SCALE-DOWN OF A BIOPROCESS SEQUENCE FOR
THE RECOVERY AND PURIFICATION OF AN
INTRACELLULAR PROTEIN

A thesis submitted for the degree of Doctor of Philosophy
by
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The performance of three scale-down unit operations for the recovery of an intracellular protein is examined as a tool to help accelerate process synthesis and development. Operations studied include, high pressure homogenisation, fractional protein precipitation, and disc stack centrifugation. The target protein is alcohol dehydrogenase from bakers’ yeast \textit{(Saccharomyces cerevisiae)}, a typical intracellular protein. The effectiveness of the scale-down process in predicting pilot scale performance is shown. It is compared with the alternative small scale system, laboratory scale. Unit operation performance is examined individually and also in a process context.

High pressure homogenisation performance is scaled down by maintaining the valve geometry, the cell concentration and the pressure drop across the valve. The scale-down high pressure homogeniser accurately predicts both product release and the resultant cell debris particle size distributions of pilot scale for the range of conditions examined. Scale-down protein release values are within 6 % of pilot scale and the maximum error in the prediction of the mean debris particle size is less than 8 %.

Disc stack centrifuge scale-down is achieved by modifying a pilot scale machine. Both the liquid and solid hold-up of the centrifuge bowl is reduced whilst still maintaining the ability to perform intermittent solids discharges. Scale-down is achieved in stages using a series of interlocking inserts to suit particular applications. Maximum scale-down gives a 76 % reduction in the separation area and a bowl volume reduction of 70 %. Separation performance of the scale-down machine closely follows the full scale version when using particulate streams of polyvinyl acetate and yeast cell debris. The particle size distribution of fine material in the supernatant stream closely predicted the pilot scale. Improved recovery performance with scale-down is seen with protein precipitates although this is small when recovery efficiencies are high.

Protein precipitations are scaled down by maintaining similar vessel geometry and the mean velocity gradient, \( G \), experienced in the vessel. With the ammonium sulphate precipitant, protein and ADH solubilities are independent of scale. With the polyethylene glycol precipitant there is over-precipitation with increasing scale. Ammonium sulphate precipitates formed at all scales (0.9-40 L working volume) give similar particle size distributions. Both ammonium sulphate and polyethylene glycol precipitates formed at scale-down (3.6 L working volume) give comparable clarification efficiencies to pilot scale.
With the ADH process sequence, the scale-down process predicts an overall product yield of 19% which is a good indication of pilot scale performance (11%). The laboratory scale process greatly over-predicts the yield (72-76%). The main reasons for disparities are differences in centrifuge performance.
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Notation

$A$ cross-sectional area ($m^2$)
$A$ absorbance (units)
$a$ constant
$a$ perpendicular distance between two centrifuge discs (m)
$B_t$ width of the spacer strip (m)
$c$ fraction of fine material in centrifuge overflow (supernatant)
$C$ concentration
$c_v$ volume concentration of aggregate ($m^3$ aggregate $m^3$ suspension)
$D$ diffusivity
$D_n$ dilution factor (-)
$d$ particle diameter ($\mu$m)
$d_c$ critical particle diameter ($\mu$m)
$d_i$ impeller diameter (m)
$d_t$ tank diameter (m)
$d_{50}$ median particle diameter ($\mu$m)
$d_{50}^*$ dimensionless median particle diameter (-)
$E$ fraction of soluble enzyme (-)
$E$ enzymatic activity (units)
$E$ electrical field strength
$E_t$ mass yield (-)
$f$ fraction of course material in the centrifuge underflow (slurry)
$f$ particle shape factor
$F$ Fraction of size distribution (-)
$F(d)$ cumulative undersize distribution
$f_i$ disc spacer strip correction factor (-)
$F_1$ force acting on a particle in a centrifugal field
$\Delta F_{\text{sup}}$ volume fraction of particles in supernatant (-)
$\Delta F_{\text{feed}}$ volume fraction of particles in feed (-)
$g$ gravity ($m s^{-1}$)
$G$ mean velocity gradient ($s^{-1}$)
$h$ particle separation distance (m)
$h$ homogeniser valve gap (m)
$I$ electrical current (mA)
$J$ collision frequency
\( K \)  dimensionless rate constant (-)
\( K \)  particle related constant (KQ theory)
\( K_A \)  rate constant (-)
\( k \)  constant (-)
\( l \)  length of centrifuge (m)
\( M \)  feed mass flow rate (kg s\(^{-1}\))
\( M_w \)  molecular weight
\( N \)  number of passes through homogeniser valve (-)
\( N \)  particle number concentration
\( N \)  Avagadro’s number
\( N_{seg} \)  segregation number
\( n \)  number of discs (-)
\( OD \)  optical density (units)
\( P \)  pressure (Pa)
\( P \)  power (W)
\( P_v \)  power per unit volume (w m\(^{-3}\))
\( P \)  fraction of protein remaining soluble (-)
\( P_o \)  power number
\( Q \)  volumetric flow rate (m\(^3\) s\(^{-1}\))
\( R \)  gas constant
\( R_f \)  valve rod radius (m)
\( R_0 \)  homogeniser valve radius at point of entry
\( r_1 \)  inner radius (m)
\( r_2 \)  outer radius (m)
\( r_e \)  effective radius of centrifuge (m)
\( R_e \)  Reynolds number (-)
\( R_m \)  maximum protein release (g kg\(^{-1}\) packed cells)
\( R_p \)  protein released (g kg\(^{-1}\) packed cells)
\( s_e \)  settling distance (m)
\( t \)  time (s)
\( t_{ma} \)  macro-mixing time (s)
\( T \)  temperature (°C)
\( T(d) \)  Grade efficiency (-)
\( T_m \)  Motor torque (N m)
\( u_0 \)  fluid velocity through the homogeniser valve (m s\(^{-1}\))
\( \Delta U \)  voltage pulse
$V$ Volume (m$^3$)
$V_b$ bowl volume (ml)
$V_{ds}$ bowl discharge volume (ml)
$V_{dl}$ disc space liquid volume (ml)
$V_{ds}$ disc space solid volume (ml)
$V_s$ solids capacity (ml)
$v_g$ velocity of a particle under the force of gravity (m$^2$ s$^{-1}$)
$v_z$ velocity of a particle under a centrifugal force (m$^2$ s$^{-1}$)
$v_z^*$ unhindered settling velocity (m$^2$ s$^{-1}$)
$v_z^*$ hindered settling velocity (m$^2$ s$^{-1}$)
w Boltzman parameter (µm)
w * Dimensionless standard deviation (-)
y normalised volume of particles of diameter, d (-)
Y yield (-)
z linear distance travelled by the particle (m)
$Z_l$ number of spacer strips per disc (-)

**Greek symbols**

$\alpha$ collision effectiveness factor (-)
$\varepsilon$ energy dissipation per unit mass (W kg$^{-1}$)
$\eta$ Kolmogorov microscale of turbulence (m)
$\varphi$ potential energy of interaction
$\mu$ fluid phase viscosity (kg m$^{-1}$ s$^{-1}$)
$\mu$ mean particle diameter (µm)
$\nu$ kinematic viscosity (m$^2$ s$^{-1}$)
$\theta$ ½ conical disc angle (rads s$^{-1}$)
$\rho$ density (kg m$^{-3}$)
$\rho_R$ electrical resistance of electrolyte
$\sigma$ geometric standard deviation (m)
$\Sigma$ Sigma factor (m$^2$)
$\omega$ Angular velocity (rads)

**Abbreviations**

ADH Alcohol dehydrogenase
C1 whole yeast cell recovery step (centrifugation)
C2  yeast cell debris recovery step (centrifugation)
C3  1st cut precipitate recovery step (centrifugation)
C4  2nd cut precipitate recovery step (centrifugation)
CD  Cell disruption valve
CR  Cell rupture valve
dw  dry weight
FV  Flat valve
KE  Knife-edge valve
PF  purification factor
PEG  polyethylene glycol
P1  1st cut precipitation step
P2  2nd cut precipitation step
PVAc  polyvinyl acetate
RCF  relative centrifugal force
RO  reverse osmosis purified water
ww  wet weight
w/v  weight per volume (kg m⁻³)
1. INTRODUCTION

Biotechnology companies need to take products to market as quickly as possible in order to maximise the return due limited patent life and other licensing issues. To do this a speedy transition from scientific discovery to technological development is essential. This and the need to gain regulatory approval, means that process development often requires a financial input equal to or even greater than that devoted to drug discovery. The development process is often expensive due to the problems of scale-up associated with the transfer from laboratory to large manufacturing scale (Datar, 1986). The gap between these two extremes of scale is usually spanned by the pilot plant by which stage process equipment tends to operate in a similar fashion to the industrial scale and hence process performance, in terms of both product quality, reproducibility and throughput, is usually similar (Fahrner, 1993).

Methods to both accelerate the development process and to reduce the associated large costs would be very welcome. The work undertaken in this thesis attempts to address these two issues by developing and testing a process sequence that is a scale-down of the pilot scale stage. This process sequence could in theory be used in conjunction with laboratory scale work to determine suitable unit operations for the process. In addition, it could help to target important experiments for pilot scale trials.

The process sequence examined is a primary purification procedure involving a number of unit operations. It is used to illustrate problems encountered with scale-down. Although it would have been ideal to have extended the process sequence to include final product purification, this was considered to be too ambitious with regard to the time frame of the study. The thesis involved the development and verification of the scale-down unit operations on an individual basis and examination of process performance by linking the individual unit operations to form the primary purification sequence mentioned above.

A context for using scale-down is discussed below. This is followed by a brief introduction to each of the unit operations to be scaled down and how they interact with each other. Afterwards, an introduction to the methods employed in scale-down and the aims of the research are discussed. Mass balance simulations are used to help determine the scale required for each unit operation in the scale-down process sequence.
1.1 Scale-down

Scale-up is a procedure for designing and building large-scale systems on the basis of the results of experiments with small-scale models. Ideally, the small scale system should produce the same results as the large scale set-up. In practice differences in performance often occur and allowances must be made. Many ways exist for scale-up and these are discussed below. Problems often associated with the small-scale model approach are that conditions employed at small scale are often not transferable to large scale and hence disparities in process performance occur. These can be very costly, particularly if they appear only in the later stages of design. A method for solving the problems of scale up is that of scale-down. This involves the simulation of full scale conditions at small scale.

Bioprocess development and optimisation is traditionally performed at pilot-scale since operating characteristics are more similar to large scale and hence successful transfer into production is more likely. However, pilot-scale development is expensive and hence any useful process data that can be obtained at a small scale with scale-down procedures will provide considerable savings. Additionally, scale-down operation is particularly important in the early stages of development when often there are only small fermentation volumes available for study. The ability to obtain useful design data at this stage will help to accelerate the development process.

1.1.1 Role of scale-down in biotechnology

Scale-down and process synthesis

The design of complex manufacturing systems involves a multiple tool approach (Young et al, 1984). The first step is to use mathematical models in the form of computer simulations to determine a design strategy. These select from all possible configurations those that will potentially meet the design requirements. These models alone cannot provide sufficient detail for a tactical decision to be made. This is particularly true of the biotechnology industry where process modelling is at an early stage of development. Here it is only through physical simulation that sufficient data can be obtained to make a final decision on the configuration to be used.

The following approach is broadly applicable to the biotechnology industry for the development of purification processes for new products. The first step could involve an expert system approach to define process components that are acceptable. Such decisions would be both product and manufacturing related. Using the acceptable process components
computer simulations could be run to determine the processes that are most likely to suit the particular product. Such simulations would require mathematical models for each unit operation and appropriate physical property data. As discussed above, computer simulations are not likely to provide sufficient information to make a decision on the processing scheme but should help to narrow down the number of options. The most likely process configurations can then be examined more closely through physical simulation. This role would be fulfilled by scale-down as this would reduce the time and costs associated with a pilot scale campaign. It may or may not be possible to make a final process configuration decision based on scale-down data. This would depend on the confidence in each scale-down unit operation of the process. But, at least, the number of process configuration options should be narrowed-down even further so that it is necessary to scale-up only a few alternatives to the pilot scale, on which basis a final decision can be made.

Scale-down and process development and optimisation
Once a decision has been made on the configuration then scale-down can be used to further define the process. This stage would involve many aspects such as, defining monitoring and control systems, optimising process variables, and highlighting areas of concern for further investigation at pilot scale. The advantages of the scale-down approach again being in saving time and costs, this being mainly laboratory rather than pilot facility based.

Scale-down and validation of virus removal/inactivation
A very important field for scale-down is the validation of virus removal and inactivation steps. These are required by regulatory bodies for biological products that come into contact with material of mammalian origin at any stage in the manufacturing process. Guidelines are provided by the Committee for Proprietary Medical Products (CPMP, 1991). To meet regulatory body approval it is not sufficient simply to show that the product is free from virus but that the process is capable of virus removal/inactivation. Thus, it is necessary to spike in virus. A panel of viruses is used to challenge the process and this often contains infectious agents such as human immunodeficiency virus and Hepatitis A virus. This work can only be performed in a suitable contained laboratory and hence cannot usually be performed at the manufacturing scale. Therefore a scale-down mimic is used (Foster and Cuthbertson, 1994; Darling and Spalto, 1996).

1.1.2 Approaches to Scale-down
When solving a scaling problem it is important to specify the kind of solution that is required. One of the key questions here is how much money is to be spent on the solution? For the
production of a bulk commodity where the product added value is low, or generic products where competition is strong, then a sophisticated and efficient process design is essential and hence investments are large. On the other hand, for speciality products, being first on the market is essential and life cycles are short, hence there is little time for research and development. Once this specification has been made then the method(s) for scaling can be determined.

There are three different kinds of phenomena important for process design, thermodynamic phenomena; micro-kinetic (intrinsic) phenomena, both of which are scale-independent; and transport phenomena which are very dependant on scale and are the only reason for scaling problems. Transport phenomena in bioprocesses are governed by two mechanisms, flow (convection) and diffusion (conduction); directly related to these are shear, mixing, mass transfer, heat transfer, and macro-kinetics. Further complications in scale-up arise from microbial factors including growth, decay and shear sensitivity.

Methods used in scaling:
- Fundamental methods
- Semi-fundamental methods
- Rules of thumb
- Dimensional analysis/regime analysis
- Trial and error.

Fundamental methods (mechanistic equations)
These involve solving micro-balance equations for mass, momentum and heat transfer and of conversion (kinetic) equations (Sweere et al., 1987). Complications occur for process design since balances must contain terms for transport in three directions and the boundary conditions are very complicated. In addition, balances for momentum transfer are coupled with those for heat and mass transfer. Lastly momentum balances are usually set up for an homogeneous fluid which is not often a realistic representation of a bioprocess fluid. Hence fundamental methods are only used for very simple cases.

Semi-fundamental methods
These methods use simplified equations, particularly for flow, which generally use one or a combination of the following models: plug flow, plug flow with dispersion, and well mixed. The semi-fundamental method in combination with rules of thumb is the most widespread method used in scaling.
Rules of thumb
These involve performance indices such as power per unit volume \((P/V)\) or speed ratio \((N_1/N_2)\). Constant power per unit volume can be used in almost every scaling situation.

Dimensional analysis
This requires dimensionless numbers to be kept constant with scaling. Keeping these constant means that the relative importance of the mechanisms influencing the process does not change during scaling. This method can be very effective but it has major drawbacks. Usually it is not possible to keep all dimensionless groups constant with scaling and hence the most important groups must be determined and the others ignored. The groups of importance are determined using regime analysis. Problems occur if a change in regime occurs during scaling since then this method is no longer valid. Geometric similarity is often maintained for scale-up from bench to pilot scale, however on transfer to industrial scale equipment configurations often alter hence invalidating this method. Another problem is that the collection of the parameters and variables for deriving the dimensionless groups can be an arbitrary process which is aided by experience. Despite the problems, dimensional analysis, combined with regime analysis and small scale experiments, is often used for scale-up problems, particularly mass, heat and momentum transfer problems.

Methodology for approaching scaling problems varies. A mechanistic approach, which involves knowing the essential mechanisms that affect the problem, often enables wide application, however, development can be lengthy and expensive. The other extreme is a trial and error approach which too can be very expensive since the lack of a mechanistic background can lead to unpleasant surprises with each scale-up which require further trial and error experimentation in order for them to be solved.

Usually, in biotechnology, it is wise to use a scaling method somewhere between mechanistic and trial and error. The approach used is generally not entirely quantitative.

1.2 Cell disruption
The importance of micro-organisms as a source of commercially useful products has been recognised for a very long time. Yet microbial chemicals produced commercially are nearly all of the extra-cellular type. However intracellular proteins with catalytic or biological activity are of growing importance for developments in enzyme technology, as well as for the production of mammalian proteins by recombinant-DNA technology. Hence the release of
these proteins, as a first step in their isolation, is critical. The aims of cell disruption are to maximise product release while avoiding denaturation and also maximising the disruption rate. In addition, avoiding the generation of fines (sub-micron particles) is a target as these may be difficult to remove and often interfere with later purification steps.

Several reviews have been published on methods of cell disruption (Engler 1985, Chisti and Moo-Young 1986, Schutte and Kula 1990, Harrison, 1991). Techniques can be split into large and small scale methods.

1.2.1 Small scale disruption

Various methods of mechanical and non-mechanical disruption are used. Non-mechanical types include enzymatic lysis; chemical lysis by alkali, detergent, organic solvent; freezing and thawing; and the use of temperature sensitive mutants. An advantage of chemical and enzymatic lysis is their ability to offer some selectivity for the release of single components from within the cells. All these methods have good potential for large scale use albeit in specific situations (Shutte and Kula 1990).

1.2.2 Large scale disruption

Large scale disruption has centred on mechanical methods, particularly high pressure homogenisers and high speed agitator bead mills. In recent years the design and performance of these machines have been improved and investigated intensively for the disruption of different microbial cells. In addition, contained, steam-sterilisable versions have been developed for handling recombinant DNA products. The bead mill is a very effective cell disrupter and has an advantage over the homogeniser in that it is able to handle filamentous organisms at high concentrations. Despite this, the homogeniser is the more commonly used machine industrially. Possible reasons are limitation of bead size and the good flexibility of homogenisers.

High speed agitator bead mill.

This machine has either a vertical or horizontal chamber containing rotating discs mounted on a motor driven shaft. The beads, occupying up to 80% of the free chamber volume, provide the grinding action. Operating characteristics are affected by number of discs and their design, agitator speed, cell type and concentration, residence time and, temperature. Cell disruption kinetics are discussed by Currie et al (1972) and Limon-Lason et al (1979).
High pressure homogeniser.
This is the most commonly used machine for large scale cell disruption (Scawen and Melling, 1985). A high pressure positive displacement pump delivers the cell suspension into a valve assembly. It passes into the valve gap, where cell disruption occurs, which consists of a valve rod pressed against a valve seat by a spring or hydraulic mechanism (Figure 1-1). After passing through the valve slit the fluid flows radially. On exiting the valve gap the fluid strikes the impact ring before leaving the valve at a relatively low velocity and pressure. Overall flow rates range from 28-100 L h\(^{-1}\) for small scale machines to as high as 40 000 L h\(^{-1}\) for large scale (Shamlou et al, 1995). Fluid velocities through the valve gap range from 50-300 m s\(^{-1}\) (Phipps, 1971). Operating pressures can be as high as 1200 barg. For a particular flow rate and operating pressure the width of the valve gap is dependent on the valve geometry. This may range from 10 \(\mu\)m for a low flow rate unit operating at high pressure to 300 \(\mu\)m for a high flow rate unit operating at low pressure (Phipps, 1975).

![Diagrammatic representation of a CD (cell disruption) valve](image)

**Figure 1-1**  
Diagrammatic representation of a CD (cell disruption) valve

1.2.3 Factors affecting disruption by high pressure homogenisation  
Factors affecting cell disruption include cell characteristics and concentration; homogeniser valve design; pressure, temperature and number of passes employed.
Cell wall structure

Cell wall composition is dependant on genetic and environmental factors. Keshavarz et al (1990) showed Gram negative bacteria to be the most easily disrupted, followed by Gram positive bacteria and then yeasts. Fungi are more resistant to homogenisation due to their thick walls but are readily disrupted with a bead mill since this exposes weakness due to the long cell length.

Growth conditions

An attempt was made by Engler (1979) to derive a general expression for disruption but he concluded that this would be misleading due to variabilities with growth conditions. Gray et al. (1972) found that E.coli batch cultures grown on a synthetic medium were more easily disrupted than those grown on complex medium. Cells grown at high specific growth rates and also cells harvested during the exponential growth phase are more susceptible to disruption (Engler and Robinson 1981). Siddiqi et al (1995a) also found that the growth rate employed during the fermentation affected the cell wall strength. It is known that stationary phase cells tend to have stronger cell walls. Conceivably, actively growing cells may direct energy to reproduction rather than the strengthening of cell walls.

Cell concentration

For baker's yeast, disruption was found to be independent of cell concentration in the range 30-600 kg/m$^3$ (Hetherington et al, 1971). For filamentous organisms cell concentration is important since high cell densities lead to blockage of the homogeniser valve.

Temperature

Increased disruption rates have been observed with higher temperatures (Hetherington et al. 1971). Attempts to correlate this with changes in the suspension viscosity have been made with inconclusive results (Keshavarz et al, 1990). However operation at higher temperatures is not usually desirable due to the thermolabile nature of many biological products.

Pressure and pass number

It is generally agreed for baker's yeast that disruption kinetics are first order and that the pressure exponent does not vary within the operating pressure range 100-500 barg (Keshavarz et al, 1990). Modern high pressure homogenisers are capable of operating at pressures greater than 1000 barg which should allow the disruption process to be accelerated since full disruption should be possible in a single pass. Siddiqi (1995a) found that at pressures greater than 800 barg it was possible to fully disrupt bakers' yeast in a single pass. The drawback to this was that cell debris formed at this pressure was smaller, and therefore
more difficult to recover by centrifugation, than when operated at 500 barg for 5 passes which gave similar levels of cell disruption.

1.3 Centrifugation

Centrifugal separation is one of the most important unit operations in processes in the chemical, pharmaceutical and food industries. Experience gained in these fields has been drawn upon to help solve the problems involved in many separation and purification steps of new biotechnological processes. Centrifugation takes advantage of the density differences between the solid and liquid phase. Centrifugal separation of biological materials is often very difficult since they tend to be structurally weak, temperature sensitive, and the phase density differences can be quite low. In general, valuable substances formed during the fermentation process are found in low concentration in a mixture with liquid and frequently solid constituents of the substrate. The concentration, isolation and purification of the valuable biologics entail the following process requirements: continuous processing of large batches, gentle product treatment, aseptic processing of pathogenic organisms, sterile product handling to avoid infection, and achievement of high yield and good economy. Centrifugal separators play key roles in many of these processes in the primary separation immediately after fermentation and further downstream in purification and isolation procedures.

1.3.1 Centrifuge types

The applications of centrifugation and the design of centrifuges to deal with these is reviewed by Axelsson (1985) and Brunner and Hemfort (1988). Five main types of centrifuges exist to deal with the varied demands presented in the biotechnology industry. Choice of separator depends on the properties of the process stream: particle size of the solid to be removed, density difference of the solid and liquid phases, solid concentration, and tendency to emulsify. The main uses of centrifuges in downstream processing are cell harvesting and washing immediately after fermentation and product recovery following extraction, crystallisation, or precipitation steps.

Tubular bowl

This has a relatively simple design. The process fluid is pumped into the centrifuge at the bottom and the solids are sedimented against the bowl wall as the fluid flows up and out of the top via an inner ring dam or a centripetal pump. The simple design allows high rotational speeds to be used and hence very good separation is possible. Additionally, very high levels of solids dewatering is possible which is very important for high value, soluble products.
However solids must be removed manually by the operator which means contact with process material is unavoidable. Aerosols can also be a problem. Hence operation of this design type is labour intensive and best restricted to process streams with low solids content and small batch volumes.

**Decanter centrifuge**

The design of the decanter is derived from the tubular bowl centrifuge with the modification that the solids discharge from the bowl is achieved by means of a conveyor screw which rotates at a different speed to the bowl. It is used primarily to concentrate solids in process liquids with high solids content. Due to the inherent imbalance of the design the centrifugal force capability is small and hence it is suitable only for large, relatively dense particles. However new machines capable of high speed operation are being piloted. The ability of decanters to handle high solids concentrations and the capability of very high levels of solids dewatering make this a very attractive option for many processes. Main uses are sludge dewatering in the waste-water industry and initial fermentation broth clarification.

**Multichamber centrifuge**

This type of centrifuge is basically a solid wall bowl centrifuge with a high solids holding capacity but manual solids discharge. The bowl is equipped with up to five concentric chamber inserts which have the effect of extending the axial flow path and increasing the area available for sedimentation. In the inner chamber it is mainly larger particles that are sedimented but in the outer chambers the centrifugal force is increased and hence finer particles are sedimented. The multichamber design uses a hydrohermetic feedzone where the feedzone is flooded. Thus material fed to the centrifuge is accelerated more slowly than with the standard feedzone were feed simply impacts on the metal surface of the centrifuge. Thus, with the hydrohermetic design, greatly reduced shear forces are experienced by particles during liquid acceleration. This results in low levels of shear breakage of particles and hence better recovery performance. The relatively low centrifugal force relative to the tubular bowl design is compensated for by the long residence time in the bowl. This type of machine is capable of relatively high levels of solids dewatering.

**Disc stack centrifuge**

Unlike the other centrifuge designs solids separation does not take place on the bowl wall but in the disc stack which consists of a series of conical discs through which the process stream travels. Discs are separated by a very small distance (~ 0.5-1.0 mm) in order to minimise the sedimentation distance. Sedimented solids, collected on the underside of the discs, travel
down the disc and collect in the solids holding space at the bowl periphery. Several variations on the basic design exist: non-solids discharging for process streams with very low solids concentrations, this is a batch operation machine; nozzle types for handling high solids content streams by enabling their continuous discharge, this is a fully continuous operation i.e. both solids and liquids are discharged continuously. This produces very wet solids. The final variant is the intermittent discharge type which is designed to handle lower solids content streams. When the solids holding space is full, ports in the periphery of the bowl are hydraulically opened and the solids are ejected at full bowl speed. Both partial (discharge of solids alone) and full (discharge of solids plus bowl liquid) are possible. In both cases solids are much wetter than those collected with the tubular bowl and decanter centrifuge designs which is less than ideal when the product is soluble. Hydro-hermetic feedzone types are available which reduced the damage to shear-sensitive particles. This type of centrifuge is widely used in the downstream processing of biotechnology-based processes.

**Tubular bowl (solids discharging) centrifuge**

A relatively new design by Carr Separations Inc. employs the high separation and dry solids capability of the tubular bowl design with an automated solids discharge mechanism. The bowl has a considerably smaller aspect ratio than tubular bowl designs which results in high wall centrifugal forces. As the bowl is constructed largely of titanium, which is considerably less dense than stainless steel, it can be operated at high speed. Solids are discharged automatically by rotating the bowl at low speed while a scraper, located in the centre of the bowl, moves toward the bowl wall. The displaced solids drop out of the bowl into a sealed chamber below. Operation is contained and the centrifuge can be cleaned and sterilised in-place.

1.3.2 **Centrifugal recovery of biological solids**

Problems with the recovery of biological solids are mainly related to the small particle size, low density difference between the particles and the continuous liquid phase, sensitivity of the solids to shear and to temperature, and denaturation at air/liquid interfaces.

**Separation of cells**

A low density difference between some cells and fermentation broth, of the order of 10 kg m$^{-3}$, combined with high viscosities in the range of 1-5 mPas can make separation difficult. This is amplified by the small size of some cells; bacteria are usually in the range of 1-3 μm, yeast are more easily sedimented due to their larger size, 4-8 μm. Fungi, although large in
comparison, can be very difficult to sediment because their high shear sensitivity leads to size
degradation.

**Separation of cell debris**

To isolate intracellular products it is necessary to disrupt the cells. Mainly, this is performed
by mechanical means, usually with an homogeniser or bead mill. Removal of microbial cell
debris is one of the most difficult downstream operations in which to achieve high efficiency
(Bonnerjea *et al.*, 1988) yet it is also very important since debris can interfere with
purification processes further downstream. An example of this is affinity chromatography
which has the potential for very high resolution purification and hence its use early on in the
purification process would be ideal. However, it is both expensive and easily fouled which
usually confines its application to end stage purification. Cell debris produced by
homogenisation has a broad size distribution some of which is below 0.2 μm. This combined
with a density difference, depending on the lipid content, of as little as 10 kg m⁻³ explains the
difficulty encountered in debris separation.

**Separation of protein precipitates**

Density and sizes of protein precipitates depend upon the protein to be precipitated, the
precipitant, and the precipitation system (Bell and Dunnill 1982). Aggregate sizes are
typically between 5 and 25 μm although they may be as little as 1 μm (Clarkson *et al.*, 1993a).
Density differences relative to the liquid phase range from 10 to 150 kg m⁻³. This may mean
that the centrifuge must be operated at very low flow rates which can cause problems due to
temperature rises unless adequate cooling is available. Additional separation problems can
occur due to high viscosity caused in the main by non-protein components, namely nucleic
acids released during cell disruption. These may be removed by the addition of nucleases for
digestion, or salts or polymers for precipitation. Other problems encountered are associated
with protein properties, the nature of proteins can lead to foaming and air entrapment
resulting in low effective settling velocities as well as protein denaturation due to the presence
of large air/liquid interfaces. High shear in the centrifuge, typically in the feed zone, can
result in aggregate breakage creating fine particles thus reducing separation efficiency. It is
difficult to determine the degree of particle breakage in the feedzone since two processes are
occurring simultaneously, namely shear breakage and particle separation. Pumping of
precipitate to the centrifuge has been shown to cause shear breakage (Hoare *et al.* 1982).
Aggregate strength is dependant on the protein, precipitant and also precipitation system used
(Section 0). Once the protein precipitate has been sedimented in the centrifuge it must then be
discharged. For this type of operation an intermittent discharge disc stack centrifuge is the
usual choice of machine since it combines abilities of good separation potential with semi-continuous operation. Protein precipitates are sticky, gelatinous and exhibit viscoelastic properties which can cause problems with discharge for this type of centrifuge. Additionally, protein precipitates are not easily de-watered hence slurries are contaminated with significant quantities of supernatant.

**Centrifugation of feeds with high solids concentration**

Feed rates calculated theoretically are generally much higher than those usable in practice due to hindered settling (Section 0) which occurs when centrifuging streams with high solids content. This results in a reduced particle settling velocity. For feed solids concentration of 0-2 % v/v practical flow rates of around two thirds the predicted flow rate are recommended. For feeds with solids concentration of around 5 % v/v feed rates of approximately half the theoretical rate are advised (Clarkson et al, 1993b).

Feed stream solids concentration is very important when choosing the centrifuge design, Figure 1-2 (Brunner and Hemfort, 1988). Solid bowl centrifuges are only suitable for low solids concentrations process streams since they must be stopped and dismantled to remove solids once the bowl is full. If however, the solid material requires low throughputs for separation and also a high concentration factor is required then a disc centrifuge with an intermittent solids discharge is more suitable, even for concentrations up to 30 % by volume. For very high solids contents streams, where particularly dry solids are required, then the scroll decanter centrifuge may well be appropriate if the separation capability is sufficient.

<table>
<thead>
<tr>
<th>Separable solids (vol %)</th>
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<th>20</th>
<th>40</th>
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<td>Chamber bowl centrifuge</td>
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<td>Solid bowl centrifuge</td>
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<td>Self–cleaning centrifuge</td>
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<td>Nozzle centrifuge</td>
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<td>Decanter centrifuge</td>
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Figure 1-2  Guide to suitable centrifuge design with respect to feed solids concentration
1.4 Protein precipitation

Protein precipitation and its subsequent recovery are important operations in industrial scale protein purification. It is used to greatly reduce the volume of process fluid to be processed further downstream and also to purify the product by reducing the levels of contaminating proteins. Englard and Seifter (1990) estimated that up to 80 % of published protein purification protocols had at least one protein precipitation step. Applications vary from primary isolation procedures (Michaels, 1990) to finishing operations (Paul and Rosas, 1990). It is possible to isolate different protein fractions by taking a number of cut points, an industrial example is blood plasma processing (Foster and Watt, 1980). More recently, multi-cut separation tends to be performed by chromatography and the uses of protein precipitation tend to be restricted to single or double cut fractionation.

Protein precipitation can be split into four separate stages. The first stage is the addition of precipitant to achieve supersaturation of the protein and the formation of nuclei by the diffusion controlled agglomeration of thousands of protein molecules (Rothstein, 1994). Nucleation is followed by perikinetic growth of nuclei to form microscopic colloidal entities termed primary particles. There is then orthokinetic growth by the collision and adhesion of primary particles to form macroscopic precipitate particles. Kinetics of the perikinetic and orthokinetic stages are discussed in Section 11.4.3.1. Collisions can lead to break-up of larger particles as well as aggregation and growth and hence the final average particle size is a balance between orthokinetic growth and hydrodynamic shear-controlled break-up. The final stage is a period of ageing of the precipitate particles. Under appropriate shear conditions the precipitate particles continue to grow in size, strength and density.

Protein precipitation is an irregular aggregation process as opposed to crystallisation which involves the regular, stepwise aggregation of monomers. Why one process occurs and not the other has been the focus of much work. Mikol et al (1990) found that protein aggregation occurred with some precipitants in protein solutions that were undersaturated. In such cases, once the solution was supersaturated, proteins were unable to crystallise and precipitation occurred. Whereas, with other precipitants the proteins remain mono-dispersed up to saturation and then crystallised once supersaturation was reached. Kadima et al (1990) suggested that when the aggregation of monomers occurs too rapidly then precipitation results rather than crystallisation.

Precipitation is performed by the addition of a reagent which causes the formation of insoluble particles of protein. Usually the intention is to isolate the protein in its original
molecular form or one that is easily returned to that form. Hence precipitants that cause irreversible denaturation are of limited interest.

In a single cut fractionation sufficient precipitant is added to bring some of the contaminating proteins out of solution but usually leaving the product protein in solution. The solid proteins would then be separated, often by centrifugation. For a two cut fractionation process more precipitant would then be added to bring the product out of solution whilst usually leaving some contaminating proteins in solution. Again, this is often followed by centrifugation to recover the product. Hence the two cut process usually enables two purification stages depending on the total protein/product solubility profiles.

1.4.1 Methods of protein precipitation

Protein solubility in various solvents is determined by the distribution of hydrophilic and hydrophobic residues on the molecule's surface. Altering the protein environment by the addition of precipitants reduces the barrier to protein association and primary protein particles are able to form. Precipitation is performed by either changing the nature of the solvent environment or by direct interaction of a reagent with the protein.

**Methods that effect the solvent environment include:**

a. Salting-out by the addition of high concentrations of neutral salts. Commonly used salts are ammonium sulphate and sodium sulphate.

b. Reduction in the dielectric constant of the solvent by the addition of miscible solvents, for example ethanol and acetone.

**Methods that involve direct interaction with the protein include:**

a. Charged polyelectrolytes. These act as flocculating agents under the appropriate pH conditions, for example CMC (carboxymethyl cellulose)

b. Polyvalent metal ions, for example calcium ions.

c. Non-ionic polymers - these are thought to act by reducing the water available for protein solvation.

d. pH adjustment - altering the pH to the iso-electric point of the protein and thereby reducing its solubility.

A review of the different methods of precipitation is given by Bell et al. (1983). Choice of precipitating agent depends on many factors: selectivity, denaturation, use of product, flammability, corrosiveness, and cost.
1.4.2 Processing aspects to protein precipitation

Protein precipitates may be recovered by filtration or more usually by centrifugation (Devereux et al, 1984). For centrifugal recovery the important properties of precipitate particles are particle size and density. A related aspect to precipitate size is particle strength. Precipitate particles are shear-sensitive (Devereux et al, 1984, McIntosh et al, 1988) and are easily broken in the high shear regions of industrial centrifuges (Bell and Brunner, 1983, Mannweiler et al, 1989). The physical characteristics of precipitates are affected by many parameters

a. type of precipitator (tubular or stirred tank)  
b. type of precipitant  
c. initial protein concentration  
d. mixing  
e. residence time in the reactor  
f. pH and ionic strength

The effect of different precipitator type has been examined by Bell and Dunnill (1982). Precipitate prepared in a tubular reactor with only a short ageing period results in large, irregularly shaped precipitates of low density that are more prone to shear damage than precipitates formed using a stirred tank reactor (STR). This is thought to be due to the region around the impeller of the STR where precipitate particles are exposed to very high shear forces which result in the survival of only dense and small, i.e. less shear-sensitive, particles. Improvement in the size and density of precipitates formed in tubular reactors can be made by using an ageing section which allows exposure to higher shear rates for short time periods. Acoustic conditioning also improves precipitate particles sedimentation characteristics (Hoare et al, 1987, Foster and Watt, 1980) by inducing turbulent flow and hence promoting aggregation of fine particles.

The effect of different types of precipitant was examined by Chan (1986) using a tubular reactor. He discovered a wide variation in mean precipitate particle diameter using different reagents for the precipitation of soya protein: sulphuric acid (12.5µm), ethanol (7.5µm), and ammonium sulphate (3.1µm).

Virkar et al. (1982), in a study of iso-electric precipitation of soya protein, examined the effect of protein concentration on the final aggregate size. No effect was discovered for
concentrations in the range 2-15 kg m$^{-3}$ but from 15-30 kg m$^{-3}$ there was a measurable increase in the mean particle diameter.

1.4.3 Precipitation reactors

The basic reactor configurations used for protein precipitation are the stirred tank reactor, used for both batch and continuous operation; and the tubular (plug flow) reactor. The stirred tank reactor usually consists of a baffled tank which is mixed by an impeller, typically a Rushton turbine. With the tubular reactor the protein stream and the precipitant stream are pumped into a static mixer (Richarz and Sporri, 1972) where mixing occurs due to turbulence created by the convoluted flow path. The static mixer has a small bulk volume so mixing is rapid, even at large scale, thus reducing problems of over-precipitation caused by localised high concentrations of precipitant (Section 0). After the mixing section, the precipitate enters an ageing section where a much reduced shear rate is encountered in order to promote particle growth.

The initial design problem is to establish the contacting requirements of the reactants for nucleation. In the case of fast reactions, which is normally true for small molecular weight precipitants, the major design consideration is to achieve rapid mixing to ensure a uniform distribution of precipitant thus preventing over-precipitation and possible protein denaturation; and for efficient heat transfer in the case of exothermic reactions. For nucleation reactions that are slower, rapid mixing is less crucial so both types of reactor may be considered.

On a small scale, high power input, and therefore rapid mixing, in a stirred vessel are easy to achieve but on a production scale the power input is restricted for economic and mechanical reasons causing mixing and mass transfer problems (Sweere et al. 1987). Hence, this type of vessel may not be suitable for precipitations with short nucleation times and a tubular type may be more appropriate. An advantage of precipitation using the tubular reactor is that it is continuous and can be fed directly to a centrifuge for solids separation. Therefore, it is possible to avoid passing the precipitates through a pump where shear damage may result (Hoare et al., 1982). Batch precipitation in a stirred tank normally requires pumping of the precipitate stream to the centrifuge unless vessel head pressure is used.

Once the nuclei have formed and grown to produce primary particles then the next stage is shear-controlled, aggregative growth to form macroscopic precipitate particles. The shear conditions promote particle collisions which can result in particle growth but also in particle
breakage. Hence, although increasing the shear rate increases the rate of particle growth the overall particle size may reduce if the balance is shifted towards particle breakage.

For both reactor designs workers have found that having high shear regions in parts of the reactor promotes aggregation (Leentvaar and Ywena, 1980). In the case of tubular reactors (Virkar et al, 1982) aggregation performance was improved by the insertion of meshes to create turbulence (Figure 1-3).

Figure 1-3 The effect of meshes on precipitate particle size in a continuous tubular reactor
Particle ageing, i.e. exposing particles to low shear intensities for extended time periods, also helps to improve particle strength (Bell and Dunnill, 1982) due to the preferential survival of tightly packed aggregates. Significant differences in particle strength between precipitates prepared in stirred vessels and tubular reactors were reported by Bell et al (1983). Precipitate prepared in the tubular reactor exhibited significantly less resistance to disruption.
A variation in precipitator design proposed for blood fractionation mixes the precipitant and protein streams in a centrifugal pump for rapid mixing prior to ageing in a tubular section and also a stirred tank (Falke et al, 1981). Rothstein (1994) proposed a design to reduce the occurrence of local precipitant excess and control the number of nuclei formed. This employed a series of static mixers before each of which precipitant was added so that the concentration of precipitant was increased slowly. An ageing section, either tubular or stirred tank is employed before the stream is returned to the static mixers for further precipitant addition. The final precipitant concentration is reached after a series of cycles. He found that the cut points were cleaner and that solids recovered by centrifugation were dryer.

1.5 Process interactions

In the past workers have mainly concentrated on single unit operations rather than considering processes as a whole and the effect that unit operations have on others further downstream. Interactions include: nucleic acids and cell debris released during cell disruption can affect fractional precipitation and chromatography stages; increased cell rupture to maximise intracellular product release can result in debris too fine to sediment which then passes through subsequent steps. Such interactions make it essential to have a process view of downstream processing. Interactions between different unit operations in general and especially those involved in protein purification are discussed below.

