The effects of fermentation scale on the cell qualities of
Saccharomyces cerevisiae and Saccharopolyspora erythraea

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Abstract

This report investigates the effect of scale-up on the cell quality of *Saccharomyces cerevisiae* and *Saccharopolyspora erythraea*. Cell quality was defined as the chemical and physical composition of a cell at a particular point during fermentation. Chemically this includes the concentration of the cells internal and externally secreted molecules, whereas physically this includes the nature of the cells structure, in terms of morphology and wall strength, and its influence on the broth rheology. Knowledge of the cell quality is useful because it can influence the operation of the fermenter and the subsequent downstream processing operations; cell quality profiles can be used to determine the best harvesting point of the fermentation, to obtain the optimum mix of cell qualities. For instance mass, heat and momentum transfer are influenced by the broth rheology which in turn may depend on the cell morphology; whereas the cells' product location and concentration determines the required scale of the fermenter and specifies and sizes the downstream processing chain.

Batch fermentations of the two organisms were carried out in defined media, using fermenters between 7 and 1500 litres scale. The fermentations were sampled regularly to produce cell quality profiles for the duration of the fermentations. The cell qualities monitored for *S. cerevisiae* were the dry cell weight, alcohol dehydrogenase and glucose-6-phosphate dehydrogenase activities, the cell soluble protein content and the cell wall strength (indirectly determined by the homogenisation of the cells). For *S. erythraea* the cell qualities monitored were the dry cell weight, the erythromycin production, the broth rheology, the cell morphology, the cell soluble protein content and the cell wall strength. The cell quality profiles of *S. cerevisiae* were found to be scale independent over the range studied. For *S. erythraea* the cell quality profiles were dependent on the operating conditions and the scale of fermentation; particularly, cell fragmentation was noted to increase with stirrer speed and fermentation scale.

The method of scale-down of fermentation appears to be suitable for reproducing the conditions and behaviour of cells from production scale fermenters in the laboratory; these models reproduce the most important mechanisms operating at the large scale. Scale-down models based on the recirculation of broth through a high shear zone, are suggested as a means of reproducing the cell quality profiles obtained in the large scale fermenters used in this study, especially to investigate the effects of shear on cell qualities such as cell wall strength and morphology.
Dedication

To my mother, Shamim Akhter Ahmed, to my late farther, Sanaullah Khan, and to my second father, Khurshid Ahmed: all of whom I am very proud of, and lucky to be their son.

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Notation

$a$, interfacial area per unit unaerated liquid volume

$A$, area

$A'$, cross sectional area of the body

$c_p$, heat capacity

$C^*$, oxygen saturation value in the air-liquid phase boundary

$C_D$, drag coefficient

$C_l$, dissolved oxygen concentration in the bulk liquid

$d_b$, sparged air bubble diameter

$D$, impeller diameter

$D$, dispersion coefficient

$D_x$, diffusivity

$D_e$, effective diffusion coefficient

$DOT$, dissolved oxygen tension

$D_r$, impeller diameter ratio

$F_D$, drag force on body

$g$, acceleration due to gravity

$h$, film heat transfer coefficient

$H_t$, impeller height

$H_L$, liquid height

$H_{pot}$, total potential head from an impeller

$I_b$, impeller blade height

$k$, thermal conductivity

$k_p$, oxygen mass transfer coefficient

$k_{la}$, volumetric oxygen mass transfer coefficient

$L$, litres

$N$, impeller rotational speed

$N_o$, impeller speed ratio

$OTR$, volumetric oxygen transfer rate

$p$, pressure

$P$, power input / ungassed power requirement

$P_g$, gassed power requirement
$P_{out}$, output pressure
$Q_s$, sparge gas flow rate
$Q_{dv}$, volumetric flow from an impeller
$Q_{ir}$, impeller pumping capacity ratio
$Q_s$, surface aeration rate
$S_{O_2}$, solubility of oxygen
$t$, temperature
$t_{ax}$, axial dispersion time
$t_b$, bubble residence time
$t_c$, liquid circulation time
$t_{con}$, time constant for conversion
$t_d$, time constant for diffusive transport
$t_f$, time constant for flow transport
$t_m$, liquid mixing time
$t_{mL}$, liquid phase oxygen mass transfer time
$t_{O_2}$, time for oxygen transfer
$t_{oc}$, oxygen consumption time
$t_{sa}$, substrate addition time
$t_{sc}$, substrate consumption time
$T$, tank diameter
$V$, volume of liquid
$V_{La}$, aerated liquid volume
$X_{O_2}$, oxygen molar fraction in the gas
$Z$, depth of liquid from the free surface

Greek symbols

$\varepsilon$, gas holdup, volume of gas per unit aerated liquid volume
$\mu$, dynamic viscosity
$\mu_a$, apparent viscosity
$\mu_c$, continuous phase viscosity
$\mu_d$, dispersed phase viscosity
$\rho$, liquid density
\( \Delta \rho \), density difference between gas and liquid
\( \rho_g \), gas density
\( \rho_m \), mean liquid density
\( \sigma \), interfacial surface tension
\( u \), velocity of flowing stream
\( u_{tb} \), bubble terminal rise velocity
\( u_s \), superficial gas velocity
\( v \), kinematic viscosity

**Dimensionless numbers**

\( N_D \), Bodenstein number
\( N_{FR} \), Froude number
\( N_{GA} \), Galileo number
\( N_{NE} \), Newton number
\( N_{Nu} \), Nusselt number
\( N_{Pe} \), Peccelet number
\( N_{Pr} \), Prandtl number
\( N_{Re} \), Reynolds number
\( N_{Sc} \), Schmidt number
\( N_{tc} \), circulation time group
\( N_{WE} \), Weber number
1 INTRODUCTION

New microbial products are usually discovered on a small scale within the petri dish or shake flask. The point of discovery marks the beginning of a process development that will include fermentation scale-up and downstream process development. Though it would be highly desirable to integrate the development of the fermentation and purification stages (Kossen, 1993), the difficulty with this can be the uncertainty in the nature of the broth that emerges from the fermentation process. The uncertainty arises because the microbial behaviour is related to the environment of the organism, which is in turn scale dependent. The scale dependency arises because thermodynamic and microkinetic phenomena (e.g. solubility and growth and product formation, respectively) are scale independent, when taken as a function of the local environmental conditions, whereas transport processes (mass, heat and momentum transfer) are scale dependent. The result is that the local environmental conditions vary with position in the fermenter, therefore the actual thermodynamic and microkinetic behaviour also become scale dependent. That is, while the substrates being consumed in the vicinity of the microorganisms have to be replaced by transport processes, the time constants for these processes increase with scale, and the population of microorganisms face an increasingly heterogeneous environment and may exhibit a range of behaviours. Thus, common fermentation scale-up methods, relying on the extrapolation of data from one scale to another, may not give an accurate reflection of the nature of the broth from the actual production scale, because they do not reproduce the environment and consequent microbial behaviour of the large scale. This is particularly the case when there is a large discrepancy in volume between experimental and production fermentation scales. If an accurate ‘description of the broth’ could be obtained from experimental equipment, then process design, scale-up, and integration should be greatly eased.

In this study cell quality is the term used to ‘describe the broth’, where quality may be defined as the chemical and physical composition of the cell at a particular point during fermentation. Chemical qualities comprise the concentration of the cells internally and externally produced molecules and the rates of its metabolic reactions; among these will be the product(s) and contaminants of the fermentation. While examples of physical qualities include the cells wall strength and morphology, and any influence this and the externally secreted molecules may have on rheology. Though the cell quality could
include any aspect relating to the cells in a fermentation, obviously only those qualities of importance to a specific fermentation need be monitored, and these depend on the individual case. Cell quality is of central importance in any fermentation process. Essentially, the quality of the cells drives not only the scale, type and nature of the fermentation equipment but also the specifics of the downstream processing operations required to purify the product(s). As illustration, an organisms shear sensitivity may determine the choice between a stirred tank and airlift reactor for the fermentation stage, while the products location and concentration in the broth, will determine the need for cell disruption and the flow rates through downstream processing, respectively. The cell quality has a clear relevance to most areas of operation yet it is not usually followed extensively, so fermentation development may lead to optimal productivity at the expense of the ease and operating efficiency of the purification process: through neglect of contaminant levels for instance.

As mentioned, cell quality may be scale dependent and difficult to predict by common scale-up methods. However, an emerging scale-up technique, known as scale-down of fermentation, may improve the prediction of cell qualities on scale-up. The scale-down method attempts to reproduce the rate limiting mechanisms of the production scale within the experimental equipment, consequently reproducing the most important environmental features for the microorganism. The scale-down method is designed to improve the accuracy and speed of the scale-up process; monitoring cell quality in scale-down equipment should also improve process integration, by identifying the point at which there is an optimal mix of qualities for productivity and downstream processing. The objective of this project was to gather cell quality information at various scales of fermentation, to provide a preliminary basis for the design of scale-down fermentations.

