} m (macromolecule) to 10^{-6} m (bacteria), increases the permeability by 10^4. Porter suggests that back diffusion of particles away from the filter surface, aided by the cross-flow action, is more important. This radial movement is termed the 'tube-pinch' effect; the particles' radial velocity is a function of their velocity across the filter, their size, and their location

$$V = CU R_e \, (r / R)^4 (x / R) \tag{21}$$

where V = radial migration velocity, C = constant, U = average fluid velocity, R_e = Reynolds number, r = particle radius, R = tube radius, and x = radial position of particle.

Increasing the cross-flow velocity for a given particle suspension, thus, will increase this radial migration, hence reducing filter fouling and increasing flux rates. Moderating this, however, is the need to operate below shear rates that will cause product denaturation (notably of proteins). Classical formulae can predict the average shear stress for a given cross-flow velocity, tube size, and fluid viscosity, thus:

$$\tau_w = \mu\gamma \qquad (22)$$

τ_w = wall shear stress
μ = fluid viscosity
γ = shear rate

and

$$\gamma = (6U/d) \text{ for rectangular channels}$$

or

$$\gamma = (8U/d) \text{ for tubes.}$$

Significant Operating Parameters

Essentially, these have been defined in the relevant theory of microfiltration presented earlier. Factors that should be considered most carefully because of their strong influence are:

1. transmembrane pressure (driving force),
2. cross-flow velocity,
3. filter characteristics—pore size, permeability, and surface nature,
4. particle concentration,
5. temperature—viscosity, and
6. hydrodynamic conditions within the flow channels—shear and turbulence.

Some of these factors may be outside the control of the process engineer, or the range of control may be constrained by the nature of the product (e.g., temperature) or other considerations, such as energy costs.

Transmembrane Pressure

It is preferable to keep the pressure difference across the filter to the minimum consistent with providing sufficient driving force to obtain reasonable solvent flux rates. As the transmembrane pressure is increased, so the solvent flux rate increases; however, above a certain pressure difference, determined by the filter and the feed suspension, there is no further increase in flux. Higher pressures cause compaction of any fouling layer of particles and lead to losses in flux performance. Provided high cross-flow velocities are maintained, the point at which the flux rate becomes pressure independent can be at quite high transmembrane pressures, allowing high flux rates.

Cross-Flow Velocity

Increasing the cross-flow or tangential velocity of the feed suspension over the filter surface will reduce the thickness of the surface fouling layer and increase the permeability of the filter. This increases the rate of solvent flux through the filter. High cross-flow velocities allow the transmembrane pressure to be increased, which also increases solvent flux. Very high cross-flow velocities, however, can cause shear precipitation and denaturation of proteins present in solution. Furthermore, the high pressure drop produced in the filter flow channels increases pumping costs, an important factor to consider for larger process scales.

Filter Characteristics

Informed filter selection can prevent many subsequent processing problems that cannot be solved by fluid management. Important filter properties to consider include:

1. Pore size—The pore size relative to the particle size determines the probability of pore blockage,
2. Overall permeability—directly related to flux rate,
3. Thickness—contributes to the filter's permeability, for a given pore size, a thick filter will have a lower flux than a thin filter, and
4. Surface character—the surface chemistry of the membrane, determined by its chemical composition, determines the type and extent of interaction with components of the feed suspension (particulate or dissolved). Fouling is an example of adverse interaction. Principal factors are the hydrophobic/philic character and the electrostatic charge on the surface. Hydrophobic filters tend to adsorb hydrophobic solutes such as antifoams and proteins, leading to formation of a fouling layer and loss in flux. Positively charged filters will tend to attract oppositely charged cells and solutes, according to the pH and ionic strength.

Particle Concentration

Flux decreases with increasing solids concentration in the feed suspension owing to the higher concentration of particles at the filter surface. However, the flux for particulate suspensions is not as concentration dependent as ultrafiltration of protein solutions. Above a critical concentration, however, there is a sudden loss in flux rate, proposed to be owing to inhibition of the back diffusion of particles away from the filter surface (*15*). Test work can determine the point at which this occurs.