1.5.1 Fermentation and product recovery

The essential increases in titre achieved as a result of strain improvement and optimisation of culture conditions have led to the situation where fermentation rather than product recovery considerations have dominated process development. Hence downstream recovery processes often prove adequate but less than ideal. Rosen (1983) pointed out that 45 % of equipment costs can be associated with product recovery compared with 14 % for fermentation. Also the ratio of recovery to fermentation costs is 2.0 for an enzyme. Hence it is important that sufficient effort is devoted to downstream as well as fermentation aspects. An essential part of this is that of process interactions. Important parameters of fermentation that affect protein recovery have been reviewed by Fish and Lilly (1984).

1. Harvest time. Enzyme titre in a batch culture may be at a maximum for no more than an hour, for example gramicidin S synthetases in Bacillus brevis (Kula et al, 1982). Reduction in titre may be due to depletion of carbon or nitrogen which can result in increased protein
turnover. In defined media, cell lysis is more likely. This can lead to enzyme inactivation and consequent product loss.

2. **Growth medium.** The type of growth medium can affect the cell wall strength which has a significant effect on cell disruption for the recovery of an intracellular product. The rate of release of β-galactosidase from *E. coli* by passage through a high pressure homogeniser was faster when the culture was grown on glycerol with mineral salts instead of a complex medium (Gray *et al.*, 1972). This is thought to be due to the availability of components in complex media for building strong cell walls which are not present in defined media.

3. **Age of culture.** The rate of protein release during disruption was highest for bacteria harvested early in the exponential phase and decreased as further growth occurred until it was several times less for cells recovered in the stationary phase (Wang *et al.*, 1979). Similarly *Candida utilis* grown at a high specific growth rate in cyclic batch culture was easier to disrupt in a high pressure flow device than when grown at a lower specific growth rate in continuous culture (Engler and Robinson, 1981).

### 1.5.2 Homogenisation and centrifugation

The physical characteristics of the feed material to the centrifuge depend on the operating parameters of the homogeniser. In particular, the total cell debris particle size distribution and viscosity of the suspension have a major effect on centrifuge performance. Homogenisation at high pressures and for repeated passes maximises product release but also leads to the production of sub-micron sized cell debris (Keshavarz *et al.*, 1990) and the release of nucleic acids and proteoglycans (Mosqueira *et al.*, 1981). These cause an increase in suspension viscosity making debris recovery more difficult. The interaction between the homogenisation step and the cell debris clarification step is shown in Figure 1-4 (Clarkson *et al.* 1993b).
Figure 1-4 Homogeniser / centrifuge interactions
The theoretical separation limit of a centrifuge can be calculated from the centrifuge critical particle diameter, $d_c$, which is a function of the design and operating conditions of the machine. In theory, cell debris particles with a diameter greater than or equal to $d_c$ will be recovered whereas particles smaller than $d_c$ will not. Figure 1-4 shows the equivalent particle diameters above which lie 90% ($d_{90}$) and 50% ($d_{50}$) of the total particle population. Figure 1-4 shows how the centrifuge throughput must be reduced in order that the $d_{90}$ line and the $d_c$ lines intercept. At a centrifuge throughput of 100 l/h the $d_c$ line intercepts with the $d_{90}$ line at a protein release of 25%. Hence for the efficient removal of cell debris the homogeniser must be operated so that most the product is not released. If the centrifuge throughput is halved then the homogeniser can be operated to give 48% protein release.

1.5.3 Protein precipitation and centrifugation

Precipitation is sometimes perceived as an unreliable process. This is because performance is affected by a number of factors one of which is the variable composition of the feed stream resulting from variations in the fermentation. The main contaminating substances that interfere with precipitation are fermentation broth, nucleic acids and cell debris (Richardson et al, 1989).

When considering the precipitation process it is essential to remember the subsequent centrifugation step for precipitate removal. This is an analogous situation to the homogenisation/centrifugation interaction. For efficient clarification of the process stream after precipitation it is important to maximise the precipitate particle size, strength, and density difference in relation to the process liquid. Different precipitation reactor designs also impact on centrifuge clarification efficiency; discussed earlier (Section 1.4.3).

1.6 Aims of research

The main aim of the research is to develop downstream processing unit operations that give similar process performance to pilot scale equivalents but which require a much reduced process material requirement. Such unit operations would work in a similar fashion to the pilot scale units in order to mimic the performance. These unit operations are described as scaled-down as opposed to small scale unit operations which may operate in a very different fashion. A very important example, and one that is addressed in this thesis, is that of centrifugation. A widely used industrial centrifuge is the intermittent discharge disc stack centrifuge. This machine has the capability of recovering fine particles in a semi-continuous
manner since when the machine's recovered solids capacity is reached it is possible to eject them without stopping the centrifuge. Such a machine does however exert considerable stresses on the process material which can damage sensitive products. A scale-down equivalent would attempt to incorporate the same stresses and thus mimic the performance, whereas the small scale equivalent, the laboratory centrifuge, imparts very little shear to the process fluid and hence tends not to damage sensitive materials so process performance tends to be unrepresentative of the industrial type.

Three methods of operation are examined here, small scale (laboratory), scale-down and pilot scale. At each scale three unit operations are considered: high pressure homogenisation for cell disruption to release the product, disc stack centrifugation for solids separation and protein precipitation for product purification. The most important unit operation in terms of scale-down is disc stack centrifugation since no small scale machine is commercially available. Small scale high pressure homogenisers are available, although they are not everyday laboratory equipment, and a suitable scale stirred tank for protein precipitation can be manufactured simply. The performance of these scaled-down unit operations must still be determined. However for disc stack centrifugation it is necessary to either modify an existing machine to reduce its material requirement or to design a new, smaller scale machine. Both approaches require the expert input of a centrifuge manufacturer.

Two approaches were taken to studying the unit operations: one was to examine each unit operation individually so that performance over a wide range of conditions could be examined. The second was to run the unit operations as a process since this is the normal context in which they would be studied when investigating a suitable purification sequence for a particular product and, additionally, it is only in a process that interactions between different unit operations can be observed.

The choice of process system involved consideration of a number of factors that are listed below. The decision was to adopt a purification process for yeast alcohol dehydrogenases (ADH), intracellular enzymes.

- Bakers yeast (Saccharomyces cerevisiae) is a widely studied micro-organism and is very important industrially.
- ADH is a typical intracellular enzyme representative of industrially useful biocatalysts (Follows et al, 1971) and hence research performed should be applicable to many different enzymes.
- The high number of centrifugation stages in this process make this the most important unit operation. This is in keeping with emphasis on centrifugation in this scale-down study.
- This enzyme and the unit operations used in the purification process have been extensively studied at UCL. Yeast and its homogenate have been well documented by Mosqueira et al (1981); the unit operation of homogenisation is well defined (Hetherington et al, 1971); and ammonium sulphate precipitation of yeast ADH has been studied by Foster et al (1976) and Richardson et al (1989).
- The process sequence involves a wide range of unit operations thus enabling a broad area of study and allows process interactions to be taken into account.
- Packed yeast is readily available commercially thus allowing efforts to be concentrated on downstream processing.

The flow diagram for the chosen process sequence is shown below (Figure 1-5).

Figure 1-5 Process flowsheet for the purification of yeast ADH.

In place of the fermentation stage blocked yeast is re-suspended to 7% (ww/v) yeast concentration, typical for a fed-batch fermentation. The suspended cells are then harvested in the first centrifugation step prior to high pressure homogenisation to release the intracellular proteins including the product. The yeast debris formed in the breakage stage is then removed
in the second centrifugation step prior to protein purification which is achieved with a two cut ammonium sulphate protein precipitation procedure. The 1st cut precipitation leaves the product (ADH) in solution but precipitates the less soluble protein contaminants. These are then removed in the subsequent clarification step. The 2nd cut precipitation leaves some of the most soluble contaminating proteins in solution but precipitates out most of the product which is harvested in the final clarification step.
2. MATERIALS AND METHODS

In this chapter details are given of equipment, preparation of materials, assays, and experimental procedures. Three scales of operation are investigated, laboratory scale, scale-down and pilot scale. Three unit operations were studied, high pressure homogenisation for cell breakage, centrifugation for solids recovery, and protein precipitation for product purification. These unit operations were studied individually in order to examine a wide range of process parameters and also in a process context so that important process parameters such yield and purity can be examined throughout the process and interactions between different unit operations can be observed. Details of the process and reasons for its choice are discussed elsewhere (Section 1.6). The methods detailed below are specific to the individual study of unit operations. Modifications of the methods for study in a process context are discussed in Section 2.2.

2.1 Methods for unit operation study

2.1.1 Equipment.

2.1.1.1 High pressure homogenisation.

The APV K3 homogeniser was used for pilot scale studies and the APV Lab60 for scale-down and laboratory scale. Typical working volumes are 10 L for the Lab 60 and 50 L for the APV K3. Specifications for the two machines are given below (Table 2-1). Valve dimensions are given in Table 2-2.

<table>
<thead>
<tr>
<th>Model</th>
<th>Lab 60</th>
<th>K3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>APV, Crawley, Sussex</td>
<td>APV, Crawley, Sussex</td>
</tr>
<tr>
<td>Flow rate (L h⁻¹)</td>
<td>60</td>
<td>280</td>
</tr>
<tr>
<td>Hold-up volume (L)</td>
<td>&lt; 1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Scale of unit</td>
<td>Small pilot scale</td>
<td>Pilot scale</td>
</tr>
<tr>
<td>Valve type and geometry</td>
<td>CD</td>
<td>CD</td>
</tr>
</tbody>
</table>

Table 2-1 High pressure homogeniser specifications
<table>
<thead>
<tr>
<th>Valve type</th>
<th>$R_o$</th>
<th>$R$</th>
<th>$X$</th>
<th>$L_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>3.85</td>
<td>4.18</td>
<td>0.7</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Table 2-2: Important valve dimensions for CD valve used in APV K3 an APV Lab60 (dimensions are given in mm).

Where $R_o$ and $R$ are the radii of the central orifice immediately before the valve gap and immediately after the exit of the valve gap respectively, $X$ is the distance from the exit of the valve gap to the impact ring and $L_d$ is the distance across the “land”. Dimensions are shown in Figure 11-5.

2.1.1.2 Centrifugation.

Laboratory scale
All experiments were performed with the Beckman J1-MI lab centrifuge (Beckman Instruments, High Wycombe, UK). This was fitted with the JS-13.1 swing-out rotor which has a maximum operating speed of 13000 rpm (21000 g).

Pilot scale
The Westfalia SAOOH-205 disc stack centrifuge (Westfalia Separators, Milton Keynes, UK) was used for all experiments. This is an intermittent discharge disc stack centrifuge with a bowl volume of approximately 0.6 L. Two disc types were used, discs with and without riser channels (Figure 2-1). Dimensions of the two disc types were similar.
Figure 2-1 Active disc (with riser channels) from SAOOH-205 disc stack centrifuge (dimensions in mm)

A cross-section through the centrifuge is shown in Figure 2-2a.
Scale-down

The SAOOH-205 disc stack centrifuge was scaled down using a series of four inter-locking conical discs (top inserts) positioned within the bowl to reduce the separation area, the bowl volume and the sedimented solids holding capacity. The inserts may be used in different combinations thus adjusting the degree of scale-down to suit a particular application. As well as the four reducing inserts, a bottom insert is used to lift the disc stack off the centrifuge bottom away from the turbulent region. A cross section through the centrifuge when fully scaled down (4 top inserts and the bottom insert) is shown below (Figure 2-2b).

![Cross section through SAOOH-205 disc stack centrifuge (a) full stack (b) full scale down (4 reducing inserts and bottom insert)](image)

Configuration of the full stack centrifuge and scale-down options are shown in Table 2-3 and Table 2-4. The bottom insert used for configurations in Table 2-3 was only used for initial work since experimental results showed that it was necessary for it to be modified in order to align the riser channels. Additionally, the height was reduced so that more discs could be fitted since this helped to equal the degree of scale-down of the separation area and the bowl volume. The type of separating disc used in the scale-down centrifuge was the same as that used in the pilot scale machine.
### Table 2-3 Scale down options for SAOOH disc stack (bottom insert used for all scale-down options but not for full stack centrifuge)

<table>
<thead>
<tr>
<th>No. reducing (top) inserts</th>
<th>Active discs</th>
<th>Bowl volume (calculated) $V_{bc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>z</td>
<td>Number</td>
</tr>
<tr>
<td>0 (full stack)</td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 2-4 Scale down options for SAOOH disc stack centrifuge with smaller, modified bottom insert (bottom insert used for all scale-down options but not for full stack centrifuge)

<table>
<thead>
<tr>
<th>No. reducing (top) inserts</th>
<th>Active discs</th>
<th>Bowl volume (calculated) $V_{bc}$</th>
<th>Solids holding volume (calculated) $V_{sc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>z</td>
<td>Number</td>
<td>% full scale</td>
</tr>
<tr>
<td>0 (full stack)</td>
<td></td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>28</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>21</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>15</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>9</td>
<td>24</td>
</tr>
</tbody>
</table>

Dimensions for inserts and active discs are given below (Table 2-5) and representations of each insert are shown in Figure 2-3, Figure 2-4 and Figure 2-5 (all dimensions are in mm).

<table>
<thead>
<tr>
<th></th>
<th>$r_1$ (mm)</th>
<th>$r_2$ (mm)</th>
<th>height (mm)</th>
<th>½ cone angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active disc</td>
<td>27</td>
<td>55</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>Top (reducing) insert 1</td>
<td>27</td>
<td>75</td>
<td>56</td>
<td>38</td>
</tr>
<tr>
<td>Top (reducing) insert 2</td>
<td>27</td>
<td>72</td>
<td>55</td>
<td>38</td>
</tr>
<tr>
<td>Top (reducing) insert 3</td>
<td>27</td>
<td>69</td>
<td>50</td>
<td>38</td>
</tr>
<tr>
<td>Top (reducing) insert 4</td>
<td>27</td>
<td>67</td>
<td>47</td>
<td>38</td>
</tr>
<tr>
<td>Bottom insert</td>
<td>27</td>
<td>55</td>
<td>44</td>
<td>38</td>
</tr>
<tr>
<td>Bottom insert (modified)</td>
<td>27</td>
<td>55</td>
<td>22</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 2-5 Dimensions of inserts for the scale down of SAOOH disc stack centrifuge (mm)
Figure 2-4 Inserts 2-4 (inserts are geometrically similar but of different sizes)
Figure 2-5 Bottom insert
2.1.1.3 Precipitation

Precipitation was performed in stirred batch reactors the dimensions of which are given in Table 2-6. The two smaller vessels were geometrically similar, the larger vessel was not. All vessels were equipped with a six-bladed Rushton turbine. Cooling was via an external jacket fed with glycol. A schematic is shown in Figure 2-6.

<table>
<thead>
<tr>
<th></th>
<th>Laboratory scale</th>
<th>Scale-down</th>
<th>Pilot scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity (L)</td>
<td>1.4</td>
<td>4.4</td>
<td>120</td>
</tr>
<tr>
<td>Tank height (cm)</td>
<td>18.0</td>
<td>27.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Vessel diameter (cm)</td>
<td>10.7</td>
<td>16.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Impeller diameter (cm)</td>
<td>4.3</td>
<td>6.4</td>
<td>15.0</td>
</tr>
<tr>
<td>Baffle diameter (cm)</td>
<td>1.1</td>
<td>1.6</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Table 2-6 Precipitation tanks (dimensions)

![Figure 2-6 Precipitation tank](image)

2.1.1.4 Pumping

Pumps were used to supply feed material to continuous centrifuges i.e. to the pilot scale and scale-down disc stack machines. For process runs two pumps were used: for the pilot scale a lobe pump (type 50NDM, SSP Pumps Ltd., Eastbourne, England). The pump flow rate was
controlled by a PID system (System 6366, Turnbull Control Systems Ltd., Worthing, East Sussex) linked to magnetic flow meter (Model MG 711/F, Turbo-Werk GmbH, Cologne, Germany). Flow rates were also checked manually. For scale-down a gear pump (Ismatec, Model MV-Z, Croydon, UK) was used. This was a metering pump and hence the flow rate, being independent of back pressure over a wide pressure range, did not require a control system. Again, flow rates were also checked manually. A peristaltic pump (model 501U, Watson Marlow Ltd., Falmouth, England) was used as the feed to both the pilot scale and scale-down disc stack centrifuges for comparing the clarification performance with yeast cell debris and protein precipitates as it was essential that both centrifuges received the same precipitate particle size distribution to enable a direct comparison. Clarkson (1994) found that there was no shear breakage of precipitates in a peristaltic pump whereas with gear pumps precipitate shear breakage was apparent (Hoare et al, 1982).

2.1.2 High pressure homogenisation

The APV Lab 60 homogeniser was used for both laboratory and scale-down studies hence homogenisation studies were concerned with the comparison of performance of this machine and the pilot scale APV K3 homogeniser. Assessment involved determining yeast cell particle size distributions and protein release for 1-5 discreet passes through the homogeniser valve. These assessments were performed at 2 operating pressures, 300 and 500 barg, and two yeast concentrations, 28 % and 60 % (packed weight per volume).

2.1.2.1 Procedures

Packed yeast (Distillers Co. Ltd, Crawley, Sussex) was suspended to the appropriate concentration in 100 mM potassium dihydrogen phosphate buffer adjusted to pH 6.5 using 4 M sodium hydroxide. This was then disrupted by discreet passes (5 passes) at a temperature of approximately 278 K. Cooling was performed with glycol at 263 K circulated through a plate heat exchanger. Unless otherwise stated, 500 barg operating pressure was used.

2.1.3 Centrifugation (disc stack)

The recovery performance of the scale-down disc stack centrifuge configurations were compared to the full stack, pilot scale machine using the grade efficiency concept (Svarovský, 1990b). A dilute, particle suspension of polyvinyl acetate (PVAc) was used for centrifuge characterisation. Such a stream is not generally representative of typical biological feed streams which tend to be considerably more concentrated and are often sensitive to shear and
temperature (Hoare et al, 1982; Bell and Dunnill, 1982; Khan et al, 1992; Dunlop and Namdev, 1994). Hence recovery performance was assessed in two ways: using the grade efficiency method, using dilute streams of PVAc and yeast cell debris, and also by determining the clarification performance of typical biological streams over a range of \( Q/Z \) values. For all methods the same stock of feed stream was used for pilot scale and scale-down experiments so that differences from one experimental run to another were removed.

Recovery performance in terms of the grade efficiency curve was determined for the full stack disc centrifuge and all stages of scale-down. For typical biological streams clarification performance was determined for the full stack and for the fully scaled down (4 inserts, 9 active discs) as this was the set-up chosen for the process runs.

2.1.3.1 Particle system for grade efficiencies.

The chosen system must have a broad particle size distribution to enable full grade efficiency curves to be generated, a range of 1-3 \( \mu \)m being sufficient. Particle density should be relatively low in order to approximate to biological solids. Particle tensile strength should be high so that no shear breakage occurs in the centrifuge since then the particle size distribution seen by the disc stack would be different to that fed into the centrifuge and a distortion to the grade efficiency curve would result. These criteria are fulfilled by polyvinyl acetate, a comprehensive review of which is given by Daniels (1983). PVAc 190 was used (kindly donated by Hoechst AG Frankfurt, Germany) which has a particle size range of 0.5 - 3.0 \( \mu \)m and a density of 1191 kg m\(^{-3}\).

Centrifuge recovery performance was also examined using a dilute suspensions of yeast cell homogenate, also by the grade efficiency method.

Procedures for particle systems

Polyvinyl acetate particle suspensions

Concentrated suspensions of polyvinyl acetate form loose aggregates which must be broken prior to use since breakage in the high shear regions of the centrifuge would lead to distortions of the grade efficiency curve. Aggregates may be broken by sonication: ultrasound applied to a liquid causes the formation of cavities, the collapse of which result in shock waves that are thought to lead to agglomerate breakage (Scawen and Hammond, 1986). The effect of ultrasound with time on PVAc 190 was determined by Mannweiler (1990). He
found that aggregate breakage was completed after a 40 minute sonication period. To be sure of full breakage suspensions were sonicated for a period of 1 h.

0.055 kg PVAc 190 (50 % solids (v/v) stock) was thoroughly dispersed in 0.8 L of water (purified by reverse osmosis, RO). Further dilution with chilled water (5 °C) gave a final volume of 8.0 L. This was sonicated for one hour. Chilled water was used since this reduced heating caused by sonication that may result in damage to PVAc particles. Prior to experimentation the sonicated PVAc was diluted into 100 L of RO water to give a final concentration of 0.055 % (w/v).

**Yeast cell debris** - Yeast was suspended to a concentration of 600 g/l (ww) in phosphate buffer (Section 2.1.2.1). This was disrupted using the APV Lab 60 high pressure homogeniser in a continuous fashion for the equivalent of 10 discreet passes at 500 barg to ensure good disruption. Other homogenisation conditions were as for Section 2.1.2.1.

For all particle systems the experimental layout was as presented in the schematic below (Figure 2-7)

![Figure 2-7 Flow sheet of experimental set-up, V1-2: diaphragm valves, V3: needle valve. F1,2: flow meters. P: pressure gauge.](image-url)

Particles were kept in suspension in the vessel during the experiment by gentle agitation (90 rpm). In each case recovery performance was measured at three flow rates to coincide with critical diameter ($d_\text{c}$) values of 1.1 μm, 1.4 μm, and 1.7 μm. The suspension was pumped to the centrifuge via two lobe pumps (type 50NDM, SSP Pumps Ltd., Eastbourne, England) arranged in parallel. This gave a flow rate range of 20-800 L/h. The pumps were controlled
independently by two magnetic-inductive flowmeters (Turbo AG, Germany - accuracy of ± 0.5 % of full scale deflection) and independent of the physico-chemical characteristics of the fluid, provided the fluid has a conductivity of at least 5 mS. In addition, flow rates were measured by hand. Ten centrifuge bowl volumes of suspension were allowed to pass through the machine before a sample was taken. Solids discharging was performed three times between each sample to ensure cross-contamination did not occur.

Samples were analysed using the Elzone particle sizer (Section 2.3.2.1).

The practical grade efficiency curve was calculated by (derived earlier, Section 11.4.2.1):

\[ T(d) = 1 - (1 - E_t) \frac{\Delta F_{\text{sup}}(d)}{\Delta F_{\text{feed}}(d)} \]

Equation 2-1

where \( \Delta F_{\text{sup}}(d) \) and \( \Delta F_{\text{feed}}(d) \) are the volume fraction of particles of diameter \( d \) in the supernatant and the feed respectively. The mass yield, \( E_t \), was determined as follows:

\[ E_t = 1 - \left( \frac{V_{\text{sup}}}{V_{\text{feed}}} \right) \]

Equation 2-2

where \( V_{\text{sup}} \) and \( V_{\text{feed}} \) are the total volume of particles in the supernatant and feed respectively. These were determined by particle sizing and counting.

2.1.3.2 Biological streams (typical concentrations)

In addition to dilute stream work, the recovery performance with more normal concentration biological streams was determined. Streams from the ADH process sequence were used which included: whole yeast cells, yeast cell debris and yeast protein precipitated with ammonium sulphate to 40 % and 60 % saturation. Additionally, yeast protein precipitated with polyethylene glycol 8000 (PEG) was examined.

Procedures for Biological streams.

Whole cells
Packed yeast was re-suspended to a concentration typical of a fed-batch fermentation, 7 % wet weight suspension in phosphate buffer (Section 2.1.2.1). Sufficient volume was processed through the centrifuge to allow one solids discharge. Clarification performance was determined by measuring \( \text{OD}_{670} \).

\[
\text{Clarification(\%)} = \left(1 - \frac{\text{OD}_{670}\text{(supernatant)} - \text{OD}_{670}\text{(well spun)}}{\text{OD}_{670}\text{(feed)} - \text{OD}_{670}\text{(well spun)}}\right) \times 100
\]

Equation 2-3

**Yeast debris**

A 60 % (ww) suspension of yeast cells was prepared in phosphate buffer to give a final volume of 20 L. This was then disrupted in the APV K3 homogeniser at 500 barg according to Section 2.1.2.1. The homogenate was then diluted to 28 % (ww/v original yeast concentration) with additional phosphate buffer. Sufficient volume was processed through the centrifuge to enable one solids discharge. Clarification performance was determined by measuring \( \text{OD}_{670} \) as for the clarification of whole cells (Equation 2-3).

**Protein precipitate streams**

20 L of 60 % (ww/v) yeast cell debris was prepared using the APV K3 homogeniser (Section 2.1.2.1). This was then diluted to 28 % (packed weight per volume) by the addition of phosphate buffer prior to clarification in the full stack SAOOH-205 disc centrifuge at 50 L/h \((Q/\Sigma = 2.3 \times 10^{-8} \text{ m s}^{-1} \text{ (corrected for centrifuge efficiency)})\)) discharging after every 90 seconds of supernatant flow until 18 L of clarified debris had been collected. This was then cooled to 278 K then precipitated to 40 % ammonium sulphate saturation (1\text{st} cut point in ADH process) by the rapid addition of saturated ammonium sulphate pre-cooled to 278 K. The temperature was maintained using chilled glycol fed to the vessel jacket. The impeller speed was set to give a mean velocity gradient \((G)\) of 44 s\(^{-1}\). Sufficient material was processed to allow one full solids discharge. Feed, supernatant and slurry streams were assayed for solid and liquid ADH enzyme and protein (Section 2.3.1). Clarification was also determined with precipitate formed at 60 % saturation (2\text{nd} cut point in ADH process). This was prepared by preparing 40 % saturation precipitated proteins as above and then clarifying in the full stack disc stack centrifuge at 20 L h\(^{-1}\) \((Q/\Sigma = 9.1 \times 10^{-9} \text{ m s}^{-1} \text{ (corrected for centrifuge efficiency)})\). The supernatant was then cooled to 278 K and then precipitated to 60 % saturation by the rapid addition of saturated ammonium sulphate. The impeller speed was set to give a mean velocity gradient of 44 s\(^{-1}\).
In addition to ammonium sulphate precipitates clarification performance was also assessed using polyethylene glycol precipitated proteins the methods for which are described in Section 9.

For all precipitates solid protein was measured for the feed and the supernatant and the clarification performance was determined using the following equation:

\[
\text{Clarification(\%)} = \left(1 - \frac{M_{\text{protein}}(\text{supernatant})}{M_{\text{protein}}(\text{feed})}\right) \times 100
\]

Equation 2-4

where \(M_{\text{protein}}\) is the mass of solid protein in the process stream.

2.1.4 Centrifugation (Laboratory)

Clarification performance for the laboratory centrifuge was determined over a range of \(Q/\Sigma\) values for a dilute stream of polyvinyl acetate (PVAc 190), and also for more typical biological streams (yeast cell debris and yeast protein precipitates formed with both ammonium sulphate and polyethylene glycol).

All experiments were performed with the Beckman J1-M1 lab centrifuge (Beckman Instruments, High Wycombe, UK) fitted with the JS-13.1 swing-out rotor which has a maximum operating speed of 13000 rpm. Open, lipless 10 ml centrifuge tubes were used; these were filled to capacity. In most cases the centrifuge was operated at 6870 rpm, equivalent to 3870 g. For larger values of \(Q/\Sigma\), which could not be met at 3870 g, the centrifuge was operated at 4000 rpm (2000g). The centrifuge has variable acceleration and deceleration rates that effect the centrifuge only in the 0-500 rpm range. For these experiments these were set to the maximum giving a 0-500 rpm acceleration time of 15 seconds and 500-0 rpm deceleration time of 30 seconds.

For all particle streams the centrifuge tubes were always filled to capacity and after each run each tube was decanted slowly. Material remaining in the tube was said to be sedimented and hence it represented the slurry stream. Material that was decanted was said to be the clarified supernatant.

Preparation of materials
PVAc 190
0.55 g of PVAc 190 (50 % solids (v/v) stock) was thoroughly dispersed in 0.4 L of water (purified by reverse osmosis, RO). This was sonicated for one hour. Chilled water was used since this reduced heating caused by sonication that may cause damage to PVAc particles. Prior to experimentation the sonicated PVAc was diluted with RO water to give a final volume of 1 L and hence a final concentration of 0.055 % (w/v). Clarification performance was determined by measuring \( OD_{670} \) as for the recovery of whole cells (Equation 2-3).

Yeast cell debris
A 60 % (ww) suspension of yeast cells was prepared in phosphate buffer to give a final volume of 2 L. This was then disrupted in the APV Lab60 homogeniser according to Section 2.1.2.1. The homogenate was then diluted to 28 % (ww/v original yeast concentration) with additional phosphate buffer. Clarification performance was determined by measuring \( OD_{670} \) and using Equation 2-3.

1st cut precipitate (ammonium sulphate)
This was prepared in a similar manner to that used for disc stack centrifuge studies (Section 2.1.3). Differences included: homogenisation was performed with the APV Lab60. Debris was clarified using the J1-MI laboratory centrifuge at 6870 rpm (3870 g) for a time equivalent to \( Q/\Sigma = 2.3 \times 10^{-8} \text{ m s}^{-1} \) (corrected for centrifuge efficiency). The precipitation was performed in a 1 L baffled tank stirred with a Rushton turbine (Section 2.1.1).

2nd cut precipitate (ammonium sulphate)
1st cut precipitate, prepared as above, was clarified in the J1-MI laboratory centrifuge (3870 g) for a time equivalent to \( Q/\Sigma = 9.1 \times 10^{-9} \text{ m s}^{-1} \) (corrected for centrifuge efficiency). Further addition of saturated ammonium sulphate raised the concentration to the 2nd cut point. Both precipitations were performed in a 1 L baffled tank stirred with a Rushton turbine (Section 2.1.1).

Yeast protein precipitate (PEG precipitant)
Method is described in Section 9.

Assays
For both PVAc and yeast cell debris particle streams clarification performance was calculated using Equation 2-3. Clarification performance of protein precipitates was determined as above (Section 2.1.3.2).
2.1.5 Protein precipitation

Batch protein precipitation performed in a stirred vessel was examined at laboratory scale, scale-down, and pilot scale (Section 2.1.1.3) for 1st cut (40% saturation) and 2nd cut (60% saturation) ammonium sulphate precipitates and polyethylene glycol precipitate (10% w/v PEG). For the 1st cut ammonium sulphate precipitate the particle size distribution was measured with time for a range of mean velocity gradients, G. For both precipitant systems a solubility profile was determined at laboratory scale. Solubility at the cut points used in the process runs were determined for laboratory scale, scale-down and pilot scale.

2.1.5.1 Preparation of clarified yeast homogenate

Laboratory scale and scale-down

Yeast was suspended in phosphate buffer to 28% (ww/v) and homogenised using the APV Lab 60 high pressure homogeniser (Section 2.1.2.1). Homogenate was clarified using a 1P tubular bowl centrifuge operated at 23000 rpm (13000 g) at a flow rate of 10 L h⁻¹ (Q/Σ = 9.1 x 10⁻⁹ m s⁻¹ - corrected for centrifuge efficiency). The centrifuge was stopped for solids removal every 9 minutes (1.5 L of feed processed).

Pilot scale

Yeast was suspended in phosphate buffer to 28% (ww/v) and homogenised using the APV K3 high pressure homogeniser (Section 2.1.2.1). Homogenate was clarified in the SAOOH-205 disc stack centrifuge (full stack) at a flow rate of 20 L h⁻¹ (Q/Σ = 9.1 x 10⁻⁹ m s⁻¹ - corrected for centrifuge efficiency). Solids were discharged every 4.5 minutes (1.5 L of feed processed). The feed to the centrifuge was cut prior to discharging in order to reduce losses to the slurry stream.

2.1.5.2 Solubility studies

Ammonium sulphate

Yeast was resuspended to 28% (ww/v) prior to disruption for 5 passes at 500 barg. It was then clarified in the 1P tubular bowl as described above before cooling to 278 K prior to the addition of ammonium sulphate dissolved in phosphate buffer (Section 2.1.2.1) to give a saturated solution which was pre-cooled to 278 K. Saturated ammonium sulphate solution has a concentration of 514.7 g L⁻¹ at 273 K and an apparent specific volume of 0.5262 (Di Jeso, 1968). The volume of saturated ammonium sulphate solution required to reach a
particular concentration in the clarified yeast homogenate was calculated using Equation 2-5 (Di Jeso, 1968).

\[ V = \frac{1000(S_2 - S_1)}{(1 - S_2)} \]

Equation 2-5

where \( V \) is the volume, in millilitres, of saturated solution that must be added to a litre of solution of fractional saturation \( S_1 \) to give a final saturation of \( S_2 \).

**Polyethylene glycol (PEG) precipitates**

Clarified yeast homogenate was cooled to 278 K prior to the addition of PEG 8000 dissolved in phosphate buffer (Section 2.1.2.1) to give a 50 % (w/v) solution which was pre-cooled to 278 K. The volume of 50 % PEG solution required to reach a particular concentration in the clarified yeast homogenate was calculated using Equation 2-6.

\[ C_2 = C_{PEG} \left( \frac{V_{PEG}}{V_F} \right) + \frac{S_1(V_F - V_{PEG})}{V_F} \]

Equation 2-6

where \( C_2 \) is the final PEG concentration, \( C_{PEG} \) is the concentration of PEG in the precipitant, \( V_{PEG} \) is the volume of precipitant added, \( V_F \) is the final volume of the precipitate stream and \( S_1 \) is the initial PEG concentration.

**Solubility profiles**

For the 0.9 L laboratory scale vessel, solubility profiles were determined for the two precipitating agents. For ammonium sulphate the concentration was increased in 5 % intervals up to a final concentration of 90 % saturation. For PEG the concentration was increased in 0.5 % intervals up to a final concentration of 17 % (w/v) PEG concentration. To allow the collection of a large number of samples and to keep the mixing conditions constant a specific volume of clarified homogenate/precipitate was removed and replaced with the same volume of precipitant in order to achieve a pre-determined precipitant concentration. The mixture was allowed to equilibrate for 3 minutes in the case of ammonium sulphate precipitates and 10 minutes for PEG precipitates (Foster, 1972) before the next precipitate removal and precipitant addition were made. From the volume removed a sample was taken for soluble and solid protein and ADH assays (Section 2.3). The temperature of clarified homogenate and precipitant was maintained at approximately 278 K. The Rushton turbine was set to 519 rpm to give a mean velocity gradient, \( G \) of 44 s\(^{-1}\).
Solubilities at different scales
Data from the solubility profiles was used to determine the cut points for the process runs. For the ammonium sulphate process a two cut process was used; 1st cut at 40 % saturation and 2nd cut at 60 % saturation. For the PEG process a single cut process was adopted at 10 % (w/v) PEG concentration.

1st cut ammonium sulphate - Clarified yeast homogenate (Section 2.1.5.1) was cooled to 278 K prior to the rapid addition of saturated ammonium sulphate solution also pre-cooled to 278 K to give a final ammonium sulphate concentration of 40 % saturation using Equation 2-5. The stirrer speed was set to give a mean velocity gradient of 44 s\(^{-1}\). Samples were taken after 3 minutes and assayed for solid and liquid protein and ADH (Section 2.3).

2nd cut ammonium sulphate - 1st cut precipitate was produced as above except that precipitate particles were aged for approximately 38 minutes (equivalent to \(Gt = 10^5\)). For laboratory scale and scale-down the precipitate was then clarified using a 1P tubular bowl at a flow rate equivalent to a corrected \(Q/\Sigma = 9.1 \times 10^{-9} \text{ m s}^{-1}\). The bowl was cleaned of solids after every 2.5 L of feed. For pilot scale the 1st cut precipitate was clarified using the SAOIH-205 disc stack centrifuge at a corrected \(Q/\Sigma = 9.1 \times 10^{-9} \text{ m s}^{-1}\) discharging solids after every 2.5 L of feed. For all scales the supernatant was cooled to 278 K prior to the addition of saturated ammonium sulphate solution to give a final concentration of 60 % saturation. The stirrer speed was set to give a mean velocity gradient of 44 s\(^{-1}\). Samples were taken after 3 minutes and assayed for solid and liquid protein and ADH (Section 2.3).

2.1.5.3 Particle size analysis of protein precipitates
In addition to solubility studies, precipitate particle sizing was performed for 1st cut ammonium sulphate precipitate. For laboratory and scale-down, precipitate particle sizes were determined for stirrer speeds equivalent to mean velocity gradients of 11, 22, 33, 44, 55 s\(^{-1}\) for a range of time intervals. For large scale (100 L vessel), measurements were made for mean velocity gradients of 44 and 100 s\(^{-1}\). The electrolyte used for precipitate size analysis was 40 % saturation ammonium sulphate in phosphate buffer (Section 2.1.2.1). Samples were diluted ten-fold in electrolyte to prevent further changes in the particle size distribution (Clarkson, 1994). Details of particle size analysis are given in Section 2.3.2.1.
2.2 Methods for process study (ADH)

The choice of process system for the investigation of scale-down involved consideration of a number of factors which are discussed earlier (Section 1.6). The adopted system was the sequence for the purification of alcohol dehydrogenase using two cut ammonium sulphate fractional precipitation. A flow diagram (Figure 1-5) and process description (Section 1.6) were discussed earlier.

The process was run at pilot scale, scale-down, laboratory scale (high ‘g’ spin) and laboratory scale (Q/Σ). The terms in brackets for the laboratory scale refer to how the centrifuge steps were operated during process runs. For “high ‘g’ spin” runs the laboratory centrifuge was operated at 12000 rpm (18000 g) for 1 h which is equivalent to Q/Σ = 1.3 x 10⁹ m s⁻¹; whereas for “Q/Σ” runs the laboratory centrifuge was operated at the same Q/Σ values that were used for the scale-down and pilot scale process runs, allowing for the efficiencies of the different centrifuge designs.

2.2.1 Equipment

Equipment used for each process run at the different scales is shown in Table 2-7. Equipment details are given in Section 2.1.1.

<table>
<thead>
<tr>
<th></th>
<th>Pilot scale</th>
<th>Scale-down</th>
<th>Lab high g</th>
<th>Lab Q/Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeniser</td>
<td>K 3</td>
<td>Lab 60</td>
<td>Lab 60</td>
<td>Lab 60</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>SAOOH (full stack)</td>
<td>SAOOH (4 inserts)</td>
<td>JL-MI</td>
<td>JL-MI</td>
</tr>
<tr>
<td>Precipitation vessel</td>
<td>100 L</td>
<td>4 L</td>
<td>1 L</td>
<td>1 L</td>
</tr>
<tr>
<td>Pump</td>
<td>50NDM</td>
<td>MV-Z</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Table 2-7 Equipment for process runs

2.2.2 Yeast re-suspension

Block yeast was re-suspended in phosphate buffer (Section 2.1.2.1) to a concentration of 7 % (ww/v), typical of a fed-batch fermentation.

2.2.3 Cell harvesting

Results of the investigation of recovery performance of whole cells using the full stack SAOOH-205 centrifuge were used to specify a suitable flow rate and hence Q/Σ value for processing at pilot scale, scale-down and for the lab (Q/Σ) process runs, taking into account
centrifuge efficiencies (Section 0). The corrected $Q/\Sigma$ used was $Q/\Sigma = 2.3 \times 10^8$ m s$^{-1}$. For the laboratory process run, which did not apply Sigma theory to the centrifugation steps (Lab 'high g'), cells were harvested at 12000 rpm for 1 h (equivalent $Q/\Sigma = 1.3 \times 10^9$ ms$^{-1}$). For the pilot scale and scale-down processes, solids discharge intervals were defined by determining solids breakthrough points (Section 2.2.8).

2.2.4 Homogenisation

Prior to homogenisation harvested cells were re-suspended to a concentration of 28 % (ww/v) using phosphate buffer (Section 2.1.2.1) in order to standardise the protein concentrations for the different process scales. For each process scale the yeast suspension was homogenised for 5 discreet passes at 500 barg (Section 2.1.2.1). For scale-down and laboratory scale, cells were homogenised in the APV Lab60 and for pilot scale the APV K3 unit was used.

2.2.5 Yeast cell debris removal

In a similar fashion to cell harvesting, a suitable $Q/\Sigma$ for debris removal was established using the results of clarification performance of yeast cell debris using the full stack SAOHH-205 disc centrifuge. Corrected $Q/\Sigma = 1.0 \times 10^8$ m s$^{-1}$. This was used for the pilot scale, scale-down and Lab $Q/\Sigma$ processes. For the Lab high g process, cells were harvested at 12000 rpm for 1 h ($Q/\Sigma = 1.3 \times 10^9$ m s$^{-1}$). For the pilot scale and scale-down processes, solids discharge intervals were defined by determining solids breakthrough points (Section 2.2.8).