In section 1.1 of this project explains what is meant by 'scale' in relation to fermentation, and identifies the point at which scale-down methods should be applied. Section 1.2 identifies the variations that occur in the environment of fermenters on scale-up ( in terms of mass, heat and momentum transfer ), and the possible reactions of the microorganisms to these changes: i.e. the changes in cell quality that may result. Section 1.3 summarises the methods available for the scale-up of fermentations, while section 1.4 details the techniques employed for the scale-down of fermentations. Sections 1.5 to 1.9 provide further information that is necessary for the interpretation of
the results and understanding of certain methods: section 1.5 describes the behaviour of the organisms used in this study, when grown in batch culture. Methods and materials used in this study are detailed in section 2.

Section 3 describes the protocol for the scale-up of *S. cerevisiae* and goes on to present the results of the cell quality profiling of this organism. Section 4 has the same structure as section 3 but details the protocol and results for *S. erythraea*. This section also details the results of fermenter mixing time investigations, which were necessary for the interpretation of scale-up effects and for the design of scale-down models.

In section 5 the results are discussed in depth. Section 5.1 discusses the reproducibility of the cell quality profiles. Section 5.2 examines the effect of scale-up on the cell qualities studied and attempts to explain the origins of any variations. Section 5.3 indicates how the cell quality profiles themselves, may be of used in process integration by deciding the best harvesting point of the fermentations to obtain the optimal mix of cell qualities for subsequent processing. The design of possible scale-down systems for studying the effects of the engineering parameters responsible for the observed scale-up effects, are discussed in section 5.4. The project conclusions are presented in section 6
1.1 Scale In Relation To Fermentation

A useful starting point is an indication of what is meant by scale, as it applies to fermentation. The origin of cell quality variations between experimental and production fermenters, and the need for scale-down in process development, can be understood by looking at the traditional activities and characteristics of what are phrased laboratory, pilot and production scales. The volumes involved at these different scales range between test tube to 10-20L, 10-10,000L and 8,000-350,000L respectively. The laboratory and pilot scales may also be referred to as small scale while the production scale may also be termed large scale: small scale essentially implying an experimental scale. The boundaries between the scales are indistinct and usually laboratory, pilot and production scale refer to different objectives rather than different sizes of operation.

1.1.1 Laboratory scale

Most often the development at this scale revolves around the identification of possible products and microorganisms that will produce them: screening. The usual tools for the job are shakers and small fermenters, which bear little resemblance to production vessels, and the objectives are just to make the process work even though the results may not be directly scaleable (Fahrner, 1993). The limitations on productivity at this scale are due to the microorganism (microkinetic effects, e.g. growth and product formation), which grows and produces at its maximum rate.

1.1.2 Pilot scale

The pilot scale of operation is the most complex phase of fermentation development because it has many roles to fulfil. The equipment that is used here often has very flexible operating capabilities and is used to carry out engineering trials, for example to determine the optimal operating conditions. The pilot plant arose as result of economic considerations and the development of the field of chemical engineering. Many man years are required to develop a new process, therefore the risk of financial loss due to the failure of the production process necessitates trials before the designed plant is constructed, in fact the pilot plant data is often used to specify the production plant (Lowenstein 1985).
The conventional approach to piloting is either to provide equipment that has geometric similarity to the production scale vessels (in the case of process optimisation or the production of new material in an existing vessel), or to provide equipment of variable geometry (e.g. by the use of different impeller types and spacings or the introduction of cylindrical inserts to change the aspect ratio) to cover the range of commonly encountered production vessel geometries. This type of plant is borne of the similarity approach to scale-up, and the equipment bears a closer resemblance to the production scale than to the laboratory scale. As scale-up usually requires the extrapolation of data, the final scale of the pilot plant must be sufficiently close to the production scale, which may require a number of pilot steps. This results in a high cost for pilot scale equipment and experimentation: up to 10% of the production plant cost (Lowenstein 1985). Productivity is usually limited by microkinetic phenomena at the lower end of this scale, while transport phenomena become increasingly important toward the higher end of this scale.

1.1.3 Production scale

If the plant is intended for a single process then the equipment that is used here is characterised by fixed capabilities of agitation, aeration and heat transfer, which have been designed for the particular process. Here transport processes dominate the productivity and are the rate limiting mechanisms, and the microorganism does not always function with its maximum capability. As the equipment usually has little flexibility of operation, it is vital that the scale-up process correctly determines the agitation, aeration and heat transfer requirements for the process at this scale. However, even if scale-up is successful in this respect, there remains the possibility that the microorganism may exhibit a varying or unexpected metabolic behaviour under the stress of the increased environmental heterogeneity: a shift in cell quality.

A traditional development process is stepwise (Figure 1.1), beginning in the laboratory and ending with pilot plant trials, and means reproducing in the production scale equipment, the results from successful fermentations made in laboratory or pilot scale equipment. This type of development may only encounter productivity and microbial limitations due to microkinetic effects as the fermentation scale is small, and fail to model the transport process limitations and heterogeneity that are liable to be
encountered at the production scale. Generalities are difficult to apply, except that fermentations where mixing is more important (e.g. aerobic and substrate fed fermentations) will suffer greater transport process limitation and be more difficult to scale-up in the traditional way. Scale-down experiments will aim to fall into the laboratory scale of operation, and incorporate characteristics of the production scale.

![Diagram](image)

**Figure 1.1:** Traditional scale up process.

(i.e. the transport process limitations). Whereas the traditional scale-up process relies on the extrapolation of data from one scale to another, and the overcoming of problems as they arise, scale-down requires the prediction and incorporation of the effects of scale-up into the initial stages of experimentation. Thus providing a more reliable indication of the effects of scale-up on the process. This should improve the data traditionally obtained from laboratory scale (e.g. screening) and reduce the number of steps at the pilot scale. The cost of pilot scale operation is such that scale-down equipment may be used to solve the bulk of the development problems, thus reducing the level of piloting that is required before full scale operation. As a consequence the cost of development should decrease, through reduced experimentation scale and due to time savings (quicker scale-up / bigger jumps in scale), giving earlier production and hence greater profitability. This should also allow more of the objectives of the process development to be met (Fahrner, 1993).

A number of limited trials will probably still be conducted at large pilot scale in order to check that the expected behaviour is observed as the cost of failure is great (e.g. plant shut down for redesign, wages of trained staff on standby, changing equipment, loss of production and time, delay in market release etc.). Also, the sample quantities required for field testing may only be produced easily at the pilot scale. Therefore the final decision on the use of intermediate stages of development (pilot
scale) is likely to be a financial rather than scientific one. Lowenstein (1985) identifies the role of pilot plants as in Table 1.1; scale-down systems may be able to address a number of the roles identified, and achieve these roles at reduced cost.

<table>
<thead>
<tr>
<th>Role of pilot plant according to Lowenstein (1985)</th>
<th>Applicability of scale-down model to pilot plant role</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Test reaction mechanism theories</td>
<td>YES</td>
</tr>
<tr>
<td>2) Prove process feasibility and reliability</td>
<td>YES</td>
</tr>
<tr>
<td>3) Obtain design information</td>
<td>YES</td>
</tr>
<tr>
<td>4) Solve scale-up problems</td>
<td>YES</td>
</tr>
<tr>
<td>5) Gain operating experience</td>
<td>A small role</td>
</tr>
<tr>
<td>6) Produce sample quantities</td>
<td>YES, sufficient for 9) but not field testing or market evaluation A small role</td>
</tr>
<tr>
<td>7) Obtain environmental and pollution abatement data</td>
<td></td>
</tr>
<tr>
<td>8) Test materials of construction</td>
<td>NO</td>
</tr>
<tr>
<td>9) Test analytical procedures and systems</td>
<td>YES</td>
</tr>
<tr>
<td>10) Train personnel</td>
<td>NO</td>
</tr>
<tr>
<td>11) Inspire confidence in management</td>
<td>Depends on the individual</td>
</tr>
<tr>
<td>12) Affords a further opportunity to find mistakes before they become expensive to correct</td>
<td>Limits the possibility of things going wrong</td>
</tr>
</tbody>
</table>

Table 1.1: Applicability of scale-down models to pilot plant functions
1.2 Scale related transport factors affecting cell qualities

In section 1 it was suggested that the variation of transport processes with fermentation scale, was the principle cause of variation between the environment of small and large scale vessels. This section will identify the origins of variations in transport phenomena between different scales of operation and the affects this may have on the cell quality. Mass, momentum and heat transfer are considered in turn (Sections 1.2.1, 1.2.2, 1.2.3, respectively), and in section 1.2.4 suggestions will be made regarding the size of equipment that should be used for scale-down studies.