Temperature

Increasing temperature reduces the viscosity of the feed solvent (for water approx 3% per °C rise), hence increasing the flux rate. Therefore, the filtration should be carried out at the highest temperature at which the product is stable.

Hydrodynamic Conditions

These are determined by the cross-flow velocity of the suspension, produced by the pumping conditions, and the geometry of the flow channels in the filter system (or stirring in a stirred cell system). Turbulent conditions are favorable in a filtration system since they reduce the thickness of the fouling layer on the filter surface and enhance mass transfer. High shear rates are also favorable within defined limits (*see* "Cross-Flow Velocity" section).

Experimental Procedures

The previous section describes the operational parameters that have most influence on process efficiency. Any experimental testwork, therefore, must address these parameters systematically. The objective of a microfiltration process is to maximize the dewatering or concentration of the feed stream in the minimum time period with minimal product loss. Minimizing the fouling caused by the particles or solutes in the feed stream is the overriding consideration when determining process design and operation. Most experimental testwork, therefore, is aimed at solving this problem. Laboratory scale work can adopt two approaches, either: mimic, as closely as possible, the full scale process, varying the important operational parameters (identified in the previous section) on a manageable scale, or study the fundamental mechanisms operating during a filtration operation (e.g., adsorption) and seek to devise techniques for minimizing those that are adverse.

Typical filter systems are shown in schematic form in Figs. 6 and 7. Figure 6 depicts a stirred cell filtration unit; Figure 7, a cross-flow filtration system.

Stirred Cell Modules

These units have reservoir volumes within the cell from 2–300 mL. Larger volume reservoirs (5–10 L) can be used to supply these kinds of module though the small filter area limits the scale of operation. Thermostatic jackets on some units allow the process temperature to be controlled. Polarization or fouling control is provided by the stirrer, located above the filter surface. Variable speed stirring allows the influence of fluid management on the process to be studied. The driving force for the filtration system, shown in Fig. 7, is nitrogen gas pressure from a pressurized cylinder. This allows precise control of the pressure gradient throughout the test

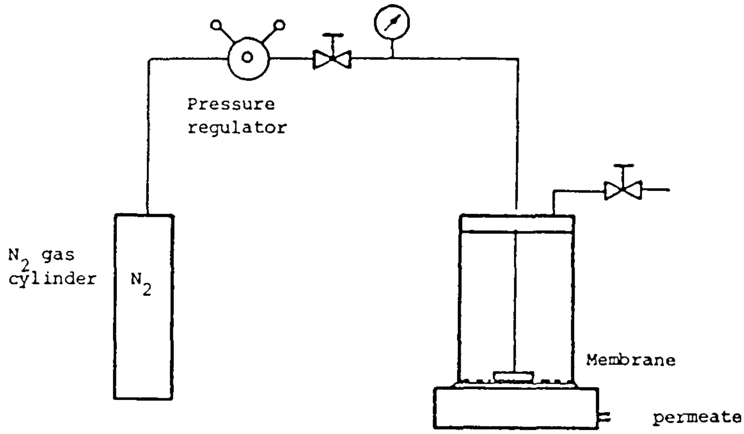

Fig. 6. Stirred microfiltration pressure cell.