2.2.6 Protein precipitation stages

Methods for process precipitation stages were the same for all process scales. Clarified yeast homogenate was cooled to 278 K in the precipitation vessel appropriate to the process scale (Section 2.2.1). The ammonium sulphate concentration was adjusted to the required saturation (40 % - 1$^\text{st}$ cut, 60 % - 2$^\text{nd}$ cut) by the addition of saturated ammonium sulphate solution, the preparation of which, and the means for calculating the required volumes is discussed above (Section 2.1.5.2). The precipitant was added rapidly in the region of the impeller shaft. The impeller speed was set to give an average shear rate of 44 s$^{-1}$ and the precipitate was aged for a time period to give a Gt value of $10^5$ (equivalent to approximately 38 minutes).
2.2.7 Precipitate recovery steps

Protein precipitate recovery was performed at a corrected $Q/\Sigma$ value of $9 \times 10^{-9} \text{ m s}^{-1}$ for the pilot scale, scale-down and Lab $Q/\Sigma$ process scales. For the Lab high g process a centrifuge speed of 12000 rpm was used for 1 h (equivalent $Q/\Sigma = 1.3 \times 10^{-9} \text{ m s}^{-1}$).

2.2.8 Breakthrough curves

For pilot scale and scale-down it is essential to determine the point at which the centrifuge solids capacity is reached so that solids discharges can be performed efficiently i.e. minimising both the loss of liquid into the slurry stream and the overflow of solids into the supernatant stream. For each of the centrifuge steps in the process, whole yeast cells, yeast cell debris and precipitate particle suspensions, the feed material was fed through the centrifuges, both pilot scale and scale-down, at the flow rate chosen for processing in the process runs. Process streams were prepared as described in Section 2.2. The supernatant was sampled at regular intervals for an extended period to ensure that solids had carried over. The percentage of solids carried over was determined using the equation:

$$\text{Carry over (\%)} = \frac{\text{OD}_{670}(\text{supernatant}) - \text{OD}_{670}(\text{well spun})}{\text{OD}_{670}(\text{feed}) - \text{OD}_{670}(\text{well spun})} \times 100$$

Equation 2-7

2.3 Assays and Instrumentation

2.3.1 Assays

For both the investigation of individual unit operations and process runs, the mass and volume of each stream was measured for the purposes of mass balancing. All streams were assayed for soluble and solid ADH activity and soluble and solid protein. The procedure for soluble and solid assays is outlined below (Figure 2-8).
2.3.1.1 Calculation of soluble protein/ADH amounts

The final amount of soluble protein or ADH for any stream is calculated as follows:

\[ \text{Total soluble protein/ADH} = \text{measured soluble conc}^a \times (1 - \text{solid fraction}) \times \text{stream volume} \]

Equation 2-8

2.3.1.2 Calculation of solid protein/ADH amounts

In calculating the total solid protein or ADH amount it is essential to take into account the volume of buffer in which the solid pellet is re-suspended for washing; hence:

\[ \text{Total solid protein/ADH} = \frac{\text{measured conc}^a \text{ in wash buffer} \times \text{vol resuspend sample}}{\text{vol of initial sample}} \times \text{stream vol} \]

Equation 2-9

2.3.1.3 Alcohol dehydrogenase activity assay.

Reaction: \[ \text{Ethanol} + \text{NAD} = \text{Acetaldehyde} + \text{NADH} + \text{H}^+ \]
Enzyme MW: 148,000 Daltons
Assay conditions: pH 8.8, temperature 25°C

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stock concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>17.15 M (absolute)</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>0.1 M</td>
</tr>
<tr>
<td>Semicarbazide HCl</td>
<td>1.0 M</td>
</tr>
<tr>
<td>Glutathione</td>
<td>0.1 M</td>
</tr>
<tr>
<td>Tris buffer</td>
<td>0.05 M</td>
</tr>
</tbody>
</table>

Table 2-8 ADH assay mixture

Semicarbazide HCl was used to remove acetaldehyde that is formed thus promoting the forward reaction (Bergmeyer, 1979).

Enzymatic activity, $E$, is expressed in terms of Units of activity per mL of solution and is defined by the following equation:

$$E = \frac{1}{\varepsilon_{340}} \frac{\Delta A}{\Delta t} \frac{V_c}{V_s} D_N$$

Equation 2-10

where $\varepsilon_{340} = 6.22 \text{ cm}^2 \mu\text{mol}^{-1}$, $\Delta A/\Delta t$ is the rate of change of absorbence at 340 nm, $V_c$ is the sample volume added to the cuvette, and $D_N$ is the dilution factor.

Samples were diluted in phosphate buffer so that the maximum absorbence rate of change did not exceed 0.6 A/min. 25μL of diluted sample was added to 1.5 mL of reagent. The rate of change of the absorbence was measured with a spectrophotometer at 340 nm over a 30 second period. The reaction rate was calculated automatically by the spectrophotometer using linear regression. Analyses were performed in triplicate with a reproducibility of ± 4%.

2.3.1.4 Bradford protein assay.

This assay is based on the colour change that occurs when Coomassie brilliant blue (G250), when in acidic solution, binds to protein (Bradford, 1976). Coomassie, when in a protonated form, is pale orange/red in colour. Proteins bind strongly to the dye forming both ionic and hydrophobic interactions. This suppresses protonation resulting in a colour change to blue. A calibration curve was produced using bovine serum albumin (BSA), 0-100 μg/ml. The
response was linear between 0.2 and 0.6 A. Hence samples were suitably diluted to keep within this range. 30 μL of diluted sample were added to 1.5 ml of reagent and the absorbence was read after five minutes at a wavelength of 595 nm. This assay is considerably more sensitive than the Lowry assay (Lowry et al, 1951) and is less prone to interference. Samples were analysed in triplicate with a reproducibility of 3 %.

2.3.1.5 Packed volumes.

Packed solids volumes were measured for each stream in order to calculate the final solid and liquid protein and ADH amounts. These were measure using calibrated 400 μl tubes. Tubes were spun at 12,000 rpm for 15 minutes in a microcentrifuge that holds the tubes in a swing-out position (Micro Centaur Lab Centrifuge, MSE, Crawley, UK). This enabled the solids level to be read easily. Samples were performed in duplicate with a reproducibility of ± 5 %.

2.3.1.6 Dry/wet weights.

Wet and dry weights were performed on all streams. Known sample volumes were dispensed into pre-weighed Eppendorf tubes in triplicate to determine the wet weight. These were then dried at 373 K and re-weighed after 24 and 48 hrs or until the weight became constant. Both sets of analyses gave a reproducibility of ± 6 %.

2.3.2 Instrumentation

2.3.2.1 Particle size analysis

This was performed using the electrical zone sensing principle developed by Coulter in 1952. An extensive literature survey on this principle can be obtained from Coulter Counter Ltd. Particle sizing studies were performed on the Elzone model 280 PC (Particle Data Ltd, Cheltenham, Glous, England). A schematic diagram of the Elzone (Figure 2-9) aids explanation of the principle. A tube with an orifice sits in a beaker which contains the sample suspended in electrolyte. An electrical current passes from an electrode situated in the beaker, through the orifice, to an electrode situated within the orifice tube. A vacuum is applied to the inside of the orifice tube thus drawing the sample through the orifice. The passage across the orifice of a particle exhibiting considerably different electrical conductivity to the suspending electrolyte results in a change in electrical resistance across the orifice that is converted by the electrode into a pulse shaped electrical signal. This is then amplified to enable the height and
the number of pulses to be recorded. The number of pulses is equal to the number of particles and the height of the pulse is related to the particle volume by the relationship:

\[ AU = \frac{E^2 f}{\rho R I} AV \]

Equation 2-11

where \( AU \) is the voltage pulse, \( AV \) is the particle volume, \( E \) is the electrical field strength, \( f \) is the shape factor, \( \rho R \) is the electrical resistance of the suspending electrolyte, and \( I \) is the electrical current.

For all analyses an 18 \( \mu \)m orifice tube was used which was calibrated with PVAc standards of sizes 2.02 \( \mu \)m and 5.0 \( \mu \)m. The calibration was checked with a 1.00 \( \mu \)m PVAc standard.

Preparation of Electrolyte
Two electrolytes were used for particle sizing. For PVAc, whole yeast cells and yeast cell debris a 10 % sodium chloride solution was used. For precipitate particle study the electrolyte was phosphate buffer (100 mM potassium dihydrogen phosphate, pH 6.5) adjusted to the final ammonium sulphate saturation in order to prevent particle dissolution or further precipitation. Electrolytes were pre-filtered in order to reduce background counts. Sartobran filter capsules were used (Sartorius AG, Gottingen, Germany) which have an average pore size of 0.1 \( \mu \)m. The large surface area of these filters enabled high filtration rates. After filtration, electrolytes were allowed to stand for 48 hours prior to use in order to enable degassing.

Analysis
Most samples were diluted in electrolyte prior to the addition of a 100 \( \mu \)l sample to 50 ml of electrolyte for the analysis. Pre-dilution of sample was usually required in order to avoid coincidence effects. This is where two or more particles traversing the orifice overlap to some degree and are counted as a single, larger particle. This can lead to an absent portion of the true distribution and also additional false data due to the larger pulses formed by partial or complete addition of signals from coincident particles. Counts with below 1 % coincidence give very accurate particle size data. An 18 \( \mu \)m orifice operated at a constant vacuum of 150 mm Hg gives a number count of 1.1 \( \times \) 10^6 particles per ml for a 1 % coincidence level. In every case sample number counts did not exceed 3.0 \( \times \) 10^5 per ml and in most cases coincidence levels did not exceed 0.1 %.
Figure 2-9 Diagrammatic representation of the Elzone model 280 PC.
3. SIZING OF THE SCALE-DOWN PROCESS SEQUENCE

The aim was to develop a scale-down process sequence that was sufficiently small to be able to be run from a 10 L fermentation. The scaled down unit operations should be similar to the pilot scale; i.e., a high pressure homogeniser should be used for cell disruption, a disc stack centrifuge for the four solid-liquid separation stages, and a stirred vessel for the two precipitation steps.

3.1 Equipment options

**High pressure homogeniser.**

The choice of homogeniser was limited to the three relatively small scale machines already available:

- **APV Micron Lab40** - This is a batch homogeniser with a very small operating capacity (40 ml).
- **APV Ranni** - A continuous mode operation high pressure homogeniser operating at 10 L h⁻¹ with a maximum operating pressure of 500 barg.
- **APV Lab60** - A small pilot scale, continuous operation homogeniser with a maximum operating pressure of 500 barg.

All three units have a similar disruption valve to the APV K3, the machine used for pilot scale studies. The operating volume of the APV Micron Lab40 is very small which would mean that the other unit operations, the disc stack centrifuge and the precipitation vessel, would also have to be small. Previous work with the APV Ranni showed wide variation in protein release (Clarkson, 1991). The APV Lab60, although it is a small pilot scale homogeniser, can be operated with as little as 1 L of material.

**Disc stack centrifuge.**

The smallest commercially available intermittent discharge machine is the SAOOH-205 (Westfalia Separators AG, Oelde, Germany) which is a pilot scale centrifuge and hence too large for scale-down work. Input from Westfalia Separators supplied a number of options:

- **TA05/DA05** - The -05 designs are scaled down centrifuges developed by Westfalia Separators. Bowl volume is approximately half that of the SAOOH-205 and there are 18 discs compared to the larger machines 37. Both the bowl diameter and disc radius are slightly reduced but energy input is maintained by operating the centrifuge at a higher speed of 12000 rpm. Unlike the SAOOH-205 neither of the scale-down machines has a centripetal pump for
supernatant discharge hence high aeration of the liquid is likely to occur. The TA version has a solid bowl and hence solids discharging is not possible whereas the DA version has two ports which enable continuous solids discharging. The solids discharge has a minimum flow rate of 35 L h⁻¹ below this problems with blockages occur. This type of discharge is only suitable for high solids concentration streams which is generally untypical of biological streams. In addition, the high solids discharge rate is unsuitable since the majority of biological solids are small and of low density and hence require a low throughput. It was thought possible to adapt the discharge system using a spring mechanism to enable intermittent solids release through the ports. Increasing the speed of the bowl would overcome the resistance of the springs and open the ports. Adapting the machines to a sliding piston discharge mechanism was not thought to be possible.

**New design** - A new small scale disc stack centrifuge could be designed which was still capable of intermittent solids discharge and supernatant removal by centripetal pump.

**Modification of the SAOOH-205** - A method was proposed to scale-down the SAOOH-205 by using a series of inserts specially made by Westfalia Separators AG. These would reduce both the separation area and bowl hold-up volume. These would enable the centrifuge to be scaled down by varying degrees to suit particular applications. The rest of the centrifuge would be unchanged hence intermittent solids discharge would still be possible and supernatant would be removed by a centripetal pump. The bowl geometry would be altered since the bowl diameter would be unchanged but the bowl height would be reduced by the inserts. Changing the bowl geometry can have an impact on recovery performance (Tomusiak, 1992).

**Precipitation vessel**

Protein precipitations will be performed in a baffled tank stirred with a Rushton turbine and cooled via an external jacket with glycol. A suitably sized vessel can be manufactured to fit the scale of the other unit operations.

**Choice of equipment**

The available scale-down disc stack centrifuges, the TA/DA05, do not offer much reduction in hold-up volume over the SAOOH-205. The ability to perform an intermittent solids discharge is important and neither machine is capable of this. Additionally, neither machine has a centripetal pump for supernatant removal. Therefore neither machine is suitable. The option of a new design would be very expensive and would take a long time to develop and,
in addition, there is no guarantee of its performance. The third option of modifying the existing SAOOH-205 centrifuge with inserts is the best option since it enables the pilot scale centrifuge to be used in various scale-down configurations and in its existing full scale configuration. The inserts can be manufactured relatively easily by Westfalia Separators AG and in a short space of time. The change in bowl geometry and lack of modifications to the feedzone may have an impact on recovery performance but this is compensated for by the ability to perform solids discharges and supernatant removal via a centripetal pump.

The choice of centrifuge is the limiting factor in defining the scale of the process. It would not be possible to scale-down the SAOOH-205 sufficiently for it to fit into the process with the APV Micron Lab40. The APV Lab60 and APV Ranni homogenisers should both be suitable in terms of process volume. The inconsistency in performance of the APV Ranni makes the APV Lab60 the preferred option. Once the centrifuge scale has been decided then the stream volumes through the process can be estimated and from this a suitably sized precipitation vessel may be designed.

3.2 Mass balance predictions

To help size the unit operations to be used for scale-down a mass balance spreadsheet was developed based on data obtained from a pilot scale run of the process (Clarkson, 1991). This was supplemented with information from a laboratory scale run and data from the literature. The spreadsheet was designed so that certain process parameters could be adjusted to determine the impact of any changes in performance with scale-down on the overall mass balance. The mass balance spreadsheet and the assumptions used are shown in Section 11.1.

Fermentation

The yeast concentration for the fermentation was assumed to be 70 g L\(^{-1}\) (ww) which is easily obtained in the fed-batch mode (Siddiqi et al, 1995a).

Centrifugation steps

Generally applied variables and assumptions:

Number of active discs and discharge volume - each centrifuge step could have any number of discs between 37 (full stack) and 5 (assumed to be the maximum possible scale-down). For the majority of cases the discharge volume was set so as to change in proportion to the number of discs. Additionally, the effect of a possible change in geometry with scale-
down was assessed since this alters the solids concentration of the slurry stream and hence the ratio of supernatant to slurry stream volume.

In all cases a full discharge, i.e. the bowl liquid content is discharged with the solids, was assumed since for centrifuges of the size of the SAOOH-205 it is not possible to perform a reproducible partial discharge where the solids are discharged and bowl liquid is retained.

Scale-down of the centrifuge may result in differences in recovery performance compared to pilot scale. Varying the recovery performance enabled the impact on process stream volumes, and hence on scale-down, to be assessed. For precipitate recovery it was assumed that total protein and enzyme were recovered equally.

For all operations, not just for the centrifugation stages, stream densities were required in order to convert the mass balance to a volume basis. This data came from the pilot scale and laboratory scale process runs.

Q/Σ was kept constant with scale-down, i.e. for each centrifugation step the Q/Σ used at pilot scale was also used for scale-down. Thus for a given number of discs a flow rate could be calculated and, for a given volume of material, a processing time was defined.

High pressure homogenisation

The degree of disruption could be varied which also affected the solids concentration of the cell debris stream. The relationship between the two was obtained from Hetherington et al (1971).

The number of passes through the homogeniser was kept constant (5 passes).

For each scale-down scenario investigated it was initially assumed that the APV Lab60 was used and hence this machine’s flow rate of 60 L h^-1 was set. If the volume of material was insufficient to operate the APV Lab60 then the APV Ranni was substituted (flow rate = 10 L h^-1).

Protein precipitation

The same cut points and ageing periods were used for scale-down as for pilot scale. Hence the time for both precipitations was fixed.

No change in solubility of protein or ADH was assumed for both cut points. Clarkson (1994) found little change in solubility at different scales for this system.

**SCENARIOS**
A range of scenarios was investigated in an effort to estimate the scale of unit operations required in order to meet the fermentation volume target of 10 L operating volume. A number of abbreviations were used:

- C1 - cell harvest centrifugation step
- C2 - cell debris clarification step
- C3 - 1st cut precipitate clarification step
- C4 - 2nd cut (product) recovery step
- H - high pressure homogenisation step
- P1 - 1st cut precipitation step
- P2 - 2nd cut precipitation step

**Pilot scale run**

Actual data for the pilot scale process run is shown in Table 3-1

<table>
<thead>
<tr>
<th>Step</th>
<th>No of discs used in centrifuge</th>
<th>No of solids discharges</th>
<th>Flow rate (L h(^{-1}))</th>
<th>Operating time (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>37</td>
<td>53</td>
<td>300</td>
<td>27</td>
</tr>
<tr>
<td>C2</td>
<td>37</td>
<td>13</td>
<td>75</td>
<td>24</td>
</tr>
<tr>
<td>C3</td>
<td>37</td>
<td>5</td>
<td>20</td>
<td>109</td>
</tr>
<tr>
<td>C4</td>
<td>37</td>
<td>6</td>
<td>20</td>
<td>148</td>
</tr>
<tr>
<td>H</td>
<td>-</td>
<td>-</td>
<td>290</td>
<td>31</td>
</tr>
<tr>
<td>P1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38</td>
</tr>
<tr>
<td>P2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38</td>
</tr>
</tbody>
</table>

**Total run time** 416

Table 3-1 Actual specification and operating times for the pilot scale process run using a fermentation volume of 135 L.

**Scenario 1**

Assuming exactly the same process performance and constant centrifuge geometry with scale-down (i.e. same centrifuge configuration for each step)

Assuming 100% cell breakage during homogenisation

Maintaining the processing times for each operation

Fermentation volume set to 10 L

The specifications required to fulfil these conditions are:

- Number of discs for each centrifugation step: 2.7
- Homogeniser flow rate: 21 L h\(^{-1}\)
Scenario 2

The minimum number of active discs in the centrifuge was set at 5 since this was believed to be the maximum feasible scale-down of the centrifuge. The maximum number of discs was set as the same as full stack centrifuge, 37.

The pilot scale run constituted a full day's work for two people. All scale-down work was to be performed by a single person and hence a shorter overall run time of 260 minutes was chosen as the maximum. Run times less than 260 minutes were considered acceptable since holding stages could be employed in order to allow for any time dependent effects.

The minimum number of discharges was set as 1 for each centrifugation stage. The number of discs used at each centrifugation step did not have to be the same.

Assuming 100% cell breakage during homogenisation,

Homogeniser flow rate was set to 60 L h\(^{-1}\) (APV Lab60).

Solving parameter was to find the smallest fermentation volume possible

<table>
<thead>
<tr>
<th>Step</th>
<th>No of discs used in centrifuge</th>
<th>No of solids discharges</th>
<th>Flow rate (L h(^{-1}))</th>
<th>Operating time (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>5</td>
<td>11.8</td>
<td>41</td>
<td>6</td>
</tr>
<tr>
<td>C2</td>
<td>5</td>
<td>2.9</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>C3</td>
<td>5</td>
<td>1.0</td>
<td>2.7</td>
<td>24</td>
</tr>
<tr>
<td>C4</td>
<td>6</td>
<td>1.1</td>
<td>3.2</td>
<td>27</td>
</tr>
<tr>
<td>H</td>
<td>-</td>
<td>-</td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td>P1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38</td>
</tr>
<tr>
<td>P2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38</td>
</tr>
</tbody>
</table>

Total run time 143

Table 3-2 Scale-down scenario for a fermentation operating volume of 4.0 L

The solution is shown in Table 3-2. In fact there are many solutions to the problem since the number of discharges for C1, C2 and C4 are not minimised. However, the only effect of increasing the number of discs for these stages would be to reduce their processing times and hence the overall processing time.

Scenario 3

The same constraints as Scenario 2 are used except:

When minimising the number of discharges then fractional, i.e. incomplete, discharges are bound to occur. In reality, this means that at the end of each centrifugation step an additional discharge is performed which contains little solids and hence is mainly liquid.

For process runs where a significant number of discharges are performed then this has an
insignificant effect on the overall mass balance, however, when the number of discharges is small this effect may be very significant particularly if only a single discharge is used. Hence, to reduce the impact of fractional discharges a minimum number of 3.0 discharges was defined.

Although it was considered acceptable for the overall run time to be less than 260 minutes a sensible minimum processing time for the centrifugation and homogenisation stages was set as 10 minutes.

<table>
<thead>
<tr>
<th>Step</th>
<th>No of discs used in centrifuge</th>
<th>No of solids discharges</th>
<th>Flow rate (L h⁻¹)</th>
<th>Operating time (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>9</td>
<td>20.0</td>
<td>73</td>
<td>10</td>
</tr>
<tr>
<td>C2</td>
<td>8</td>
<td>5.4</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>C3</td>
<td>5</td>
<td>3.0</td>
<td>2.7</td>
<td>72</td>
</tr>
<tr>
<td>C4</td>
<td>6</td>
<td>3.0</td>
<td>3.2</td>
<td>77</td>
</tr>
<tr>
<td>H</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>P1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38</td>
</tr>
<tr>
<td>P2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38</td>
</tr>
</tbody>
</table>

Total run time 259

Table 3-3 Scenario 3: minimum fermentation volume fulfilling all constraints = 12.1 L

The constraints prohibit a fermentation volume of 10 L. Operating C1 and C2 with more discs is possible but this would simply reduce the overall run time.

**Examination and testing of assumptions**

Homogenisation - All scenarios assumed complete cell breakage. If the scale-down homogeniser were not to break all cells then product stream volumes through the process would be reduced. Even if the small scale homogeniser did under-perform there should be little difference in performance after 5 passes at 500 barg due to the nature of the protein release curve which begins to level-off after the 2nd pass (Hetherington, 1971). The worse case was assumed to be 90% cell breakage. Scenario 3 was re-run with the reduced disruption and the new minimum fermentation value was calculated as 12.7 L. Additionally all scenarios assumed a flow rate of 60 L h⁻¹ for cell homogenisation, i.e. it was assumed that the APV Lab60 was always used. For all scenarios the volume of material to be homogenised fell within the operating range of this machine and hence, as this is the machine of choice, it is a fair assumption. The impact of changing the homogeniser flow rate is simply to alter the processing time, it does not effect stream sizes.
Recovery efficiency - All scenarios assumed that recovery efficiency of precipitate at C3 and C4 will remain constant with scale-down. Mannweiler (1990) found that in scaling down a centrifuge, by blanking of the disc stack to reduce the number of active discs but leaving the rest of the centrifuge unchanged, improved recovery performance results compared to the full stack for shear-sensitive solids but performance is similar for shear-insensitive solids. Hence for the ADH process, improvement in recovery of precipitates for C3 and C4 might be expected but recovery of whole cells and cell debris for stages C1 and C2 respectively should be unaltered. The effect of up to 60% improvement in recovery performance for stages C3 and C4 was assessed and is shown in Table 3-4. Improving the recovery efficiency at C3 and C4 by the same degree results in a reduced fermentation volume requirement in order to fulfil the constraint of 3 full discharges at each centrifugation step. Eventually the limiting centrifugation step is transferred from C3 to C4 and improvement in recovery performance from 40% to 60% leads to no further reduction in the fermentation volume.

<table>
<thead>
<tr>
<th>Improvement in recovery performance (%)</th>
<th>Minimum fermentation volume (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.1</td>
</tr>
<tr>
<td>10</td>
<td>11.5</td>
</tr>
<tr>
<td>20</td>
<td>11.0</td>
</tr>
<tr>
<td>40</td>
<td>10.0</td>
</tr>
<tr>
<td>60</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Table 3-4 Change in minimum fermentation volume requirement with improvement in recovery performance for Scenario 3.

Ratio of bowl volume to solids holding volume - Scaling down the SAOOH-205 with inserts will result in a change in geometry which could alter the relative volumes of the bowl and the solids holding space. The effect of increasing the solids holding volume to total bowl volume ratio is to reduce the number of discharges at the centrifugation stage concerned but, the supernatant stream volume is bigger since reducing the number of discharges means reduced fluid wastage to the slurry stream. This results in an increase in the number of discharges in centrifugation stages further downstream. However, the overall effect is to reduce the number of discharges and therefore an increase in the fermentation volume is required to compensate (Table 3-5).
Solids holding volume : total bowl volume ratio | Required fermentation volume (L)
---|---
1 | 12.1
1.1 | 12.3
1.2 | 12.4
1.3 | 12.6

Table 3-5 Change in minimum fermentation volume requirement with change in the ratio of solids holding volume to total bowl volume for Scenario 3.

Another effect of the change in geometry with scale-down may be to alter the ratio of number of discs to the bowl/solid volume. This will not affect the partitioning of the feed stream to slurry and supernatant over centrifugation steps but will affect the minimum fermentation volume if a set number of solids discharges is to be maintained (Table 3-6).

Separation area : bowl volume ratio | Required fermentation volume (L)
---|---
1.0 | 12.1
0.9 | 12.7
0.8 | 14.3
0.7 | 16.4

Table 3-6 Change in minimum fermentation volume requirement with change in the ratio of separation area to total bowl volume for Scenario 3.

Protein precipitation ageing time - This was kept constant with scale-down at 38 minutes. Precipitate particle strength has been correlated with the ageing time and the mean velocity gradient (Bell and Dunnill, 1982). A value of $G_t = 10^5$ was found to be ideal and this is what was used for the pilot scale run. The work on particle strength was performed at one scale and, therefore, it is unknown how well this will scale-down.

By combining the factors above, the best and worst case scenarios are shown in terms of the minimum fermentation volume required to fulfil the criteria described in Scenario 3 (Table 3-7).
### Table 3-7 Minimum fermentation volume required to satisfy the constraints of Scenario 3 based on best and worst case conditions

<table>
<thead>
<tr>
<th></th>
<th>Best</th>
<th>Worst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenisation efficiency</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>Improvement in precipitate recovery performance at C3 and C4 (%)</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Separation : bowl volume ratio</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Solids holding volume : bowl volume ratio</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Required fermentation volume (L)</td>
<td>8.7</td>
<td>19.4</td>
</tr>
</tbody>
</table>

#### 3.3 Summary

Two approaches to scale-down have been used to reduce the fermentation requirement to run the ADH process. One route, to scale-down the equipment, was not sufficient to reach the target fermentation volume and hence, a second approach, to minimise the amount of material within defined limits, was also used. Together a minimum fermentation volume requirement of 12.1 L was defined. There are likely to be a combination of factors that occur with scale-down some of which will help to minimise the fermentation volume, namely improved precipitate recovery performance, and some will increase the required volume, namely changes in the bowl geometry; where exactly the balance will lie is uncertain.

Sizing of the scale-down process involves a considerable number of inter-related factors which would be very difficult to assess without the aid of the mass balance spreadsheet. It enables the cumulative effects of different variables to be assessed rapidly in the form of scenarios. A drawback to the spreadsheet itself is that it is only as good as the information on which it is based. Any change in performance with scale-down could lead to unforeseen process interactions which could dramatically alter the mass balance. It is however, very useful to assess the impact of changes in the centrifuge bowl geometry.

The 10 L fermentation volume target may not be met but it is likely that the value will be reasonably close. For other processes it should be easily met since the ADH process has low solids concentration streams and precipitate recovery is poor. The precipitate particles formed by ammonium sulphate are particularly small and therefore difficult to recover (Clarkson, 1994). With the disc stack centrifuge, only fractional recoveries are possible and hence considerably more material must be processed before a solids discharge can be performed. Since the limiting fermentation volume is defined in terms of the number of discharges then the volume will be higher than for a more easily recovered precipitate. The fermentation volume required for a particular centrifuge based process will depend on the solids.
concentration of the centrifuge feed streams and the efficiency of recovery. For very dilute streams a large fermentation volume would be required but the saving relative to pilot scale would be same.
4. RESULTS OF HIGH PRESSURE HOMOGENISATION STUDIES

Summary
Experimental data comparing high pressure homogeniser performance at pilot scale, APV K3 (minimum working volume of 20 L) and scale-down, APV Lab60 (minimum working volume of 1.5 L) is given in this chapter and compared with data in the literature for the APV Micron APV Micron Lab40 (40 mL working volume), an ultra scale-down device. Important process parameters for this unit operation are intracellular protein release and debris particle size distribution. These were examined for a range of operating conditions, namely at two different operating pressures (300 and 500 barg) and two different yeast concentrations (28 and 60 % ww/v) and for a range of discreet passes through the homogeniser (1-5 passes). In all cases the degree of protein release and the debris particle size distributions obtained with the APV K3 were closely matched by the scale-down APV Lab60 device. The effect of any variation of the debris particle size distributions with scale-down on subsequent centrifugal clarification was assessed using a theoretical model describing recovery performance in a disc stack centrifuge. In all cases the predicted recovery performance at scale-down was ± 3 % of pilot scale. Results show that for the operating conditions examined homogeniser performance was independent of scale. Important criterion for cell breakage are examined in order to simplify scale-up/down.

Introduction
Common hosts for the large-scale manufacture of biologicals, such as *Escherichia coli* and *Saccharomyces cerevisiae*, tend not to excrete products (Middelberg, 1995) and hence effective methods of cell disruption are required. Large-scale methods frequently use mechanical means, the most popular being high pressure homogenisation and bead milling (Middelberg, 1995, Harrison et al, 1991, Schutte and Kula, 1990).

Important process parameters for assessing homogeniser performance are protein release, since this is an indicator of product release, and debris particle size distributions, as these impact on unit operations downstream, namely, centrifugation and or microfiltration for debris removal and also chromatography steps. For the case of debris removal very fine debris particles may affect microfiltration transmission rates and for the centrifugation step very low flow rates may be needed in order to achieve an adequate separation. Additionally, chromatography steps are often vulnerable to fine particulates leading to premature column blockage. Problems associated with cell debris fragments have been discussed by Hearle et al (1994), Hoare and Dunnill (1989), Mosqueira et al (1981), Gray et al (1973). It is therefore
very important that the scale-down homogeniser reproduces not only the product release but also breakage characteristics of the pilot scale machine.

**Experimental**

Cells were resuspended to either 28 % or 60 % (ww/v) in phosphate buffer (Section 2.1.2.1) and homogenised at either 300 barg or 500 barg for 5 discreet passes using the APV K3 (pilot scale) and APV Lab60 (scale-down) homogeniser units. Samples were taken after each pass for protein assays (Section 2.3.1.4) and for particle size analysis (Section 2.3.2.1). For particle size analyses samples were diluted sufficiently in the electrolyte (10 % NaCl, Section 2.3.2.1) so that coincidence effects were normally below 0.1 %.

To determine the effect of differences in debris particle size distributions on centrifugation a semi-empirical model based on the Rosin-Rammler distribution was used. Using the yeast cell debris particle size distributions as an input the model predicts the recovery performance for a disc stack centrifuge.

**Valve geometry**

The APV K3 and APV Lab60 homogenisers were equipped with an identical CD (cell disruption) valve (Figure 1-1). Data obtained with the APV Micron Lab40 is also discussed, this machine is equipped with a SV valve. Valve dimensions are given below:

<table>
<thead>
<tr>
<th>Model</th>
<th>Throughput, Q (L h⁻¹)</th>
<th>( R_o ) (m)</th>
<th>( R_m ) (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APV Lab60</td>
<td>60</td>
<td>3.85 x 10⁻³</td>
<td>4.18 x 10⁻³</td>
</tr>
<tr>
<td>APV K3</td>
<td>280</td>
<td>3.85 x 10⁻³</td>
<td>4.18 x 10⁻³</td>
</tr>
<tr>
<td>APV Micron Lab40</td>
<td>28</td>
<td>1.65 x 10⁻³</td>
<td>2.05 x 10⁻³</td>
</tr>
</tbody>
</table>

Table 4-1 Homogeniser valve dimensions

**Results**

Figure 4-1 shows the particle size distribution of yeast cells prior to homogenisation and the debris particle size distributions for the APV K3 and the APV Lab 60 homogenisers for one set of conditions. Disruption of whole cells causes particle breakage which leads to a shift in the distribution to the left. The homogenate distributions are fairly similar but the APV Lab 60 distribution is shifted slightly to the left. Instead of representing each particle size distribution graphically, which would take a considerable amount of space and also make comparisons difficult, it is possible to fit a Gaussian distribution to every particle distribution (Figure 4-1 - solid lines). Gaussian distributions are represented by the equation:
\[ y = y_0 + \frac{A}{\pi} \frac{e^{-\frac{(d-\mu)^2}{2w^2}}}{w \sqrt{2}} \]

Equation 4-1

where, \( y \) is the normalised volume of particles of diameter \( d \), \( \mu \) is the mean diameter and \( w = 2\sigma \). \( w \) is equal to the width of the particle size distribution at half the maximum volume. For normalised yeast cell and yeast cell homogenate size distributions this can be simplified since \( y_0 = 0, A = 1 \). Hence,

\[ y = \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{(d-\mu)^2}{2w^2}} \]

Equation 4-2

Hence, for a range of diameters, it is possible to define the particle size distribution with only two factors, \( \mu \) and \( \sigma \). Therefore, these two factors may be used to facilitate the comparison of performance, in terms of particle size distributions, of the two homogenisers, with \( \mu \) representing the position of the distribution in terms of the mean diameter and \( \sigma \) representing the spread of the distribution. In all cases the Gaussian fit gave a very low \( \chi^2 \) value which translated to > 99% matching of the fit to the data. Figure 4-2 (a) shows the change in mean diameter for the APV K3 with operating pressure and yeast concentration over a range of passes. The higher the operating pressure the smaller the mean diameter i.e. more particle breakage is occurring. At the higher yeast concentration for the same operating pressure the mean particle diameter is larger which suggests that the cells are less vulnerable when the suspension is more concentrated. This may be due to the higher fluid viscosity associated with higher yeast concentrations. Hence most breakage occurs at the higher pressure and lower yeast concentration and the least breakage occurs with the opposite conditions. The spread of the debris particle distributions increases as the mean diameter reduces (Figure 4-2 (b)). Figure 4-3 shows the same data for the APV Lab60 and similar trends are seen i.e. the higher operating pressure and lower yeast concentration gave the smallest mean diameter. The spread of the debris distributions appeared to be less sensitive to yeast concentration than the APV K3 but for both homogenisers the higher pressure led to greater distribution spread. Figure 4-4 compares the mean diameter of the APV K3 and APV Lab60 at 28 % (ww/v). These matched fairly well for the two machines particularly for 300 barg (average mismatch of \( \mu \): 300 barg, 1.7%; 500 barg 7.3%). In terms of particle spread the matching of data was also good. At 60 % yeast concentration the APV K3 and APV Lab60 also showed similar
performance although the APV K3 data tended to have a wider spread and the mean particle diameter was consistently smaller at 500 barg operating (Figure 4-5).

Comparing values of \( \mu \) and \( \sigma \) enables differences in the particle distributions for the two homogenisers to be seen but the relevance of this to a process context is unclear. As discussed earlier debris particles can have a big impact on unit operations further downstream and for this particular process the major impact will be on the centrifugation stage after homogenisation which is used to remove the cell debris. To assess the impact of differences between the debris particle size distributions of the APV K3 and the APV Lab60 a model was used to predict centrifuge recovery performance. This was based on the Rosin-Rammler distribution function (Svarovsky, 1990b) and is given below:

\[
T(d) = 1 - \exp\left(-0.865 \left(\frac{d}{d_c}\right)^{2.08}\right)
\]

Equation 4-3

This describes the separation performance of a disc stack centrifuge (Mannweiler, 1990). \( T(d) \) is the separation efficiency of a particle of diameter, \( d \), and \( d_c \) is the critical diameter.

Inputs to the model are the centrifuge specifications, in order to determine the Sigma value, the particle size distribution and the density and viscosity of the feed suspension and feed volumetric flow rate through the centrifuge. With this information, the fraction of particles recovered (mass basis) can be determined. There are two possible scenarios for a cell debris centrifugation step: one is debris removal from the liquor as for the purification of a soluble product for which a model prediction of 90 % recovery of yeast cell debris was used. The other scenario is the separation of intracellular particles more easily recovered than cell debris, an example being the recovery of inclusion bodies. In this case the majority of the debris would pass into the supernatant (Jin et al, 1994). Here, a model prediction of 10 % debris removal was employed. Using both scenarios means that both ends of the debris particle size distributions will be examined in the comparison of scales, i.e. small and large particles. Size distributions for the APV K3 were used as the input to the model and the flow rates required for 10 % and 90 % recovery were determined for all operating pressures, yeast concentrations and number of passes. Size distributions for the APV Lab60 were then inputted to the model using the flow rates established for the APV K3 in order to determine the recovery performance (Figure 4-6). For 90 % recovery the APV Lab60 predictions were
within ± 2 % total recovery of pilot scale. For the case of 10 % recovery the APV Lab60 predicted recoveries generally within ± 1 % total recovery.

In addition to particle size analysis the total protein release was also measured for all conditions. At the higher operating pressure there is greater total protein release for both the APV K3 and APV Lab60 (Figure 4-7). Additionally, protein release after each pass is higher for the lower yeast concentration for both homogenisers except for 500 barg where this is only true for the first 2-3 passes since protein release is almost complete by this stage for both concentrations. This follows the trends for particle breakage except that the breakage process continues even when total protein release has been reached. Figure 4-8 compares protein release for the two homogenisers and in all cases results are very similar; the largest mean disparity between pilot scale and scale-down being less than 6 %.

In a process context, when making operational decisions it may be necessary to compromise when defining the conditions for homogenisation since high operating pressures and multiple passes that are desirable for full product release may produce very fine debris which may be very difficult to remove. Hence a compromise may have to be reached between product release and debris removal. This is discussed more fully by Siddiqi et al (1991) and Zhou and Titchener-Hooker (1997).

**Discussion**
By maintaining the valve geometry and reducing the hold-up volume it is possible to mimic closely the performance of the pilot scale APV K3 device, both in terms of debris particle size distributions and protein release, over a range of operating conditions. Siddiqi et al (1997) has shown that this method is effective even with a very small scale device which requires only 40 ml of material. The drawback to this approach to scale-down is that it is only valid when the valve geometry is kept constant. With different valve configurations protein release varies for a given operating pressure (Keshavarz-Moore et al, 1990). In order to scale-down disruption performance between different valve designs it is necessary to understand, and be able to describe mathematically, the mechanism of disruption.

Several theories have been postulated which are discussed earlier (Section 11.4.1.2) and it is likely that more than one of these mechanisms may be involved. Studies on milk homogenisation to break fat globules suggested a link with turbulence (Walstra, 1969) since it was possible to relate globule size to the operating pressure using Kolmogoroff's theory of locally isotropic turbulence. The Reynolds number is indicative of the degree of turbulence.
and is constant for a particular homogeniser (assuming constant flow rate) and constant fluid properties i.e. it is independent of the pressure drop across the valve and the valve gap width. This is explained as follows:

\[ \text{Re} = \frac{\rho u_o h}{\mu} \]

Equation 4-4

where \( \rho \) and \( \mu \) are the density and viscosity of the fluid, \( h \) is the valve gap and \( u_o \) is the fluid velocity through the valve.

\[ u_o = \frac{Q}{2\pi R_o \mu} \]

Equation 4-5

where \( Q \) is the volumetric flow rate and \( R_o \) is the orifice radius at the point of entry. By combining equations Equation 4-4 and Equation 4-5:

\[ \text{Re} = \frac{\rho Q}{2\pi R_o \mu} \]

Equation 4-6

Several workers have studied the effect of Reynolds number on homogeniser performance. Phipps (1971) examined fat globule breakage in milk at turbulent Reynolds numbers and also at laminar Reynolds numbers which were achieved by reducing the flow rate through the homogeniser valve and by increasing the viscosity of the fluid. Disruption performance was found to be independent of the Reynolds number. A study by Siddiqi et al (1997) comparing an homogeniser that operated in the turbulent region with one that operated in the laminar region showed similar disruption of bakers yeast. Additionally, fluid viscosity changes with the number of passes through the homogeniser valve since this results in cell breakage which increases the viscosity due to the release of intracellular components such as nucleic acids (Cordes et al, 1990). Flow conditions for the APV Lab60 and the APV K3 are shown below (Table 4-2). Additionally, data is also shown for the APV Micron Lab40 device since protein release data is included later for comparison. Calculation of Reynolds numbers required density and viscosity data. Density data was taken from Mosqueira et al (1981) and viscosity data was calculated using a model (Siddiqi, 1997) based on data from Mosqueira et al (1981). Details of the model are given in Section 11.3.
<table>
<thead>
<tr>
<th>Pressure (barg)</th>
<th>Yeast concentration, % (ww/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>APV Lab60</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>500</td>
</tr>
<tr>
<td>APV K3</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>500</td>
</tr>
<tr>
<td>APV Micron Lab40</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>500</td>
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</tbody>
</table>

Table 4-2: Reynolds numbers at different yeast concentrations in APV Lab60, APV K3 and APV Micron Lab40 at 300 and 500 barg for conditions studied.