Transport phenomena are governed by two transport mechanisms, flow (by convection and turbulence) and diffusion (conduction during laminar flow and stagnant transport). If the time constants for flow, diffusion and conversion were to remain constant on scale-up there would be no problems. However, an examination of these processes shows that flow and diffusion will vary with scale (Kossen and Oosterhuis, 1985). For example, the time constant for flow transport is given by,

\[ t_f = \frac{\text{Flow path length}, L \ (m)}{\text{Flow velocity}, \nu \ (m \ / \ s)} \]  

(1.1)

Therefore in order to maintain constant flow transport characteristics between two scales, scale-up (increase in L) requires a proportionate increases in velocity. However, this requires constant stirrer speed on scale-up (as \( \nu \approx L \times \text{Stirrer speed}, \ N, \ s^{-1} \)), which in turn requires Power input per unit volume, P/V, to increase with the square of the reactor dimensions (P/V \( \approx L^2 \)). In reality P/V is kept more or less constant on scale-up, therefore flow transport is bound to suffer (\( t_f \approx L^{23} \)). Similarly, the time constant for diffusive transport is given by,

\[ t_d = \frac{L^2}{\text{Diffusivity}} \]  

(1.2)

Diffusive transport is bound to suffer with scale increase because diffusivities can not be made to change at the same rate as scale (length), therefore diffusion limited processes
will be greatly influenced by scale. Conversely, the conversion time constant is obtained from,

\[ t_{\text{con}} = \frac{\text{Reactant concentration}}{\text{Reaction rate}} \]  

(1.3)

Because the microbes are always converting substrates at the maximum rate allowed by their environment, \( t_{\text{con}} \) is relatively constant. From the foregoing, scale-up will result in an increasing influence of transport processes on fermenter performance, and an increasing heterogeneity in the environment of the microorganisms. Additionally, the options on improving these processes on the large scale, by increased stirrer speed or gas flow for instance, are limited by the sensitivity of the microorganisms to their environment (e.g. shear damage) and of the controllability of the bioreactor in relation to the operating conditions (e.g. foaming).

The important question is at what scale does the dominance of transport phenomena control or limit a fermentation, and whether this scale falls within the desired operating scale for a particular process? If, for example, transport processes limitations occur outside the pilot scale but within the production scale (Sections 1.1.2 & 1.1.3), then a traditional scale-up process will fail to anticipate these, with unknown consequences for cell quality.

Apart from differences in the transport characteristics with scale, there are other factors that may vary. These may be the use of different grades of substrate and methods of sterilisation, and effects due to the difference in the physical size of fermenters. These factors can also cause the environment and cell qualities to vary with scale, and are discussed in the following sections.
1.2.1 Mass transfer

In large scale aerobic fermentations the most important mass transfer process involves the supply of adequate amounts of oxygen to meet the demands of cell growth and product formation. The problem arises from the low solubility of oxygen (e.g. 1.2mmol/L at 25°C in water and at 1 atmosphere pressure). Additionally, fermentation scale-up prediction is hampered by important differences in mass transfer between small and large scale reactors; for example the expected equilibrium concentration of oxygen in the broth (Section 1.2.1.2).

1.2.1.1 Basic concepts; The mass transfer process

Oxygen supply is a purely physical process which requires oxygen transfer from a rising air bubble into the liquid broth, and through the liquid into the respiring cell. A number of transport resistances are involved in oxygen supply; a schematic of the process is shown in figure 1.2. Any fermentation process that affects the transport resistances will affect the overall mass transfer process of oxygen. Mass transfer variation may be expected on scale-up (Section 1.2); the tendency being for reduced oxygen supply on scale-up. The cell behaviour, and therefore quality, may change as a result of the altered availability of oxygen, depending on the necessity of oxygen by the organism. Some microorganisms will not grow without oxygen, whereas others may show alternative metabolic behaviour under oxygen limitation (e.g. Clark et al., 1995) and therefore a different quality to that desired from scale-up. The mass transfer variation between scales is most often due to the difference in size, nature and behaviour of gas bubbles, or the stagnant liquid films that surround them. The variation in these parameters resulting from differences in operating conditions and medium components between scales.
The respective resistances to oxygen transport are

1) Diffusion from the bulk gas to the gas / liquid interface.
2) Movement through the gas / liquid interface.
3) Diffusion through the liquid film surrounding the gas bubble to the relatively well mixed bulk liquid.
4) Transport through the bulk liquid to the stagnant liquid film surrounding the cell / cell aggregate.
5) Diffusion through the second stagnant liquid film.

6) Diffusive transport into the cellular floc / mycelium.
7) Diffusion through the cell aggregate.
8) Active transport into the cell.
9) Diffusion to reaction site.

NB. Where cells do not form aggregates steps 6 & 7 are not involved. It has also been known for cells to adsorb to the stagnant liquid films surrounding air bubbles and in these situations 4 to 7 are negated: here the oxygen concentration seen by the cells is not the bulk oxygen concentration.

**Figure 1.2:** Transport path of oxygen from a rising air bubble to a respiring cell (Bailey and Ollis, 1986).
1.2.1.2 Mass transfer equations

The oxygen mass transfer rate per unit unaerated liquid volume is given by an equation of the following form,

\[
\text{Volumetric oxygen transfer rate} = \frac{\text{Oxygen mass transfer coefficient}}{\text{( liquid stagnant film )}} \times \frac{\text{Mass transfer area}}{\text{per unit unaerated liquid volume}} \times \text{overall concentration driving force}
\]

\[
OTR = k_i \cdot \frac{A}{V} \cdot \left( C^* - C_t \right) \tag{1.4}
\]

In practice \( k_i \) and \( A/V \) are difficult to determine separately, so they are usually combined as \( k_l a \) (where \( a = A/V \)), the volumetric mass transfer coefficient; hence the familiar form,

\[
OTR = k_l a \cdot \left( C^* - C_t \right) \tag{1.5}
\]

An immediate discrepancy is evident between small and large scale, that is the choice of the concentration driving force. The value of \( C^* \) depends on the location at which it is measured. For small fermenters the gas and liquid phases are considered to be well mixed and \( C^* \) can be taken as the value that is in equilibrium with the exit oxygen. However, at large scale the gas flows through the fermenter with relatively little back-mixing, in which case a plug flow model is better, and a log-mean value for the concentration driving force is more appropriate (Equation 1.6). Thus the experimental prediction of OTR may vary from the actual production scale value.

\[
\left( C^* - C_t \right)_{\text{log mean}} = \frac{\left( C^*_{\text{inlet}} - C_t \right) - \left( C^*_{\text{outlet}} - C_t \right)}{\ln \left( \frac{C^*_{\text{inlet}} - C_t}{C^*_{\text{outlet}} - C_t} \right)} \tag{1.6}
\]

With the concentration driving force limited by the low solubility of oxygen, \( k_l a \) controls the oxygen transfer process. \( k_i \) itself varies little, though it is affected by \( a \), which depends on bubble size (\( d_b \)), the bubble rise velocity and the gas holdup. In turn
these parameters depend upon the vessel geometry and broth properties (Table 1.2). The physical properties of the broth are determined by its chemical and cellular composition. For example, the surface tension depends on the nature of the ionic species and the presence of surfactants. The viscosity may be greatly affected by extracellular metabolic products, and the growth morphology of the microorganism.

<table>
<thead>
<tr>
<th>Vessel geometric factors</th>
<th>Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nature of the gas distributor and agitator</td>
</tr>
<tr>
<td></td>
<td>Fermenter internals (e.g. baffles and cooling coils)</td>
</tr>
<tr>
<td>Broth physical properties</td>
<td>Viscosity</td>
</tr>
<tr>
<td></td>
<td>Density</td>
</tr>
<tr>
<td></td>
<td>Surface tension</td>
</tr>
<tr>
<td></td>
<td>Diffusivity of oxygen in liquid</td>
</tr>
</tbody>
</table>

**Table 1.2: Factors affecting the volumetric oxygen transfer coefficient**

Dimensional analysis of gas-liquid contacting systems yields correlations for \( k_f \alpha \) of the form \( N_{SH} = f( N_{RE}, N_{SC} ) \), such as the following correlations (Equations 1.7 and 1.8, Sideman et al., 1966).