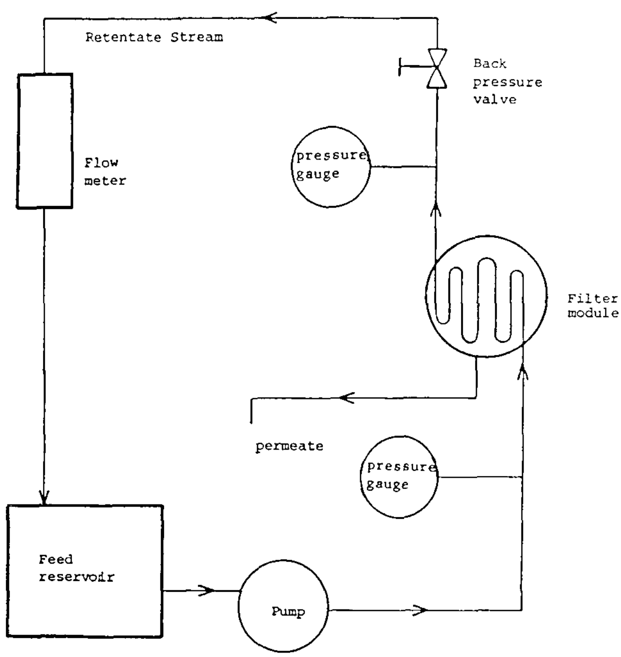

Fig. 7. Tangential flow filtration system.

and avoids pump effects, such as pressure pulses and shear damage, to the feed suspension.

Solvent flux is measured by timed collection of the permeate, measured as weight or volume collected per unit time (usually expressed as $L/m^2/h$). These units allow numerous small scale tests to be made to study the influence of most of the operational parameters listed in the previous section. Most importantly, they can be used to assess the compatibility of membranes and particular feed streams for sterile filtration of liquids and small volume processes to remove cells and other micron-sized particles.

The pressure and time dependency of the process should be assessed by tests that should last at least until a steady, limiting flux rate for the given conditions is reached. Typical pressure gradients for microfiltration are between 1 and 4 bar (15–60 psi); it is often found that above a certain pressure differential, there is no increase in permeate flux (termed the pressure independent region). Furthermore, the extent of permeate removal (dewatering) obtained is often reduced under high pressure conditions owing to increased fouling. The optimum filtration conditions often exist at the onset of the pressure-independent region with maximal fouling control (stirrer speed, cross-flow velocity).

Cross-Flow Filtration Modules

A wide variety of module and filter types are currently available (*see* next section) that use tangential fluid flow as the mechanism for controlling the extent of fouling. The range of process conditions under which a system can be operated are determined by its design and the filter material.

Thus, inorganic microfilters can be operated at higher transmembrane pressure and cross-flow velocities than the majority of 'plastic' membranes. Similar experimental considerations apply for cross-flow filtration tests as for stirred cell tests. The pressure and tangential flows here are normally produced by a pump (peristaltic or gear type normally) working against the filter and a back pressure valve (*see* Fig. 7). Temperature control is effected by controlling the temperature of the feed reservoir and lagging all the process components (or by inline heat exchanger). Typical operating pressures vary from 5-60 psi (0.3-4 bar), with cross-flow velocities up to 4 ms^{-1} (calculated from flow rate/channel area). Flux rates for microfilters vary between 20 and 300 L m^2/h. Higher flux rates can be obtained at higher temperatures; plastic filters made of polysulphone or polypropylene are stable to 60°C, inorganic filters to over 100°C. However, protein denaturation and precipitate formation, leading to product loss as well as filter fouling, places an upper limit on the operating temperature, perhaps as low as 20°C.

Permeate measurements to calculate flux rates can take several forms, including: inline flowmeter on permeate line, timed volume measurement—using a timed fraction collector, and weight of collected permeate (of known density).

It must be stressed, as in the "Stirred Cell Modules" section, that the test duration must allow the system to reach the limiting or steady-state flux for the given operating conditions. For production purposes, obviously the process runs to a defined completion point, e.g., maximum concentration factor reached, required volume of permeate, processing

Table 4
Chemical Cleaning Agents for Microfilters

NaOH	0.1*M*
NaCl	1-2*M*
NcOCl	<200 ppm
Pepsin	1%
Trypsin	1%
Detergents (Alconox, Sparkleen)	0.1%
Urea	6*M*
HCl	0.1*M*
HNO_3	0.25-0.3%