For Re < 250 the flow is considered to be laminar and for Re > 250 the flow is turbulent (Phipps, 1975). Thus, in all cases, the flow in the APV Lab60 was laminar whereas for the APV K3 the flow was turbulent, and for the APV Micron Lab40 both types of flow existed. The disruption performance of the three homogenisers was very similar (discussed earlier and Siddiqi et al, 1997) which suggests that the fluid flow regime does not affect cell disruption.

A mechanism for disruption proposed recently (Shamlou et al, 1995) is cell elongation due to the velocity gradient across the valve. The valve geometry of the APV Micron Lab40 (SV valve) is slightly different to the CD valve used in both the APV K3 and APV Lab60 but if elongation is the mechanism of disruption then the velocity through the valves should be similar since the elongational rate is proportional to the inlet velocity (Shamlou et al, 1995). In order to calculate the fluid velocity through the valve it is necessary to determine the valve gap width, $h$. This cannot be measured easily since the design of the valve prevents access and in addition the gap is very small (10-300 μm depending on the scale of operation and the operating pressure). However, Phipps (1975) developed a Benouilli related equation to calculate $h$ from the pressure drop across the valve, the valve dimensions and the flow rate. He found that this correlated well with experimental valve lift data (McKillop et al, 1955). The pressure drop, or energy losses, are defined by three terms; due to the contraction at the entrance to the valve gap, friction across the surfaces of the valve, and expansion at the outlet. The function describing the friction term differs depending on the flow regime and hence two equations may be defined, one for laminar flow and one for turbulent flow:

For laminar flow:
\[ P = \frac{\rho}{4} \left( \frac{Q}{2\pi R_0 h} \right)^2 + \frac{6\rho v Q}{\pi h^3} \ln \frac{R_f}{R_o} + \frac{\rho}{2} \left( \frac{Q}{2\pi R_f h} \right)^2 \]

**Equation 4-7**

where \( P \) is the pressure drop across the valve, \( Q \) is the volumetric flow rate, \( R_f \) and \( R_o \) are the radii of the valve rod and the orifice at entry respectively, \( \rho \) and \( \nu \) are the fluid density and kinematic viscosity respectively, and \( h \) is the valve gap width.

For turbulent flow:

\[ P = \frac{\rho}{4} \left( \frac{Q}{2\pi R_0 h} \right)^2 + \frac{5\rho \nu^{3/5}}{h^3} \left( \frac{Q}{2\pi} \right)^{7/5} \left( \frac{1}{R_o^{5/3}} - \frac{1}{R_f^{5/3}} \right) + \frac{\rho}{2} \left( \frac{Q}{2\pi R_f h} \right)^2 \]

**Equation 4-8**

Phipps (1975) cautioned care in their application since generally it is not possible to know the precise flow pattern in a closed homogeniser valve.

Using these equations a prediction was made of the valve gap widths (Figure 4-9) for the different homogenisers. For the APV Lab60 laminar flow alone is shown since \( Re < 250 \) for all the conditions examined. For the APV K3 only the turbulent profile is shown and for the APV Micron Lab40 machine, which was operated in laminar and turbulent flow, predictions are shown for both flow types. The curves show that the valve gap width decreases as the pressure increases and that the gap width for the APV K3 is larger than for APV Lab60 for the corresponding pressure, this being due to the higher flow rate of the APV K3. Additionally the curve for the APV Micron Lab40 (turbulent flow) lies above that for laminar flow because of the additional friction losses associated with turbulence.

Once the valve gap widths have been calculated it is possible to determine the maximum velocity through the valves by dividing the homogeniser volumetric flow rate by the valve gap cross-sectional area (Figure 4-10). The profile for the APV Lab60 and the APV Micron Lab40 for laminar flow match well with the velocity profile of the APV K3 for turbulent flow. However, experiments with the APV Micron Lab40 using 1% (ww/v) yeast concentration give protein release results very similar to higher concentrations (Siddiqi et al, 1997). Yet at 1% (ww/v) concentration the flow through the APV Micron Lab40 is turbulent due to the much reduced fluid viscosity. The velocity profile for the APV Micron Lab40 with turbulent flow is very different to the other profiles which suggests that the elongation theory
is incorrect or, the method for calculating the gap width, and hence the gap velocity, is incorrect.

A commonly used scale-up method is power dissipation per unit volume which gives a measure of the energy available for cell disruption. This may be calculated from:

$$P_v = \frac{P \cdot Q}{\pi(R^2 - R_0^2)h}$$  \hspace{1cm} \text{Equation 4-9}

where $P_v$ is the power dissipated per unit volume, $P$ is the operating pressure. An alternative scale-up method is the average shear rate, or mean velocity gradient ($G$) which can be calculated from the following equation:

$$G = \frac{P_v}{\mu}$$  \hspace{1cm} \text{Equation 4-10}

Figure 4-11 and Figure 4-12 show how $P_v$ and $G$ change with operating pressure for the three homogenisers assuming constant fluid viscosity and density. Similarly to the plot of velocity through the valve with pressure, the profiles for the APV Lab60 (laminar), APV Micron Lab40 (laminar) and the APV K3 (turbulent) are similar but very different to the plot for the APV Micron Lab40 (turbulent). Hence, neither correlation appears useful for describing cell disruption.

Figure 4-13 shows the fraction of protein remaining intracellular that is released for a given pass against the velocity through the valve. There is a considerable amount of scatter and no identifiable trend to the data suggesting that there is no link. Figure 4-14 shows the same protein release data but here it is plotted against the mean velocity gradient, $G$. Most of the data lies on the same line except for the 1% yeast concentration, i.e. low viscosity, runs. This suggests that $G$, which is strongly influenced by the fluid viscosity, is also unsuitable. Figure 4-15 shows a similar plot but against dissipated power per unit volume, $P_v$. This appears to give a fair correlation with protein release. $P_v$ takes into account $R_w$, $R$ and $h$. Hence for a flat valve, which has a larger $R/R_w$, the calculated $P_v$ value would be lower and this corresponds with the lower disruption efficiency of this valve design (Keshavarz-Moore et al, 1990). To determine how well $P_v$ described disruption with different valve configurations the disruption
data of Keshavarz-Moore et al (1990) was examined where four different valve geometry's were considered. Valve dimensions and protein release over the 1st pass are given below:

<table>
<thead>
<tr>
<th>KE</th>
<th>FV</th>
<th>CD</th>
<th>CRF</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_0$ (mm)</td>
<td>3.25</td>
<td>3.05</td>
<td>3.85</td>
</tr>
<tr>
<td>$R$ (mm)</td>
<td>3.34</td>
<td>4.49</td>
<td>4.33</td>
</tr>
<tr>
<td>Protein release (1st pass), %</td>
<td>63</td>
<td>34</td>
<td>51</td>
</tr>
</tbody>
</table>

Table 4-3 Valve dimensions and protein release data from a study by Keshavarz-Moore et al (1990). Yeast concentration, 45 % (ww/v), operating pressure, 450 barg. KE, knife edge; FV, flat valve; CD, cell disruption; CRF, cell rupture. All valves used a flat valve rod.

Protein release plotted against $P_v$, given in Figure 4-16, shows a trend of increasing protein release with $P_v$ but it is not a linear relationship as suggested earlier.

**Conclusion**

The cell disruption process is not fully understood yet by maintaining the valve geometry and operating at the same pressure drop across the valve it is possible to accurately scale-down both protein release and the debris particle size distribution. However, better understanding of the disruption process is required in order to scale-down from one valve geometry to another.

Many mechanisms have been proposed for cell disruption yet none of these have been shown to be universally applicable. A likely mechanism is cell elongation due to the rapid fluid acceleration upon entering the valve gap but the evidence to prove this theory is lacking. This may be due to the inadequacy of the expression, derived by Phipps (1975), to calculate the valve gap width. Further testing of this expression is required and could be achieved by calibrating the valve adjustment mechanism in order that $h$ may be determined accurately and directly, without the need for calculation.
Figure 4-1 Volume based yeast cell homogenate particle size distribution after 5 passes for 60% (ww/v) yeast concentration at 500 barg operating pressure. Closed symbol, APV K3; open symbol, APV Lab60. ■, □, yeast cell homogenate, ×, whole yeast cells. Solid lines, Gaussian fits to data.
Figure 4-2: Change in mean diameter and sigma value in APV K3 with operating pressure and yeast concentration and number of pass. (a) mean diameter, μ; (b) sigma, σ. ■, 300 barg operating pressure, 28 % (ww/v) yeast concentration; ●, 500 barg operating pressure, 28 % (ww/v) yeast concentration; ▲, 300 barg operating pressure, 60 % (ww/v) yeast concentration; ▼, 500 barg operating pressure, 60 % yeast concentration.
Figure 4-3: Change in mean diameter and sigma value in APV Lab60 with operating pressure and yeast concentration and number of pass. (a) mean diameter, $\mu$; (b) sigma, $\sigma$. □, 300 barg operating pressure, 28 % (ww/v) yeast concentration; ○, 500 barg operating pressure, 28 % (ww/v) yeast concentration; Δ, 300 barg operating pressure, 60 % (ww/v) yeast concentration; ▽, 500 barg operating pressure, 60 % yeast concentration.
Figure 4-4: Change in mean diameter and sigma value at 28 % (ww/v) yeast concentration with operating pressure and number of passes. Closed symbol, APV K3; open symbol, APV Lab60; (a) mean diameter, $\mu$; (b) sigma, $\sigma$. ■, □, 300 barg; ○, ●, 500 barg. Average mismatch between APV K3 and APV Lab60: mean diameter, 300 barg, 1.7 %; 500 barg, 7.3 %; sigma, 300 barg, 7.9 %; 500 barg, 4.8 %.
Figure 4-5: Change in mean diameter and sigma value at 60 % (ww/v) yeast concentration with operating pressure and number of passes. Closed symbol, APV K3; open symbol, APV Lab60; (a) mean diameter, μ; (b) sigma, σ. ■, □, 300 barg; ●, ○, 500 barg. Average mismatch between APV K3 and APV Lab60: mean diameter, 300 barg, 1.4%; 500 barg, 3.1%; sigma, 300 barg, 14.7%; 500 barg, 7.6%.
Figure 4-6: Predicted recovery performance of yeast cell debris homogenised with the APV Lab60 using the RRSB centrifuge model at flow rates determined using the same model with yeast cell debris homogenised with the APV K3 for a mass recovery of (a) 90 %, (b) 10 %.  

- **Black**, 28 % (ww/v) yeast concentration, 300 barg homogenisation operating pressure;  
- **Light gray**, 28 % (ww/v) yeast concentration, 500 barg homogenisation operating pressure;  
- **Dark gray**, 60 % (ww/v) yeast concentration, 300 barg homogenisation operating pressure;  
- **White**, 60 % (ww/v) yeast concentration, 500 barg homogenisation operating pressure.
Figure 4-7: Change in protein release with number of passes at different yeast concentrations and operating pressures. Closed symbol, APV K3; open symbol, APV Lab60; ■, □, 28 % (ww/v) yeast concentration, 300 barg operating pressure; ●, ○, 28 % (ww/v) yeast concentration, 500 barg operating pressure; ▲, △, 60 % (ww/v) yeast concentration, 300 barg operating pressure; ▼, ▽, 60 % (ww/v) yeast concentration, 500 barg operating pressure.
Figure 4-8: Change in protein release with number of passes at different operating pressures, (a) 60 % (ww/v) yeast concentration; (b) 28 % (ww/v) yeast concentration. Closed symbol, APV K3; open symbol, APV Lab60. ■, □, 300 barg operating pressure; •, ○, 500 barg operating pressure. Average mismatch between APV K3 and APV Lab60: 60 % (ww/v), 300 barg, 5.9 %; 500 barg, 2.4 %; 28 % (ww/v), 300 barg, 4.7 %; 500 barg, 4.8 %.
Figure 4-9 Change in valve gap width with operating pressure, at constant fluid viscosity and density. ———, APV Lab60; . . . . , APV K3 (turbulent flow); ———, APV Micron Lab40 (laminar flow); ———, APV Micron Lab40 (turbulent flow).
Figure 4-10 Change in maximum velocity through the valve with operating pressure, at constant fluid viscosity and density, — — —, APV Lab60; ·····, APV K3 (turbulent flow); ·····, APV Micron Lab40 (laminar flow); ·····, APV Micron Lab40 (turbulent flow).
Figure 4-11 Change in dissipated power per unit volume with operating pressure, at constant fluid viscosity and density. ——, APV Lab60; ———, APV K3 (turbulent flow); ———, APV Micron Lab40 (laminar flow); ———, APV Micron Lab40 (turbulent flow).

Figure 4-12 Change in mean velocity gradient with operating pressure, at constant fluid viscosity and density. ———, APV Lab60; ———, APV K3 (turbulent flow); ———, APV Micron Lab40 (laminar flow); ———, APV Micron Lab40 (turbulent flow).
Figure 4-13 Fraction of protein remaining intracellular that is released in a pass through the homogeniser valve versus the velocity through the valve. Data is shown for the pilot scale APV K3, the scale-down APV Lab60 and the laboratory scale APV Micron Lab40 (data courtesy of Siddiqi, 1997). Data points are shown by numbers, in groups of five representing 1-5 passes through the homogeniser in that order: 1-20, APV Lab60; 1-5, 300 barg 28 % (ww/v); 6-10, 300 barg 60 % (ww/v); 11-15, 500 barg 28 % (ww/v); 16-20, 500 barg 60 % (ww/v). 21-40, APV K3; 21-25, 300 barg 28 % (ww/v); 26-30, 300 barg 60 % (ww/v); 31-35, 500 barg 28 % (ww/v); 36-40, 500 barg 60 % (ww/v). 41-70, APV Micron Lab40; 41-45, 300 barg 1 % (ww/v); 46-50, 300 barg 10 % (ww/v); 51-55, 300 barg 45 % (ww/v); 56-60, 500 barg 1 % (ww/v); 61-65, 500 barg 10 % (ww/v); 66-70, 500 barg 45 % (ww/v).
Figure 4-14 Fraction of protein remaining intracellular that is released in a pass through the homogeniser valve versus the mean velocity gradient experienced in the valve. Data is shown for the pilot scale APV K3, the scale-down APV Lab60 and the laboratory scale APV Micron Lab40 (data courtesy of Siddiqi, 1997). Data points are shown by numbers, in groups of five representing 1-5 passes through the homogeniser in that order: 1-20, APV Lab60; 1-5, 300 barg 28 % (ww/v); 6-10, 300 barg 60 % (ww/v); 11-15, 500 barg 28 % (ww/v); 16-20, 500 barg 60 % (ww/v). 21-40, APV K3; 21-25, 300 barg 28 % (ww/v); 26-30, 300 barg 60 % (ww/v); 31-35, 500 barg 28 % (ww/v); 36-40, 500 barg 60 % (ww/v). 41-70, APV Micron Lab40; 41-45, 300 barg 1 % (ww/v); 46-50, 300 barg 10 % (ww/v); 51-55, 300 barg 45 % (ww/v); 56-60, 500 barg 1 % (ww/v); 61-65, 500 barg 10 % (ww/v); 66-70, 500 barg 45 % (ww/v).
Figure 4-15 Fraction of protein remaining intracellular that is released in a pass through the homogeniser valve versus the dissipated power per unit volume experienced in the valve. Data is shown for the pilot scale APV K3, the scale-down APV Lab60 and the laboratory scale APV Micron Lab40 (data courtesy of Siddiqi, 1997). Data points are shown by numbers, in groups of five representing 1-5 passes through the homogeniser in that order: 1-20, APV Lab60; 1-5, 300 barg 28 % (ww/v); 6-10, 300 barg 60 % (ww/v); 11-15, 500 barg 28 % (ww/v); 16-20, 500 barg 60 % (ww/v). 21-40, APV K3; 21-25, 300 barg 28 % (ww/v); 26-30, 300 barg 60 % (ww/v); 31-35, 500 barg 28 % (ww/v); 36-40, 500 barg 60 % (ww/v), 41-70, APV Micron Lab40; 41-45, 300 barg 1 % (ww/v); 46-50, 300 barg 10 % (ww/v); 51-55, 300 barg 45 % (ww/v); 56-60, 500 barg 1 % (ww/v); 61-65, 500 barg 10 % (ww/v); 66-70, 500 barg 45 % (ww/v). Regression coefficient, R = 0.8.
Figure 4-16 Fraction of protein remaining intracellular that is released in a pass through the homogeniser valve versus the dissipated power per unit volume experienced in the valve. Yeast concentration, 45 % (ww/v), operating pressure, 450 barg; ■, KE valve; ○, FV valve; △, CRF valve; ▼, CD valve.
5. RESULTS OF DISC STACK CENTRIFUGATION STUDIES

Summary
A method is described for the scale-down of a disc stack centrifuge which reduces the number of separating discs and also the liquid and solid hold-up of the centrifuge bowl to enable operation with a reduced process material requirement. Scale-down is achieved in stages using a series of interlocking inserts to suit particular applications. Maximum scale-down gives a 76% reduction in the separation area and a bowl volume reduction of 70%. Separation performance of the full stack machine and scale-down variants is compared using the grade efficiency concept. Dilute polyvinyl acetate and bakers' yeast homogenate particle suspensions are used for the comparison. Initial experiments with polyvinyl acetate showed improved recovery performance with scale-down. Poor alignment of the riser channels of the bottom insert with the outlets of the distributor was found to be the cause. After modification of the bottom insert the grade efficiency curves for both polyvinyl acetate and yeast cell debris produced by the scale-down variants closely followed the curves for the full stack machine. This resulted in supernatants of the same particle volume size distribution and concentration when using scale-down methodology to mimic the full scale operation. Additionally, performance was determined with particle suspensions at solids concentrations characteristic of process streams. Recovery of whole yeast cells was complete (~100% recovery) for both the scale-down and full scale centrifuges even at the capacity flow rates of the scale-down and full scale centrifuges. Yeast cell debris recovery was very similar at both scales of operation but with protein precipitates the scale-down centrifuge showed improved recovery performance at low clarification efficiencies.

Introduction
The smallest available disc stack centrifuge is still a pilot scale machine and hence requires a significant amount of process material in order to operate. Typically this is well in excess of 10 L of fermentation broth. The ability to run with a reduced process material requirement is crucial in certain applications, for example, in the early stages of process development when fermentations tend to be still at a small scale. Additionally, as processes are developed further, extensive trials are required to identify desired operating conditions. If sufficiently relevant data can be obtained at a reduced scale then there would be significant cost benefits to this approach.
Previous centrifuge scale-down work has concentrated on scaling down the separation area by reducing the number of active discs (Mannweiler and Hoare, 1992; Rumpus, 1997). This was achieved by blanking off most of the disc stack leaving as little as one tenth the number of active discs. The recovery of a dilute suspension of polyvinyl acetate particles in the full stack centrifuge could be closely matched using a scaled-down version of the same centrifuge. This work highlighted the importance of careful positioning of the active discs. When positioned at the bottom of the disc stack, recovery performance was inferior to the full stack machine. This was attributed to turbulence associated with this region of the centrifuge where the feed stream enters the settling region causing re-entrainment of sedimented solids. Re-positioning of the active discs at the top of the blanked-off disc stack resulted in recovery performance superior to the full stack machine probably due to additional sedimentation as particles flow through the solids holding space before reaching the active discs. Positioning of the active discs a small distance off the bottom of the centrifuge, above the turbulent region, resulted in recovery performance very similar to the full stack machine. Blanking-off of the disc stack was the only centrifuge modification made. Hence, although the bowl was slightly reduced in volume, the solids holding space was unaffected so the amount of process material required was similar to the full stack version.

This work examines the feasibility of scaling down in stages the hold-up volume of the centrifuge in parallel with the separation capacity. This is with a view to reducing the amount of material needed to predict the solid-liquid separation of a full stack machine. The advantage of being able to scale down in stages is that the centrifuge can be set-up to suit a particular application, for example, for a process sequence which employs more than one centrifugation step where the volumes of material to be processed at each stage differ.

Results and discussion

Dilute streams

Polyvinyl acetate (PVAc)

Figure 5-1a shows the change in recovery performance with PVAc 190 over a range of flow rates for the full stack centrifuge. Steady state is achieved within 7-8 bowl volume changes with a slightly more rapid approach to steady state at higher flow rates. Figure 5-1b compares non-steady state recovery performance of the full stack centrifuge (37 active discs) with the fully scaled-down centrifuge (9 active discs) for the same $Q/\Sigma$ value of $5.2 \times 10^8$ m s$^{-1}$. Again the same trends were observed. The results compare with dye-tracer pulse studies reported previously (Mannweiler and Hoare, 1992) where 8-9 bowl volume changes were needed to reach steady state. The results reported here are significant as they confirm that the
approach to steady state for particulates is not substantially different to that of the fluid. For all runs at all scales, samples were taken after 10 bowl volumes of material had been passed through the centrifuge to ensure that steady state had been reached.

As a reference line an experimental, semi-empirical fit to data obtained with the Westfalia BSB 7-47-476 disc stack centrifuge using polyvinyl acetate (Brunner and Molerus, 1979) was employed for each grade efficiency figure. This fit is based on the Rosin-Rammler distribution function (Mannweiler, 1990, Svarovsky, 1990b) and is given below.

\[
T(d) = 1 - \exp \left( -k \left( \frac{d}{d_c} \right)^n \right)
\]

Equation 5-1

where, \( T(d) \) is the separation efficiency of a particle of size \( d \), and \( d_c \) is the critical diameter, \( k \) and \( n \) are constants which appear to depend on the process material (For polyvinyl acetate \( k = 0.865, n = 2.08 \)).

The grade efficiency curve was plotted as a function of the dimensionless term \( d/d_c \) since this enables runs performed at different operating conditions to be compared (Figure 5-2). The data follows Equation 5-1 for all values of \( d/d_c \) studied. Scale-down recovery performance was assessed in a similar fashion for the same range of critical diameters. A shift of the practical grade efficiency curve to the left was seen with increasing scale-down, i.e. recovery performance improved with the degree of scale-down (Figure 5-3). Additionally, the curve appeared to be steeper which resulted in lower values of \( T(d) \) for low values of \( d/d_c \) for scale-down configurations with 1-3 top inserts. The centrifuge separation area is scaled down to a greater extent than the bowl volume and the disparity between the two increases with increasing scale-down. Therefore, the residence time in the centrifuge for a given \( Q/\Sigma \) or \( d_c \) increases with increasing centrifuge scale-down. A plot of mass yield, \( E_r \) (fraction of material sedimented - mass basis) against the residence time for a given \( d_c \) shows a clear relationship; the longer the residence time in the centrifuge the more solids are sedimented (Figure 5-4a). It is generally accepted that liquid in the periphery of the bowl is mostly stagnant and that only the liquid around the disc stack is actually flowing (Mannweiler, 1990). Examination of the scale-down configurations showed a misalignment between the riser channels of the bottom insert and outlets from the distributor. It is likely that this was causing liquid to be forced outwards into the normally stagnant area and thus enabling sedimentation of solids directly into the solids holding space thus explaining the additional sedimentation with longer bowl
residence times. Modification of the bottom insert to correct the misalignment resulted in the mass yield being independent of the bowl residence time (Figure 5-4).

Although the modified bottom insert corrected the problem of change in recovery performance with scale-down it was decided to further modify it by reducing its height so that more discs could be fitted and hence the degree of separation area scale-down was more similar to the degree of bowl scale-down. The reason for this being that the disparity between separation area and bowl volume scale-down resulted in extended operating times which would have the effects, in a process context, of allowing more dewatering of sedimanted solids due to the longer times between solids discharges and also increasing liquid temperature which is not ideal for biological solids because they are often temperature-sensitive.

Grade efficiency curves with the modified, smaller bottom insert are shown in Figure 5-5. All of the scale-down variants gave similar grade efficiency curves to the full stack machine although there was slightly more scatter in the data.

**Yeast cell debris**

Recovery performance was also determined using yeast cell debris. Figure 5-6 shows the grade efficiency curves for the centrifuge with a full stack. In this case the curve changes with flow rate (or critical diameter) with greater grade efficiency values at higher flow rates for equivalent values of $d/d_e$. Comparison with the reference line (Equation 5-1) shows greater apparent efficiency at higher values of $d/d_e$ but reduced efficiency at lower values. Additionally, negative grade efficiency values are seen for low values of $d/d_e$ for the higher flow rates. The lower flow rate studied also indicates an approach to negative values of $T(d)$. Negative values are indicative of particle breakage occurring in the centrifuge feedzone. However, breakage of yeast cell debris particles in the centrifuge is unlikely since they have already experienced considerably greater stresses during homogenisation. This is discussed later.

Figure 5-7 shows the grade efficiency curves for yeast cell debris for each of the scale-down variants. All show the same trend as the full stack with the higher flow rates (higher $d_e$ values) giving greater values of grade efficiencies for similar values of $d/d_e$ and also the greater negative values of $T(d)$ at low values of $d/d_e$. Again, both of these effects can be attributed to break-up of aggregates leading to higher apparent removal of large particles and to generation of fines which were not present in the feed. Comparison of grade efficiency curves for the
full scale and scaled-down centrifuges for equivalent flow rates per gap show good agreement (Figure 5-8). This suggests that the extent of deviation from the reference curve is due to flow through the disc stack itself, where fluid flow rates are the same at different scales and is not due to the centrifuge feedzone where fluid flow rates are different at different scales. Shear break-up of aggregates in the feedzone would be readily reversible whilst shear break-up in the actual separating region, i.e. the disc gap, would not be due to the simultaneous separation process. The removal of the large aggregates would tend to stabilise the residual fines in the supernatant due to the lower aggregation frequency (Smoluchowski, 1917). Figure 5-9 shows the increased volume of small particles in the supernatant relative to the feed which indicates particle breakage in the centrifuge.

For an ideal particle-liquid system there is no shift in the grade efficiency curve with flow rate; additionally the curve does not show negative values of $T(d)$. The yeast homogenate system obviously is not ideal but it is a more realistic feed stream in terms of processing. The grade efficiency curves, which are used for process design, of the full stack machine are closely mimicked with scale-down which implies that this machine is suitable for this application. Figure 5-9 also shows that the volume and distribution of particles in the supernatant for full scale and scaled-down centrifuges are very similar. For clarity, data is shown only for the $d_c$ value of 2.7 μm but a similar matching of results at different scales was obtained for other critical diameter values. In process development terms it is very important that the scale-down centrifuge mimics the pilot scale in terms of the distribution and volume of particles in the supernatant stream since such particles often impact detrimentally on other unit operations downstream (Grey et al, 1973; Hearle et al, 1994; Hoare and Dunnill, 1989; Mosqueira et al, 1981). Thus the scale-down centrifuge will highlight these problems at an early stage instead of later during pilot trials.

**Concentrated streams**

Recovery performance was also examined with higher solids concentration streams, more typical of a process context. In this case data was shown as the clarification efficiency (probability scale) versus $Q/L$ (log scale). A probability scale was used for the clarification efficiency axis since this linearises a Gaussian distribution which is the usual distribution form for biological solids.

**Whole yeast cells**

Table 5-1 shows recovery performance of whole yeast cells with the full stack and full scale-down configurations. Recovery was complete even at the capacity flow rate of the centrifuge.
due to the large cell size, high density difference between the cells and the suspending liquid
and the relatively low fluid viscosity.

**Table 5-1 Recovery of whole yeast cells (28 % (ww/v)) in SAOOH-205 disc stack centrifuge**

<table>
<thead>
<tr>
<th>$Q/\Sigma$ (m s$^{-1}$)</th>
<th>Full stack (37 discs)</th>
<th>Scale-down (9 discs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.8 \times 10^{-7}$</td>
<td>100.1 %</td>
<td>99.8 %</td>
</tr>
<tr>
<td>$1.2 \times 10^{-7}$</td>
<td>99.8 %</td>
<td>99.9 %</td>
</tr>
<tr>
<td>$6.1 \times 10^{-8}$</td>
<td>99.6 %</td>
<td>102.3 %</td>
</tr>
<tr>
<td>$3.1 \times 10^{-8}$</td>
<td>101.0 %</td>
<td>100.0 %</td>
</tr>
<tr>
<td>$2.3 \times 10^{-8}$</td>
<td>102.0 %</td>
<td>99.5 %</td>
</tr>
<tr>
<td>$1.5 \times 10^{-8}$</td>
<td>99.6 %</td>
<td>98.8 %</td>
</tr>
</tbody>
</table>

**Yeast cell debris**

Figure 5-10 shows the recovery of yeast cell debris with $Q/\Sigma$ for full stack and full scale-
down configurations. Partial recoveries are possible within the flow rate range of the
centrifuges mainly because the particle size is much reduced compared to whole cells. In
addition, the density difference between the solid and liquid phase is reduced and the fluid
viscosity is increased due to the release of cell contents, particularly nucleic acids
(Mosqueira, 1981). Full stack recovery tends to be slightly better than scale-down but only by
as little as 1-2 % which is within experimental error.

**Protein precipitates**

Protein precipitate recovery reveals significant differences in performance, with the scale-
down configuration showing improved recovery performance for both ammonium sulphate
and PEG protein precipitates although this tends to be significant only for low clarification
efficiencies which are generally of less interest. The cause of the difference in clarification is
thought to be the feedzone where the fluid entering the centrifuge is accelerated to the speed
of the bowl and hence this is a region of high shear. This section is unaltered with scale-down
and hence the flow rate through this section is reduced with scale-down. Precipitate particles
are shear sensitive and tend to be broken in the feedzone (Bell and Brunner, 1983, Bell et al,
1996) which leads to a lower than expected recovery performance. This has lead to
manufacturers altering the feedzone design in order to reduce the shear stresses experienced
(Mannweiler, 1990). It is possible that the reduced flow rates through this section associated
with scale-down result in a more gradual acceleration of the precipitate stream which causes
less precipitate particle damage and hence improved recovery performance. Despite the differences in recovery the scaled down centrifuge still gives a good indication a full stack performance particularly for high clarification efficiencies. Recovery of 1st cut (40 % saturation) ammonium sulphate precipitate was superior to 2nd cut (60 % saturation) for both full stack and scaled down centrifuges. This is probably due to the increased viscosity and the reduced density difference between particles and suspending liquid associated with the higher ammonium sulphate concentration resulting in a reduced settling velocity. Recovery of ammonium sulphate precipitates is significantly worse than for PEG precipitates due to the much smaller particle size.

The semi-hermetic feedzone design of the SAOOH-205 is thought to provide several locations of high shear where particle breakage is likely (Van der Linden, 1987). The first of these is the lock-nut which secures the centrifuge bowl to the spindle. The feed stream, which has no angular momentum, hits the lock nut which is rotating at the speed of the centrifuge bowl. This is not thought to cause significant damage to the feed material since the contact time is very short. The second location is the accelerator ribs of the distributor where the majority of the acceleration of the feed material to the bowl speed is likely to occur. Other regions of the distributor are also thought to play a part. How significant the different regions of the feedzone are in particle breakage is unknown and may also vary depending on the operating conditions. The feed flow rate and back-pressure on the centrifuge affect the liquid depth in the centrifuge feedzone and if a sufficiently high feed rate or back-pressure is used then the liquid level can rise sufficiently to conceal the lock nut thus effectively altering the feedzone characteristics to those of a hydro-hermetic design which has been shown to result in considerably less breakage of shear-sensitive particles (Mannweiler, 1990).

In order to calculate the shear rates experienced in the feedzone of the centrifuge it is necessary firstly to calculate the dissipated power per unit volume. But in order to do this the volume of liquid present in the feedzone must be known which is not the case and, as explained above, this volume changes with flow rate and back-pressure. Since precipitate particles tend to be difficult to recover, the flow rates used are very low in terms of the operating capacity of the centrifuge. Hence, it is likely that there is little change in the volume of material in the feedzone over the range of flow rates used for both full scale and scale-down studies and hence dissipated power per unit volume levels should be similar. This would suggest that the level of shear breakage would be similar at both scales. Another possible explanation for the disparity in recovery between the two scales is the additional time it takes for feed material to pass from the high shear region of feedzone, through the
lower shear regions of the feedzone, and to the disc stack at scale-down due to the reduced flow rates. This may enable more re-aggregation of precipitate particles and hence a larger precipitate particle size would be presented to the disc stack thus explaining the improved recovery performances.

**Conclusion**

The scale-down inserts used in this study reduce the separation area by reducing the number of active discs and reduce the liquid and solid hold-up volume thus enabling the centrifuge to be run with less process material. Recovery performance of dilute streams of polyvinyl acetate and yeast cell debris in the full scale machine was closely mimicked at all the stages of scale-down.

Scaling down the centrifuge with inserts results in a change in the bowl geometry since the bowl height is reduced with scale-down but the bowl diameter is unchanged. This might have been expected to alter the fluid flow patterns in the centrifuge bowl which in turn could affect recovery performance, however this does not seem to have occurred. Recovery performance of higher solids concentration streams was similar for shear-insensitive solids but for shear-sensitive solids, i.e. protein precipitates, scale-down recovery is superior probably due to the centrifuge feedzone not being scaled down. A mechanical redesign of the feedzone may remedy this problem but this is a difficult and expensive undertaking which would involve a redesign of much of the centrifuge and also a better understanding of the exact reasons for the disparity in recovery performance between full scale and scale-down. This requires further research.

An advantage of scaling down by placing inserts into a pilot scale machine is that the bowl speed and diameter are unchanged and hence dewatering of collected solids should be similar to the full stack machine. Additionally, the solids discharge mechanism is unaffected. This is important as this is another region where shear damage of biological materials can occur. The shear associated with the rapid passage of materials through the solids discharge ports plus impaction and shear in the solids catcher chamber wall can all cause damage to sensitive materials. This was not the case with materials tested here. The ability to scale down in stages simply by adding an additional insert allows the centrifuge to be tailored to a particular application/process. A suitable scale-down set-up would be defined by the processing time available, the $Q/£$ to be used and also the volume of material to be processed.
This study only considers solids recovery but an equally important aspect of centrifuge performance is solids dewatering since this has a significant impact on process yield. This is a particularly important issue for disc stack centrifuges which tend to produce relatively wet solids. This issue is addressed later (Section 8). Additionally, it is important that scale-down centrifuge performance be examined in a process context with other scaled-down unit operations so that its effect on important process parameters, such as yield, and interactions with other unit operations may be examined (Section 8). If successful then a scaled-down process sequence should provide valuable information and be an important additional tool for process development.

Despite a 70% reduction in bowl volume, operation of the scale-down disc stack centrifuge still required a significant amount of process material. In light of this, and the likely limitations in the degree of any further scale down, an ultrascale down centrifuge was developed and performance examined (Section 6).
Figure 5-1: Change in the recovery performance of SAOOH-205 disc stack centrifuge sedimenting polyvinyl acetate particles ($\rho = 1190 \text{ kg m}^{-3}, \mu = 0.001 \text{ Pa s}$) with number of bowl volumes passed. Figure 5-1 (a), SAOOH-205 with full stack configuration (37 active discs) - effect of flow rate, $Q$ (L h$^{-1}$): ■, 20; ○, 45; ▲, 75; V, 100; ♦, 120; +, 150; ×, 180. (b) SAOOH-205 with full stack and full scale-down (as in Figure 2), operated at same $Q/2$. $(Q/2 = 5.2 \times 10^8 \text{ m s}^{-1})$. Key: ■, full stack: 37 active discs, bowl volume = 0.44 L, $Q = 100 \text{ L h}^{-1}$; ○, full scale-down: 9 active discs, bowl volume = 0.13 L, $Q = 24 \text{ L h}^{-1}$.
Figure 5-2: Grade efficiency curve for SAOOH-205 disc stack centrifuge with full stack configuration (37 active discs) using a polyvinyl acetate particle system, PVAc 190 ($\rho = 1190$ kg m$^{-3}$, $\mu = 0.001$ Pa s). Critical diameter, $d_c$ (µm): ■, 1.1; ○, 1.4; ▲, 1.7.
Figure 5-3: Grade efficiency curves for SAOOH-205 disc stack centrifuge with scale-down configurations using a polyvinyl acetate particle system ($\rho = 1190 \, \text{kg m}^{-3}$, $\mu = 0.001 \, \text{Pa s}$).

Critical diameter, $d_c$ (μm): ■ 1.1; ○ 1.4; ▲ 1.7. %%% Equation 5-1. All versions contain bottom insert plus for Figure 5-3a: 1 top insert, 22 discs, $Q$, L h$^{-1}$; ■ 140, ○ 235, ▲ 315. Figure 5-3b: 2 top inserts, 15 discs, $Q$, L h$^{-1}$; ■ 100, ○ 160, ▲ 235. Figure 5-3c: 3 top inserts, 9 discs, $Q$, L h$^{-1}$; ■ 60, ○ 95, ▲ 140. Figure 5-3d: 4 top inserts, 3 discs, $Q$, L h$^{-1}$; ■ 20, ○ 30, ▲ 45.
Figure 5-4: Mass yield for $d_c = 1.7 \mu m$ against the reciprocal of the liquid bowl residence time for SAOOH-205 disc stack centrifuge using polyvinyl acetate particle system ($\rho = 1190 \text{ kg m}^{-3}, \mu = 0.001 \text{ Pa s}$). Number of active discs; ■, 37 (full stack); ○, 28, 1 top insert; ▲, 21, 2 top inserts; ▼, 15, 3 top inserts, ●, 9, 4 top inserts. Figure 5-4 (a) original, unmodified bottom insert; (b) bottom insert with modified riser channels. Solid lines show trends to the data.
Figure 5-5: Grade efficiency curves for SAOOH-205 disc stack centrifuge with scale-down configurations using a polyvinyl acetate particle system ($\rho = 1190 \text{ kg m}^{-3}, \mu = 0.001 \text{ Pa s}$).

Critical diameter, $d_c$ (μm); ■, 1.1; ○, 1.4; ▲, 1.7. Equation 5-1. All versions contain modified bottom insert plus for Figure 5-5a: 1 top insert, 28 discs, $Q$, L h$^{-1}$; ■ 180, ○ 300, ▲ 440. Figure 5-5b: 2 top inserts, 21 discs, $Q$, L h$^{-1}$; ■ 140, ○ 220, ▲ 330. Figure 5-5c: 3 top inserts, 15 discs, $Q$, L h$^{-1}$; ■ 100, ○ 160, ▲ 235. Figure 5-5d: 4 top inserts, 9 discs, $Q$, L h$^{-1}$; ■ 60, ○ 90, ▲ 140.
Figure 5-6: Grade efficiency curve for SAOOH-205 disc stack centrifuge with full stack configuration (37 active discs) using a yeast cell debris particle system ($\rho = 1052$ kg m$^{-3}$, $\mu = 0.001$ Pa s). Critical diameter, $d_c$ (µm); ■, 1.7; ○, 2.2; △, 2.7.
Figure 5-7: Grade efficiency curves for SAOOH-205 disc stack centrifuge with scale-down configurations using yeast cell debris particle system ($\rho = 1052$ kg $m^{-3}$, $\mu = 0.001$ Pa s). Critical diameter, $d_c$ ($\mu m$): ■, 1.7; ○, 2.2; ▲, 2.7. §§§§§ Equation 5-1. All versions contain bottom insert. Figure 5-7a: 1 top insert, 28 discs, $Q$, L h$^{-1}$; ■ 120, ○ 200, ▲ 290. Figure 5-7b: 2 top inserts, 21 discs, $Q$, L h$^{-1}$; ■ 90, ○ 150, ▲ 220. Figure 5-7c: 3 top inserts, 15 discs, $Q$, L h$^{-1}$; ■ 66, ○ 107, ▲ 157. Figure 5-7d: 4 top inserts, 9 discs, $Q$, L h$^{-1}$; ■ 40, ○ 64, ▲ 94.
Figure 5.8: Grade efficiency curves for SAOOH-205 disc stack centrifuge with scale-down configurations using yeast cell debris particle system ($\rho = 1052 \text{ kg m}^{-3}, \mu = 0.001 \text{ Pa s}$). Number of active discs; ■, 37 (full stack); O, 28, 1 top insert; ▲, 21, 2 top inserts; ▽, 15, 3 top inserts, ♦, 9, 4 top inserts. Figure 5.8a, $d_c = 1.7 \text{ \mu m}$, flow rate/active disc ($Q/n$) = 4.3 L h$^{-1}$; Figure 5.8b, $d_c = 2.2 \text{ \mu m}$, $Q/n = 7.0 \text{ L h}^{-1}$; Figure 5.8c, $d_c = 2.7 \text{ \mu m}$, $Q/n = 10.5 \text{ L h}^{-1}$. 
Figure 5-9: Particle size distributions for yeast cell debris feed to the centrifuge and supernatant for full stack (37 active discs) and full scale-down (4 top inserts, 9 active discs). For both centrifuges $d_c = 2.7 \ \mu$m; ■, yeast cell debris feed; ▲, full stack supernatant, $Q = 390 \ \text{L h}^{-1}$; △, scale-down supernatant, $Q = 94 \ \text{L h}^{-1}$. Solid lines show trends to the data.
Figure 5-10: Recovery curves for SAOOH-205 disc stack centrifuge using yeast cell homogenate (28 % (ww/v)). Number of discs; ■, 43 (full stack); ○, 9, 4 top inserts. Each point is the average of 3 assays and the error bars show the maximum and minimum values. Solid line shows the trend to the data.
Figure 5-11: Recovery curves for SAOOH-205 disc stack. Closed symbol, full stack centrifuge (43 discs); open symbol, fully scaled down (9 discs), 4 top inserts. ▲, △, 1st cut ammonium sulphate; ▼, ▽, 2nd cut ammonium sulphate precipitate; ■, ○, polyethylene glycol precipitate (10% w/v). Each point is the average of 3 assays and the error bars show the maximum and minimum values.
6. RESULTS OF LABORATORY CENTRIFUGATION STUDIES

Summary
The scale down disc stack centrifuge gave very good predictions of pilot scale performance, however the amount of process material required was still quite larger. In view of likely limitations in any further scale down, an ultrascale down centrifuge was developed and its performance determined.