For stirred tank reactors, STR:

\[
\frac{k_f \alpha D^2}{D} = A \left( \frac{\mu}{\rho \cdot D} \right)^\alpha \left( \frac{\mu \cdot \nu_s}{\sigma} \right)^\beta \left( \frac{D^2 \cdot N \cdot \rho}{\mu} \right)^\gamma \left( \frac{\mu_c}{\mu} \right)^\delta
\]  

(1.7)

For airlift reactors, ALR:

\[
\frac{k_l \cdot d_b}{D} \text{ or } \frac{k_f \alpha \cdot d_b^2}{D} = B \left( \frac{\mu}{\rho \cdot D} \right)^\alpha' \left( \frac{\mu \cdot \nu_s}{\sigma} \right)^\beta' \left( \frac{d_b \cdot v_b \cdot \rho}{\mu} \right)^\gamma'
\]

(1.8)

Additional dimensional groups may be incorporated to account for geometric variables: of which \( (H_f/T_f) \), \( (D/T) \) and \( (H_f/H_L) \) are examples. The equations are often
simplified for a given fluid system and geometry so that $k_i\alpha$ can be related to the most important operating variables: agitation ($N$), aeration ($\nu_s$) and power input (Equations 1.9 & 1.10).

For STR:  
$$k_i\alpha = A_i \nu_s^B \cdot \frac{P_s}{V} = A_i \nu_s^B \cdot \left(\frac{P_s}{V}\right)^\gamma$$  
(1.9)

For ALR:  
$$k_i\alpha = B_i \nu_s^B \cdot \nu_b^\gamma \cdot d_b^{\gamma - 2}$$  
(1.10)

The restricted conditions for the derivation of these simplified equations explains to some extent the great variety in the reported values of the empirical exponents. Also the STR correlations only apply to small scale vessels, and need to be modified for the large scale by accounting for the extra power draw of multiple impeller vessels.

Though a theoretical analysis describes STRs and ALRs by the different equations above, Andrew (1982) suggests that virtually all large STRs fall in the category of free bubble rise reactors (i.e. ALRs) because the mass transfer coefficient is relatively unaffected by the turbulence conditions in the fermenter. Andrew (1982) only classifies small STRs as high turbulence reactors: the importance of gas flow rates ($\nu_s$) and bubble size ($d_b$) in large STRs is therefore underestimated. This notion is supported by Oosterhuis and Kossen (1984), with their use of a five compartment model to describe oxygen transfer in a 19$m^3$ (liquid volume) dual turbine bioreactor. They proposed two agitated compartments, each in the shape of a torus incorporating part of the impeller blades ($k_i\alpha \propto \left(\frac{P_s}{V}\right)^\alpha \cdot \nu_s^B$), and two free bubble rise sections ($k_i\alpha \propto \nu_s^B$); the nature of the fifth compartment was not clearly indicated. They suggest that such a model is justified where the time constant for oxygen transfer ($1/k_i\alpha$) is smaller than the liquid circulation time ($t_e$); that is the vessel is poorly mixed compared to oxygen transfer. For the opposite situation the correlations reported by Sideman et al. in 1966 (Equations 1.9 & 1.10), will apply. Manfredini et al. (1983) also used a compartment model, to describe oxygen transfer in non-Newtonian fermentations in a 112$m^3$ fermenter with four turbines. The model consisted of four well mixed compartments in series. While the simplified correlations may be sufficient for the determination of $k_i\alpha$s in experimental vessels, the prediction of realistic $k_i\alpha$ values for the production scale is not simple from the acquired data.

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1.2.1.3 Bubble size

Bubble size is important in determining the interfacial area per unit aerated liquid volume ($a$), the gas holdup, $\varepsilon$ (volume of gas per unit aerated liquid volume) and $k_i$. The connection to $a$ is easily seen; for the same holdup and gas flow rate, smaller bubbles present a larger surface area to volume ratio for oxygen transfer (Equation 1.11).

$$a = \varepsilon \cdot \frac{6}{d_b} \quad (1.11)$$

where $\varepsilon = \frac{Q}{V_{la}}$ \hspace{1cm} (1.12)

Disregarding other factors, smaller bubbles give higher $k_i\varepsilon$ values. The gas holdup is affected by virtue of a lower rise velocity for small bubbles, which reduces the bubble terminal velocity, $v_{tb}$:

$$v_{tb} = \frac{d_b \left( \rho - \rho_g \right)^2 \cdot g}{18 \mu} \quad (1.13)$$

The effects of bubble size on $k_i$ are more complex and set an optimum value for bubble diameter, $d_b$, by determining the type of boundary layer around the gas bubble (Van't Reit, 1983). In perfectly clean systems the boundary layer around a moving gas bubble should remain mobile. However, trace impurities present in all systems mean that small bubbles have rigid immobile interfaces surrounded by a stagnant liquid film. Gas transfer through these boundary layers is by molecular diffusion along a linear concentration gradient, which results in low values of $k_i$ (approximately $10^{-4}$ m/s for bubble diameters less than 2mm in air water dispersions). Large bubbles by contrast, have mobile interfaces and no liquid is entrained in the film layer. Mass transfer here is described by Higbie's penetration theory and leads to higher values of $k_i$ (approximately $4 \times 10^{-4}$ m/s for bubble diameters greater than 3.5mm). The outcome is an optimal value of $k_i$ and $k_i\varepsilon$ around a $d_b$ of 2.5mm in water. The agitation and aeration conditions in a fermenter are controlled to maintain a similar mean bubble size, however this size is almost transitional between small and large bubble behaviour (i.e. rigid and mobile gas-liquid interface behaviour) and can be easily unbalanced by conditions within the fermenter (e.g. presence of surfactants), and hence fermentation scale.
1.2.1.4 Scale related factors affecting oxygen mass transfer

Oxygen mass transfer is affected by a large number of variables, many of which interact with each other, to alter the concentration gradient or mass transfer coefficient.

Temperature

Temperature rise affects both oxygen solubility and diffusivity in the liquid phase; whilst the former is reduced the latter is increased. The dominant effect is on solubility, so temperature rise reduces oxygen mass transfer. Solubility is related to temperature through the following equation:

\[ C^* = \frac{468}{31.6 + t} \]  \hspace{1cm} (1.14)

The solubility of oxygen in water (at 1 atmosphere pressure) falls dramatically between 15-30°C, from 1.54 to 1.16 mmoL/L. However, temperature control is not generally considered a problem in fermenters, except where very large volumes of fast growing microorganisms are involved. Manfredini et al. (1983) found no axial or radial temperature gradients in 112 m³ fermenter with non-Newtonian broths. In this case there were no cooling coils in the vessel, where they are present they may lead to poor mixing in certain regions of the fermenter. Temperature gradients arising from poor mixing will have marked effects upon the oxygen supply process, and could lead to gradients in dissolved oxygen or localised oxygen supply problems.

Pressure

Pressure (P_out) affects the oxygen transfer rate by altering the value of C*, which alters the concentration driving force for oxygen transfer (Equation 1.5), according to the following equation (Crueger and Crueger, 1984):

\[ C^* = \frac{1}{S_{O_2}} \left( P_{out} + Z \rho_m \right) Y_{O_2} \]  \hspace{1cm} (1.15)
The equation also contains a term to take account of hydrostatic pressure effects $(Z, \rho_m)$. The hydrostatic effect will depend on the holdup, $\varepsilon$ (which affects the mean liquid density, $\rho_m$), according to the following relationship:

$$\rho_m = \rho (1 - \varepsilon) + \rho_{g.e} \approx \rho (1 - \varepsilon)$$  \hspace{1cm} (1.16)

Laboratory and pilot scale fermenters often differ from production fermenters in their aspect ratios; typical height-to-diameter ratios are 1-2:1 for laboratory and pilot scale, and 2-6:1 for production scale vessels. Even in the case where geometric similarity is maintained there will be a considerable hydrostatic head at increased scale. As OTR usually exceeds the vertical bulk mixing rate at large scale, there will be a resultant dissolved oxygen tension (DOT) profile. Manferdin et al. (1983) reported axial DOT gradients as a result of hydrostatic pressure in a 112m$^3$ fermenter even though water was used in their study, which should give fast bulk mixing due to a low viscosity.

The actual effects of the increased or varying dissolved oxygen on the fermentation itself, will depend on the microbial system involved. Yang and Wang (1992) increased DOT by using fermenter head space pressurisation. They compared the effects that this had on a bacterium (Escherichia coli) and an algae (Oochromonas malhamensis). The increased OTR generally improved the cell growth rate and viability of the organisms. However, the algae suffered reduced cell growth at high levels of oxygen enhancement, which they concluded was due to oxygen toxicity; the effect of hydrostatic pressure on this organism would then depend on the level of oxygen enrichment achieved. Varder and Lilly (results summarised by Lilly, 1983) cycled the fermenter head pressure to simulate the DOT gradients in a large scale penicillin fermentation. They found the penicillin production rate fell to a value lower than expected from the mean DOT value, so this organism was sensitive to heterogeneity in oxygen concentrations.