Table 5
Membrane Filter Materials

Material	Pore sizes (μm)	Comments
Plastics		
Cellulose acetate, nitrate	0.2 5.0	Analytical uses, can be autocalved hydrophilic.
PTFE (Teflon)	0.2, 1.0	Low fouling, autoclavable, solvent resistant.
PVDF	0.2, 0.45	Reasonable chemical resistance (e.g., cleaning agents), can modify surface groups.
Polysulphone	0.1, 0.2, 0.45, 0.65	Very common, hard wearing (1-2 yr life), chemically resistant, usually hydrophobic, can be sulphonated to make hydrophilic 'sterilizable.'
Others		
PVC, nylon, cellulose, polypropylene, polyester, and polycarbonate	(0.1-10 μm pores)	
Inorganics		
Carbon/zirconia, sintered stainless steel, and alumina	0.1-30 μm	Robust, heat stable, costly, high flows and pressures possible, usually tubular units.

Table 6
Membrane Module Types[a]

Laboratory Scale		Comments	Suppliers
Stirred cell	Disk filters	10–500 mL batch continuous filtration possible limited filter area.	Domnick Hunter Sartorius Millipore Amicon Gelman
Cross-flow unit spiral, radial, or zig-zag flow channels	Disk filters	Continuous filtration, usually batches of a few liters.	As above
Process scale			
Flat sheet		Wide range of module types and sizes. Multiunit systems for large scale usage. Filters in cassette form, 0.5 m^2 area is typical. Developed from UF units	Millipore Dorr-Oliver Sartorius Rhone-Poulenc
Tubular		Wider bore tubes: 6–25 mm diam. Tubulent flow, usually plastic supported membranes, some inorganic filters.	Membrana PCI Anotec SFEC Abcor
Hollow-fiber		0.2–1.5 mm id fibers. Many fibers (up to 3000) in a single module—up to 5 m^2 area.	Amicon Microgon
Pleated		Membrane cast onto support and covered in turbulence promoting mesh. Pleated around central permeate tube.	Gelman

[a](17).

time limit, and so on. In a production situation, it is likely that the process time will be substantially longer than the time taken to reach the limiting flux rate. Thus, initial flux rates alone are meaningless to any process design exercise.

If high flux rates are to be maintained over long periods of time, some form of membrane cleaning process must be incorporated into the operating cycle. Membrane cleaning is a complex science in itself, and can involve physical, chemical or biological treatments, or a combination of all these. Physical methods include: water washing, backflushing and recycling, sponge ball treatment, and ultrasonic treatment. Heat treatment of inorganic filters can also remove foulant layers. Chemical cleaning preparations are numerous; some are listed in Table 4.

Manufacturers provide guidelines on compatibility between their membranes and cleaning agents and recommend specific agents or specialist commercially-available formulations. With a well optimized process, users should expect to attain concentration factors of about 10-fold (e.g. 100 L $\rightarrow$ 10 L) before flux rates become diminishingly low.

Equipment

A wide range of filter types, materials, and configurations are now available. Microfiltration equipment has been primarily developed from reverse osmosis and ultrafiltration equipment; in many cases, the equipment is identical, only the filter pore size is different. Tables 5 and 6 summarize most of the modules currently available; detailed information is available from manufacturers. Micro- or ultrafiltration membranes can be used for small particle filtration applications; often, ultrafilters are used to provide a molecular sieving operation, as well as particle removal. The most commonly used filters are the 0.2 and 0.45 μm pore size plastic filters, either in flat sheet or hollow fiber form. Although 0.45 μm filters allow higher initial flux rates, often 0.2 μm filters are used since they tend to show reduced fouling for 1 μm particles, such as bacterial cells, and can sustain higher flux rates and concentration factors.

Recently-developed inorganic filters tend to have larger pore sizes (8–15 μm) and exhibit higher flux rates with high cross-flow velocities. A major advantage is their resistance to steam sterilization. The nature and quantity of product adsorption to inorganic filters is an important factor in filter choice, which merits careful consideration.

Correct equipment choice can only be made following a certain amount of test work to check its suitability for the specific process application. Never rely entirely on manufacturers claimed performance data since these are often produced with 'ideal' or at least 'clean' process streams.

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