A method is shown for the adaptation of Sigma theory (Ambler, 1959, Trowbridge, 1962) describing recovery performance in a laboratory centrifuge in order to predict the performance of a pilot scale, industrial disc stack centrifuge. The technique can easily be used to predict the recovery performance of other industrial centrifuge types. Using this method, recovery performance was determined firstly for an ideal, dilute suspension of polyvinyl acetate particles and then for realistic biological streams of yeast cell debris and protein precipitates. Recovery of polyvinyl acetate particles was found to be well predicted; likewise for yeast cell debris although improved recovery was seen with the laboratory centrifuge for low values of Q/Σ. The high shear conditions associated with the disc stack centrifuge, as opposed to the low shear environment of the laboratory design, evidently leads to the breakage of debris aggregates and poor performance. Recovery of protein precipitates in the laboratory centrifuge over-predicted performance in the disc centrifuge to an even greater degree due to the highly shear sensitive nature of precipitate particles. Methods to improve the laboratory prediction of clarification of shear-sensitive particles in industrial centrifuges are discussed.

Introduction
It would be very useful if a laboratory centrifuge could be used to predict the performance and provide material representative of the industrial scale. This would help in specifying the centrifugation step, i.e. help in determining a suitable industrial centrifuge type for a particular application or whether or not a centrifuge step is in fact appropriate.

Laboratory centrifuges are capable of very efficient clarification which cannot generally be matched by an industrial device. However, by the application of engineering principles to laboratory centrifugation it may be possible to mimic performance of the larger scale. Ideally, it would be best to use a small scale industrial machine. However, taking the example of the
Sigma theory, which is used to describe solid-liquid separation performance, may be applied to a laboratory centrifuge. The solids separation ability of a particular centrifuge operating at a defined flow rate is given by the ratio $Q/\Sigma$, which is a ratio of the volumetric flow rate to the equivalent separation area of the centrifuge. The equation defining $Q/\Sigma$ for a laboratory centrifuge quoted in the literature [Ambler, 1959; Trowbridge, 1962] is only appropriate for constant speed operation. It does not take into account the acceleration and deceleration stages which can add significantly to the overall $Q/\Sigma$ value. This is particularly true when operating the laboratory centrifuge at $Q/\Sigma$ values typical of industrial machines and hence an adaptation of this equation is required. The effectiveness of this approach is assessed here using various particulate feed streams.

**Theory**

The equation, derived earlier (Section 11.4.2.2), defining recovery performance for a laboratory centrifuge is:

$$Q = \frac{2\ln\left(\frac{2r_2}{r_1 + r_2}\right)}{\omega^2 t}$$

Equation 6-1

where $r_1$ and $r_2$ are the distances from the centre of rotation to the liquid surface and the surface of the sedimented cake respectively, $\omega$ is the angular velocity about the centre of rotation, and $t$ is the total time of centrifuging. The volumetric flow rate, $Q$, is not an obvious term for a batch centrifuge but it enables comparison with continuous feed flow centrifuges. For a batch centrifuge, $Q$ is defined as:

$$Q = \frac{V}{t}$$

Equation 6-2

where $V$ is the volume of material in the centrifuge tube and $t$ is the run time of the centrifuge at constant speed.

Equation 6-1 applies for operation at constant speed only and must be adapted in order to allow for run-up and run-down of the centrifuge bowl. The acceleration and deceleration periods are assumed to be represented by linear equations and these are shown diagrammatically (Figure 6-1). The time period $t_a-0$ is equivalent to the acceleration period of the centrifuge; $t_b-t_a$ is equivalent to the constant speed stage; and $t_c-t_b$ is equivalent to

\[127\]
The contribution of each stages is shown in Figure 6-2, where the distance travelled \( r_a \), \( r_b-r_a \) and \( r_c-r_b \) occur during \( t_0 \), \( t_b-t_a \), \( t_c-t_b \) respectively.

\[
\omega = \frac{\omega_0 t}{t_a} \quad \omega = \omega_0 \left(1 - \frac{t - t_b}{t_c - t_b}\right)
\]

Figures 6-1 Laboratory centrifuge (angular velocity)

The fundamental equation for particle motion in a centrifuge tube is given by:

\[
\frac{dr}{dt} = \frac{v_s \omega^2 t}{g}
\]

Equation 6-3

where \( v_s \) is the settling velocity of a particle due to gravity.
Hence the general equation for the three phases of the centrifugation process is given by:

\[
\frac{\dot{r}}{r} = \int_{t_i}^{t_f} \frac{v_g \omega^2}{g} dt
\]

Equation 6-4

where,

\[\omega = f(\omega_o, t)\]

Equation 6-5

Substituting for the three different conditions, centrifuge acceleration, steady state, and centrifuge deceleration, and then integrating allows three equations to be described:

\[
\ln \left( \frac{r_a}{r} \right) = \frac{v_g \omega_o^2}{g} \frac{t_a}{3}
\]

Equation 6-6

\[
\ln \left( \frac{r_b}{r_a} \right) = \frac{v_g \omega_o^2}{g} \left( t_b - t_a \right)
\]

Equation 6-7

\[
\ln \left( \frac{r_c}{r_b} \right) = \frac{v_g \omega_o^2}{g} \int_{t_i}^{t_f} \left( 1 - \frac{t - t_b}{t_c - t_b} \right)^2 dt
\]

Equation 6-8

where \(\omega_o\) is the angular velocity once the centrifuge has reached the operating speed.

Combination of Eq 4 with the integral of Eq 5 gives:

\[
\ln r_c - \ln r_a = \frac{v_g \omega_o^2}{g} \left( t_b - t_a \right) + \left( \frac{t_c - t_b}{3} \right)
\]

Equation 6-9

Combining Eq 3 and Eq 6 and rearranging gives:

\[
\ln r_c - \ln r = \frac{v_g \omega_o^2}{g} \left( \frac{t_a}{3} + \left( t_b - t_a \right) + \left( \frac{t_c - t_b}{3} \right) \right) = \ln \left( \frac{r_c}{r} \right)
\]

Equation 6-10

For 50 % recovery of particles, assuming them all to be of the same size, then,

\[
r = \frac{r_2 - r_1}{2} \quad \text{and} \quad r_c = r_2
\]

Equation 6-11

Hence,
\[
\ln\left(\frac{r_c}{r_j}\right) = \ln\left(\frac{2r_2}{r_2 - r_1}\right)
\]

**Equation 6-12**

Rearranging:

\[
\ln\left(\frac{2r_2}{r_2 + r_1}\right) = \frac{\nu g \omega_0^2}{3g} \left( t_c + 2t_b - 2t_a \right)
\]

**Equation 6-13**

If \( y \) and \( 1-x \) are defined as the fraction of the overall centrifugation time for acceleration and deceleration respectively then, \( t_b = xt_c \) and \( t_a = yt_c \), then,

\[
\ln\left(\frac{2r_2}{r_2 + r_1}\right) = \frac{\nu g \omega_0^2}{3g} t_c \left( 1 + 2x - 2y \right)
\]

**Equation 6-14**

Substituting Equation 6-2 into Equation 6-14 gives:

\[
\ln\left(\frac{2r_2}{r_2 + r_1}\right) = \frac{\nu g \omega_0^2}{3g} \frac{V}{Q} \left( 1 + 2x - 2y \right)
\]

**Equation 6-15**

Rearranging

\[
Q = 2\nu g \frac{\omega_0^2 V}{6g \ln\left(\frac{2r_2}{r_2 + r_1}\right)} \left( 1 + 2x - 2y \right)
\]

**Equation 6-16**

\[
\Sigma = \frac{Q}{2\nu g} = \frac{\omega_0^2 V}{6g \ln\left(\frac{2r_2}{r_2 + r_1}\right)} \left( 1 + 2x - 2y \right)
\]

**Equation 6-17**

\[
\frac{Q}{\Sigma} = \frac{6g \ln\left(\frac{2r_2}{r_2 + r_1}\right)}{\omega_0^2 \left( 1 + 2x - 2y \right) V}
\]

**Equation 6-18**
Equation 6-19

\[ \frac{Q}{\Sigma} = \frac{6g \ln \left( \frac{2r_2}{r_2 + r_1} \right)}{\omega_e^2 (1 + 2x - 2y)t_c} \]

For a disc stack centrifuge \( \Sigma \) is given below. The equation is derived earlier (Section 0).

\[ \Sigma = \frac{2\pi n \omega^2 \left( r_o^3 - r_i^3 \right)}{3g \tan \theta} f_i \]

Equation 6-20

where \( n \) is the number of active discs, \( r_i \) and \( r_o \) are the inner and outer disc diameters respectively, \( \theta \) is the disc half conical angle, and \( f_i \) is a correction factor to allow for lost separating area due to spacer strips on each disc. The correction factor is defined as,

\[ f_i = 1 - \left( \frac{3Z_i B_i}{4\pi r_2} \right) \times \frac{1 - \left( \frac{r_i}{r_o} \right)^2}{1 - \left( \frac{r_i}{r_o} \right)^3} \]

Equation 6-21

where \( Z_i \) and \( B_i \) are the number and width of spacer strips respectively.

Results and Discussion

The bowl speed profile with time for the Beckman J2-MI laboratory centrifuge is shown in Figure 6-3 for the example of \( Q/\Sigma = 2.0 \times 10^7 \) m s\(^{-1}\). Up to the maximum speed used (6870 rpm - this corresponds to 3870g which is the same as the mean centrifugal force experienced in the disc stack centrifuge) the curves are linear except for the initial acceleration phase (0-500 rpm) and the end of the deceleration phase (500-0 rpm). The contribution of these two regions to the \( Q/\Sigma \) value was small (< 1 %) for the \( Q/\Sigma \) range studied and may be ignored. Therefore the acceleration and deceleration profiles may be approximated using linear equations as described in the theory section and shown in Figure 6-3. For this example, the duration of constant, maximum speed operation (6870 rpm), \( t_a-t_o \), is 11 seconds. With variation of \( Q/\Sigma \), \( t_a-t_o \) will change but the duration of the acceleration and deceleration stages, \( t_a \) and \( t_c-t_b \) respectively, will remain constant.
The laboratory centrifuge was operated at 6870 rpm (3870 g) to give the same mean centrifugal force as the SAOOH-205 disc stack centrifuge as recommended by Ambler (1952). In theory, however, the centrifugal force experienced in the laboratory centrifuge should not affect the recovery performance as long as $Q/\Sigma$ is maintained.

Figure 6-4 shows the importance of the acceleration and deceleration stages to the overall $Q/\Sigma$ value. The larger the $Q/\Sigma$ value the more essential it is to apply the correction. The normal operating range for the SAOOH-205 disc stack centrifuge is 50-300 L h$^{-1}$ (approximate $Q/\Sigma$ range: $2 \times 10^8$ m s$^{-1}$ to $2 \times 10^7$ m s$^{-1}$). Flow rates much below this are not viable due to excessive temperature rises which tend to damage biological products. Flow rates much higher are rarely used since biological products tend to be relatively small and of low density and thus require low flow rates for efficient separations. For this $Q/\Sigma$ range the contribution of the acceleration and deceleration stages to the overall $Q/\Sigma$ is 10-58 % when operating the laboratory centrifuge at the same average centrifugal force as the SAOOH-205 disc stack centrifuge (3870 g).

For large values of $Q/\Sigma (> 2.4 \times 10^7$ m s$^{-1}$) it was not possible to operate the laboratory centrifuge at 3870 g as there was insufficient time for the centrifuge to reach the operating speed before the desired $Q/\Sigma$ value was reached. In such instances a lower speed, 4000 rpm (1312 g), was adopted. Clarification performance at the two different speeds was assessed using the polyvinyl acetate particle system (Figure 6-5). This is a shear-insensitive particle system with a relatively broad particle distribution (approx. 0.6-3.8 μm) making it ideal for centrifuge characterisation. For the same $Q/\Sigma$ the recovery performance at the two different speeds was closely matched. Error bars show that with reduced clarification there is more spread to the data. This is an inherent aspect of the decanting technique and is apparent in all the figures. With partial clarification there is a gradient of solids concentration in the liquid phase which is at its highest near the bottom of the centrifuge tube. Thus, the additional decanting of a single drop can have a large impact on the overall clarification performance. In a process context it would be usual to examine the $Q/\Sigma$ range for which solids clarification efficiency is high and in such circumstances this effect is much reduced.

When comparing the clarification performance of a disc stack centrifuge to the laboratory centrifuge a correction factor must be used since not all the assumptions made when describing solid-liquid separation by Sigma theory are met for the disc stack centrifuge (Willis and Fitch, 1973). A wide range of values is quoted in the literature, 0.4-0.73 (Ambler, 1959; Frampton, 1963; Morris, 1966; Murkes and Carlsson, 1978). Few of these
workers detail there methods and hence it is difficult to explain the wide range of values. Frampton, however, suggested that different disc centrifuge bowl geometries may account for the range of efficiency factors and a study by Tomusiak (1992) examining the recovery performance of different disc centrifuge designs with the same feed material concluded the same. Comparison of clarification performance of the SAOOH-205 with that of the Beckman J1-MI laboratory centrifuge using polyvinyl acetate shows very good agreement for the entire range of \( Q/\Sigma \) values examined (Figure 6-6) when adopting an efficiency factor of 0.4 for the disc stack centrifuge. Hence, efficiency factors of 0.4 and 1.0 were used for the SAOOH-205 and the Beckman J1-MI centrifuges respectively throughout this study.

For yeast cell debris (Figure 6-7) good agreement is shown for higher values of \( Q/\Sigma \) but for the lower values the laboratory centrifuge shows slightly better clarification although error bars do overlap. If this is a real phenomenon then poorer clarification associated with the disc stack centrifuge may be due to shear breakage of loosely associated aggregates of debris in the high shear regions of this centrifuge type which remain intact in the low shear environment of the laboratory machine. Evidence for this was found in earlier work (Section 5 and Maybury et al, 1998). It is unlikely that differences in debris recovery performance are due to different debris particle size distributions produced by the two homogenisers since work by Siddiqi et al (1997) showed then to be very similar. It is not possible to extend the data of the SAOOH-205 to lower values of \( Q/\Sigma \) in order to test the similarity in clarification efficiency to the Beckman J1-MI since the disc centrifuge is already at its lower flow rate operating limit.

For protein precipitates there are significant differences in clarification efficiency between the two centrifuge types, the Beckman J1-MI laboratory centrifuge giving superior clarification performance (Figure 6-8, Figure 6-9). This is most likely due to shear damage of sensitive precipitate particles in the high shear regions of the disc stack centrifuge. It is unlikely to be due to differences in particle properties of precipitates prepared at different scales (Laboratory scale, 1L precipitation vessel; Pilot scale, 120 L precipitation vessel) since material prepared at the 4 L and 120 L scales gave comparable recovery performances in the fully scaled down disc stack centrifuge (Section 7). Additionally, results of clarification at laboratory scale of material prepared at both 1L and 120 L scale agreed well (Figure 6-8, Figure 6-9).

For ammonium sulphate precipitated proteins the laboratory centrifuge predicts that the 2nd cut precipitates are easier to recover than the 1st cut. With the disc stack centrifuge the reverse
trend is seen. This is probably due to the larger mean size of 2nd cut precipitates compared to 1st cut (Clarkson, 1994). The difference in particle size is probably lost with the disc stack centrifuge due to shear breakage of particles. Thus, it is the reduced viscosity of the 1st cut precipitates (lower ammonium sulphate concentration) that dominates and hence recovery is higher with the disc stack centrifuge.

The difference in protein precipitate recovery performance between the two machines is less for the ammonium sulphate precipitates than for polyethylene glycol precipitates. Precipitate particles are made up of primary particles which are typically 1μm in size (Bell et al, 1983). Ammonium sulphate precipitates, made up of these primary particles, are only a little bigger, 1-3 μm, and hence breakage would probably have less effect on recovery performance than it would for the larger PEG precipitate particles (Figure 6-10).

**Conclusion**

Adaptation of the Sigma equation for a laboratory centrifuge to take into account the contribution of the acceleration and deceleration stages is very important when trying to mimic the recovery performance of a disc stack centrifuge. Failure to do this results in a calculation error for $Q/\Sigma$ of 10-58 % for the normal working flow rate range for the disc stack centrifuge. This error could be much reduced by operating the laboratory centrifuge at a much reduced centrifugal force so that to obtain a certain $Q/\Sigma$ a much longer run time would be required thus reducing the contribution of the acceleration and deceleration phases to the overall $Q/\Sigma$ value. The drawback to this approach is that the laboratory centrifuge is no longer exerting the centrifugal force experienced in the disc stack centrifuge. For prediction of clarification performance this may not be important but if the laboratory centrifuge were also to be used to mimic solids dewatering then this is likely to be significant.

Accurate prediction of disc stack centrifuge clarification performance using the laboratory centrifuge is achieved for shear-insensitive particles when using an efficiency factor of 0.4 for the disc stack centrifuge. The efficiency factor is required to allow for inappropriate assumptions made in the Sigma theory describing recovery performance in a disc stack centrifuge.

For particles that are shear-sensitive, which is true of many biological products, then there can be a very large error between the laboratory prediction and the disc stack clarification performance. To make the laboratory centrifuge a broadly applicable predictive tool it will be necessary to develop a device to mimic the conditions experienced in industrial centrifuges.
namely high shear and in some cases air-liquid interfaces. One approach to the problem of shear would be to design a device to determine the shear sensitivity of particles in terms of changes in the particle size distribution. This information could be used to predict recovery performance in industrial machines. The drawback of this approach is that it does not provide material representative of the industrial scale for further investigations on unit operations downstream. A better approach would be to design a device that mimics the shear conditions of the industrial machines and so providing material which could then be used in the laboratory centrifuge. This could provide information about industrial scale recovery performance and also provide process material which would be of a quality representative of industrial scale.

Here the laboratory centrifuge was used to predict only the recovery performance of a disc stack centrifuge. It would be a much more useful tool if it could also be used to predict the partitioning of the liquid phase between the supernatant and slurry streams. This would allow a mass balance to be performed over the operation enabling the determination of important process information such as product yield and purity. The following protocol could be employed:

- The laboratory centrifuge is operated at a defined $Q/\Sigma$ in order to mimic recovery performance of the disc stack machine. The supernatant is decanted.
- Material remaining in the tube, the slurry, is spun at an RCF equivalent to that experienced at the disc stack bowl wall for a length of time that would be required to fill the solids space of the disc stack centrifuge. This time could be calculated with measurements of the feed stream solids concentration, the recovery performance (volume basis), the flow rate through the disc stack centrifuge and determination of its solids capacity. This would mimic the centrifugal force and the time for which that force was experienced by the solids and hence the degree of solids dewatering. After this spin decanted liquid should be added to the supernatant stream. The liquid content of solids should be representative of material obtained from a partial discharge (only solids discharged) of a disc stack centrifuge. To mimic stream partitioning when full discharges (both solids and bowl liquid discharged) are to be used then supernatant should be added back to the slurry stream in the ratio of disc stack centrifuge solids holding space volume to the bowl liquid volume.

The ultrascale down centrifuge is likely to be a very useful process development tool. With development it should be capable of indicating industrial centrifuge performance. The
advantage of ultrascale over scale down is that much smaller process material volumes are required making it possible to use earlier on in the process development procedure and making it viable to test a wider range of process options. In addition it is likely to be an inexpensive tool that is widely available whereas the scale-down approach would require greater investment.
Figure 6-3 Change in centrifuge speed with time for the Beckman J2-MI laboratory centrifuge in order to give $\alpha = 2.0 \times 10^{-7} \text{ m s}^{-1}$, ■, acceleration; ○, deceleration. Solid lines through the data for acceleration and deceleration stages are linear regression fits ($r = 0.997$ for both fits). Data points below 500 rpm are ignored for linear fitting. Drop lines (at 500 rpm and 6870 rpm) show limits of constant centrifuge acceleration and deceleration.
Figure 6-4 Contribution of acceleration and deceleration stages to the overall $Q/\Sigma$ when operating the laboratory centrifuge at the same average centrifugal force as the SAOOH-205 disc stack centrifuge (3870 g). Shaded area: normal operating range of a disc stack centrifuge for biological products.
Figure 6-5 Comparison of recovery performance at 1312 g and 3870 g of polyvinyl acetate particle system with the Beckman J2-MI laboratory centrifuge. Each data point represents the average of 3 assays and the error bars show the maximum and minimum values. Solid line is an apparent linear regression fit to the data ($r = 0.97$)

■, 1312 g (4000 rpm); ○, 3870 g (6870 rpm).
Figure 6-6 Comparison of recovery performance in a disc stack and laboratory centrifuge with polyvinyl acetate 190. Each data point represents the average of 3 assays and the error bars show the maximum and minimum values. Solid line is an apparent linear regression fit to the data ($r = 0.96$)

■, SAOOH-205 disc stack centrifuge; ○, Beckman J2-MI laboratory centrifuge.
Figure 6-7 Comparison of recovery performance of yeast cell debris (28% (ww/v)). Each data point represents the average of 3 assays and the error bars show the maximum and minimum values. The solid line is an apparent linear regression fit to the Beckman laboratory centrifuge data (r = 0.94).

■, SAOOH-205 disc stack centrifuge; ○, Beckman J2-M1 laboratory centrifuge.
Figure 6-8 Comparison of clarification performance in a laboratory and a disc stack centrifuge using ammonium sulphate precipitated protein; ■, ●, disc stack centrifuge (precipitate prepared in 120 L vessel, flow rate range: 15-80 L h⁻¹); □, ○, Beckman J2-MI laboratory centrifuge (precipitate prepared in 1 L vessel, $t_c$: 178-3782 s); ⊗, ⊘, Beckman J2-MI laboratory centrifuge (precipitate prepared in 120 L vessel); ■, □, △, 1st cut precipitate (40 % ammonium sulphate saturation); ●, ○, ⊘, 2nd cut precipitate (60 % ammonium sulphate saturation); (b) protein precipitates formed by the addition of polyethylene glycol (10 % w/v). Each data point represents the average of 3 assays and the error bars show the maximum and minimum values. Solid lines show trends to the data.
Figure 6-9 Comparison of clarification performance in a laboratory and a disc stack centrifuge using polyethylene glycol (10 % w/v) precipitated protein; ■, disc stack centrifuge (precipitate prepared in 120 L vessel, flow rate range: 12-80 L h⁻¹); O, laboratory centrifuge (precipitate prepared in 1 L vessel, tᵣ: 175-2769 s); ⊙, laboratory centrifuge (precipitate prepared in 120 L vessel). Each data point represents the average of 3 assays and the error bars show the maximum and minimum values. Solid lines show trends to the data.
Figure 6-10 Particle size distribution of protein precipitates after 38 minute ageing period (prepared in a 1 L vessel, mean velocity gradient, $G = 44$ s$^{-1}$); ■, polyethylene glycol precipitant (10 % w/v); ○, ammonium sulphate precipitant (40 % saturation).
7. RESULTS OF PROTEIN PRECIPITATION STUDIES

Summary
Precipitations were scaled down by maintaining similar vessel geometry and the mean velocity gradient experienced in the vessel. With ammonium sulphate precipitant, protein and ADH solubilities were independent of the scale of operation. With polyethylene glycol (PEG) precipitant there appeared to be over-precipitation with increase in scale. Ammonium sulphate precipitates formed at all scales (0.9-40 L working volume) gave similar particle size distributions. Both ammonium sulphate and PEG precipitates formed at scale-down (3.6 L working volume precipitation) gave comparable clarification efficiencies to pilot scale.

Introduction
Precipitation is an important operation for protein purification with a wide range of applications including food proteins and enzymes. Various methods exist which are discussed earlier (Section 1.4). Fractional precipitation, which is used to purify a target protein from as many as a thousand contaminating proteins, is investigated here. Important aspects of precipitation performance are solubility, particularly for fractional precipitation where a slight shift in the solubility curve can have a dramatic effect on performance, and precipitate particle characteristics which can influence the subsequent particle separation procedure (Bell et al, 1983).

Experimental
Packed yeast was resuspended to 28 % (ww/v) in phosphate buffer and homogenised at 500 barg using the APV K3 (pilot scale precipitations) or the APV Lab60 (scale-down and laboratory scale precipitations). Debris was clarified using the SAOOH-205 (full stack) for pilot scale precipitations and with the 1P tubular bowl centrifuge for scale-down and laboratory scale. $Q / \Sigma = 9.1 \times 10^{-9} \text{ m s}^{-1}$ (corrected for centrifuge efficiency) was used for both centrifuges to ensure a consistent level of debris removal. 1st cut precipitate was clarified to provide material for the 2nd cut precipitate studies. Clarification was achieved in the: SAOOH-205 disc stack (full stack) centrifuge for pilot scale precipitations, SAOOH-205 disc stack (full scale down, 9 active discs) for scale-down, and Beckman J2-M1 laboratory centrifuge for laboratory scale precipitations. All clarifications were performed at $Q / \Sigma = 9.1 \times 10^{-9} \text{ m s}^{-1}$. 
Solubility profiles of ADH and protein were determined for both ammonium sulphate and PEG precipitations at the 0.9 L working volume scale. For the chosen cut points solubilities were determined for pilot scale (40 L working volume), scale-down (3.6 L working volume) and laboratory scale (0.9 L working volume).

The particle size distributions of 1st cut ammonium sulphate precipitates were determined using the Elzone 280 PC particle sizer for a range of shear rates for all precipitation scales. Additionally, clarification performance of the scale-down disc stack centrifuge was determined with both 1st cut ammonium sulphate and PEG (10 % w/v) precipitates prepared at the 40 L (pilot scale) and 3.6 L (scale-down) working volume scales.

**Results and discussion**

**Protein and ADH solubility**

**Ammonium sulphate studies**

To ensure that all loss of protein and ADH from the soluble phase was due to reversible precipitation and that assay error could be eliminated, a mass balance was performed for every solubility step, i.e. both solid and liquid components were assayed for both ADH activity and total protein. Mass balances were within 10 % in all cases but since soluble assays appeared to give the most reproducible results these were used for all figures. There was no loss of protein or ADH over the range of the solubility curve which implies that reversible, non-denaturing protein precipitation was occurring.

The solubility curve for protein and ADH (Figure 7-1) was determined at the laboratory scale (0.9 L working volume). Each sample was assayed in triplicate and results were within ± 5 % for soluble assays.

Protein and ADH solubilities at the 1st and 2nd cut points (40 % and 60 % ammonium sulphate saturation respectively), given in Table 7-1, show very similar values for the different scales of operation. This agrees with Clarkson et al (1992) who found that the protein and ADH solubility curves changed little with change of scale, biomass concentration and degree of cell debris clarification. Foster (1972) studied the effects of debris loading on the solubility profiles for debris concentrations of 1-5 % (v/v) and found no significant difference. This equated to debris carry-over into the supernatant of 4-20 % which easily covers the range applicable to this study where debris carry-over was 10 % for all scales. Richardson (1987) did detect a shift in the solubility profile due to the presence of debris but this was for the extreme cases of clarified and unclarified homogenate. The shift in the
solubility profiles was attributed to the higher initial starting concentrations of ADH and protein. The composition of the feed stream to the 2nd cut precipitation was different for different scales due to varying levels of 1st cut precipitate clarification (Pilot scale, 40%; scale-down, 60%; laboratory scale 75%). This however did not affect the solubility of the 2nd cut point which is in agreement with the results of Clarkson (1994).

<table>
<thead>
<tr>
<th></th>
<th>Pilot scale (40 L working vol)</th>
<th>Scale-down (3.6 L working vol)</th>
<th>Lab scale (0.9 L working vol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st cut (40%) Protein</td>
<td>0.70 ± 0.037</td>
<td>0.67 ± 0.030</td>
<td>0.65 ± 0.030</td>
</tr>
<tr>
<td>saturation</td>
<td>ADH</td>
<td>0.97 ± 0.045</td>
<td>0.96 ± 0.045</td>
</tr>
<tr>
<td>2nd cut (60%) Protein</td>
<td>0.28 ± 0.010</td>
<td>0.26 ± 0.014</td>
<td>0.30 ± 0.008</td>
</tr>
<tr>
<td>saturation</td>
<td>ADH</td>
<td>0.05 ± 0.004</td>
<td>0.07 ± 0.004</td>
</tr>
</tbody>
</table>

Table 7-1 Fraction of protein and ADH remaining soluble at 1st and 2nd cut points for pilot scale, scale-down, and laboratory scale; working volumes, 40 L, 3.6 L and 0.9 L respectively.

In continuous precipitation studies Foster et al (1976) found that different contacting procedures can result in different final equilibria positions and that the more 'violent', i.e. the more rapid, the initial contacting of precipitant and clarified homogenate the greater the degree of over-precipitation. Over-precipitation occurs when the initial protein concentration exceeds the protein solubility at the initial salt concentration (Foster et al, 1976; Iyer and Przybycien, 1994). Imperfect mixing leads to transient contact of regions of locally high salt and high protein concentrations both of which result in additional precipitation. Whether or not over-precipitation occurs depends on the relative rates of mixing and protein precipitation. If mixing is more rapid than the rate of precipitation then the transient conditions of high salt and protein concentrations will no longer exist by the time the precipitation occurs and hence no over-precipitation should result. Hence over-precipitation is more likely to occur for rapid precipitations, such as with salts, or at large scale where mixing times are extended. Factors that affect over-precipitation include the rate of agitation, the reactor geometry, the rate of addition of precipitant and the feed geometry i.e. where the fluid is fed.

For all three scales of operation examined the mean velocity gradient, $G$, was kept constant. Since:

$$ G = \sqrt{\frac{P}{\mu V}} $$

Equation 7-1
and the fluid viscosity is relatively constant with shear then it follows that the power input per unit volume is maintained at the different scales. As the size of vessel increases the macro-mixing time increases with constant power per unit volume. For vessels of similar geometry to those used in this study the mixing time can be determined using the following correlation (McCabe et al, 1986):

\[ t_{ma} = \frac{36}{N} \]

Equation 7-2

where \( t_{ma} \) is the time for complete (99 %) macro-mixing to be achieved, and \( N \) is the impeller speed. This equation is valid for \( Re > 10^5 \), a condition which is met for all vessel sizes. For the vessels in this study this gave macro-mixing times of:

<table>
<thead>
<tr>
<th>Operating volume</th>
<th>40 L</th>
<th>3.6 L</th>
<th>0.9 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{ma} )</td>
<td>9.4 s</td>
<td>5.1 s</td>
<td>4.2 s</td>
</tr>
<tr>
<td>( Re )</td>
<td>32 000</td>
<td>11 000</td>
<td>6 000</td>
</tr>
</tbody>
</table>

These times, which do not include micro-mixing times, exceed the reaction time for the precipitation which is approximately 1s for salt precipitations (Parker and Dalgleish, 1977). Hence, over-precipitation with increase in scale might be expected. The calculated macro-mixing times are for 99 % homogeneity, to achieve say 90 % homogeneity would take considerably less time which might explain why no over-precipitation is observed at the different scales. With further scale-up macro-mixing times would extend further and hence over-precipitation may become a problem. Iyer and Przybycien (1994) found that the degree of precipitation is most strongly influenced, assuming constant protein concentration, by the precipitant addition rate and by maintaining a constant dimensionless addition rate over-precipitation due to different mixing conditions can be minimised. This approach may be necessary if further scale-up is to be considered.

Polyethylene glycol (PEG) studies

The solubility profiles at 0.9 L operating volume for ADH and protein are given in Figure 7-2. Error bars, showing variation between replicate assays, were usually within 5 %. A single cut procedure was adopted, at 10 % (w/v) PEG concentration, since a second cut point would provide no further purification of the ADH. At pilot scale (40 L working volume) and scale-down (3.6 L working volume) clarified homogenate was precipitated directly to 10 % w/v. Protein and ADH solubilities are shown in Figure 7-2. There appears to be a trend of over-
precipitation with increased scale but variation is relatively large and there is significant error bar overlap. The scales of operation examined with PEG precipitations are the same as for ammonium sulphate precipitations for which there was no over-precipitation. Foster (1972) found that protein precipitations with PEG were less prone to over-precipitation than with ammonium sulphate. This is probably due to the much reduced precipitation rate with PEG, equilibrium being reached in about 10 minutes compared to almost instantaneous equilibrium with ammonium sulphate. Solubilities with PEG precipitations is dependent on the cell debris loading (Richardson, 1987) and hence any reduction in the degree of clarification with scale-up would explain the results. However, care was taken to ensure the degree of debris clarification was similar (discussed in more detail in Section 6). Taking all this into account, it is difficult to say difference in solubilities with scale-up were due to over-precipitation or experimental error.

Precipitate particle properties
Figure 7-3 shows the volume based particle size distribution for 1st cut ammonium sulphate protein precipitates (40 % ammonium sulphate saturation) aged at 44 s⁻¹ for different process scales. The figure shows that, at this particular shear rate, particle breakage is dominant since with increasing time the proportion of larger and smaller particles reduces and increases respectively. Similar data was also obtained for mean velocity gradients of 11, 22, 33, 55, 100 s⁻¹. Instead of presenting data for each shear rate separately it is combined to give Figure 7-4 which is a plot of the mean velocity gradient against the volume of the precipitate particle size distribution above 1.2 μm. It would have been ideal to plot against the mean diameter or even d₅₀, which is more appropriate to centrifugation, but unfortunately the Elzone particle sizer was unable to capture the full particle size distribution. Figure 7-4 shows very good agreement between the different scales which implies that the mean velocity gradient is an effective method for maintaining the particle size distribution with scale-down.

In addition to the precipitate particle size other important physical properties are the particle density and strength. The particle strength must be maximised in order to limit the degree of particle breakage in the high shear region of the centrifuge feedzone. High particle density will also help improve centrifugal recovery efficiency. For batch ageing of a precipitate suspension the improvement in aggregate strength is dependent on the level of shear and the residence time in the vessel. Bell and Dunnill (1982) incorporated these parameters into the ageing group, Gₜ, known as the Camp number. They found that values of Gₜ ≥ 10⁵ are required for maximum particle strength. Figure 7-5 compares the clarification efficiency of the scaled down SAOOH-205 disc stack centrifuge with protein precipitate feed streams
prepared under the same conditions at pilot scale (40 L working volume) and scale-down (3.6 L working volume). Unfortunately, the laboratory scale precipitation provided too little material to investigate clarification performance. For both ammonium sulphate and PEG precipitates clarification performance is very similar for the two different precipitation scales. This does not mean that the precipitate particles necessarily had similar particle size, density or strength when entering the centrifuge but that these properties were similar once the precipitate suspensions had passed through the centrifuge feedzone where a degree of particle breakage occurs (Section 5).

**Conclusion**

Scale-down of protein precipitation by maintaining the mean velocity gradient and similar vessel geometry results in very similar particle size distributions for the range of mean velocity gradients tested and for the vessel size range examined. It is uncertain if this method would be sufficient if a wider range of vessel scales were to be examined since the range of shear rates experienced in a stirred tank increases with increasing scale. Other important particle properties, namely density and strength were not examined directly but similar recovery performances with the scaled down SAOOH-205 centrifuge for both ammonium sulphate and PEG precipitates suggest that these also scale-down well with constant mean velocity gradient. Similar solubilities of ADH and protein with ammonium sulphate achieved at all scales may not be so simple to achieve if further scale-up were to be examined due to the increased macro-mixing time that occurs when vessels are scaled up with constant power per unit volume. Over-precipitation with scale up seemed to occur for PEG precipitates although these results oppose the findings of Foster (1972). Investigation of the scaling method proposed by Iyer and Przybycień (1994), who found that precipitant addition rate to be the most influential factor on over-precipitation (assuming constant protein concentration), could help where over-precipitation is likely.
Figure 7-1 Protein and ADH solubility with ammonium sulphate concentration for the 0.9 L precipitation vessel (working volume). ■, total protein; ○, ADH. Lines are Boltzmann fits to the data but are present only to show the trend. Data points are the mean values and error bars show the range of values.
Figure 7-2 Protein and ADH solubilities at cut point (10 % (w/v) PEG) shown in relation to solubility curves for protein and ADH for 0.9 L vessel (working volume); ■, ADH solubility,
pilot scale at 10% cut point (40 L working volume); □, ADH solubility, scale-down at 10% cut point (3.6 L working volume); ●, protein solubility, pilot scale at 10% cut point (40 L working volume); ○, protein solubility, scale-down at 10% cut point (3.6 L working volume); †, ADH solubility curve (0.9 L working volume); ×, protein solubility curve (0.9 L working volume). Lines through data points are to show trends. Data points are the mean values and error bars show the range of values.
Figure 7-3 Volume based particle size distribution of yeast protein precipitate particles (40 % ammonium sulphate saturation, 1st cut point). Particles are aged at a mean velocity gradient, $G$ of $44 \text{s}^{-1}$. Particle size distribution after: 6 minutes (square symbols), 12 minutes (round symbols). Symbols: solid, pilot scale (40 L operating volume); open, scale-down (3.6 L operating volume); open with cross, laboratory scale (0.9 L operating volume). Lines show data trend for 6 and 12 minutes.
Figure 7-4 Fraction of 1\textsuperscript{st} cut precipitate (40 % ammonium sulphate saturation) particle size distribution (volume basis) above 1.2 \( \mu \text{m} \) after an ageing period of 18 minutes (normalised to the data for laboratory scale, \( G = 11 \text{ s}^{-1} \)) ■, Pilot scale (40 L operating volume); ○, scale-down (3.6 L operating volume); ▲, laboratory scale (0.9 L operating volume).
Figure 7-5 Clarification efficiency of the fully scaled down (4 top inserts, 9 active discs) SAOOG-205 disc stack centrifuge with yeast protein precipitates: (a), 1\textsuperscript{st} cut (40 % saturation ammonium sulphate); (b), PEG precipitate (10 % w/v). Precipitates prepared at: ■, pilot scale (40 L working volume); O, scale-down (3.6 L working volume). Ammonium sulphate precipitates were aged at $G = 44\text{ s}^{-1}$ for $Gt = 10^5$, PEG precipitates were aged at $G = 22\text{ s}^{-1}$ for $Gt = 10^5$. 
8. RESULTS OF ALCOHOL DEHYDROGENASE PROCESS STUDY

Summary
The scale-down process sequence highlighted the unsuitability of ammonium sulphate as the precipitant when using a disc stack centrifuge for solids recovery. This enables an early decision to be taken with regard to the type of centrifuge or the type of precipitant to be used in the large scale process. The ultrascale down process failed to identify the problems with precipitate recovery with scale-up.

Of the three unit operations examined centrifugation was the most significant cause of differences in process performance for the different scales of operation. The performance of the homogenisation and protein precipitation steps was similar at all scales. The mean protein and ADH concentration after cell disruption was 35.5 mg mL$^{-1}$ ($\sigma = 1.1$ mg mL$^{-1}$) and 550.0 U mL$^{-1}$ ($\sigma = 24.6$ mg mL$^{-1}$) respectively. The mean proportional precipitation of protein and ADH at the 1$^{st}$ cut point was 0.23 ($\sigma = 0.02$) and 0.08 ($\sigma = 0.01$) respectively; and at the 2$^{nd}$ cut point, 0.67 ($\sigma = 0.02$) and 0.92 ($\sigma = 0.02$) respectively. Process performance was most significantly affected by differences in recovery performance, particularly of precipitates, and in the degree of dewatering of recovered solids. Efficient solids separation and high levels of dewatering at the laboratory scale resulted in high overall yields (72-76 %) whereas less efficient performance of the pilot scale disc stack centrifuge gave yields of 11 % (full solids discharges) and 16 % (partial solids discharges). This level of performance was more closely matched by the scale-down sequence: 19 % (full solids discharges), 34 % (partial solids discharges). Disparities between scale-down and pilot scale were mainly due to improved precipitate recovery performance. The scale-down sequence was also accurate in predicting other important aspects of process performance at pilot scale; namely product concentration and purification. When Sigma theory was applied to the laboratory centrifuge then laboratory scale prediction of pilot scale performance was improved.