The effects of increased hydrostatic pressure may be further complicated by the increase in CO$_2$ solubility that results. The effects of an increase in dissolved CO$_2$ on a penicillin fermentation were demonstrated by Ju et al. (1991). They sparged the fermenter with CO$_2$ enriched air, which resulted in an increase in the hyphal branching frequency of P. chrysogenum: at a concentration of 20% CO$_2$ in the inlet air, the growth morphology of the organism was altered from diffuse to pelleted. The increase in
branching frequency reduced the apparent viscosity of the broth which improved \( k_A \) by the following relationship:

\[
k_A = \text{Constant} \cdot D_e^{2/3} \cdot \mu_s^{-0.33}
\]  

(1.17)

The benefits of the improved \( k_A \) were only slight, and only at low concentrations of \( CO_2 \) in the influent air (5%). At higher \( CO_2 \) concentrations the reduced oxygen transfer through the pellet dominated the mass transfer process and the overall cell growth and penicillin production were severely reduced. Though the concentrations involved in the work of Ju et al. (1991), may be higher than would be expected to result simply by hydrostatic pressure enhancement, other systems may show greater sensitivity to \( CO_2 \) levels. Creuger and Creuger (1984) report a 33% drop in sisomicin yield resulting from the inclusion of just 1% \( CO_2 \) in the influent air of this fermentation. Again the effects are system dependent, and extremely difficult to predict where a change in hyphal morphology is concerned. The last example illustrates how easily the balance of a successful fermentation may be shifted by a slight variation in the environment of certain organisms on scale-up.

**Rheology**

Increased viscosity leads to reduced \( k_A \). Viscosity may increase on scale-up due to the use of complex media components, and generally may be altered if the morphology of a filamentous organism is different between scales, or if the production of excreted molecules by the organism varies.

**Media Components**

Bubble coalescence and therefore bubble size determination is a balance between the surface tension which restores the sphericity of bubbles and the turbulent shear forces which reduce bubble size. Whereas in clean systems the bubble size can be controlled by agitation and aeration (the shear forces), additives interfere with the balance and influence the mean bubble size and the bubble size distribution. The particular additive is an important factor in determining the outcome of its addition.

One of the major problems in fermentation development is that different grades of media are often used at different scales. Experimental stages may use refined chemicals, whereas economics may dictate that the production fermenter uses crude carbon and
nitrogen sources (e.g. molasses and malt extract for the former and corn-steep liquor, yeast extract and soy meal for the latter). Variations in the media components are likely to affect oxygen transfer.

Though additives may reduce the solubility of oxygen (1.2 mmoL/L in pure water at one atmosphere and 25°C compared to 0.71 mmoL/L for a 2.0M NaCl solution) it is difficult to generalise about the overall effect of different chemicals. The result depends upon the extent to which they alter $k_l$ compared to $a$. Most additives reduce $k_l$ with respect to water by either increasing the viscosity of the liquid phase (thus increasing the depth of the stagnant liquid film), by adsorbing to the gas liquid interface (and increasing the interfacial resistance) or by a combination of the two (the resistances are indicated in figure 1.2). Crude extracts are complex substrates and prove to be indefinable. Many of the biochemicls that they contain are amphiphatic and spontaneously adsorb to two phase interfaces and behave like surfactants.

Additives can be separated into two classes, coalescence inhibitors and coalescence promoters. The inhibitors reduce the mean bubble diameter and therefore increase $a$ (Equation 1.11). As smaller bubbles have lower rise velocities (Equation 1.13), the gas holdup may also increase which will reduce P/V and therefore $k_l a$ (Equation 1.9). In coalescence inhibition the bubbles produced by the agitator or dispersing device are largely preserved: thus bubble size is independent of agitator speed for instance. The coalescence promoters produce the opposite effects.

Keitel and Onken (1982) demonstrated that inhibition was dependent on the concentration of the inhibiting agent. The mean bubble diameter got smaller and the bubble size distribution got tighter as the inhibitor concentration was increased. At a particular inhibitor concentration the value of $a$ levelled off and the system became saturated. These effects were due to the use of soluble inhibitors which function by having to surround the gas bubble. The surfactants reduce the surface mobility of the bubbles which reduces $k_l a$, as in the case of small bubbles with rigid interfaces (Section 1.2.1.3). Keitel and Onken (1982) also found that different additives gave different levels of activity; the enhancement of $a$ was in the following order: polyvalent ions > monovalent ions > mono carboxylic acids > n-alcohols > glycols and ketones. They concluded that the greater the difference in character between the hydrophobic and hydrophilic regions the stronger the coalescence inhibition.

Benedek and Heideger (1971) reported similar results with NaCl solutions and the ionic surfactant sodium dodecyl sulphate (SDS); both inhibited coalescence and $a$
was independent of stirrer speed. NaCl doubled $k_a$ at a concentration of 0.3mol/l, by virtue of a six fold increase in $a$. Even though $a$ increased by a similar amount with 40ppm SDS, there was a minimal increase in $k_a$, indicating a significant decrease in $k_i$. Interestingly they recorded an increase in $a$ with the addition of antifoam; $a$ was dependent on the stirrer speed indicating that the system was non-coalescing; antifoam reduced $k_a$ overall.

The use of antifoam in a clean system by Benedeck and Heideger (1971) does not illustrate its likely behaviour in a real fermentation; here it would probably reduce $a$ by promoting coalescence. The need for only small quantities of antifoam to illicit the full effects is consistent with the mechanism of coalescence promotion described by Prins and van't Reit (1987). They also describe how proteins adsorb to gas bubbles to inhibit bubble coalescence and breakup, creating 'foam'. Large scale fermentations are likely to use crude sources of nitrogen for economic reasons; these sources will usually contain some soluble proteins which could lead to foaming in the vessel. Though a certain degree of foam (interfacial area) is desirable, antifoam is added to guard against foam blow-out; especially as large vessels may have a high superficial gas velocity. Foam is not controlled easily so excess antifoam is commonly added, with a consequential reduction in $k_a$. Apart from the cell quality changing as a result of the use of a different medium grade affecting oxygen supply, the quality (the cells enzymatic composition for instance) is likely to change just by the cells adaptation in order to be able to consume the different metabolites.

**Surface aeration**

This phenomenon relates to the oxygenation of a fermentation broth by entrainment of gas from the free liquid surface. The extent to which surface aeration contributes to the aeration of a fermenter depends partly on the depth to which liquid at the free surface is carried and partly on the area of the free surface relative to the area achieved by the holdup (i.e. the total interfacial area). Whereas shake flasks solely use surface aeration to supply oxygen to the growing culture, common industrial fermenters receive little benefit from gas entrainment at the free surface. The concern in scale-up is that surface aeration may add to the mass transfer performance of small vessels, say less than 20L operating volume, so correlations based on these vessels may overestimate the capabilities of larger fermenters.
Nienow et al. (1979) studied surface aeration in a single agitator vessel containing 18L tap water. Though surface aeration was high in the absence of sparging it rapidly decreased with increasing gas flow rate. Increased agitator speed at low sparge rates improved the surface aeration. A possible explanation for the low rates of surface aeration under sparged conditions in their study, is that the impeller was placed relatively close to the bottom of the vessel: less than one impeller diameter. This would have reduced the power drawn by the impeller and the maximum surface turbulence, hence maximum surface aeration, possible with their set up. However, they did employ an impeller of a large diameter (D / T = 0.5: which is larger than the standard 0.33) and this would have helped with surface aeration.

Veljkovic and Skala (1989) studied the effects of stirrer speed, sparge rate, power draw and the number of impellers on the surface aeration intensity, $\alpha_{SA}$ (ratio of surface aeration rate to gas sparge rate), in a 14L fermenter. They identified two surface aeration regimes around a critical power draw. Below the critical power level, the surface aeration rate was low, as gas entered the liquid by diffusion. The rate increased by the use of more impellers, as this improved convection in the upper region of the liquid. Above the critical power level, the surface aeration occurred by gas entrainment, and depended on the power input and not the number of impellers. In both regimes the surface aeration rate increased with stirrer speed and reduced with increasing sparge rate. They suggest the following correlation for use above the critical power value, where surface aeration was most intensive:

$$\alpha_{SA} = 6.2 \times 10^{-14}.P_g^{1.5}.Q^{-2.2}$$  \hspace{1cm} (1.18)

As $P_g \propto N^3$ (Equation 1.25),

$$\alpha_{SA} \propto N^{4.5}.Q^{-2.2}$$  \hspace{1cm} (1.19)
Other factors

Metha and Sharma (1971) investigated the effects of various geometric dimensions on $a$ and $k_a$. Some of their results have important implications upon the scale of operation.

Liquid height ($H$) to tank diameter ($T$) ratio:

Their results indicate that $a$ is inversely proportional to $H/T$ for sparged contacting with single impellers. The increase in liquid height above the impeller leads to a sharp drop in surface aeration also. In the extreme the vessel behaves as a two compartment system, with a lower stirrer region and upper bubble column; extra impellers are required to counteract this. Additional impellers can only be added when there is sufficient separation for them to function efficiently (Hudcova et al., 1989); the use of more than one impeller can have other consequences (see below).