Introduction
Previously, the performance of the scale-down and laboratory scale of each of the three unit operations, high pressure homogenisation, protein precipitation and centrifugation, has been examined individually and compared to the pilot scale. In this chapter the three unit operations are examined together, in a sequence, using the Alcohol dehydrogenase (ADH) process as a basis (Figure 8-1). Reasons for the choice of process are given earlier (Section
This approach enables important process information such as product yield and purification to be examined and also any interactions between the different operations can be seen. Thus, the aim of this chapter was to determine how well the pilot scale performance, in a process context, could be predicted when the amount of material available was limited and thereby restricting the investigations to small scale systems, laboratory scale and scale-down. The laboratory process was run in two ways which differed only for the centrifugation stages. For one laboratory process (Lab 'high g') the centrifuge steps were run at high RCF for a long period. The second approach applied the adapted Sigma theory concept used in Section 6 to each of the centrifugation steps so that the same $Q/Z$ values (corrected for centrifuge efficiency) used for pilot scale and scale-down runs were employed. The second approach might be expected to be a better mimic of pilot scale performance, however, the first approach was deemed justified since it is widely used in the literature (Foster et al, 1976; Richardson et al, 1990). The scale-down and pilot scale processes were also run in two fashions, the difference being in the type of solids discharge employed, with both partial and full discharge methods being investigated. With partial discharges the disc stack centrifuge bowl is held open only long enough for solids to be discharged whereas the bowl liquid is retained. With full discharges the bowl is held open for longer and both solids and bowl liquid are discharged.

![Fermentation/Resuspension, Harvesting, Disruption, Debris Removal, Fractional Precipitation, Waste, Solid Product](image_url)

Figure 8-1 Flowsheet of ADH process sequence (process is operated in a series of batch stages).
Equivalent fermentation volumes (assuming 7 % ww/v final yeast concentration) for each process scale are shown below (Table 8-1).

<table>
<thead>
<tr>
<th></th>
<th>Laboratory scale</th>
<th>Pilot scale</th>
<th>Scale-down</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation volume</td>
<td>3 L</td>
<td>150 L</td>
<td>10 L</td>
</tr>
</tbody>
</table>

Table 8-1 Equivalent fermentation volume required to run each process

**Experimental**

The flow diagram for the process is shown in Figure 8-1. In place of the fermentation stage blocked yeast was re-suspended to 7 % (ww/v) yeast concentration, typical for a fed-batch fermentation. Using commercially available yeast enabled efforts to be concentrated on downstream processing. The suspended cells were then harvested in the first centrifugation step. The concentration of the cell slurry was adjusted to 28 % (ww/v) prior to high pressure homogenisation to release the intracellular proteins including the product (ADH). The yeast debris formed in the breakage stage was then removed in the second centrifugation step prior to primary protein purification which was achieved with a two cut ammonium sulphate precipitation procedure. The 1st cut precipitation leaves the majority of the product in solution but precipitates the less soluble protein contaminants. These are then removed in the subsequent clarification step. The 2nd cut precipitation leaves some of the most soluble contaminating proteins in solution but precipitates out most of the product which is harvested in the final centrifugation step.

Process equipment is shown below (Table 8-2). Equipment details are given in Section 2.1.1. Operating conditions for each unit operation are shown in Table 8-3.

<table>
<thead>
<tr>
<th></th>
<th>Laboratory scale</th>
<th>Pilot scale</th>
<th>Scale-down</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeniser</td>
<td>APV Lab 60</td>
<td>APV K 3</td>
<td>APV Lab 60</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>Beckman J2-MI</td>
<td>Westfalia SAOOH-205</td>
<td>Westfalia SAOOH-205 (with inserts)</td>
</tr>
<tr>
<td>Precipitation vessel (operating volume)</td>
<td>0.9 L</td>
<td>40 L</td>
<td>3.6 L</td>
</tr>
<tr>
<td>Feed pump to centrifuge</td>
<td>N/A</td>
<td>SSP Pumps, model 50NDM lobe pump</td>
<td>Ismatec gear pump, model MV-Z gear</td>
</tr>
</tbody>
</table>

Table 8-2 Process equipment
Table 8-3 Operating parameters for process runs (Q/Σ values are corrected for centrifuge efficiency) - see Figure 8-1 for equipment codes.

<table>
<thead>
<tr>
<th></th>
<th>Lab 'high g'</th>
<th>Lab 'Q/Σ'</th>
<th>Scale-down</th>
<th>Pilot scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenisation</td>
<td>Pressure</td>
<td>500 barg</td>
<td>500 barg</td>
<td>500 barg</td>
</tr>
<tr>
<td></td>
<td>No. passes</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Precipitation</td>
<td>G s⁻¹</td>
<td>44</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Gt</td>
<td>10⁵</td>
<td>10⁵</td>
<td>10⁵</td>
</tr>
<tr>
<td>C1 (whole cells)</td>
<td>Q/Σ (m s⁻¹)</td>
<td>1 x 10⁹</td>
<td>2.3 x 10⁻⁸</td>
<td>2.3 x 10⁻⁸</td>
</tr>
<tr>
<td>C2 (debris)</td>
<td>Q/Σ (m s⁻¹)</td>
<td>1 x 10⁹</td>
<td>1.0 x 10⁻⁸</td>
<td>1.0 x 10⁻⁸</td>
</tr>
<tr>
<td>C3 (P1)</td>
<td>Q/Σ (m s⁻¹)</td>
<td>1 x 10⁹</td>
<td>9.0 x 10⁻⁹</td>
<td>9.0 x 10⁻⁹</td>
</tr>
<tr>
<td>C4 (P2)</td>
<td>Q/Σ (m s⁻¹)</td>
<td>1 x 10⁹</td>
<td>9.0 x 10⁻⁹</td>
<td>9.0 x 10⁻⁹</td>
</tr>
</tbody>
</table>

Results and discussion

Mass balancing

To discover any discrepancies in assays a complete mass balance was performed on each process run at the different scales. This involved the measurement of mass and volume and determining the soluble and solid protein and ADH concentration for each stream. With these measurements there are three possible sources of error: error in the measurement of stream mass and volumes, error in the assay, and sample error due to heterogeneity of process streams. Another possible source of discrepancy, but not error, is loss of ADH/protein. Loss of enzyme activity is feasible due to the harsh environment experienced during processing, for example, high shear and air/liquid interfaces associated with centrifugation and batch protein precipitation. Losses may also occur through protease action. None of these however, should affect the total protein assay. Hence, the measurement of protein is very important for establishing the reasons for any mis-balances in ADH activity.

In all cases errors in stream masses and volumes were within 1%. Protein and ADH balances over each unit operation are shown below (Table 8-4). The starting point for Table 8-4 is the second centrifugation step since this is the first unit operation over which it is possible to perform balances because it is only in the previous operation, high pressure homogenisation, that intracellular components are released. It was assumed that there was no change in the solubility of protein and ADH over centrifugation steps thus enabling liquid and solid components to be balanced separately. Over the disc stack centrifugation stages there were significant temperature rises and the solubility of protein and ADH in ammonium sulphate is
known to be temperature dependent. However, Clarkson (1994) found that once the precipitate is formed then subsequent solubility is not affected by changes in temperature within the range experienced during centrifugation. This result was confirmed by mass balance studies performed on the different process scales (Table 8-4) which showed no shift in the solubility over centrifugation stages. Over the precipitation stages there were changes in solubility and hence a total balance for protein and ADH must be used. The mean error for individual protein and ADH mass balances for the different scales was: laboratory scale, 2.4 %; scale-down, 3.4 %; pilot scale, 3.8 %. Therefore, as the scale increases so did the error in the assay balances. This was partly due to the smaller error in measurement of stream masses and volumes at smaller scale but also possibly due to increased process stream heterogeneity with increase in scale.

Alsaffar (1994) examined the stability of ADH in homogenate and found that for pH 6.5 the enzyme was stable for the duration of the stability study (6 hours). For this study, the time between homogenisation and assay analysis was kept below 6 hours. He also concluded that the degree of debris clarification did not affect enzyme stability. This is important since the degree of debris clarification was not constant for the different process scales. For all the process runs an attempt was made to maintain the temperature at 4 °C. For the laboratory scale runs this was possible but for scale-down and pilot scale temperature rises occurred over the disc stack centrifugation stages. In all cases the peak temperature did not exceed 20 °C and process material was cooled immediately afterwards. Alsaffar (1994) found that even at 25 °C ADH is stable for up to 3 hours. Clarkson (1994) found that ADH is also stable for the length of the study (8 h) when suspended in 40 % and 60 % saturation ammonium sulphate solutions even at 25 °C.
Table 8-4 Results of protein and ADH balances in terms of fraction accounted for, bold values show errors > ± 5%.

<table>
<thead>
<tr>
<th></th>
<th>Lab (high g)</th>
<th>Lab (Q/L)</th>
<th>Scale-down (full)</th>
<th>Scale-down (partial)</th>
<th>Pilot (full)</th>
<th>Pilot (partial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2 soluble protein</td>
<td>0.96</td>
<td>0.96</td>
<td><strong>0.92</strong></td>
<td>0.95</td>
<td><strong>0.92</strong></td>
<td>0.96</td>
</tr>
<tr>
<td>C2 soluble ADH</td>
<td>1.02</td>
<td>1.01</td>
<td>1.01</td>
<td>0.97</td>
<td><strong>0.91</strong></td>
<td><strong>0.94</strong></td>
</tr>
<tr>
<td>C3 soluble protein</td>
<td>0.99</td>
<td>1.02</td>
<td>0.95</td>
<td>0.99</td>
<td><strong>0.93</strong></td>
<td>1.00</td>
</tr>
<tr>
<td>C3 soluble ADH</td>
<td>0.97</td>
<td>1.00</td>
<td>1.02</td>
<td>0.96</td>
<td><strong>0.94</strong></td>
<td>1.01</td>
</tr>
<tr>
<td>C3 solid protein</td>
<td>1.02</td>
<td>0.97</td>
<td><strong>0.93</strong></td>
<td>0.95</td>
<td>0.97</td>
<td>0.96</td>
</tr>
<tr>
<td>C3 solid ADH</td>
<td>0.97</td>
<td>1.02</td>
<td>1.03</td>
<td>0.96</td>
<td>1.04</td>
<td>1.01</td>
</tr>
<tr>
<td>C4 soluble protein</td>
<td><strong>0.88</strong></td>
<td>0.97</td>
<td>0.99</td>
<td>1.00</td>
<td>1.01</td>
<td>0.97</td>
</tr>
<tr>
<td>C4 soluble ADH</td>
<td>0.99</td>
<td>1.00</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>C4 solid protein</td>
<td>1.03</td>
<td>0.98</td>
<td>1.04</td>
<td>1.02</td>
<td><strong>0.98</strong></td>
<td><strong>1.07</strong></td>
</tr>
<tr>
<td>C4 solid ADH</td>
<td>0.98</td>
<td>0.99</td>
<td>1.00</td>
<td>1.04</td>
<td>0.99</td>
<td><strong>1.09</strong></td>
</tr>
<tr>
<td>P1 total protein</td>
<td>1.02</td>
<td>1.01</td>
<td>0.95</td>
<td>1.03</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>P1 total ADH</td>
<td>0.99</td>
<td>0.97</td>
<td>1.04</td>
<td>1.05</td>
<td>1.02</td>
<td>1.00</td>
</tr>
<tr>
<td>P2 total protein</td>
<td>1.03</td>
<td>0.98</td>
<td>1.03</td>
<td>1.03</td>
<td>0.98</td>
<td>0.95</td>
</tr>
<tr>
<td>P2 total ADH</td>
<td>0.99</td>
<td>0.97</td>
<td>0.98</td>
<td>1.05</td>
<td><strong>0.93</strong></td>
<td>0.95</td>
</tr>
</tbody>
</table>

There was no significant trend of loss/gain of protein/ADH occurring in more than one of the process runs which suggests that ADH was stable under the processing conditions.

**Process performance**

Important aspects of performance for this process are:

Clarification of yeast cell debris - residual solids can impact on the performance of other unit operations (Gray et al, 1973)

Product purification factor (in terms of protein) - this is affected by:
- protein and ADH solubility at P1 and P2
- recovery performance at C3
- extent of solids dewatering at C4

Process yield - this is affected by:
- the degree of cell disruption during homogenisation
- recovery performance and dewatering of recovered solids at centrifuge stages C2 and C3
- ADH solubility at P1 and P2
recovery performance at C4.

Product concentration factor (defined as the ADH concentration in the product stream divided by the ADH concentration in the homogenate stream) - this is affected by:

- the degree of cell disruption
- recovery performance at C2
- ADH solubility at P1 and P2
- extent of solids dewatering at C4

In order to understand the reasons for differences in yield, purity and concentration through the process it is necessary to first have a grasp of differences in performance of each unit operation at different scales:

**Homogenisation**

The degree of cell disruption is the first possible source of differences. It impacts on the yield and can also affect protein solubility during precipitation since the degree of precipitation is dependent on the initial protein concentration (Foster, 1972). The protein concentration can also affect precipitate particle size (Clarkson, 1994) and particle strength (Twineham et al, 1984) and, consequently, can influence centrifugal recovery performance. The mean protein and ADH concentration after cell disruption was 35.5 mg mL$^{-1}$ ($\sigma = 1.1$ mg mL$^{-1}$) and 550.0 U ml$^{-1}$ ($\sigma = 24.6$ U ml$^{-1}$) respectively. This represents a maximum variation between the different process scales of 6% and 10% for protein and ADH respectively. Assay error would account for differences in the protein concentration but the increased variation with ADH is probably due to differences in the quality of the starting material i.e. age/condition of the yeast cells.

**Centrifugation**

**Sedimentation performance** - This impacts both on product yield and purity. For all scales recovery of whole yeast cells was complete since the cells are large and relatively dense in comparison to the suspending liquid thus making them relatively easy to recover (Figure 8-2). For yeast cell debris recovery performance was approximately 90% (volume basis) in all cases apart from Lab (high g) where full recovery was attained as it also was for 1$^{st}$ and 2$^{nd}$ cut precipitates. Recovery of 1$^{st}$ cut precipitate showed higher sedimentation for the Lab ($Q/\Sigma$) scale compared to pilot scale which is as expected since the low shear conditions in the laboratory centrifuge should result in less particle breakage and hence higher recoveries. Recovery at scale-down was superior to pilot scale but inferior to the Lab ($Q/\Sigma$) which again is probably due to the reduced shear conditions in the feedzone compared to pilot scale but,
obviously, a higher shear environment than in the laboratory centrifuge. Recovery of 2\textsuperscript{nd} cut precipitate followed a similar trend of the highest shear conditions of the pilot scale centrifuge resulting in the lowest recovery, the lowest shear conditions of the Lab (Q/Σ) process gave much higher recovery and the scale-down centrifuge between the two. The Lab (Q/Σ) centrifuge showed better recovery performance of 2\textsuperscript{nd} cut precipitate compared to 1\textsuperscript{st} cut precipitate which is the reverse situation found at both pilot scale and scale-down. Possible reasons for this turnaround are given in Section 6. As might be expected little difference in clarification was observed for partial versus full discharge process runs. The impact of these trends in recovery performance are discussed below.

**Solids dewatering** - For this process solids dewatering influences the product yield, product purity and the product concentration factor. There were large differences in dewatering for the different process scales mainly due to differences in centrifuge design. Laboratory centrifuges are capable of very high levels of solids dewatering. The Lab (high g) process tended to give drier solids compared to Lab (Q/Σ) since the centrifuge in the former process was operated at a higher angular velocity and for a longer period. The disc stack centrifuge based processes were run either with full discharges or in a manner to simulate partial discharges. It is not possible to perform consistent partial discharges with a disc stack centrifuge as small as the SAOHH-205 since the discharge process is very rapid. Instead, the centrifuge was dismantled prior to discharging and the solids were removed manually. This gave relatively dry solids, but slightly wetter than with laboratory processes. When operating with full discharges liquid in the centrifuge is also discharged and hence the solids are much wetter. The higher the solids concentration of the centrifuge feed stream the more frequent solids discharges must be and hence a greater proportion of the liquid phase is lost the slurry stream. The relative proportions of liquid and solids in the slurry stream for the full stack centrifuge was different to the scale-down centrifuge. This is because discharged liquid is not solely from the centrifuge bowl, which is scaled down, but also from the centripetal pump chamber and feedzone, both of which are not scaled down. This is discussed in Section 11.2.

The degree of solids dewatering can have indirect influences on process performance, for example, for the recovery of whole cells from fermentation broth poor dewatering of the cell slurry results in the retention of significant levels of broth components. Varga (1997) found that antifoam associated with recombinant yeast cells recovered using a disc stack centrifuge (full discharges) affected the solubility profile of ammonium sulphate precipitations of intracellular proteins and that a washing step had to be introduced. He concluded that better
dewatering of the recovered cells would remove sufficient antifoam to avoid the requirement for cell washing.

The laboratory based processes and the partial discharge processes gave high levels of solids dewatering when recovering whole yeast cells (Table 8-5). Good dewatering was possible since the cells are compressible and pack well. The scale-down (partial discharge) gave very similar levels of dewatering to pilot scale (partial discharge). This is because the bowl diameter is kept constant with scale-down and hence the centrifugal force acting on the solids which causes dewatering is also kept constant. Solids recovered with the scale-down centrifuge (full discharge) were wetter than those from the pilot scale machine for reasons discussed above. The different levels of dewatering of recovered cells have no implications for this investigation since the starting material, packed yeast, was recovered from buffer, not fermentation broth, and the cell concentration after recovery was adjusted to 28% (ww/v) before homogenisation for all process scales.

<table>
<thead>
<tr>
<th></th>
<th>Lab high g</th>
<th>Lab Q/Σ</th>
<th>Scale-down</th>
<th>Scale-down (full)</th>
<th>Pilot scale (partial)</th>
<th>Pilot scale (full)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% liquor removed</td>
<td>100</td>
<td>99</td>
<td>98</td>
<td>85</td>
<td>98</td>
<td>92</td>
</tr>
</tbody>
</table>

Table 8-5 Solids dewatering during whole yeast cell recovery - values are normalised to the original packed yeast (term in brackets refers to type of solids discharge). Data is normalised to Lab high g results.

Protein precipitation

The degree of precipitation of protein and ADH at the two cut points (Figure 8-3) affects both the product yield and the degree of purification. These are discussed in detail below. The mean proportional precipitation of protein and ADH at the 1st cut point was 0.23 (σ = 0.02) and 0.08 (σ = 0.01) respectively; and at the 2nd cut point, 0.67 (σ = 0.02) and 0.92 (σ = 0.02) respectively. The degree of debris and 1st cut precipitate clarification was not the same for all processes and this should have an influence on solubility profiles. This is discussed earlier (Section 7).
Recovery of yeast cell debris

Recovery of yeast cell debris is shown in Figure 8-2. All processes showed a sedimentation performance of 90% excluding the Lab (high g) process which recovered all debris. Thus, by the application of Sigma Theory debris recovery in the pilot scale centrifuge was closely mimicked by both small scale systems, Lab (Q/2) and scale-down.

Product purity

Figure 8-4 shows the product purity (in terms of protein), otherwise known as the specific ADH activity for each process stream. For each process scale values are normalised to the homogenate stream purity which was very similar for each of the runs (15 U mg⁻¹). For the debris separation step (Figure 8-4a) specific ADH activity was independent of recovery performance and dewatering since both the ADH and the protein are in the liquid phase and hence always remain in the same ratio. Therefore values remain around 1.0 for both the C2 slurry and supernatant. The 1ˢᵗ cut precipitate stream (Figure 8-4b) should have given the same specific activity as the C2 supernatant stream as long as no product denaturation has occurred during precipitation. This is because the only difference between the streams is the addition of ammonium sulphate. This causes a change in the phase of the product and protein but since the specific activity is based on total quantities, i.e. solid and liquid, no change in specific activity should result. All process scales gave values around 1.0 apart from the scale-down process (full discharge) which gave a slightly raised specific ADH activity. This can be explained by examining the ADH and protein balances over the 1ˢᵗ precipitation step (P1) which show both a significant gain in ADH and a loss of protein probably due to an unrepresentative sample or assay error.

The C3 slurry stream showed a reduction in the specific activity for all process scales which is as expected since the 1ˢᵗ cut precipitation solidifies considerably more protein than ADH (Figure 8-3) which should then be recovered in the subsequent, C3, centrifugation step. The Lab (high g) process gave the lowest specific activity followed by the Lab (Q/2) and pilot scale and scale-down partial discharge processes. Significantly higher activities were seen for full discharge processes. How small the specific ADH activity is for the C3 slurry stream depends on the degree of dewatering of the solids. Since most of the ADH and protein is still soluble, and hence is in the liquid phase, it does not take much liquid entrainment to raise the specific activity significantly. Hence the dryer solids of the laboratory and partial discharge processes gave significantly lower activities compared to the much wetter solids of the full discharge processes. Thus the Lab Q/2 process is able to mimic the purity of the partial discharge pilot process alone. The scale-down partial discharge process also mimics the pilot
scale (partial discharge) well and the full discharge version highlights the trend of increased slurry specific activity but the value compared to pilot scale is significantly higher due to the extra liquid that is discharged with solids, discussed earlier.

A drop in the specific activity for the C3 slurry stream should be matched by a rise in the activity of the supernatant stream although this should be much less obvious since the majority of ADH and protein remains in the liquid phase and the solids concentration is relatively low in the supernatant. In fact, it is only for the lab (high g) process, where all precipitate is recovered (Figure 8-2) that a significantly raised specific activity can be seen. The exception to this is the scale-down process (full discharge) which gave a falsely high activity due to a large imbalance (loss) in both soluble and solid protein (Table 8-4). This conclusion is re-enforced by the specific activity value for 2nd cut precipitate, which should be the same as the value for C3 supernatant but which is in fact significantly lower. All other process runs show C3 supernatant specific activities close to 1.0 due to significant carryover of solid protein into the supernatant (Figure 8-2).

For the 2nd cut precipitate the specific activities were comparable to the C3 supernatant values which was as expected, as long as no ADH degradation has occurred, since the only difference was the addition of ammonium sulphate. At the 2nd cut precipitation 90-97 % of the total ADH is precipitated compared to only 64-70 % of the total protein (Figure 8-3) thus the C4 slurry streams should give specific activities above 1.0 and the greater the solids are dewatered the greater the specific activity should be. Sensitivity to dewatering levels will be fairly low since little ADH and protein is left in solution by this stage of the process. All process scales gave activities above 1.0.

The laboratory scale processes both gave high activities which ties in with good solids dewatering associated with this type of centrifuge. For the pilot scale and scale-down processes however, the partial discharge runs, which gave relatively dry solids, gave lower activities than the full discharge runs. This is the reverse of the expected trend but can be explained by apparent superior recovery of solid protein in comparison to solid ADH for the partial discharge runs (Figure 8-2, recovery: pilot scale, ADH, 22 %, protein, 26 %; scale-down, ADH, 42 %, protein 45 %). Superior recovery of one component over another was probably not a real effect since it did not occur consistently for any process and, more likely, it was due to assay error or sample heterogeneity. The scale-down (full discharge) process gave a similar specific ADH value to the pilot scale.
It is difficult to make meaningful comparisons of specific activities for C4 slurry since values are relatively insensitive to the levels of dewatering and hence assay errors become significant. Figure 8-5 shows the effect of solids dewatering and recovery performance on entrainment of soluble protein, and hence liquor, with recovered solids for the C4 centrifugation step. For full discharges liquid entrainment was the dominant effect. Again, scale-down showed more soluble protein entrainment compared to pilot scale due to proportionally more liquid being discharged with solids.

The specific activity of the C4 supernatant streams (Figure 8-4) is dependent on the solids recovery performance over this step. After the 2nd cut precipitation only 4-11% of the ADH was left in the soluble form whereas 30-36% of protein was still soluble. Hence well clarified C4 supernatant will have a low specific activity, however, where clarification is poorer the activity will be increased. The lowest activity occurred with the Lab (high g) process since all solids were recovered. This is followed by the Lab (Q/Z) process which also had a very high solids recovery (ADH, 86%, protein, 85%). The pilot scale and scale-down processes showed much higher specific activities due to the carry-over of solids, which consisted mainly of ADH rather than protein after P2, masking the purification effect on the specific activity. This pilot scale effect was predicted well by scale-down but poorly by both laboratory processes.

Overall the lab (Q/Z) process was a better predictor than the lab (high g) process of the specific activities of the pilot scale (partial discharge process) since recovery performances were more similar and the recovered solids tended to be wetter. The prediction was not very good for the final (C4) centrifugation step since recovery performance was far superior to pilot scale. Laboratory processes were unable to predict the effects of full discharges on the specific activity since laboratory centrifuge slurries were well dewatered even for the lab Q/Z process. Scale-down (partial discharge) accurately predicted the specific activities encountered at pilot scale (partial discharge). With full discharges the scale-down process highlighted the trend encountered at pilot scale (full discharge) but further modification to the scale-down centrifuge is necessary to reduce liquid loss with discharge and hence enable a more accurate prediction. Differences between the specific activities of different processes were often small, particularly for the streams of importance i.e. the product streams, and hence comparisons are susceptible to assay error and other sources of error. It was only in clear-cut situations such as for stream C3 slurry and C4 supernatant where solids dewatering and recovery performance differences between the processes had a significant effect on the
specific activity that confident conclusions could be drawn. Even then care must be taken to ensure that mass balances are accurate.

To summarise the purification efficiencies of the different processes the overall potential and actual purification factors are shown in Table 8-6.

<table>
<thead>
<tr>
<th>Purification factor (PF)</th>
<th>Lab high g</th>
<th>Lab Q/Σ</th>
<th>Pilot (full)</th>
<th>Pilot (partial)</th>
<th>Scale-down (full)</th>
<th>Scale-down (partial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential</td>
<td>1.59</td>
<td>1.44</td>
<td>1.36</td>
<td>1.39</td>
<td>1.72</td>
<td>1.40</td>
</tr>
<tr>
<td>Actual</td>
<td>1.51</td>
<td>1.46</td>
<td>1.61</td>
<td>1.17</td>
<td>1.39</td>
<td>1.17</td>
</tr>
</tbody>
</table>

Table 8-6 Overall purification factor for ADH relative to protein, PF_{potential} is the product of P1 and P2 purification factors, PF_{actual} is the specific ADH activity of the product stream relative to the homogenate. Potential purification is a measure of the purification achievable by the precipitation stages alone and does not take into account the performance of the subsequent centrifuge step, i.e. complete solids recovery and solids dewatering is assumed.

The potential purification factor is the product of the individual factors for the 1st and 2nd cut precipitations. For the 1st cut precipitation the purification factor is the total soluble ADH activity in the 1st cut precipitate stream divided by the total soluble protein concentration of that stream. Values are normalised to the feed, C2 supernatant stream. For the 2nd cut the purification factor is the total solid ADH in the 2nd cut precipitate stream divided by the total solid protein of that stream and then normalised to the feed. Hence the potential purification factors assume complete dewatering and full recovery of solids, and therefore are independent of centrifuge performance. The Actual purification factor for each stream is the specific ADH activity of the product stream, C4 slurry, divided by the specific activity of the homogenate stream. Hence the Actual purification factor takes into account solids dewatering of recovered solids. It is independent of recovery performance unless solid ADH and protein are recovered at different efficiencies.

Both the laboratory centrifuge processes showed slightly lower actual purification compared to potential due to some entrainment of liquid with recovered solids during the centrifugation stages C3 and C4 but also, in the case of Lab (high g), due to small errors in the mass balance. The pilot scale (full discharge) process was the only one to show a higher purification in actuality compared to potential. This was due to a better recovery of solid protein compared to solid ADH for the 1st cut recovery step (C3) and the reverse situation for the 2nd cut precipitate step (C4). Both of these improved the actual purification sufficiently to counter the effects of liquid entrainment with solids which shift the purification in the opposite direction. All other process scales showed a lower actual purity compared to
potential which was the expected result. A larger drop would be expected for full discharges compared to partial discharges due to the additional liquid entrainment with solids. This, however, was not the case due to a combination of small errors in the assays through the processes.

Product Yield
Figure 8-6 shows the step and overall yield for each process. For the centrifugation steps the yield is calculated as the total ADH activity in the product stream (the product stream is the supernatant stream for C2, C3 and the slurry stream for C4) divided by the total ADH activity in the centrifuge feed stream. For the precipitate streams the yield is calculated as follows: for the 1st cut precipitate the yield is defined as the total soluble ADH in the precipitate stream divided by the total soluble ADH in the feed stream (C2 supernatant); for the 2nd cut precipitate stream the yield is defined as the total solid ADH in the precipitate stream divided by the total soluble ADH in the feed stream (C3 supernatant).

For the debris separation step all processes gave the same recovery performance (90 %) except the Lab (high g) process for which all debris was recovered. Thus despite the dryer solids of the Lab (high g) process (Figure 8-7) the extra loss of liquid associated with the higher debris recovery resulted in only a slightly improved yield over this step. Both of the laboratory processes over-predicted the yield for both the partial and full discharge pilot scale process since in both cases more liquid, and hence product, was lost to the slurry stream. The scale-down (partial discharge) process accurately predicted pilot scale yield over the debris clarification step (C2). The scale-down (full discharge) process under-predicted the yield due to the additional liquid loss with solids discharging that is discussed earlier. Large losses in yield for the full discharge runs were due to the high solids concentration of the homogenate stream which required a significant number of solids discharges and hence much liquid loss to the slurry stream. Figure 8-7 shows loss in yield both corrected and uncorrected for solids recovery performance. The corrected values show predicted yield losses assuming full solids recovery. Yield losses for partial discharge processes were similar but slightly higher than for laboratory processes due to slightly wetter solids. For all process scales much greater loss in yield was seen for the debris recovery step in comparison to the 1st cut precipitate step due to the higher solids loading of the homogenate stream. Solids concentration of the homogenate streams were approximately 15 % compared to 6 % for the 1st cut precipitate streams. With full discharges, yield losses were greatly increased due to the additional loss of bowl liquid during the discharge process.
Yield over the 1st cut precipitate step was similar at all scales due to similar levels of product precipitation. For the C3 centrifugation step yields were similar for all scales with perhaps a tendency to higher yields for the laboratory and partial discharge processes. Yields for the Lab (high g) and scale-down (partial discharge) processes were slightly depressed due to apparent slight losses in ADH over this step in both cases (Table 8-4). The difference in yield between full and partial discharge processes was smaller for C3 than for C2 because the solids fraction of the precipitate stream was less and the recovery performance was poorer which meant that less solids discharges were required.

The yield values for the 2nd cut precipitation are simply the proportion of soluble ADH that is precipitated (Figure 8-3). Unlike the 1st cut, the feed streams to the 2nd cut were different for the different processes due to the different degrees of 1st cut clarification during the C3 step. The presence of pre-formed precipitate particles may effect the 2nd cut precipitation in two ways: firstly the solubility equilibrium would tend to be pushed towards the soluble phase and hence less precipitation would be expected for the pilot scale and scale-down systems and secondly particle characteristics, namely size and density, may be affected since the precipitate particles already present may act as a seed encouraging fewer and larger particles to be formed. This effect may be negligible since there is still significant levels of debris left which could also act as a seed and also larger precipitate particles would tend to be broken due to the microscale of turbulence. In actuality, the level of precipitation was fairly similar at all scales.

For the final centrifugation step the product was mainly in the solid form and hence it is recovery performance which had the dominant effect on the yield. Both laboratory processes gave high recoveries and hence high yields and are a poor indicator of the pilot scale processes which gave the worst recoveries and so the lowest yields. The scale-down processes gave improved yields relative to pilot scale due to improved precipitate recovery but still gave a good indication of the problems to be encountered with scale-up.

**Product concentration**

Along with product yield and purity, another important process parameter is the product concentration factor. The recovery process examined here is a primary purification sequence in that further purification would be necessary to produce a high purity product. A key aim of primary purification is to concentrate the product so that smaller scale high purification unit operations can be employed thus saving on capital and running costs. Figure 8-8 shows the overall product concentration factor which is defined as the product concentration in the
product stream divided by the product concentration in the homogenate stream. The laboratory processes gave similar values since product dewatering for C4 is comparable. Both significantly over-predicted the concentration factor achieved at pilot scale for both full and partial discharges. The partial discharge processes gave lower product concentration factors than the laboratory scale processes due to wetter C4 slurry streams. The pilot scale (partial discharge) gave a greater but comparable value to that obtained at scale-down which is explained by the wetter slurry solids (Figure 8-5). The scale-down (full discharge) process gave a good prediction of pilot scale but a slightly lower product concentration due to the additional liquid discharged with solids discussed earlier.

**Conclusion**

The unit operation that had the most significant effect on process performance at pilot scale was disc stack centrifugation. The pilot scale centrifuge gave very poor recovery performance of ammonium sulphate precipitates. This problem was highlighted by the scale-down process sequence but not by the ultrascale down process sequence. The scale down process, when used early on in process development, would have highlighted the need to change either the precipitant or the type of centrifuge in order to improve process performance. The effect of change of precipitant to polyethylene glycol on the process performance was investigated (Section 9).

The homogenisation and precipitation stages had only a small effect on the differences in process performance at different scales. This might be expected since the design of equipment and mode of operation was similar for all scales. It could be argued that laboratory based studies would preclude the use of high pressure homogenisation and that sonication would be a likely alternative. Such a device would give very different disruption performance (Maybury, 1996). The centrifugation stages were much more influential due to differences in solids recovery and solids dewatering performance.

The laboratory processes tended to predict the dewatering of the pilot process (partial discharges) fairly well but not so for the full discharge process which is important as there is significant impact on the yield, product purity and concentration. A method to adapt the laboratory process to allow for full discharges is discussed earlier (Section 6). The scale-down centrifuge also closely predicted the dewatering of the pilot process (partial) and gave a good indication of the effects of full discharge operation. However, scale-down of certain parts of the centrifuge outside of the bowl are required to prevent too much liquid loss with discharged solids which has a significant impact, particularly on the yield, over the C2 step.
The solids concentration of the homogenate stream (15 %) is however, unsuitably high for a disc stack centrifuge. Lower solids concentration streams, more suited to the disc stack centrifuge, would give a much reduced disparity between scale-down and pilot scale.

The lab (high g) process over-predicted pilot scale solids recovery at every step which impacted significantly on the prediction of yield. The lab (Q/£) process accurately predicted pilot scale debris recovery performance but over-predicted precipitate recovery, particularly the 2nd cut, and hence greatly over-predicted the overall yield. The scale-down centrifuge likewise over-predicted pilot scale precipitate recovery performance but by a much reduced amount. Ammonium sulphate precipitates are not ideally suited to recovery in a disc stack centrifuge due to their small size and relatively low density. Thus only partial recoveries are possible even at very low flow rates. This leads to a much larger disparity in performance between scale-down and pilot scale compared to at higher recovery performances (Section 5). Normally a centrifuge step is run for full solids recovery and hence the situation encountered with the ADH process is artificial and therefore it could be argued that the only requirement of the scale-down system in this case is to show that the process is not suitable for scale-up and this it clearly fulfils. An alternative system has been examined that is more suited to disc stack centrifugation (Section 9).

This particular process was fairly insensitive to process interactions since solubilities at both precipitation stages were not affected by differences in the feed streams, namely different degrees of debris and precipitate carryover. If polyethylene glycol had been used as the precipitate then solubility differences would have been expected (Foster, 1972). Additionally, if the process had been carried on into a chromatography step then significant differences in performance could be anticipated due to greatly different degrees of debris carryover. Good chromatography performance would be anticipated for the Lab (high g) process whereas fouling problems would be likely with the other processes which may require another unit operation, such as filtration, in order to rectify.

Mass balance data needs to be of a very high standard to enable confident comparison between different processes. In most cases data presented here balanced to within 4 % yet this can provide sufficient error to make comparisons difficult where there are only small differences in process performance parameters. This was particularly true for comparison of specific ADH activity data (purification). The low purification factors attainable with the ammonium sulphate precipitation did not help in identifying differences in performance between the different process scales.
The combination of better prediction of pilot scale recovery performance and solids dewatering at scale-down compared to laboratory scale gives much more accurate process information in terms of yield, product concentration but less clearly so for product purification, mainly due to the low purification factors attainable with the ADH process. Proposed modifications to the scale-down centrifuge discussed above and in Section 5 would make the scale-down system an even more useful tool for predicting larger scale performance. Application of Sigma theory to the laboratory centrifuge, something which is not widely reported, does make it more useful in predicting large scale performance but further modifications discussed in Section 6 are required.
Figure 8-2 Fraction of solids sedimented at each of the four centrifugation stages in the ADH process: Lab (high g); Lab (Q/2); Pilot scale (full discharge); Pilot scale (partial discharge); Scale-down (full discharge); Scale-down (partial discharge).
Figure 8-3 Fraction of the total protein and ADH precipitated at each cut point. P1, 1st cut precipitation (40% ammonium sulphate saturation); P2, 2nd cut precipitation (60% ammonium sulphate saturation); P1, Lab (high g); P1, Lab (Q/2); P1, Pilot scale (full discharge); P1, Pilot scale (partial discharge); P2, Scale-down (full discharge); P2, Scale-down (partial discharge).
Figure 8-4 Specific ADH activity (total ADH / total protein) relative to the homogenate for each stream; Lab (high g); Lab (Q/2); Pilot scale (full discharge); Pilot scale (partial discharge); Scale-down (full discharge); Scale-down (partial discharge).
Figure 8-5 Percentage of soluble protein in the feed entrained with recovered solids for C4 product precipitate recovery. Results are shown uncorrected and corrected for solids recovery performance: \( \text{Lab (high g)} \), \( \text{Lab (Q/2)} \), \( \text{Pilot scale (full discharge)} \), \( \text{Pilot scale (partial discharge)} \), \( \text{Scale-down (full discharge)} \), \( \text{Scale-down (partial discharge)} \). Correcting for solids recovery performance is achieved by dividing the value for entrained solid protein by the fraction of solids recovered.
Figure 8-6 Product yield (ADH) normalised to the homogenate stream at each step through the process and overall for each run; , Lab (high g); , Lab (Q/2); , Pilot scale (full discharge) , Pilot scale (partial discharge); , Scale-down (full discharge); , Scale-down (partial discharge).
Figure 8-7 Loss in yield due to liquid entrainment with sedimented solids during centrifugation stages C2, cell debris separation, and C3, 1st cut precipitate recovery. (a), uncorrected for solids recovery performance; (b), corrected for solids recovery performance: , Lab (high g); , Lab (L/2); , Pilot scale (full discharge); , Pilot scale (partial discharge); , Scale-down (full discharge); , Scale-down (partial discharge). Correcting for solids recovery performance is achieved by dividing the yield by the fraction of solids recovered.
Figure 8-8 Product concentration factor (ADH concentration in the product stream (c4 slurry) / ADH concentration in the homogenate stream).
9. PURIFICATION OF ALCOHOL DEHYDROGENASE USING POLYETHYLENE GLYCOL PRECIPITANT.

Summary
Experimental results were combined with mass balance simulations to compare the ability of small scale systems (laboratory scale and scale-down) to predict the process performance of a pilot scale equivalent over a range of operating conditions. The process used was for the primary purification of alcohol dehydrogenase (ADH) from baker's yeast which involved a polyethylene glycol (PEG) precipitation step. The laboratory scale process predicted a yield of ~90% and a purification factor of 1.9 - 2.2 for the range of conditions studied. This compared well with the yield obtained at pilot scale (83%); however, unlike the laboratory system, there was barely any product purification at pilot scale (purification factor: ~ 1.0). The scale-down system proved to be a more accurate mimic since predictions for process yields were within 6 - 12% and the purification factor within 0.2 - 0.4 of pilot scale performance.

Introduction
In the previous chapter the performance of a pilot scale purification process sequence was compared to a scale-down equivalent. In this case, the pilot scale performance was determined first and then the scale-down system was run using the same operating parameters. Although this is a useful comparison, the approach that would be used in practice would be to operate the small scale system first, and from the results obtained, operating conditions for the large scale process would be chosen. This latter approach was used in this study. Additionally, process performance was assessed for a range of operating parameters.

Two approaches to small scale studies are examined, ultrascale-down and scale-down, and their effectiveness in predicting the performance of an equivalent pilot scale system is determined. The process used for the comparison is for the purification of alcohol dehydrogenase from *Saccharomyces cerevisiae* using polyethylene glycol precipitant. The process flowsheet is shown in Figure 9-1. The key difference between the two small scale systems was the centrifugation unit operation. The ultrascale-down system used a laboratory centrifuge (Section 6) which requires only millilitres of biomaterial. A scale-down disc stack centrifuge (Maybury et al, 1998) was used for the scale-down system. Several litres of material were required for this machine.
Materials and methods

High pressure homogeniser equipment
Ultrascale-down - APV Lab60 (APV Manton Gaulin, Everett, Mass, USA) flow rate, 60 L h⁻¹.
Scale-down - as for ultrascale-down.
Pilot scale - APV K3 (APV Manton Gaulin, Everett, Mass, USA) flow rate, 290 L h⁻¹.