Stirrer speed ($N$):

When the geometry, superficial gas velocity, and $H$ are kept constant, two operating regimes can be identified in relation to $N$. At low $N$, $a$ is small and gas flow controlled and changing $N$ has little effect. At high $N$ (above a critical speed, $N_0$), $a$ and $k_a$ increase linearly with $N$; $a$ is now impeller controlled and changing gas flow rate has little effect. Metha and Sharma indicate $N_0$ to be quite high (approximately 500rpm: $T = 12.5$ cm, $D = 5.8$ cm), suggesting that large fermenters operate in the low $N$ regime and only very small fermenters can operate in the high $N$, high $k_a$, regime (similar to the suggestion of Andrew, 1982: Section 1.2.1.2).

Increasing $T$ (between 12.5-70 cm):

At constant $D/T$, $a$ is proportional to $ND/T^{0.5}$; this implies that $a$ would increase with overall dimensions, even with geometric similarity of vessels, if stirrer speed could be kept constant. However, constant $N$ is unrealistic.

Type of impeller:

Radial flow impellers give higher $k_a$ values than axial flow impellers, for the same $D/T$ and $N$. Also the $k_a$ achieved by radial flow impellers increases more sharply with $N$. However there is no difference between different impellers for the same power input (van't Riet, 1979). Practically, large fermenters may use combinations of axial and
radial flow impellers to improve bulk mixing (e.g. in viscous fermentations); \( k_{L}a \) may be lower under these circumstances especially if the lower impeller, the one closest to the sparger, is of the axial flow type.

**Number of impellers:**

As indicated earlier, the use of more than one impeller (e.g. as vessel scale increases) can lead to compartmentalisation of the fermenter. The presence and nature of the concentration gradient depends upon the relative rates of oxygen transfer and fluid mixing (if \( t_{m} \gg \) time constant for oxygen transfer, an oxygen concentration gradient results), the hydrodynamics in different parts of the fermenter and the type of impeller used (radial flow impellers give the highest degree of compartmentalisation).

In the experiments of Manfredini et al. (1983) they found four well mixed compartments using four impellers. Each compartment had a different, but radially uniform, DOT profile; this resulted in an axial oxygen concentration gradient. In this high viscosity system the OTR was slow compared to the liquid circulation time. The gas bubbles experienced hindered coalescence and small bubbles, with low rise velocities, recirculated to give an STR type of compartment.

Oosterhuis and Kossen (1984) found axial and radial DOT profiles in their dual impeller fermenter. In contrast they found five different oxygen transfer compartments. In the low viscosity system OTR is fast compared to \( t_{c} \); most of the oxygen transfer is near the impeller where the small bubbles are formed. Elsewhere, strong coalescence means bubbles rise rather than recirculate, therefore the outlying regions behave as bubble columns.

Some possible effects of oxygen concentration gradients on microorganisms have been indicated earlier in this section; in terms of mass transfer correlations the models that describe the behaviour of compartmented fermenters are different to those of fermenters (usually small vessels) behaving as single units. Thus differences in the oxygen supply at different scales of fermentation can cause cell quality changes, and scale-up predictions may not be valid for large production vessels when based on pilot scale equipment.
1.2.2 Momentum transfer

In the interest of creating a homogenous environment for cell growth the vessel contents has to be thoroughly mixed. Where substrates are added, such as oxygen during sparging and growth limiting nutrients in fed-batch and continuous fermentations or where acid and alkali are used for pH control, these must be rapidly and evenly distributed. Similarly, temperature homogeneity and control within the volume of the broth, has to be achieved via the limited surfaces of the jacketed walls or internal coils, which requires liquid movement to and away from these surfaces. The objective of momentum transfer in a bioreactor is to obtain good mixing and gas dispersion, good mass and heat transfer are implicit, the momentum transfer problems may be viewed as ones of mixing.

Though mixing may be achieved in a non-flowing system simply by diffusion of mass along a concentration gradient and heat along a temperature gradient, the rates would obviously be uneconomic. When fluid elements move at different relative velocities during laminar flow, there is a flow of material down the velocity gradient: from high to low velocity. As the constituent material of the fluid has both mass and velocity it also has momentum (mass×velocity = momentum, kg m/s) and ultimately the velocity gradient results in a net momentum transfer perpendicularly to the flow direction. If the flowing material has variations in composition and temperature, movement of material down the velocity gradient will also effect mass and heat transfer but at enhanced rates to static diffusion.

The kinetic energy is introduced into the fermenter by the expansion and rise of the compressed sparged gas (ALR and STR) and movement of the impeller(s) (STR). The inertial component of the fluid, its density, promotes momentum transfer whereas the frictional component, its viscosity, dissipates the momentum as internal thermal energy: "heat".

Turbulent mixing in a fermenter involves three processes operating a different scales. Firstly, convective transport, also called recirculating or bulk flow, resulting from the pumping action of the impeller for instance. This flow component is responsible for the mean flow velocity of a turbulent stream. Secondly, eddy diffusion, local turbulent mixing due to the bulk motion of large groups of molecules that extract momentum from the convective flow and dissipates it sideways. Finally, molecular diffusion, spreading
out caused by relative molecular motion that leads to homogeneity on the molecular scale; each process reduces the non-uniformities to a particular degree. In turbulent mixing the former two mechanisms dominate the mixing; though the ultimate degree of uniformity is achieved by the latter process, bulk flow and eddy diffusion greatly increase its rate by increasing the surface area for the process.

The quality of a mixture can be described by the scale and intensity of the non-uniformities (Brodkey, 1981). The scale denotes the average distance between centres of maximum difference in the property concerned, which for turbulent mixing corresponds to the size of the eddies and is reduced by eddy break-up. The smallest scale of segregation produced by the turbulent eddies is still large compared to molecular dimensions. Intensity represents the range or variance of properties existing in a mixture. This does not reduce until the scale of the non-uniformities falls below the sample size or until molecular diffusion reduces the spread of the properties.

1.2.2.1 Fluid dynamics

Though turbulent flow is by far the most common flow behaviour in fermenters and the transport rates due to it far exceed those of laminar flow, a consideration of laminar flow is very useful for describing shear forces within fluids. Taking the example of fluid flow in a pipe, a characteristic flow and shear pattern result (Figure 1.3). The gradient at any point on the velocity profile gives the fluid shear rate, $\gamma$. Multiplying $\gamma$ by the fluid viscosity (momentum diffusivity for Newtonian fluids: Bradshaw, 1971) gives the fluid shear stress, $\tau$ (Equation 1.20):

Shear stress within laminar flowing fluid, $\tau = \mu \frac{dv}{dy}$.  

\[ \text{Shear stress within laminar flowing fluid, } \tau = \mu \frac{dv}{dy}. \]  

\[ \text{Equation 1.20} \]

**Figure 1.3**: Velocity and shear rate profiles for laminar flow in a pipe
As mentioned earlier mixing in a tank is bought about by $\gamma$ (differences in velocity between different layers of fluid): breakup of particles, bubbles and aggregates is due to $\tau$. As the velocity gradient is equivalent to a momentum gradient the shear stress can be regarded as a momentum flux (Beck and Mutzall, 1975).

The average shear rate produced by an impeller is only a function of stirrer speed (shear rate increasing with N); so long as N remains constant on scale-up so does the average shear rate. However, N usually decreases with scale up therefore $\tau$ and $\gamma$ suffer (poorer gas dispersion and mixing). On the other hand maximum shear rate is a function of both N and D and tends to increase with scale-up (Oldshue, 1983), which can result in cellular damage. Additionally, in larger tanks there is a greater range of shear rates than in smaller tanks: greater heterogeneity of shear. Van Suijdam and Metz (1981b) indicate fluid shear to be of primary importance in the abrasion of fungal pellets. This is because the pellets are of roughly the same density as the suspending medium so they follow the fluid streamlines. Particle-stirrer and particle-particle collisions would only be important if the pellet densities were substantially different to that of the medium (i.e. high inertial effects). They suggested the following model for determining the stable particle diameter against stirrer speed and diameter, for turbulent agitation of mycelial pellets (Equation 1.21). This was based on the assumption that the breakup and cohesive forces for the hyphae in a pellet, had a constant ratio. The model agreed well for the agitation of Penicillium chrysogenum pellets.

$$\text{Particle diameter} = \text{Constant} \times N^{-1.2} \times D^{-0.8} \quad (1.21)$$

Fluid shear damage is most likely for microorganisms that have a large size, which is usually the result of a mycelial morphology, because the organisms may cross fluid streamlines rather than be contained within them. For these organisms $P/V$ (stirrer speed, N) would have to remain within reasonable limits to avoid cell damage (a reduction in the particle size). This is counter to the requirements of mass transfer in filamentous fermentations, which often have a high viscosity, and therefore require greater power input to reduce the boundary layers that reduce mass transfer.

**Stresses within turbulent flows**

Different stresses are associated with the different mechanisms operating during turbulent mixing. The convective flow applies stresses that are parallel to the flow
direction and are known as linear stresses. The eddy motion exerts turbulent ("Reynolds") stresses; these are the shearing stresses that are responsible for bubble break-up. As in the case of laminar flow the stresses are indicative of momentum transfer, being either forward or perpendicular, respectively.

Eddies

The size of the turbulent eddies is determined by the scale of the equipment at the upper limit (impeller diameter for STRs) and by the fluid viscosity at the lower limit; the smallest eddies being in the range of 20-100μm (Bailey & Ollis, 1986). The scale of eddies decreases by vortex stretching (Bradshaw, 1971). The different sizes of eddy contain different levels of energy, which is transferred from the large to small eddies by inertia. As the rate of transfer of energy through the eddies is large compared to the rate of change of their energies they can be regarded as permanent structures, being in statistical equilibrium with one another (Hinze, 1959).

Considering gas dispersion, eddies that are larger than the bubbles will capture them and transport them to a different position. Smaller eddies will have no interaction as they rapidly lose their energy through viscosity. However, eddies of a comparable size to the gas bubbles will be responsible for bubble break-up. This would suggest that as vessel dimensions increase the proportion of the turbulent eddies that are responsible for bubble breakup decreases, as the scale of the eddies also increases. Put another way, the balance between breakup and coalescence forces shifts toward the latter within an increasing volume of the broth on scale-up. This is also evident from the increase in the range and the maximum shear rates with scale, whilst average shear rates remain constant (for constant N on scale-up) or decrease (Oldshue, 1983); that is to say while shear rates in large vessels are higher at the impeller tips they are lower at the walls compared to small vessels. Where shear thinning broths, or broths with a yield stress, are involved apparent viscosity may be very low near the impeller yet high enough near the walls and at points of stagnation to be flowing laminarly (transport by diffusion) or even be static. The effect is compounded by the improved dissipation of turbulence by higher viscosity, which means the size of the smallest eddies increases, requiring more transport by diffusion and producing larger nutrient gradients and stresses as a result.
1.2.2.2 Considerations for mixing in impeller agitated fermenters

**Impeller types**

The choice of impeller made within a fermenter has significant effects on the capabilities and characteristics of the vessel because of variations in power draw, the discharge flow patterns and the balance between flow and turbulence they create. Impellers may be classified into three categories based on the flow patterns (Oldshue, 1983).

i) Axial flow impellers, e.g. marine impeller.
These create an orderly flow pattern and draw less power than most other impellers of an equivalent diameter and running at the same speed, which results in low torque for a given power input. As the majority of the flow from this type of impeller passes via its cross section and the blade tips are very narrow, the impeller creates a low shear environment. The marine impeller is often used for operations requiring bulk mixing or suspension of solids and are poor with respect to gas dispersion.

ii) Radial flow impellers, e.g. disk ("Rushton") turbine.
These may have a variable number of blades that can be flat or curved and may have a disk or be open. Owing to a pressure differential between each side of the impeller open turbines do not have a true radial pumping action, pumping up or down while discharging radially. Disk turbines do pump in a true radial fashion so long as they are not to close to the vessel bottom, liquid surface or each other. Radial flow impellers draw more power than axial flow impellers for a given speed and diameter as they are less streamlined: they have a high projected area in the direction of movement. The result is high turbulence to flow ratio. The variety that incorporate a disk are preferred for gas dispersion as they prevent gas rising up the impeller shaft, a zone of low shear and flow resistance, consequently they have a higher flooding limit.

iii) Mixed flow impellers, e.g. pitched blade turbine.
These are predominantly axial flow in type but have a strong radial component of flow also. They are better at gas dispersion than radial flow impellers due to their flat blade tips and higher liquid discharge via the tips. Generally their properties are intermediate between the other two types of impeller and it is more difficult to model their behaviour.
Which ever type of impeller is used the width of the discharge stream will
determine the ratio of longitudinal (convective flow associated) to perpendicular (shear
associated) momentum transfer. This is why radial discharge impellers give more shear
than flow and why axial flow impellers are the reverse. For a particular impeller type as
scale increases there is a corresponding increase in the width of the discharge stream;
however, as the average discharge velocity does not increase proportionately, the fluid
stream has a greater flow/shear ratio: i.e. the impellers becomes poorer for gas
dispersion on scale-up.

**Effects of baffling**

Baffles reduce the tendency of the broth to swirl allowing turbulence to occur at
lower impeller speeds, hence higher gassed power draw and better gas dispersion. The
turbulence itself is steady (seen as a constant power number at high Reynolds number)
which gives predictable loading on the agitator motor. However, the turbulence at the
liquid surface of a fully baffled vessel may be low, and result in poor mixing-in of liquids
added on to the surface, and entrainment of gases (foam) and floating solids. In these
instances reduced baffling allows for a surface vortex that draws material toward the
agitator.

**Impeller pumping capacity**

The volumetric flow from an impeller, \( Q_i \) \((m^3/s)\) is a function of impeller shape,
size (D), speed (N) and the shape of the vessel and fittings. The flow area for axial flow
impellers is their cross section and for radial flow impellers is the cylindrical ring
described by the rotation of the outer edges of the blades. The value of this parameter
may be determined by equation 1.22: flow usually increases with scale (D).

\[
\text{Volumetric flow from an impeller,} \quad Q_i = \text{constant} \times N \cdot D^3 \tag{1.22}
\]

The principal dimensionless number quantifying flow is the flow number, \( N_Q \), which is
given simply by,

\[
\text{Dimensionless flow number,} \quad N_Q = \frac{Q_i}{N \cdot D^3} \tag{1.23}
\]
Impeller power requirement

Though in aerobic fermentations we are necessarily concerned with gassed power requirements, the correlations are empirical and can give large differences between calculated and measured values for the gassed power (Ashley, 1994); however, useful insights into scale-up issues may be gained from a consideration of the ungassed power requirement (the principal effect of gassing is to reduce power input by reducing the bulk density and by streamlining the impellers). The power requirement is a function of impeller shape, size (D), speed (N), the fluid properties and the shape of the vessel and fittings. For a given system the power requirement depends on the size of the resistances to fluid motion due to density, viscosity and interfacial forces, which resist fluid flow near the bubble surface (fluid motion at the surface of the gas bubble reduces the film thickness—figure 1.2—to enhance the concentration driving force for gas-liquid mass transfer). For back-mixing of the gas phase gravitational and buoyancy forces are considered as resistances (acting on density differences).

The ungassed power requirement during viscous / laminar flow is independent of fluid density and is given by,

\[ P = K \cdot \mu \cdot N^2 \cdot D^3 \quad \text{K ranges from 0.32 to 6.6 for a six blade turbine} \quad (1.24) \]

Conversely the power requirement during turbulent flow is viscosity independent (the secondary motion in the fluid, the turbulence, provide the resistance to motion) and is given by,

\[ P = K' \cdot \rho \cdot N^3 \cdot D^5 \quad \text{K' ranges from 33.0 to 71.0 for six blade turbines} \quad (1.25) \]

Even if viscosity increases during the fermentation, for example during a filamentous fermentation, the power requirement will be unchanged.

The power requirement can be expressed in an analogous method to pumping situations because mixers give both a pumping effect and velocity head; the power requirement can be written as (Oldshue, 1983),

\[ P = Q \cdot \rho \cdot H_{pot} \quad \text{H}_{pot} \text{ is the total potential head produced} \quad (1.26) \]
This is a useful representation because $H_{pot}$ is indicative of the turbulence (small scale motion) in the jet stream of the impeller.

At constant power input and for geometrically similar vessels, scale affects $N$ and $Q_i$ by the following degrees:

\[
N_r = \left( \frac{1}{D_r} \right)^{5/3} \tag{1.27}
\]

\[
Q_{ir} = D_r^{4/3} \tag{1.28}
\]

That is to say that the larger impeller runs at lower speed and produces more flow for an equivalent power input. Turbulence, as indicated by $H_{pot}$, is proportional to $N^2 D^2$ (substituting Equation 1.22 and 1.25 into 1.26) and varies with $1/D^{4/3}$ (Equation 1.28); therefore if constant power per unit volume is the scale up criterion this would result in a decrease in turbulence, $H_{pot}$, at the larger scale according to Equation 1.26. The increase in scale is accompanied by a transition from a dispersion controlled environment (high shear, small bubbles, high interfacial area) to a coalescence controlled environment (low shear, large bubbles, low interfacial area), which has poorer oxygen mass transfer capabilities.