Centrifuge equipment
Ultrascale-down - Beckman J2-MI laboratory centrifuge (Beckman Instruments (UK) Ltd, High Wycombe, UK) equipped with a JS-13.1 swing out rotor.
Scale-down - Westfalia SAOOH-205 disc stack centrifuge (Westfalia Separator AG, Oelde, Germany) with reducing inserts: 4 top inserts, bottom insert, 9 active discs.
Pilot scale - Westfalia SAOOH-205 disc stack centrifuge: 37 active discs.
Protein precipitation equipment
A baffled tank stirred with a Rushton turbine was used for all scales:
Ultrascale-down - working volume = 0.9 L, \( D_T = 0.11 \) m, \( D_I = 0.04 \) m.
Scale-down - working volume = 3.6 L, \( D_T = 0.16 \) m, \( D_I = 0.06 \) m.
Pilot scale - working volume = 40 L, \( D_T = 0.50 \) m, \( D_I = 0.15 \) m.

Materials
Bakers’ yeast was supplied by The Distillers Co. Ltd. (Sutton, Surrey, UK). All chemicals, unless specified otherwise, were obtained from Sigma Chemical Co. Ltd. (Poole, Dorset, UK).

Preparation of yeast cell homogenate
Bakers’ yeast was resuspended to 28 % (ww/v) in 0.1 M KH\(_2\)PO\(_4\) buffer (buffer adjusted to pH 6.5). This was homogenised at 500 barg for 5 discreet passes. Homogenate temperature was maintained at 278 K.

Cell debris clarification
Cell debris was clarified at \( Q/\mu_L = 1.0 \times 10^{-8} \) m s\(^{-1}\) for all process systems.

Solids breakthrough profiles were determined for the continuous-flow disc stack centrifuges (scale-down and pilot scale) in order to determine time intervals for solids discharges. Full solids discharges (bowl solids and liquid are discharged together) were performed in all cases.

The laboratory centrifuge (ultrascale-down system) was operated at an RCF of 3870g which is the mean RCF experienced in the disc stack of the SAOOH-205 disc stack centrifuge (scale-down and pilot scale). The centrifuge tubes used were open, lipless 10 ml tubes filled to capacity. After the centrifuge run the tubes were decanted slowly. The supernatant stream was defined as the decanted material whilst the slurry sediment stream was defined as the material remaining in the tube.

Protein precipitation
For all precipitations the temperature was maintained at 278 K by cooling with glycol through the vessel jacket. The mean velocity gradient, \( G \), was held constant at 44 s\(^{-1}\). Protein and ADH solubility curves were determined using the 1.0 L laboratory vessel by the step-
Wise addition of PEG in the form of a 50 % (w/v) solution containing 0.1 M KH₂PO₄ buffer (adjusted to pH 6.5). There was a 10 minute interval between each addition to allow for protein solubility equilibration.

For all process runs, 50 % (w/v) PEG solution was added to rapidly to the clarified homogenate to give a final PEG concentration of 10 % (w/v).

Precipitate clarification
Clarification performance was determined for a range of Q/μS values.

Calculation of overall product yield and purity
Yield over the homogenisation step, \( Y_H \):
\[
Y_H = \frac{ADH_H}{ADH_{in}}
\]
Equation 9-1
Yield over the debris clarification step:
\[
Y_{C_1} = 1 - (SF_H \times E_{C_1} \times LF_{C_1,super})
\]
Equation 9-2
Yield over the precipitation step:
\[
Y_P = \frac{ADH_{C_1,super}}{ADH_P}
\]
Equation 9-3
Yield over precipitate clarification step:
\[
Y_{C_2} = 1 - (SF_P \times E_{C_2} \times LF_{C_2,super})
\]
Equation 9-4
The overall product yield, \( Y_f \), is given by:
\[
Y_f = Y_H \times Y_{C_1} \times Y_P \times Y_{C_2}
\]
Equation 9-5
Eqs 2 to 6 enable a mass balance to be established for ADH through the process. Using the same equations, but substituting data for protein in place of ADH, allows a similar mass balance to be determined for protein. From the full mass balance the overall purity, \( P_f \), can be calculated:
Results and discussion

Figure 9-2 shows protein release with number of passes at 500 barg and 28 % (ww/v) yeast concentration for the small and large scale high pressure homogenisers. Protein release is similar for all passes. Protein and product (ADH) release have been shown to follow similar trends (Siddiqi et al, 1997). Release data after 5 passes (96 % release at small scale, 94 % release at large scale) was used as inputs to the mass balance.

To enable mass balance calculations the solids concentration of the feed streams to centrifugation steps $C_1$ and $C_2$ were determined and the results are shown in Table 9-1.

<table>
<thead>
<tr>
<th></th>
<th>Laboratory scale</th>
<th>Scale-down</th>
<th>Pilot scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_1$</td>
<td>0.14</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>$C_2$</td>
<td>0.10</td>
<td>0.10</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table 9-1: Solids volume fraction (SF) of the yeast cell homogenate stream (feed stream to $C_1$) and the precipitate stream (feed stream to $C_2$)

Solids concentrations for both streams were similar for all process scales. Also required for mass balancing is the degree of liquid that is entrained with recovered solids during the centrifugation stages. For the disc stack centrifuges (full stack and scale-down) full solids discharges were assumed. In this case, the fraction of liquid entrained with discharged solids is largely dependent on the recovered solids and liquid capacity of the centrifuge bowl. These volume ratios were calculated from a machine drawing of the centrifuge bowl and are shown in Table 9-2. For the laboratory centrifuge the volume of liquid entrained with sedimeted solids depends on the spin time and speed of the centrifuge. For the purposes of this study a typical example was used for all cases (Table 9-2).

<table>
<thead>
<tr>
<th>$V_S/V_L$</th>
<th>Laboratory scale</th>
<th>Scale-down</th>
<th>Pilot scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.0</td>
<td>0.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 9-2: Ratio of discharged solids volume to entrained liquid volume for the 3 centrifuge options.
It was assumed that the centrifuge was operated at $Q/\mu \Sigma = 1.0 \times 10^4$ m s$^{-1}$ for the debris clarification step for all process scales. Figure 9-3 shows clarification efficiency for a range of $Q/\mu \Sigma$ and inset are shown the recovery efficiencies at the different scales for the chosen $Q/\mu \Sigma$. All process scales gave a recovery efficiency of approximately 70%. These data were used as inputs to the mass balance spreadsheet.

ADH and protein solubility data with PEG precipitant is shown in Figure 9-4. From this data a precipitation cut point of 10 % (w/v) was chosen for all process scales. Inset are the fractions of ADH and protein remaining soluble at the different scales at 10 % (w/v). The inset data was used as inputs to the mass balance spreadsheet.

Figure 9-5 shows precipitate recovery performance for a range of $Q/\mu \Sigma$. From this data, recovery efficiencies between 90 % and 99.9 % were chosen for the two small scale systems and the corresponding pilot scale recovery efficiencies (i.e. at the same $Q/\mu \Sigma$) were read from the data. These are shown in Table 9-3. These data were used as inputs to the mass balance spreadsheet.

<table>
<thead>
<tr>
<th>Laboratory scale</th>
<th>Pilot scale</th>
<th>Scale-down</th>
<th>Pilot scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.9 %</td>
<td>5.0 %</td>
<td>99.9 %</td>
<td>99.95 %</td>
</tr>
<tr>
<td>99.5 %</td>
<td>2.0 %</td>
<td>99.5 %</td>
<td>99.9 %</td>
</tr>
<tr>
<td>99.0 %</td>
<td>0.2 %</td>
<td>99.0 %</td>
<td>98.0 %</td>
</tr>
<tr>
<td>95.0 %</td>
<td>0.0 %</td>
<td>95.0 %</td>
<td>85.0 %</td>
</tr>
<tr>
<td>90.0 %</td>
<td>0.0 %</td>
<td>90.0 %</td>
<td>59.0 %</td>
</tr>
</tbody>
</table>

Table 9-3: Corresponding pilot scale centrifuge precipitate recovery efficiencies to small scale system (laboratory scale and scale-down) centrifuge recovery efficiencies. Values taken from Figure 9-5

Thus operating conditions for process runs were fixed for the cell homogenisation, debris clarification and protein precipitation stages but changes in centrifuge operation for the precipitate clarification step were examined and the effect of yield and purity discussed.
Mass balance predictions

Laboratory scale mimic

Yield

Figure 9-6(a,b) shows the overall yield and purity for the laboratory scale over the range of $Q/fL$ values coinciding with laboratory scale precipitate recoveries of 90 - 99.9 % and the corresponding pilot scale values for the same $Q/Σ$ range. The laboratory system gives an overall yield of approximately 90 % which increases slightly with $Q/Σ$ due to reduced recoveries and therefore less loss of solid-associated liquid to the slurry stream. This can be seen as a fairly good indicator of pilot scale performance where a yield of approximately 83 % was attained. Pilot scale yield also increases slightly with $Q/Σ$ for the same reasons. The reason for the very good yield at pilot scale is the very poor precipitate recovery performance which means that very few solids discharges are required so little liquid, and hence yield, is lost. Nearly all losses associated with the pilot scale runs are due to liquid loss during the homogenate clarification step whereas at laboratory scale both centrifugation steps are important sources of loss in yield.

The factors that effect process performance are: the degree of product release during cell disruption, solids recovery performance and the degree of solids dewatering over the centrifugation steps, and the degree of product and protein insolubilised during the protein precipitation step. The mass balance simulations for pilot scale can be re-run substituting in one of the small scale systems performance characteristics, i.e. either solids dewatering, recovery performance or protein/product solubility, in order to determine the major source of performance disparity between pilot scale and the small scale mimic.

Substituting the recovery performance of the laboratory scale into the pilot scale simulation leads to much reduced yields due to the additional loss of liquid, and hence product, to the slurry stream during precipitate clarification. Substituting the solids dewatering performance of the laboratory scale into the pilot scale simulation leads to improved yields since even less solid-associated liquid, and hence product, is lost. The final variation, which substitutes precipitate solubility of the laboratory scale into the pilot scale simulation has little impact on process yield differences even though over-precipitation of ADH occurs at pilot scale. This is because the solid ADH remains in the supernatant due to very poor pilot scale solids recovery.
Thus the laboratory scale yield is similar to pilot scale due to two counterbalancing effects, recovery and solids dewatering, that on their own affect the yield considerably.

Purity
The laboratory process gives a high product purification factor due to efficient removal of precipitated protein (Figure 9-6b). This is a poor indicator of pilot scale performance which shows barely any change in the purity compared to the homogenate due to very low precipitate recoveries.

Substituting the recovery performance of laboratory scale into the pilot scale simulation leads to a purification factor superior to the laboratory scale. This is due entirely to the over-precipitation at pilot scale which solidifies relatively more protein than ADH and thus raising the purification factor. The degree of solids dewatering has no impact on the purification factor. The very poor recovery performance at pilot scale is the dominant factor in determining the purification since this negates any differences in precipitation performance.

Scale-down mimic
Yield
The scale-down mimic under-predicts pilot scale yield by 6 - 12% (Figure 9-6c). The accuracy of the prediction decreases as precipitate recovery performance decreases due to an increased disparity in recovery performance between pilot scale and scale-down (Figure 9-5). The increased disparity, seen at higher $Q/\Sigma$ values, is not very significant since high clarification efficiencies are usually important in processing terms i.e. centrifuge steps tend to be run with efficient solids separation in mind.

Substituting the recovery performance of the scale-down centrifuge into the pilot scale simulation results in a much closer yield prediction of pilot scale. This shows that differences in solubility and solids dewatering appear to be the dominant reasons for the difference in performance. The other two re-run simulations back-up this conclusion since when the retained scale-down performance parameter is either solubility or solids dewatering then the deviation from pilot scale performance is significant.

Purity
Product purity depends on the degree of precipitation and also precipitate recovery performance. This explains why the scale-down prediction of purity is below that of pilot scale when recovery performances are similar since over-precipitation at pilot scale solidifies...
relatively more protein thus giving a purer product (Figure 9-6d). However, as pilot scale recovery performance becomes gradually worse in comparison to scale-down then this effect is negated since a significant proportion of solids pass into the supernatant thus lowering the purification factor relative to the scale-down prediction. The improved recovery performance of scale-down results in an increased purification factor relative to pilot scale. This effect is counterbalanced by over-precipitation at pilot scale which results in a relatively lower purification factor for scale-down. The actual scale-down position is a balance of these two effects. Wetter recovered solids at scale-down, as expected, has no influence on the purification factor.

Conclusions

Operating the pilot scale system in a manner determined to give good performance at laboratory scale results in similar process yields but virtually no purification of the product. Both yield and purity are important to the overall process performance. This implies that the laboratory centrifuge is a poor mimic of pilot scale performance due to large differences in centrifuge performance, in terms of both solids recovery and dewatering, and also precipitation performance since the small scale system does not mimic the over-precipitation of pilot scale.

The scale-down system gives a fairly good prediction of both yield and purity over the range of conditions examined. The prediction would be less accurate if it was extended to larger Q/Σ values where recovery performance is lower but this is of less importance since centrifuge steps are normally run for efficient solids separation. Over the region of high recovery performance the difference in solids separation between scale-down and pilot scale is very low and hence modification to correct any differences could be argued to be unnecessary. It is important, however, to correct the differences in the liquid content of recovered solids since this can have a significant impact on yield predictions particularly when feed stream solids concentrations are high and hence more solids discharges are required.

Adaptation of the operation of the laboratory centrifuge to improve its predictive abilities would include adding back supernatant to recovered solids in order to allow for the poorer dewatering of the large scale centrifuge and also the shearing of feed material to mimic the conditions experienced in the disc stack centrifuge feedzone. The problems with the scale-down centrifuge are less severe as this system is already a useful tool for predicting pilot scale performance. However, the proportion of liquid discharged relative to solids must be
reduced by scaling down the centripetal pump chamber, discussed earlier. Additionally, adaptation of the feedzone to mimic the shear conditions in the full stack device would be useful for correcting for differences in recovery performance of shear sensitive solids.

The problems of over-precipitation may be mimicked by adjusting the agitation rate to give a similar micro and macro-mixing rate at the small scale (Iyer and Przybycien, 1994; Rothstein, 1994). It should be noted that the scale-down system did show some over-precipitation relative to the laboratory scale and hence was a closer mimic of pilot scale performance.
Figure 9-2: Change in product release with number of passes. Homogeniser operated at 500 barg using 28 % (ww/v) yeast suspension, O, scale-down / laboratory scale; ■, pilot scale.
Figure 9-3: Clarification of yeast cell debris, O, laboratory scale; Δ, scale-down; ■, pilot scale. Each data point is the average of 3 assays and the error bars show the maximum and minimum values.
Figure 9-4 Precipitation of ADH and total protein with polyethylene glycol, +, laboratory scale ADH solubility profile; ×, laboratory scale protein solubility profile; ▲, scale-down ADH solubility; △, scale-down protein solubility; ■, pilot scale ADH solubility; □, pilot scale protein solubility. Each data point is the average of 3 assays and the error bars show the maximum and minimum values.
Figure 9-5 Clarification efficiency of different centrifuges (corrected for centrifuge efficiency) using yeast protein precipitate formed by PEG precipitation (10% w/v final concentration); ○, Beckman J2-M1 laboratory centrifuge (precipitate prepared in 1 L vessel); ■, SAOHH-205 disc stack centrifuge, full stack, 37 active discs (precipitate prepared in 100 L vessel); △, SAOHH-205 disc stack centrifuge, full scaled down, 4 top inserts, 9 active discs (precipitate prepared in 4 L vessel). Each data point is the average of 3 assays and the error bars show the maximum and minimum values.
Figure 9-6 Overall process yield and purification factor at pilot scale using small scale system results to define large scale operating parameters: (a, b), laboratory scale; (c, d), scale-down. Importance of different sources of error, namely different centrifuge recovery performance and solids dewatering, plus differences in product and protein solubility; between small and large scale are shown. X, small scale performance; +, pilot scale performance; □, Pilot scale performance with precipitation performance of the small scale substituted; ○, Pilot scale performance with precipitate recovery performance of the small scale substituted; Δ, Pilot scale performance with the centrifuge solids dewatering performance of the small scale substituted.
10. CONCLUSIONS AND FUTURE WORK

The scope of the study included:

1. Development and performance verification of scaled down unit operations: high pressure homogenisation, protein precipitation and disc stack centrifugation.
2. Examination of the performance of these unit operations in a process context using the primary purification of alcohol dehydrogenase as the test process.
3. Development of an ultrascale down centrifuge unit operation in order to enable process running using very small scale fermentations.
4. Application of scale-down and ultrascale down to process synthesis and process development.

The findings of these are discussed in turn and summarised.

1. Development and performance verification of scaled down unit operations

High pressure homogenisation for cell disruption - There are two important aspects to homogeniser performance for cell disruption: the degree of product release and the resultant cell debris particle size distribution. Both of these were accurately scaled down from the APV K3 homogeniser (~50 L working volume) to the APV Lab60 (~2 L working volume) and even to the APV Micron Lab 40 (~40 ml working volume). Scale-down was achieved by maintaining the valve geometry, the cell concentration and the pressure drop across the disruption valve. The fact that large pilot scale cell homogeniser performance can be accurately predicted by a device that requires only 40 ml of material means that a very small scale process could be devised if this were the limiting size unit operation. In theory, a fermentation of less than 1 L working volume may be used to accurately predict performance at the pilot scale. In practice, the performance of other unit operations may not scale down as well as the high pressure homogeniser.

Scaling problems do occur when different valve designs are used. This situation might arise if a large scale homogeniser were already in place but no small scale equivalent was available. To solve this problem, a better understanding of the cell disruption process and of the fluid flow profiles in the disruption valve is required. At present there are many theories of the cell disruption process yet evidence that any one process is the sole cause of disruption is lacking. The situation would be helped by a more rigorous understanding of the flow patterns in the disruption valve. In most cases the need for this may be avoided since switching the valve
type in any machine is usually a simple process and thus enabling accurate scale-up of disruption performance by maintaining the valve geometry.

Disc stack centrifugation (SAOOH-205 centrifuge) - Scale-down was achieved by modifying a pilot scale centrifuge. Bowl inserts reduced the liquid and recovered solids capacity in stages to suit particular applications. Recovery performance was scaled down by keeping constant the feed volumetric flow rate to separation area ratio \( (Q/\Delta) \).

The scale-down centrifuge accurately predicted the recovery performance of the full stack machine when using shear insensitive particulate streams of polyvinyl acetate (PVAc) and yeast cell debris provided that careful attention was paid to the alignment of the scale-down inserts. The scale-down centrifuge also accurately predicted the recovery performance of the much larger Westfalia CSA-8 machine, a large pilot scale centrifuge. With shear-sensitive protein precipitates the scale-down centrifuge tended to over-predict recovery performance. This was thought to be due to the reduced shear conditions in the feedzone of the scale-down centrifuge.

Modification of the feedzone of the scale-down centrifuge is required in order to maintain the degree of particle shear damage occurring at pilot scale. At present, the particle shearing mechanisms that take place are not fully understood and thus further work is required before a scale-down methodology for the feedzone can be devised. The flow patterns in the regions of the feedzone that are thought to be involved in particle breakage need to be mapped. The differences in recovery performance between pilot scale and scale-down are only significant when the overall recovery efficiencies are low - less than 90%. This is not the normal region of operation for a centrifuge since the aim is usually complete, or near complete, solids recovery. For separation efficiencies of protein precipitates of greater than 90%, then the difference between scale-down and pilot scale recovery performance was small. This could not be said for the small scale alternative to the scale-down machine, the laboratory centrifuge.

Discharged solids from the scale-down centrifuge were slightly wetter than those from the pilot scale machine. This was due to the discharge of liquor from regions of the centrifuge that were not scaled down, namely the centripetal pump chamber. For process streams with low solids content streams, then this has little effect on process performance, however, it becomes more significant as the stream solids concentration increases. This problem should
be fairly easily rectified by the use of an insert to reduce the liquid hold-up of the centripetal pump chamber and is recommended as an immediate action.

The disc stack centrifuge was the unit operation that limited the extent of scale-down of the ADH process volume. The target volume, equivalent to a 10 L fermentation volume, was achieved not simply by unit operation scale-down but also by careful operation of the process. This latter approach could also be used at pilot scale and then the difference in the amount of material required for the two scales of operation would be only 4-fold, equal the degree of centrifuge scale-down. Further centrifuge scale-down would enable processes to be run from even smaller fermentation volumes. It should be possible to further scale-down the SAOOH-205 machine by using more inserts. This would however, increase the impact of the non-scaled down sections of the centrifuge on process performance. Hence, these issues would also have to be addressed. It may prove more feasible to design a new small scale centrifuge from scratch rather than by further modification of the SAOOH-205. This is an issue that requires the input of the centrifuge manufacturer.

**Protein precipitation** - Protein precipitate particles formed at the scale-down level had a similar particle size distribution and showed similar recovery performance in the disc stack centrifuge to those formed at pilot scale. This was achieved by maintaining the mean velocity gradient with scale-down.

Protein and product solubility are also important concerns for protein precipitation scale-down. There was no apparent change in solubility with scale for ammonium sulphate precipitations yet with polyethylene glycol precipitations there appeared to be over-precipitation with scale-up. Ideally, for precipitation scale-up/down, both the macro- and micro-mixing times should be kept constant in order to keep protein/product solubility constant, however, it is impossible to maintain both when changing scale. As yet, very little data has been published on the scale-up of protein precipitation. A method proposed by Iyer and Przybycien (1994) which involved maintaining a dimensionless precipitant feed addition rate may prove to be useful although this has as yet only been tested at small scale.

2. Examination of scale-down unit operation performance in a process context.

Scale-down process examination of the purification process for ADH with ammonium sulphate highlighted the problem with precipitate recovery encountered at pilot scale. From this information it would be possible to decide early to modify the process in some way in order to make the process more suitable to large scale operation. The decision made was to
change the type of precipitant from ammonium sulphate to polyethylene glycol (PEG). The scale-down process not only showed that this modified process was suitable for pilot scale operation but also accurately defined the operating conditions required for efficient operation. Thus, at an early stage and with reduced quantities of process material, a successful process synthesis decision was made.

3. Development of an ultrascale down centrifuge operation

A four-fold reduction in the volume of process material required at pilot scale was made with the scale-down process. The unit operation limiting the degree of scale-down was the centrifuge step. Both high pressure homogenisation and protein precipitation have been successfully scaled down to a degree that allows very small process material volumes to be used (discussed earlier). Further disc stack centrifuge scale-down may be possible but it is likely to be limited on mechanical grounds or at least would require a significant investment to develop successfully. Due to this, an alternative approach was adopted which used a laboratory centrifuge to which engineering scale-down principles were applied. Such a machine has the advantage of being more readily used by industry since it is already widely used for purposes other than process synthesis/development. This ultrascale down centrifuge resulted in accurate prediction of pilot scale centrifuge recovery performance for shear-insensitive solids but over-predicted performance of shear sensitive solids such as protein precipitates. Hence in the processes examined, which included shear sensitive streams, it was a poor predictor of pilot scale performance and failed to identify the problems encountered at pilot scale. A method was proposed to enable more accurate prediction of pilot scale performance using ultrascale down equipment.

Most purification processes involve chromatographic stages for final product treatment although these were not examined in this study. The performance of such stages is very sensitive to any solids present in the process stream. Both the ultrascale down and scale-down processes accurately mimicked the degree of debris loading after the debris separation step. The laboratory process did not accurately mimic this. Also the ultrascale down process failed to accurately mimic solids loading of the process streams when shear-sensitive solids were involved. Therefore, only the scale-down process is likely to mimic larger scale performance for a complete process when shear-sensitive solids are encountered.

Due to the very small quantities of material required by the ultrascale down process, another application would be to monitor a large scale process during its operation and to use these results to optimise its operation. Additionally it could be used to identify batch to batch
fermentation differences and enable the large scale purification process to be set-up accordingly.

4. Application of scale-down and ultrascale down technology to process synthesis/development

This study has highlighted the potential of both scale-down and ultrascale down processes. Initial work verified the performance of the individual unit operations. Application to process work highlighted the effectiveness of the small scale processes in foreseeing processing problems at larger scales. The modified process, using PEG, was used to verify the usefulness of the scale-down process sequence for solving large scale processing problems before they are encountered. Also the PEG chapter highlights the approach required for efficient process synthesis/development studies. The most important thing to note is that it is not sufficient to simply run the small scale process with a single set of operating conditions and hope to predict all possible problems to be encountered with scale-up. It is essential to examine a range of operating variables from which a suitable operating window for the larger scale can be determined.

The major role for scale-down systems is narrow down the range of suitable unit operations for a particular product, highlight problems which may be encountered and also to determine operating windows. Scale-down studies are not a replacement for the larger scale since scale-down is not a perfect mimic, they are a targeting mechanism.

Summary

This study has proved the effectiveness of the scale-down approach and has highlighted the potential of ultra scale-down. Both are very important tools for process development and process synthesis. The speedy transition required from product discovery to manufacture requires a reliable and systematic approach, this is discussed in the introduction. An integral part of this approach is filled by scale-down and ultra scale-down technology.

The next logical step, excluding the future research proposals mentioned above, is to broaden the range of scale-down unit operations that are available. This will enable the process synthesis and development approach that has been discussed to be realised and in doing so will provide an invaluable tool to the bioprocess industry.
11. Appendices

11.1 Mass balance spreadsheet

The mass balance spreadsheet is shown using numbers from a given scenario and also with formulas.
| A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S |
| 1 | Dry weight composition | (A)  | (D) | Centrifugation 1 | Centrifugation 2 | Homogenisation | Centrifugation 2 | AmmonSO4 | | | | | | | | | | |
| 2 | | | | stream 1 | stream 2 | stream 3 | stream 4 | stream 5 | | | | | | | | | | |
| 3 | | | | Conc. conc. mass | Conc. conc. mass | Conc. conc. mass | Conc. conc. mass | Conc. conc. mass | | | | | | | | | | |
| 4 | | | | gel slurry water | gel slurry water | gel slurry water | gel slurry water | gel slurry water | | | | | | | | | | |
| 5 | Icl protein | 40.00% | | | | | | | | | | | | | | | | |
| 6 | solid protein | 0.00% | | | | | | | | | | | | | | | | |
| 7 | DNA | | | | | | | | | | | | | | | | |
| 8 | Other solubles | | | | | | | | | | | | | | | | |
| 9 | Total % | | | | | | | | | | | | | | | | |
| 10 | Pred. pH | 6.28 | | | | | | | | | | | | | | | | |
| 11 | Precipitate recovery | 33.62% | | | | | | | | | | | | | | | | |
| 12 | Total sol protein | 101.67 | | | | | | | | | | | | | | | | |
| 13 | Solid protein | | | | | | | | | | | | | | | | |
| 14 | Cell debris | | | | | | | | | | | | | | | | |
| 15 | Int water | | | | | | | | | | | | | | | | |
| 16 | % cell debris | | | | | | | | | | | | | | | | |
| 17 | ADH leaching | 66.02% | | | | | | | | | | | | | | | | |
| 18 | Ret. slurry | 1.09% | | | | | | | | | | | | | | | | |
| 19 | Volume | 1399.13 | | | | | | | | | | | | | | | | |
| 20 | Flow rate (L/Min) | | | | | | | | | | | | | | | | |
| 21 | Time (mins) | | | | | | | | | | | | | | | | |
| 22 | Cell Recovery | 100 | | | | | | | | | | | | | | | | |
| 23 | Homogenisation | 67 | | | | | | | | | | | | | | | | |
| 24 | Debris removal | 92 | | | | | | | | | | | | | | | | |
| 25 | Precipitation 1 | 38 | | | | | | | | | | | | | | | | |
| 26 | Precipitation 2 | 38 | | | | | | | | | | | | | | | | |
| 27 | Product recovery | 9 | | | | | | | | | | | | | | | | |
| 28 | Total time | 185 | | | | | | | | | | | | | | | | |
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| 100 | | | | | | | | | | | | | | | | |

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Mass balance spreadsheet for a typical scenario
Mass balance spreadsheet showing formulae
11.2 SAOOH-205 disc stack centrifuge - initial characterisation

11.2.1 Summary
The bowl geometry is not constant with scale-down which might impact on recovery performance. There is a volume of liquid that adds to the solids discharge which originates from outside the bowl and hence is not reduced with scale-down and so dilutes the slurry stream. This impacts on the ratio of supernatant stream volume to slurry stream volume. Geometry changes will also result in extended discharge intervals which may alter solids water content in certain situations. The full stack centrifuge showed a temperature rise in the supernatant stream relative to the feed of 1.8 °C compared to 5.2 °C for the fully scaled down centrifuge.

11.2.2 Introduction
Little data is available from the manufacturer concerning dimensions the SAOOH-205 disc stack centrifuge such as bowl volume and solids holding volume. No data exists for the scale-down configurations. Using machine drawings and measurements direct from the centrifuge parts, calculations were made to determine dimensions of the centrifuge which can affect the centrifuge performance. These included: the total bowl volume, the volume of the disc stack and the solids holding space volume.

For biological products it is often important for the temperature to be closely controlled usually at, or near to, 0 °C (Foster and Watt, 1980). In an industrial centrifuge, such as the disc stack, there are several sources of heat transfer to the process stream:

1. Mechanical - heat from the clutch, gear and bearings conducted through the centrifuge spindle
2. Liquid friction during acceleration of the process stream in the centrifuge feedzone
3. Air friction on the rotating bowl
4. Liquid friction on the centripetal pump during supernatant discharge from the bowl
5. Liquid friction on discharged slurry through the solids discharge ports and on the catcher chamber wall.

Scale-down of the disc stack centrifuge has been achieved by adapting a pilot scale machine. Since the separation is scaled down so is the flow rate but the residence time in the centrifuge is not scaled-down by the same degree since, although the bowl volume is smaller, the feed-
zone and the centripetal pump chamber are unchanged. Thus there is more time for heat transfer to occur.

11.2.3 Materials and methods

Measurement of discharge volumes

The discharge volume was determined for each centrifuge configuration by pumping water through the centrifuge. The water feed supply was stopped prior to discharge to prevent additional water entering the discharged stream and thus giving a falsely high measurement. Also the supernatant line was dismantled to prevent reverse flow of liquid into the bowl during discharge which would also cause a falsely high measurement.

Measurement of heat transfer

Water, at room temperature, was fed to the full stack (43 active discs) and the fully scaled down (4 top inserts, 9 active discs) at \( Q/\Sigma = 9.8 \times 10^9 \text{ m s}^{-1} \). Feeding to the centrifuge was continued until the supernatant temperature reached steady state. For both runs the centrifuge had not been used for 24 h previously and hence was at room temperature.

11.2.4 Results and discussion

Calculated bowl volumes and measured discharge volumes for SAOOH-205 disc stack centrifuge and all scale-down configurations are shown in Table 11-5. All volumes were calculated from machine drawings except the discharge volume which was a measurement of the volume of water released per solids discharge during centrifuge running. With a disc stack centrifuge as small as the SAOOH-205 a solids discharge releases not only the contents of the solids area but also the liquid in the bowl. This is because the discharge process is very rapid at this scale; for a larger centrifuge it is possible to discharge just the solids as discharge times are longer (partial discharge). The disc space solid volume \( (V_{sa}) \) is the volume occupied by the disc stack, this reduces with scale-down as the number of discs reduces. The disc space liquid volume \( (V_{sl}) \) is the unoccupied volume between the discs which is the same as the disc volume since the gap between the discs is the same as the disc thickness. The solids holding capacity \( (V_s) \) is the unoccupied volume from the bowl periphery to the outer edge of the disc stack. The total bowl volume is calculated by adding \( V_{sa} \) and \( V_s \). Column 9 of Table 11-5 shows the difference in volume between the discharge volume and the bowl volume. This gives a fairly consistent value of 110-120 ml for all centrifuge configurations which is due to water outside the bowl, probably from the centripetal pump chamber and also the feedzone. These areas are not scaled down. This will have an impact on process runs since the ratio of supernatant stream volume to slurry stream volume will reduce with scale-down. The impact
of this will be small for streams with a low solids concentration but it will be more significant for more concentrated streams.

Column 10 shows the ratio of disc separation area to the solids holding volume normalised to the full stack centrifuge. This ratio defines the time between solids discharges for a particular separation process since the rate of solids accumulations is defined by the flow rate through the centrifuge and the flow rate to separation area ratio is kept constant with scale-down. The other important factor being the volume of the solids holding space. This ratio tends to reduce with time which will mean an extended interval between solids discharges. This may lead to additional dewatering of collected solids depending on their rheology. This may have an effect on the process yield if the solids are discharged in a partial manner but the effect would not be noticed if full discharges were performed. In some cases additional dewatering may alter the ease with which solids are discharged. It will also enable additional heating of collected solids from the bowl wall.

The ratio of bowl volume to solids holding volume normalised to the full stack centrifuge (column 11) is a measure of bowl geometry changes with scale-down. Changes in geometry may have an impact on the fluid flow characteristics in the bowl which in turn may alter recovery performance. This ratio does change a little with scale-down, particularly for the full scale-down variant (4 top inserts, 9 active discs).

The temperature rise in water fed through the full stack and the scale-down centrifuge are shown below (Table 11-4).

<table>
<thead>
<tr>
<th>Feed temperature (°C)</th>
<th>Supernatant temperature (°C)</th>
<th>Temperature rise (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full stack (43 discs)</td>
<td>20.7</td>
<td>22.5</td>
</tr>
<tr>
<td>Full scale-down (9 discs)</td>
<td>20.7</td>
<td>25.9</td>
</tr>
</tbody>
</table>

Table 11-4 Temperature rise across the SAOOH-205 disc stack centrifuge for the full stack and fully scaled down variants.

11.2.5 Conclusion

Problems with the scale-down centrifuge that may be anticipated include differences in the recovery performance between full stack and scale-down with shear-sensitive solids since the feedzone design is unaltered. Additionally, changes in yield might be expected due to greater
liquid loss to the slurry stream with scale-down. How severe these problems will be is
difficult to predict and will be dependent on the materials being processed.

The temperature rise across the scaled down centrifuge is significantly larger than for the full
stack centrifuge and this may have an impact on process performance for temperature
sensitive products. This is due to the increased residence time in the centrifuge at scale-
down. The temperature rise across the full stack centrifuge is small although this is not the
case if the feed stream to the centrifuge is pre-cooled. Clarkson (1994) measured a significant
8 °C temperature rise with the SAOOH-205 full stack centrifuge when processing a stream
fed at 5 °C. Thus cooling is required for both the full stack and scale-down centrifuges
although neither machine has this capability. Larger centrifuges can be specified with frame
cooling, centripetal pump cooling and direct bowl cooling (Hemfort, 1988). The latter, which
is the most important, is achieved by spraying cooled water directly onto the bowl wall. The
drawback of this approach is the dilution of collected solids. Although the cooling can be
turned off during solids discharges a significant proportion of solids remain in the catcher
chamber and these would be diluted by the cooling spray. An alternative method would be to
use liquid nitrogen which would vaporise upon contact with bowl and hence would not
interfere with discharged solids. This, however, may cause stressing of the centrifuge bowl.
Table 11-5: Specifications of SAOOH-205 disc stack centrifuge - full stack and scale-down configurations (volumes in ml)

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. top inserts</td>
<td>Bottom insert</td>
<td>No. discs $(n)$</td>
<td>Disc space solid volume $(V_{ds})$</td>
<td>Disc space liquid volume $(V_{dl})$</td>
<td>Solids holding capacity $(V_s)$</td>
<td>Bowl volume $(V_b)$</td>
<td>Discharge volume $(V_{dis})$</td>
<td>$V_{dis} - V_b$ (normalised)</td>
<td>$z/V_s$</td>
<td>$V_s/V_b$</td>
</tr>
<tr>
<td>0</td>
<td>No</td>
<td>43</td>
<td>189</td>
<td>189</td>
<td>250</td>
<td>439</td>
<td>550</td>
<td>111</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>Yes</td>
<td>32</td>
<td>141</td>
<td>141</td>
<td>200</td>
<td>341</td>
<td>460</td>
<td>119</td>
<td>0.93</td>
<td>0.97</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>24</td>
<td>106</td>
<td>106</td>
<td>151</td>
<td>257</td>
<td>374</td>
<td>117</td>
<td>0.92</td>
<td>0.97</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>17</td>
<td>75</td>
<td>75</td>
<td>115</td>
<td>190</td>
<td>308</td>
<td>118</td>
<td>0.86</td>
<td>0.94</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>9</td>
<td>40</td>
<td>40</td>
<td>89</td>
<td>129</td>
<td>248</td>
<td>119</td>
<td>0.59</td>
<td>0.83</td>
</tr>
</tbody>
</table>
11.3 Homogenate viscosity

Homogenate viscosity depends on the cell concentration and the fraction of cells that are broken. There is a linear relationship between the fraction of cells disrupted and the fluid viscosity which is attributed to the release of intracellular polysaccharides (Mosqueira et al, 1981).

Using the data of Mosqueira et al (1981), Siddiqi (1997) developed an empirical equation relating apparent viscosity ($\mu$) to protein release ($R_p$). The model was based on experimental data for 45% yeast but was found to extrapolate well for yeast concentrations of 1-60%.

\[
\mu = \mu_{\text{max}} - \exp\left(\ln\left(\frac{\mu_{\text{max}} - \mu_{\text{min}}}{k \cdot R_p}\right)\right)
\]

Equation 11-7

\[
\mu_{\text{min}} = \mu_{\text{init}} = 0.000129C + \mu_{\text{water}}
\]

Equation 11-8

\[
\mu_{\text{max}} = 0.03 \text{ Pa s}, \quad k = 0.0029 \text{ for packed bakers' yeast}, \quad \mu_{\text{water}} = 0.001 \text{ Pa s}
\]

where, $R_p$ is the amount of soluble protein released (mg per ml of initial packed yeast suspension); $\mu$ is the apparent viscosity of the suspension (Pa s); $C$ is the initial yeast concentration (% ww/v); $k$ is a constant; and subscripts max, min and init refer to the maximum, minimum and initial (whole) cell suspension values respectively.
11.4 Theory

11.4.1 Theoretical aspects of high pressure homogenisation

11.4.1.1 Kinetics of cell disruption in a high pressure homogeniser.

There are two important aspects to cell disruption, protein release since this is a measure of product release, and debris particle size distribution since this impacts on other unit operations downstream (Siddiqi et al, 1991).

Protein release

Process variables include operating pressure, design of the valve unit, temperature, cell type and concentration and the number of passes (Hetherington et al, 1971; Kula and Schutte, 1987). Disruption was found to follow first order kinetics with respect to the number of passes and may be described by the general equation:

\[
\ln \left( \frac{R_m}{R_m - R_p} \right) = k . N = k . N . P^a
\]

Equation 11-1

where \(R_m\) is the maximum soluble protein available for release, \(R_p\) is the soluble protein released after \(N\) number of discrete passes, \(K\) is a dimensionless rate constant which, as shown, is principally a function of the pressure, \(P\), across the valve seat; \(k\) is a constant which is a function of temperature and possibly a function of cell concentration; \(k\) and the pressure exponent \(a\) are dependant on cell type. For baker's yeast a pressure exponent of 2.9 was determined by Hetherington et al (1971). Other models have been developed to predict the degree of cell disruption (Middelberg et al 1994).

Kinetics of enzyme release from baker's yeast have been shown to be first order, but the rate of enzyme release varies with its location within the cell (Follows et al. 1971). Acid phosphatase which is located outside the cell membrane is released faster than the overall protein release, which itself is faster than fumerase which is reported to be present in the mitochondria. Water soluble intracellular enzymes and other globular proteins are stable with respect to shear (Virkar et
al. 1982) and no significant activity loss for tested enzymes has been detected after several passes through the homogeniser.

**Kinetics of cell debris particle size reduction with homogenisation**

Most models that have been developed for cell disruption are based on protein release and hence the effect of operating conditions on the generation of cell debris is largely unknown. This data is essential for the purposes of designing and specifying suitable unit operations for debris removal.

Siddiqi et al (1995b) related the cell particle size distribution to the operating pressure and the number of passes. The two parameter model was based on a Boltzmann type equation:

$$F(d_{N,P}) = 1 - \left[ \frac{1}{1 + \exp \left( \frac{d - d_{50}}{w} \right)} \right]$$

**Equation 11-2**

where $F(d)$ is the cumulative undersize distribution (volume basis), $d_{50}$ is the median diameter of the homogenate size distribution, $w$ is the Boltzmann parameter. Both the $d_{50}$ and the parameter $w$ were found to be related to the number of passes and the operating pressure and the following relationships were developed from experimental data:

$$\ln \left( \frac{1}{d_{50}^*} \right) = \frac{k}{N^{0.4} \Delta P}$$

**Equation 11-3**

where $d_{50}^*$ is the dimensionless $d_{50}$, $k$ is a constant and $\Delta P$ is the operating pressure minus the threshold pressure below which little or no cell disruption occurs. For commercial packed yeast cells, $P_{\text{threshold}} = 116$ barg.

$$w^* = -2.3d_{50}^* \quad d_{50}^* < 0.33$$

$$w^* = 5.5d_{50}^* - 2.4 \quad d_{50}^* \geq 0.33$$

**Equation 11-4(a,b)**

where $w^*$ is the dimensionless standard deviation.
Fluid mechanical stresses due to turbulent flow. A model has been developed based on this mechanism (Doulah and Hammond, 1975). In fully turbulent flow the important criterion is the ratio of the particle size to the size of the smallest eddies. The theory is discussed in Section 0 and reviewed in detail elsewhere, (Tomi and Bagster, 1978a, Cherry and Papoutsakis, 1986). Using an equation derived by Tambo and Hozumi (1979), Shamlou et al (1995) estimated that the force exerted on a yeast cell due to turbulence was 2.2 μN which is considerably less than the 40-90 μN reported to be necessary for the rupturing of yeast cells (Roberts et al, 1994).

Collision between yeast cells and valve walls (Impingement). This was first proposed by Engler and Robinson (1981) and also by Keshavarz-Moore et al (1990) who derived an equation to estimate the maximum force exerted on a cell which was calculated to be less than 1 μN and hence should be insufficient to cause any serious damage to the yeast cell wall. For milk processing there is a general consensus that the impact ring (Figure 1-1) does not affect oil droplet disruption (McKillop et al, 1955, Pandolfe, 1982) whereas yeast cell disruption is markedly improved when an impact ring is used (Engler and Robinson, 1981, Keshavarz Moore et al, 1990). Altering the distance to the impact ring had a pronounced effect on the disruption of yeast cells but much less of an effect on the disruption of *E. coli* which suggests that different mechanisms may be important for different cell types and/or possibly cell sizes (Kleinig and Middelberg, 1994).

Forces due to cavitation. High velocities in the valve result in areas of low pressure which may lead to cavitation. Pandolfe and Kinney (1983) proposed that cavitation and turbulence are the only important mechanisms in cell disruption. Phipps (1974) showed that cavitation did not play a role in the disruption of oil droplets.

Compressive and decompressive forces due to the rapid pressure rise and fall through the valve. Engler (1979) showed that yeast cell disruption due to rapid release of pressure by itself is not significant.
• Elongational forces due to velocity gradients across the valve (Phipps 1971, 1975). Using a specially constructed device he found that the breakage of oil droplets during milk homogenisation was independent of the type of flow, i.e. laminar or turbulent. He also found that similar disruption occurred for conditions that excluded cavitation. He proposed that droplet breakage was due to the velocity gradients through the valve which distorted the droplets into long thread-like structures which then broke into smaller droplets if the velocity gradient was sufficiently steep. Shamlou et al (1995) calculated that flow in two regions of the valve approximated to the required flow type. These were the area close to the valve rod and next to the surface of the impact ring.

Development of a complete mathematical description of the complex flow field in the valve gap and a better understanding of yeast cell properties is required in order to determine the role of the different mechanisms of cell disruption.

11.4.2 Theoretical aspects of centrifugal separation

11.4.2.1 Separation efficiency

The recovery performance of a centrifuge can be characterised by the separation efficiency which is usually described in terms of the grade efficiency concept discussed below (Svarovsky, 1990a). This is applicable to all solid-liquid separation equipment whose separation performance does not change with time if all operational variables are kept constant.

Mass balance equations

A separator is fed with a liquid stream containing suspended solid particles which it then separates from the liquid phase. Assuming no accumulation of solids within the separator then:

$$ M = M_f + M_c $$

Equation 11-6

where $M$ is the mass flow rate of the feed, $f$ is the fraction of fine material in the overflow, $c$ is the fraction of coarse material in the underflow. Provided there is no change in particle size within the separator i.e. no agglomeration or breakage then the mass balance will also apply to any size
fraction. Thus for size \( d \), the mass of particles of size \( d \) in each stream is equal to the mass flow rate multiplied by the particle fraction:

\[
M \frac{\Delta F}{\Delta d} = M_e \frac{\Delta F_e}{\Delta d} + M_f \frac{\Delta F_f}{\Delta d}
\]

Equation 11-7

where \( F \) is the fraction of the size distribution.

**Total efficiency**

The total efficiency of separation, \( E_t \), is defined as the ratio of the mass of particles separated, \( M_e \), to the total mass of solids fed into the separator:

\[
E_t = \frac{M_e}{M_e + M_f} \quad \text{or} \quad E_t = 1 - \frac{M_f}{M}
\]

Equation 11-8

Equation 11-7 can be rewritten:

\[
\frac{\Delta F}{\Delta d} = E_t \frac{\Delta F_e}{\Delta d} + (1 - E_t) \frac{\Delta F_f}{\Delta d}
\]

Equation 11-9

**Grade efficiency**

Separation performance of most equipment is size dependant i.e. different sizes are separated with different efficiencies. Hence the total efficiency, \( E_t \), is dependant on the size distribution which makes it material dependant and therefore is unsuitable as a general criterion of efficiency. A more useful concept is the grade efficiency, \( T(d) \), which takes into account the separation efficiency for each particle size:

\[
T(d) = \frac{(M_e)_d}{(M)_d}
\]

Equation 11-10
Substituting:

\[ T(d) = \frac{M_c}{M} \left( \frac{\Delta F_c}{\Delta d} \right) = E_f \frac{\Delta F_f(d)}{\Delta F(d)} \]

Equation 11-11

where \( \Delta F(d) \) is the feed particle size distribution.

More usefully the grade efficiency can be expressed in terms of the feed and the fine product:

\[ T(d) = 1 - (1 - E_f) \frac{\Delta F_f(d)}{\Delta F(d)} \]

Equation 11-12

Particle size distributions can be expressed as a number distribution (number of particles), an area distribution (surface area of particles), and as a volume distribution (volume of particles). For constant particle density the volume distribution is proportional to the mass distribution. In this thesis the volume distribution is always used unless specified differently.

If the grade efficiency curve is plotted against particle diameter, \( d \), then it will move along this axis depending on the physical properties of the suspending liquid and of the particles. The grade efficiency is plotted against the dimensionless parameter \( d/d_c \) in order to standardise this axis so that the grade efficiency curve is a representation of the separator's performance regardless of process stream since \( d_c \), the critical diameter, takes into account the physical properties of the particle stream.

11.4.2.2 Sigma Theory

Sigma theory was developed by Ambler (1952, 1959, 1961). It enables a centrifuge to be described in terms of a separation area. It is independent of process parameters.

In centrifugation a high degree of clarification is usually important and hence the behaviour of the smallest particles in the system is usually the controlling factor. Therefore viscous resistance is of
prime importance although turbulent resistance is also a factor. Now, the effective force acting on a particle in a centrifugal field, $F_1$, is given by:

$$F_1 = (m - m_1) \omega^2 r$$

Equation 11-13

where $m$ is the mass of the particle, $m_1$ is the mass of fluid displaced by the particle, $\omega$ is the angular velocity, $r$ is the distance of the particle from the axis of rotation.

If the particle is a sphere, the force is given by:

$$F_1 = \frac{\pi}{6} d^3 \Delta \rho \omega^2 r$$

Equation 11-14

where $d$ is the particle diameter, and $\Delta \rho$ is the density difference between the particle and the suspending fluid ($\Delta \rho = \rho - \rho_s$).

The force opposing the sedimentation of the particle is given by Stokes' law, assuming that the particle is a small inert sphere in a dilute suspension and laminar flow conditions.

$$F = 3 \pi \mu d v_s$$

Equation 11-15

where $\mu$ is the fluid phase viscosity, $v_s$ is the velocity of the particle moving through it. When the force causing sedimentation reaches equilibrium with the resisting force, i.e. when $v$ becomes a constant then:

$$v_s = \frac{\Delta \rho d^2 \omega^2 r}{18 \mu}$$

Equation 11-16

When the particle is in the gravitational field then:
The simplest form of a continuous centrifuge is a rotating cylinder with end caps (Figure 11-2). Fluid is fed from one end and discharged from the other. \( v_s \) is the velocity with which the particle approaches the bowl wall. If the thickness of the liquid layer, \( s \), is small relative to the radius of the cylinder then \( v_s \) will be approximately constant and the distance the particle will settle during the time the fluid in which it is suspended is in the centrifuge bowl is given by:

\[
x = v_s t = \frac{\Delta r d^2 \omega^2 r}{18 \mu} \cdot \frac{V}{Q}
\]

Equation 11-18

where \( t \) is time, \( Q \) is the volumetric flow rate of the feed, \( V \) is the volume of liquid in the bowl: \( \pi r_l^2 - r_i^2 \).

If \( x \) is greater than the initial distance of the particle from the wall then it will be sedimented. In an ideal system when \( x = s/2 \), half the particles of diameter \( d \) will be removed from suspension and half will not. By substituting and rearranging Equation 11-18:

\[
Q = \frac{\Delta \rho d^2}{9 \mu} \cdot \frac{V \omega^2 r}{s}
\]

Equation 11-19

from which the critical diameter for the removal of half the particles can be determined:

\[
d = \left( \frac{9 \mu Q}{\Delta \rho} \cdot \frac{s}{V \omega^2 r} \right)^{0.5}
\]

Equation 11-20

Equation 11-19 can be rewritten as:
\[ Q = 2v_s \Sigma \]

Equation 11-21

in which,

\[ v_s = \frac{\Delta \rho d^2 g}{18 \mu} \]

Equation 11-22

and

\[ \Sigma = \frac{V \omega^2 r_e}{g s_e} \]

Equation 11-23

where \( r_e \) is the effective radius of the centrifuge, and \( s_e \) is the settling distance. \( \Sigma \) is an index of centrifuge size.

Theory for different centrifuge types are discussed below:

**Bottle (laboratory) centrifuge**

This is a batch centrifuge used for processing small volumes (Figure 11-1)
Equation 11-16 and Equation 11-17 may be rearranged to give:

\[ V_g = V_g \frac{\omega^2 r}{g} = \frac{dr}{dt} \]


Equation 11-24

If \( r \) is distance of the particle form the axis of rotation, \( r_1 \) is the radius of the liquid surface in the bottle, \( r_2 \) is the radius at the surface of the sedimented cake, and \( t \) is the total centrifuging time then a rearrangement and integration gives:

\[ \int_{r}^{r_2} \frac{dr}{r} = \int_{0}^{t} \frac{V_g \omega^2}{g} dt \]


Equation 11-25

from which,

\[ \ln \frac{r_2}{r} = \frac{V_g \omega^2 t}{g} \]


Equation 11-26

At the point where half the particles are sedimented assuming them all to be the same size then:

\[ A (r-r_1) = A (r_2-r) \]


Equation 11-27

where \( A \) is the cross-sectional area of the bottle and therefore:

\[ r = \frac{r_2 + r_1}{2} \]


Equation 11-28

and
\[ t = \frac{V}{Q} \]

Equation 11-29

is the effective flow rate through the bottle. Substituting and rearranging:

\[ Q = 2 \nu_s \frac{\omega^2 V}{2g \ln \left( \frac{2r_2}{r_1 + r_2} \right)} \]

Equation 11-30

from which:

\[ \Sigma = \frac{Q}{2 \nu_s} = \frac{\omega^2 V}{4.6g \log \left( \frac{2r_2}{r_1 + r_2} \right)} \]

Equation 11-31

or more conveniently:

\[ \frac{Q}{\Sigma} = \frac{4.6g \log \left( \frac{2r_2}{r_1 + r_2} \right)}{\omega^2 t} \]

Equation 11-32

The time required to sediment all particles of diameter \( d \) can be determined by integrating \( dr/r \) in Equation 11-25 between the limits \( r_1 \) and \( r_2 \). This may be regarded as the special case when \( r = r_1 \), and:

\[ \ln \frac{r_2}{r_1} = \frac{\nu_s \omega^2 t}{g} \]

Equation 11-33
For spherical particles, from Equation 11-16 and rearranging:

\[ t = \frac{18 \mu \ln \left( \frac{r_2}{r_1} \right)}{\Delta \rho d^2 \omega^2} \]

Equation 11-34

**Tubular bowl centrifuge**

This type of centrifuge is a simple high speed design with a manual solids discharge (Figure 11-2).

![Figure 11-2 Particle in a tubular bowl centrifuge](image)

From Equation 11-24 the radial velocity due to centrifugal force is given by:

\[ \frac{dr}{dt} = \frac{\nu \omega^2 r}{g} \]

Equation 11-35
Linear velocity due to the flow of liquid through the bowl is assumed to be uniform across its cross-section and is given by,

\[ \frac{d\bar{E}}{dt} = \frac{Q}{\pi(r_2^2 - r_1^2)} = \frac{QI}{V} \]

Equation 11-36

from which,

\[ \frac{d\bar{r}}{dz} = \frac{V \cdot \nu_g \omega^2 r}{Qg} \]

Equation 11-37

Integration of this equation between the limits \( r_1 = r \), at \( z = 0 \), and \( r = r_2 \) at \( z = 1 \), gives,

\[ \ln \left( \frac{r_2}{r} \right) = \frac{VV \cdot \nu_g \omega^2}{Qg} \]

Equation 11-38

The 50 % cut-off corresponds to \( \pi(r_2^2 - r^2) = \pi(r^2 - r_1^2) \)

Equation 11-39

and

\[ r = \left( \frac{r_2^2 + r_1^2}{2} \right)^{0.5} \]

Equation 11-40

By rearrangement, and using Equation 11-40, Equation 11-38 becomes:
\[ Q = 2V \frac{V \omega^2}{g} \frac{1}{\ln \left( \frac{2r_2^2}{r_2^2 + r_1^2} \right)} \]

Equation 11-41

for which the approximation, within a maximum error of 4%, is,

\[ Q = 2V \frac{V \omega^2}{g} \left( \frac{3/2 r_2^2 + 1/2 r_1^2}{r_2^2 - r_1^2} \right) \]

Equation 11-42

From Equation 11-42, and Equation 11-21 and \( \pi(r_2^2 - r_1^2) \),

\[ \Sigma = \frac{2\pi \omega^2}{g} \left( \frac{3/4 r_2^2 + 1/4 r_1^2}{r_2^2 - r_1^2} \right) \]

Equation 11-43

Disc stack centrifuge

Sedimentation in this type of centrifuge can be treated theoretically by analysing the space between two adjacent discs on the assumption that the flow in such a machine is uniformly distributed in all such spaces.
Figure 11-3 Schematic diagram of a single disc gap in a disc stack centrifuge. Fluid enters disc gap at \( r_2 \) and leaves at \( r_1 \). The disc half angle, \( \theta \), and gap width, \( a \), are also shown. Particle, \( P \), experiences a centrifugal motion, due to the angular velocity of the centrifuge (\( \omega \)), and also moves with the fluid to give a resultant trajectory which depends on the particle settling velocity and the point of entry between the discs.

The particle \( P \) has 2 motions:

1. Centrifugal force moves it away from the lower disc,

\[
\frac{dy}{dt} = \frac{v_g \omega^2 r}{g} \cos \theta = \frac{v_g \omega^2}{g} \left( r_2 - x \sin \theta \right) \cos \theta
\]

Equation 11-44

where \( \theta \) is the half conical disc angle

2. The particle is swept along by the fluid flow from \( r_2 \) to \( r_1 \).

If there are \( n \) spaces between the discs in a given rotor and the total throughput is \( Q \), then the flow between each pair of discs is \( Q/n \), where \( n \) is the number of discs minus one, and the average velocity across the distance \( a \), between and normal to the adjacent discs at radius \( r \) is \( Q/2\pi nr \).
The velocity will be zero at the disc surface and reaches a maximum somewhere between them. Let \( f(y) \) be the ratio of liquid velocity at point \( y \) to the mean velocity. Then,

\[
\frac{dx}{dt} = \frac{Q}{2\pi nr} f(y) = \frac{Qf(y)}{2\pi n a (r_2 - x \sin \theta)}
\]

Equation 11-45

and from Equation 11-44 and Equation 11-45

\[
\frac{dy}{dx} = \frac{2\pi n a v_x \omega^2}{Qg} \left( r_2 - x \sin \theta \right)^2 \cos \theta \frac{f(y)}{f(y)}
\]

Equation 11-46

Upon integration:

\[
\int_{y_2}^{y_1} f(y) dy = \frac{2\pi n a v_x \omega^2}{3Qg} \left( r_2^3 - r_1^3 \right) \cot \theta
\]

Equation 11-47

where \( y_2 \) is the value of \( y \) at \( x = 0 \) where the particle enters the space between the two discs and \( y_1 \) is the value \( y \) at \( x = (r_2 - r_1) \csc \theta \) where the particle would leave the disc stack if it were not sedimented and it will just be sedimented at \( y_1 = a \).

Now,

\[
\int_{y_2}^{a} f(y) dy
\]

Equation 11-48

is the fraction of flow between \( y_2 = y \) and \( y_1 = a \) and particles of diameter \( d \) in this part of the flow will be sedimented. When the fraction equals a half then 50 % of the particles will be sedimented. At this cut-off,
\[ Q = \frac{4\pi n v_e \alpha^2}{3gC} (r_2^3 - r_1^3) \cot \theta \]

Equation 11-49

in which \( C \) is the average value for the integral of \( f(y) \), and,

\[ \Sigma = \frac{Q}{2v_k} = \frac{2\pi n \omega^2 (r_2^3 - r_1^3)}{3gC \tan \theta} \]

Equation 11-50

The value and significance of \( C \) in Equation 11-50 is not completely understood. Assuming flow between adjacent discs is symmetrical and laminar then \( C \) equals unity only when either all or half the particles of diameter \( d \) are sedimented; i.e. when the value of \( y \) at \( x = 0 \) is either zero or \( a/2 \). The value of \( C \) is significantly different from unity when the particle starts from any other position and may be greater than unity even when starting from the \( a/2 \) position if the flow pattern is not laminar which can occur when solids accumulate, due to surface imperfections, or by the vector effect of centrifugal force.

Equation 11-50 assumes that the entire area of the disc is available for separation. In reality the area available is reduced due to the spacer strips on each disc which maintain the gap between adjacent discs. Thus, a correction factor, \( f_i \), is required:

\[ f_i = 1 - \left( \frac{3Z_i B_i}{4\pi r_2} \times \frac{1 - \left( \frac{r_1}{r_2} \right)^2}{1 - \left( \frac{r_1}{r_2} \right)^3} \right) \]

Equation 11-51

where, \( Z_i \) is the number of spacer strips per disc and \( B_i \) is the width of the spacer strip (Mannweiler, 1990). Equation 11-51 assumes that the spacer strips run the full length of the disc, i.e. from \( r_1 \) to \( r_2 \).
11.4.2.3 Alternative theories

Forsberg of Alfa Laval, while performing tests on milk skimming found a relationship between centrifuge parameters and separation capacity (Axelsson, 1985). He looked at the worst possible case: a particle at the upper surface of a disc at \( r_2 \) being sedimented on the next disc at \( r_1 \), where \( r_1 \) and \( r_2 \) are the inner and outer disc radii respectively. He arrived at the following relationship:

\[
KQ = \frac{3.6}{100^k} \left( \frac{2\pi}{k + 2} \right) w^{2k} n \cot \theta (r_2^{2+k} - r_1^{2+k})
\]

Equation 11-52

He found the best fit with test data when \( K = 0.75 \). \( r_1 \) and \( r_2 \) are expressed in cm. The usual form of this equation is that given by Sullivan and Erikson (1961):

\[
KQ = 280 \left( \frac{N}{1000} \right)^{1.5} n \cot \theta (r_2^{2.75} - r_1^{2.75})
\]

Equation 11-53

where \( N = \) bowl speed (revolutions per minute).

\( KQ \) is the product of the volumetric feed flow rate and a particle-related constant. This is very similar to Sigma theory. By comparison \( K \) is inversely proportional to \( v_2 \). \( K \) is determined by experiments on a disc bowl of known geometry the results of which can then be applied for scale-up.

A method developed by Gupta (1981) scales up based on grade efficiency plots and three dimensionless parameters. Bohman and Murkes (1969) have developed similar uniformity and equivalence plots.

11.4.2.4 Experimental/observed separation in a disc centrifuge

Differences in actual separation performance compared to that predicted by the equations above can be explained by the following (Sokolow, 1971):

1. Flow through the disc space is not always laminar over the whole gap width and radius.
2. Particle sedimentation may be reversed, i.e. re-mixing can occur.

3. Solids loading at the entrance and the fluid flow to the discs is not regular for the whole disc stack.

4. Hindered settling effects - these are due to particle-particle interactions in concentrated streams.

The settling velocity as calculated using Equation 11-16 is only valid for very dilute streams. With more concentrated streams the settling velocity is reduced due to hindered settling. When the solids concentration exceeds approximately 2 % then the boundary flow layers for adjacent particles overlap when considering the mean distance, \( l_m \) between particles (Figure 11-4). This causes a displacement of liquid in the opposite direction to the settling particles thus reducing the settling velocity (Richardson and Zaki, 1954).

![Figure 11-4 Unhindered and hindered settling](image)

Using mono-sized, rigid, spherical particles they derived an empirical relationship between the solids concentration and the terminal settling velocity:

\[ v_z^* = v_z(1-c_v)^\alpha \]

Equation 11-54
where \( v_z^* \) and \( v_z \) are the hindered unhindered settling velocities respectively and are given by Equation 11-16; \( c_y \) is the volume concentration of aggregate in suspension and \( \sigma \) is a geometric factor. For rigid, spherical particles \( \sigma = 4.6 \), whereas for non-rigid, non-spherical particles \( \sigma \) can vary between 10 and 100 which can make a very large difference when compared to Stokes law. Clarkson et al (1993b) found that flocculated yeast suspensions gave \( \sigma \) values between 12 and 20.

The differences discussed above cause a deviation from the grade efficiency curve as it is predicted by Stokes law (Figure 11-5). For a dilute suspension of polyvinyl acetate (PVAc) particles the grade efficiency approaches unity at approximately 2.5 times the critical diameter. Note that further deviations occur when non-dilute feed suspensions are used.

![Grade efficiency curve](image)

**Figure 11-5 Grade efficiencies for the ideal (based on Stokes law) and for a disc centrifuge (using a dilute suspension of PVAc)**

11.4.3 Theoretical aspects of protein precipitation

11.4.3.1 Kinetics and mixing effects in protein precipitation

Protein precipitation may be divided into two stages governed by different mechanisms: primary aggregation (perikinetic growth) which is where insolubilised protein molecules form initial aggregates through Brownian motion induced collisions; secondary aggregation (orthokinetic growth) is the mechanism by which primary and subsequent particles aggregate when they reach a size when the fluid motion influences their growth.
Perikinetic growth

For a mono-sized dispersion the initial rate of decrease of the particle number concentration may be described according to the theory of Smoluchowski (1917):

\[- \frac{dN}{dt} = K_A N^2\]

Equation 11-55

where $N$ is the particle number concentration, and $K_A$ is the rate constant which is determined by the diffusivity, $D$, and the particle diameter, $d$:

\[K_A = \frac{8\pi D d}{\tau}\]

Equation 11-56

Equation 11-55 and Equation 11-56 apply up to a limiting particle size defined by the fluid motion. Typical sizes ranging from 0.1 μm for high shear fields to 10 μm for low shear fields.

Orthokinetic aggregation

When a suspension of particles greater than approximately 1 μm diameter is sheared then the fluid motion will result in particle collision and hence aggregation. Aggregate break-up may also occur due to collisions, contact with solid surfaces, or through fluid shear. The growth process can be described for a suspension of uniformly sized, spherical particles of diameter, $d$, in a uniform shear field with a mean velocity gradient, $G$. The initial decrease in particle number concentration, $N$, is given by, (Ives 1978):

\[- \frac{dN}{dt} = \frac{2}{3} \alpha N^2 G d^3\]

Equation 11-57

where $\alpha$ is the collision effectiveness factor.
Even though orthokinetic aggregation has no effect on protein solubility it is essential that it is considered since it affects the particle size and density which, in turn, affect the subsequent centrifugal recovery process.

11.4.3.2 Fractional precipitation

Falconer and Taylor (1946) developed a graphical procedure to study the recovery of protein product from mixtures where the product is a significant part of the overall protein content and where just a few protein species are present. This has been used successfully for the final stages of purification however it is unlikely to be useful for the currently relevant situation of cell extract fractionation. Firstly, the method relies on a discontinuity in the total protein precipitation profile at the onset of precipitation of the desired enzyme which is unlikely to occur in a complex system due to the overlapping solubilities of all the background proteins. Secondly, the diagram is not suited to a double-cut process.

Dixon and Webb (1961) suggested representing the fractionation of a product protein with respect to a contaminating protein as plots of solubility curve gradient versus precipitant concentration. This method can be used to reduce the degree of contamination with fractional precipitation. It is however, a laborious technique and also the fractionation performance is quantified only with respect to specific, individual contaminants as opposed to the total protein contamination.

An alternative approach proposed by Richardson et al (1990) is the fractionation diagram. This eliminates the precipitant concentration by plotting the enzyme and protein solubilities against each other to give the process equilibrium curve (Figure 11-6):
The objective, as with all phase equilibrium unit operations, is to superimpose an operating line on the equilibrium curve so that the required process conditions may be obtained from the intercept of the two. For fractional precipitation the operating tie-line can be obtained by considering the parameters which specify the degree of purification (enrichment) and the quantity of product obtained; i.e. the purification factor \(PF\) and the yield \(Y\). For a double-cut fractionation these are defined by:

\[
PF = \frac{E_1 - E_2}{P_1 - P_2}
\]

Equation 11-58

\[
Y = (E_1 - E_2)
\]

Equation 11-59

where \(E\) and \(P\) are the fractions of enzyme and protein remaining in solution respectively following the first (1) and second (2) cuts. The above theory assumes an ideal equilibrium stage.
In reality there will be an offset from the equilibrium curve due to two opposing effects. Firstly the quantity of enzyme and protein remaining soluble after each cut will be reduced due to soluble material trapped within the precipitate. Secondly, any non-recovered precipitate will oppose this effect by being included in the soluble phase.

11.4.4 Scale-down of unit operations of the ADH process

11.4.4.1 Scale-down of a high pressure homogeniser

It is not always necessary to fully understand the mechanism of a particular operation in order to scale it successfully and this is certainly true of high pressure homogenisation. Several mechanisms have been proposed to describe the breakage mechanism in the valve (Section 11.4.1.2) but this is still an area of debate. Cell breakage is probably due to several mechanisms and the importance of different mechanisms may vary depending on such factors as cell type and size (Kleinig and Middelberg, 1994). Despite this lack of knowledge high pressure homogenisation has been successfully scaled over a wide size range; units with flow capacities from 100 to 20 000 L h⁻¹ were shown to give similar milk homogenisation performance. Likewise, a study by Siddiqi et al (1997) comparing homogenisers over the flow rate range 28 to 300 L h⁻¹ showed very similar disruption of yeast cell debris in terms of both protein release and debris particle size distributions. The smallest machine they studied, with a flow rate of 28 L h⁻¹, was the APV Micron Lab40 which has an operating volume of 40 ml and works in a batch fashion. The disruption valve for the Lab40 is shown below (Figure 11-5). The scale-up principles in both cases were maintaining the operating pressure across the valve and keeping similar valve geometry.

Important valve dimensions are:

- land width (Ld)
- impact distance (X)
- valve rod dimensions (Rr)
- valve gap width (h)

Important criteria for scaling in terms of fluid flow may be:

- power dissipation per unit volume
- fluid velocities
- velocity gradients

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Geometrical terms are shown in Figure 11-5.

![Figure 11-5 Micron Lab40 disruption valve (SV)](image)

11.4.4.2 Scale-down of centrifugation

Different theories for the scaling of centrifugal separation are discussed in Section 11.4.2. The most commonly used method, and that which is discussed below, is Sigma Theory which was proposed by Ambler (1959).

Use of Sigma theory for scale-down

This is an application of the theory described in section 11.4.2.2. For a given particle system whose $v_g$ is a constant, $Q/\Sigma = 2v_g$ = constant, therefore it should be possible to compare various centrifuges on the basis:

$$\frac{Q_1}{\Sigma_1} = \frac{Q_2}{\Sigma_2} = \frac{Q_3}{\Sigma_3} = etc.$$  

Equation 11-60
In practice this is not always the case. With tubular bowls over a fairly wide range of proportions of bowl diameter to bowl length the determined $\Sigma$ values were between 97 and 98 % of the calculated values (Ambler, 1959). For disc centrifuges the observed $\Sigma$ value was 55 % of the theoretical value (Ambler 1959, 1961; Frampton, 1963). The low efficiency of the disc stack centrifuge is not a criticism of this type of machine but merely an indication of the effect of non-symmetrical flow patterns that the theory does not take into account.

The important point is that data, in terms of $Q/\Sigma$, should not generally be extrapolated from the performance of one centrifuge type to another. In addition, the efficiency factors vary with different reporters; for example, for a disc stack centrifuge: 55 %, as mentioned above, 73 % (Mureres and Carlsson, 1978); 40 % (Axelsson, 1985); and 45 % (Morris, 1966). This may be due to different centrifuge bowl geometry’s (Tomusiak, 1992).

When dealing with small particles which exhibit Brownian motion then the diffusion constant;

$$D = \frac{RT}{3\pi\etaNd}$$

Equation 11-61

where $N$ is Avagadro’s number, $R$ is the gas constant and $d$ is the particle diameter; may be significant and the applied centrifugal force must be great enough to overcome the threshold effect before the laws of sedimentation apply. In addition, with small particles extrapolation should not be made over too wide a range of centrifugal force even when dealing with centrifuges of similar dimensions (Ambler, 1959).

**Scale-down of a disc stack centrifuge**

Mannweiler and Hoare (1992) scaled down a disc stack centrifuge with the application of Sigma theory. With a large pilot scale centrifuge (Westfalia BSB 7-47-476, bowl volume 3.0 L., full stack 72 discs) they reduced the number of discs in the stack from 72 by degrees and studied the effect of this, the throughput capacity and the location of the active discs on the grade efficiency plotted against $d/dc$ since any changes can be attributed directly to a change in the hydrodynamic behaviour of the fluid passing through the centrifuge. They noted that the grade efficiency curve was shifted depending on the position of the active discs in the stack. When the active discs were placed at the top of the disc stack the grade efficiency curve was shifted to the right, i.e. an
improvement in separation performance occurred. This is believed to be due to the separation of solids directly into the sediment holding space. The grade efficiency plot was shifted to the left, i.e. separation efficiency was reduced, when the active discs were positioned at the bottom of the disc stack. This was thought to be due to the turbulent region at the bottom of the centrifuge causing re-entrainment of sedimented solids. Since the aim was to mimic a full stack centrifuge the active discs were raised off the bottom of the disc stack by the minimum distance required to avoid turbulence effects around the distributor. Placement of the active discs in this position also minimised solids separation directly into the sediment holding space prior to entry into the disc stack. The grade efficiency curves are shown in Figure 11-. These show that separation performance is independent of flow rate and closely follows the recovery performance of the full stack centrifuge.
Similar work was carried out by Rumpus (1997) using a smaller disc stack centrifuge (Westfalia SAOOH, bowl volume 0.6 L full stack 37 discs) in an attempt to reduce the amount of process fluid required. The results concurred with those found by Mannweiler and Hoare (1992) with the larger BSB 7-47-476 machine in that placement of the active discs (1/4 stack) just above the bottom of the centrifuge was critical if the performance of the full stack configuration was to be mimicked. Similarly to Mannweiler's work, only the number of active discs was reduced; the bowl volume was unchanged which meant that the amount of process fluid required to operate at scale-down was similar to the full stack centrifuge.
11.4.4.3 Scale-down of protein precipitation

There are important aspects to the scale-down of protein precipitation: product/protein solubility and precipitate particle characteristics. Solubility is affected by the contacting conditions of the precipitant and protein streams. For many precipitations, solubility is relatively insensitive to contacting conditions and hence scale-down efforts can be concentrated on the particle characteristics. In the case of fractional precipitation there is usually more concern with reproducible solubility behaviour since the cut points are usually at the steep points in the solubility curve and hence any deviation in the precipitant concentration can cause very large changes in the solubility.

Rate of precipitation

Where the solubility behaviour is important to the process then the rate of precipitation may play a significant role in defining the final solubility at different scales. Foster et al (1976) found a significant shift in the fractionation lines with different contacting procedures. They postulated that these shifts might be due to differences in localised precipitant concentrations. In a normal phase equilibrium situation this would not cause a shift in the solubility as the over-precipitated protein should re-dissolve once the regions of high concentration have been dispersed. Foster proposed the existence of pseudo-equilibria due to an energy barrier to dissolution of the precipitate. This may be due to a greatly reduced surface free energy which results in a negligible dissolution rate in comparison to the overall time of the precipitation. Evidence for this theory was found by Hoare (1982); he diluted casein precipitates in precipitant of the same concentration in order to determine their size by Coulter counter. In normal phase equilibria some re-dissolving of the precipitate would be expected in order to maintain the solubility equilibrium, however, this was not observed experimentally.

At large scale, mixing times are reduced and hence the pseudo-equilibrium effect can affect the solubility. If the rate of mixing is rapid in comparison to the rate of precipitation then the precipitant should be well distributed before any significant precipitation has occurred and hence the solubility profile should be very similar to the small scale. If, however, the mixing rate is similar to or lower than the precipitation rate then the solubility profile will be shifted and over-precipitation will occur at large scale.

The rate of protein aggregation (Section 11.4.3.1) is dependent on physical factors such as the protein diffusion rate which are independent of process considerations. It is also dependent on the
square of the protein concentration and hence over-precipitation with scale-up is more likely at higher protein concentrations. The other important factor is the mixing.

**Rate of mixing - effect on solubility**

Mixing is dependent on two mechanisms: macro-mixing or bulk mixing which reduces the scale segregation between the protein solution and the precipitant; and micro-mixing which reduces the degree of segregation.

Macro-mixing takes place in two stages. If the precipitant is added rapidly then the first stage is to distribute the precipitant in large packets throughout the vessel. These packets are then broken up by the mechanism of turbulent shear. This mechanism reduces the precipitant packet size to that of the size of the turbulent eddies. Once this limiting size has been reached then turbulence has no further effect.

At the same time that macro-mixing is occurring molecular diffusion of the precipitant and the protein species will occur. This micro-mixing brings the two species into intimate contact. Until the precipitant comes into intimate contact with the protein species there will be no precipitation. The diffusion rates of low molecular weight precipitants, such as salts, will be very rapid in comparison to high molecular weight proteins and in such a case the protein may be considered to be stationary. However, for high molecular weight polymeric precipitants which form viscous solutions then the diffusion rate of the protein species may be relatively significant.

Macro- and micro-mixing occur simultaneously, however, since micro-mixing is a diffusion controlled process, the mass transfer rate is also dependent on the surface area available. Therefore, it is more efficient once the eddies have reached their limiting size. If the macro-mixing time is significant then significant micro-mixing may occur during this period leading to over-precipitation and pseudo-equilibria. The extent of over-precipitation is dependent on the rate of precipitant addition, the concentration of the precipitant solution and the intensity of mixing. Over-precipitation is often irreversible and may lead to protein denaturation (Przybycien and Bailey, 1991).

Naumann (1974) developed a relationship for determining the relative rates of macro- and micro-mixing which he termed the segregation number:
where $D$ is the species diffusivity and $R$ is the limiting drop size. It is assumed that the macro-mixing time, $t$, is equal to the vessel residence time (continuous operation) which does not necessarily follow.

Alternatively, it is possible to measure the time for combined macro- and micro-mixing and compare this with the calculated time for micro-mixing alone. Khang and Levenspiel (1976) measured the time required for concentration fluctuations to drop within a certain amplitude of the final value due to combined macro- and micro-mixing. For turbine mixers:

$$\left( \frac{N}{K} \right) \left( \frac{d}{D} \right)^{2.13} = 0.5 \quad \text{for} \quad \text{Re} > 2 \times 10^3$$

Equation 11-63

$$K = \frac{\ln(A/50)}{t}$$

Equation 11-64

where $N$ is the turbine speed, $d$ is the turbine diameter, $D$ is the tank diameter and $t$ is the time for concentration fluctuations to drop within $A$ % of the final value.

A theoretical mixing time for micro-mixing alone can be determined by calculating the time required for the concentration at the centre plane of an infinitely extended slab to reach a proportion of the final averaged concentration by the mechanism of unsteady state diffusion (Bourne and Dell’Ava, 1987). The width of the slab may be assumed to be approximated by the Kolmogorov turbulent micro-scale of mixing, $\eta$.

$$\eta = \left( \frac{\nu^3}{\varepsilon} \right)^{\frac{1}{4}}$$

Equation 11-65

where $\nu$ is the kinematic viscosity and $\varepsilon$ is the energy dissipation per unit mass and is given by:
\[ \varepsilon = P_o N^3 d^5 / V \]

Equation 11-66
where \( P_o \) is the power number which is determined as a function of the Reynolds number from standard correlations (Rushton et al, 1950). \( V \) is the volume of fluid.

Fick’s law for unsteady state diffusion during micro-mixing gives:

\[ \frac{dC}{dt} = D \frac{d^2 C}{dy^2} \]

Equation 11-67
where \( C \) is the precipitant concentration within the slab and \( D \) is the diffusion coefficient and \( y \) is the distance in the direction of diffusion. From Equation 11-67 the time required for the concentration to approach within a defined degree of the final precipitant concentration can be determined and hence the micro-mixing time can be approximated.

Equation 11-63 and Equation 11-66 show that both the macro- and micro-mixing times may be reduced by increasing the agitation rate. However, there may need to be a compromise since high agitation rates can lead to oxidation and shear associated damage of proteins (Thomas and Dunnill, 1979). Over-precipitation can be reduced by the gradual re-solubilisation of proteins during the final stage of precipitation, precipitate particle ageing (Watt, 1970).

**Rate of mixing - effect on the physical properties of precipitate particles**
Apart from protein solubility other important aspects to precipitation scale-down are the physical properties of the precipitate particles which include: final particle size, strength and density. These are determined by the operating conditions employed during the perikinetic and orthokinetic stages as well as the particle ageing stage (Chan et al, 1986, Nelson and Glatz, 1985, Przybicien and Bailey, 1989). Precipitate physical properties are less important than solubility issues if over-precipitation and denaturation are problems. In such cases mixing issues dominate the design (Hoare et al, 1983).

**Precipitate growth**
Turbulent flow in a stirred tank is generally non-homogeneous and non-isotropic (Levins and Glastonbury, 1972) for which a simple characterisation can be made with the energy dissipation per unit mass, ϵ. It can be measured and approximated and has been found useful for scale-up (Tomi and Bagster, 1978a). From this the mean point velocity gradient is derived, G,

\[ G = \left( \frac{\epsilon}{\nu} \right)^{1/2} \]

Equation 11-68

where ν is the kinematic viscosity. This can be re-written to give:

\[ G = \left( \frac{P}{V\mu} \right)^{1/2} \]

Equation 11-69

where \( P \) is the power input to the stirred vessel, \( V \) is the vessel liquid volume and \( \mu \) is the viscosity. A simplified version of Smoluchowski's (Smoluchowski, 1917) orthokinetic aggregation (Section 0) where collision frequency, \( J_\gamma \), between particles of size \( d_i \) and \( d_j \) is given by:

\[ J_\gamma = \frac{G(d_i + d_j)^3 n_i n_j}{6} \]

Equation 11-70

where \( n \) is the particle count per unit volume. Hence, the rate of aggregation is directly proportional to \( G \) and therefore a particle size may be defined by \( G \) and increasing \( G \) means a reduced time is required to attain a particular size. This is only true at low shear rates where hydrodynamic break-up is not important (Glatz et al, 1986). A practical upper limit of shear exists for each particle size (Tomi and Bagster, 1978b). Experiments cycling the shear rate show that the precipitate growth process is largely reversible (Tomi and Bagster, 1978a).

Turbulent breakage

Several mechanisms are thought to cause aggregate break-up including erosion due to shear, collisions and pressure gradients across the aggregate; these are discussed in detail by Bell et al (1983). The rate of breakdown of aggregates is strongly dependent on the shear rate (Hoare et al, 1982). Bell (1982) measured the precipitate particle size after 600s for vessels of 0.27 L, 0.67 L and 200 L for a range of values of \( G \). Good agreement between the different scales was found for
$d_{90}$ but not such good agreement for $d_{95}$ For centrifugal separation it is the fine end of the particle
size distribution, i.e. the $d_{90}$, that is important and hence this may be a useful scaling rule in such a
case. The $d_{90}$ was similar at different scales even for high values of the mean velocity gradient
when particle breakage was significant.

The other important physical properties of precipitate particles are strength and density. No work
is published studying these properties at different scales. Tambo and Hozumi (1979), studying
clay flocculated with aluminium sulphate, found a direct relationship between floc strength and
density. Bell and Dunnill (1982) examined the strength of precipitate particles aged at a range of
shear rates but for varying lengths of time. They found a good relationship between the degree of
breakage and the ageing parameter $Gt$. Maximum particle strength was achieved at $Gt = 10^5$ for all
shear rates studied although particles aged at higher shear rates showed reduced dependence on
$Gt$. Whether this relationship will hold at different scales is uncertain since the range of shear rates
experienced in a vessel changes with scale.
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