The power draw characteristics of an impeller are given by the dimensionless power number, $N_P$, which is a function of Reynolds number ($N_{RE}$), Froudes number ($N_{FR}$, for unbaffled vessels) and all geometric ratios (e.g. (T/D), (H/T) etc.). Empirically,

\[
N_P = \text{Constant} \cdot (N_{RE})^a \cdot (N_{FR})^b \cdot \text{ (geometric ratio, gr )}^c \cdot \text{ (gr )}^d \text{, etc.} \tag{1.29}
\]

which, for geometric similarity and full baffling, reduces to,

\[
N_P = \text{Constant} \times N_{RE}^a \tag{1.30}
\]
A log-log plot of \( N_p \) vs. \( N_{RE} \) shows \( N_p \) to be constant under turbulent conditions (\( N_{RE} > 10^4 \)) in a fully baffled vessel (Rushton et al., 1950), thus,

Turbulent power number, \( N_p = \frac{P}{\rho \cdot N^3 \cdot D^3} = \text{Constant} \)  \hspace{1cm} (1.31)

The power number is a ratio of external force exerted by the impeller to inertial force exerted by the liquid. This may also be considered as a pressure coefficient,

\[
\frac{\Delta p}{\rho \cdot v^2} = \frac{\text{pressure difference causing flow}}{\text{inertial force}} \hspace{1cm} (1.32)
\]

(\( \Delta p \) is related to the power consumption: and \( v = ND \), is a measure of the tip speed) and as a drag coefficient (\( C_D \)) or friction factor,

\[
C_D = \frac{F_D \cdot g}{(\rho \cdot v^2 / 2 \cdot A')} \hspace{1cm} (1.33)
\]

As, \( v \propto ND \), \( A' \propto D^2 \) and \( P \propto N \cdot F_D \cdot D \), substitution yields the familiar \( N_p \) equation. Bearing the latter relationship in mind, the more streamlined an impeller is the lower is its \( N_p \).

### 1.2.2.3 Determination of circulation and mixing times in STRs and scale up effects

The circulation time, \( t_c \), may be described by the average time required for a fluid element to complete one circulation around the tank or a circulation loop. Various methods may be used for determining this value experimentally, the response of a conductivity probe to an ionic tracer is a fairly common one.

Holmes et al. (1964) studied \( t_c \) and the pumping capacity in a turbine stirred and baffled tank, as these parameters are indicative of the convective flow. Their definition of pumping capacity as (\( \nu_r \) the radial flow velocity averaged over \( I_b \) and the impeller radius): \[
Q_i = \pi \cdot D \cdot I_b \cdot \nu_r \hspace{1cm} (1.34)
\]
reduces to the familiar form of Equation 1.22 by assuming \( u_r \) to be proportional to the blade tip speed, \( \pi ND \), and indicates clear increase in pumping capacity with scale (increased \( D \) and \( I_h \)). Holmes et al. (1964) point out that \( u_r \) at any radius from the impeller, is proportional to \( 1/\text{radius} \), so the pumping action decays as the discharge stream recedes from the impeller: this is the effect of turbulence. They used the following description of \( t_c \):

\[
tc, \text{ the residence time in a circulation loop averaged over all the streamlines } = \frac{\text{average circulation loop length}}{\text{average loop velocities}}
\]  

(1.35)

In the turbulent region (constant \( N_p \) zone) \( t_c \) is proportional to \( 1/N \) and,

\[
t_c \approx 1/N\left( \frac{T}{D} \right)^2
\]  

(1.36)

which they used to define a dimensionless circulation time group, \( N_{tc} \), as:

\[
N_{tc} = N \times t_c \left( \frac{D}{T} \right)^2 = constant
\]  

(1.37)

The mixing time, \( t_m \) (time required to homogenise the tracer), in various vessel sizes was estimated by following the conductivity of the liquid upon addition of an ionic tracer. \( t_m \) was estimated quite accurately from the conductivity trace, by observing the number of peaks until homogenisation: this took five circulations in the equipment used, giving:

\[
t_m = 5t_c
\]  

(1.38)

Their experiments used a liquid depth equivalent to \( T \) with the impeller at mid depth, Uhl and Gray (1986) point out that a turbine with a clearance of \( T/3 \) gives the fastest mixing by virtue of greater interference between the upper and lower circulation zones produced by a such an impeller, and this may modify Equation 1.38.

Work on the same system (Voncken et al., 1964) to quantify the dispersion of the tracer during circulation, which is dependent on molecular diffusion, indicated
increased D/T ratio to improve dispersion also. This is manifested by a reduction in the Bodenstein number, $N_B$, ($N_B = \frac{\nu_{\text{mean}} \times l_c}{\mathcal{D}}$; $l_c$ is the characteristic length-circulation loop length in this case-and $\mathcal{D}$ the dispersion coefficient). Vonken et al. (1964) point out that time is the important factor in dispersion and the majority of dispersion occurs in the circulation loops rather than in the impeller zone; notably dispersion is independent of $N$.

Joshi et al. (1982) suggest that $t_c$ may be estimated by determining the length of the longest circulation loop, as illustrated below, and dividing by the average circulation loop velocity.

![Marine impeller](image1.png)

![Disk tubine](image2.png)

**Figure 1.4:** Typical circulation loops in agitated vessels.

The mixing time follows by assuming that 4-5 circulations are required for complete mixing. The estimates of Pandit and Joshi (1983) are good but are more accurate for larger vessels, where the ratio of flow to turbulence to is greater. Estimates for pitched blade turbines were unreliable due to strong secondary flow patterns. The disparity between circulation loop length (linear dimensions) and loop velocities on scale-up will obviously lengthen circulation and mixing times.

Sparging air into agitated vessels operating in the turbulent regime leads to increased blend times (Einsele and Finn, 1980). The introduction of air reduces the bulk density of the broth hence power draw (Michel and Miller, 1962) and consequently the pumping capacity; additionally the impeller is streamlined by the gas cavities behind the blades. Einsele and Finn (1980) also indicated unfavourable interactions between the gas bubbles and turbulent eddies. The sensitivity of larger equipment to sparging may be higher because their mixing behaviour is strongly dependent on pumping action,
it is also worth noting that sparging at low stirrer speeds may improve mixing (Pandit and Joshi, 1983); sparger type can have an influence here as well. This probably corresponds to the region where $k_f a$ is gas flow controlled (Section 1.2.1.4: Other factors).

As intimated in the section on mass transfer (Section 1.2.1.4), the media quality usually suffers with scale, and if this leads to coalescence inhibition (e.g. increased viscosity or reduced surface tension) then $t_m$ is increased, again by a reduction in power due to reduced bulk density (increased holdup).

**Considerations for mixing times**

Hansford and Humphery (1966) demonstrated the importance of the degree of mixing for continuous bakers yeast fermentation yield. They were able to show that poor mixing of the growth limiting nutrient reduced the yield at all dilution rates and that low dilution rates, where the proportion of the substrate used for maintenance is high, further reduced the yield. The 5L vessel that they used would be regarded as having a small $t_m$; evidently the utilisation of glucose is very fast and the cells in a region of high substrate immediately absorb it, leading to starvation elsewhere. The region in which the growth limiting nutrient concentration is exceeded is commonly called the feed zone.

Feed zone effects were investigated by Namdev et al. (1992) with aerobic fed batch bakers yeast fermentations in a 1L fermenter with long external circulation loop. The feed was injected intermittently into the beginning of the recycle loop; loop residence times were varied between 0.5-12 minutes. They reported little effects for reasonable recirculation rates but suggested that simultaneous oxygen limitation would have been a better model for a production vessel.

The existence of a feed zone effect ultimately depends on the response time of the organism to high and low nutrient concentrations compared to the residence time within the a high nutrient zone. The response period is illustrated schematically below:

```
  Residence time in the feed zone  t_s  t_f  Period
                                     A
  Response time to high [nutrient]  t_1  t_2
                                     B
  Response time to low [nutrient]   t_3  t_4
                                     C
  Response period of the organism  t_5  t_6
                                     D
```
If period B is longer than A then no response is observed. The feed zone effect may also depend on the concentration and volume of the feed added; a high viscosity feed and large volume would require more time to dissipate, in effect increasing the residence time of the microorganism within the feed zone.

Consideration of the following two comments is bound to shed light on the problem of scale up and mixing. Uhl and Gray (1966) indicate $t_m$ to have the following functional relationship,

$$t_m = f \{ N_{RE}, N_{FR}, N_{SC}, \Delta p/\rho \}$$  \hspace{1cm} (1.39)

In light of which equal mixing performance as scale ($D$) increases for geometrically similar vessels, will only be achieved if density or viscosity increase; as the increase of either of these parameters is usually not significant $t_m$ is bound to increase with scale. Oldshue (1983) and Penny & Tatterson (1983) point out that to maintain $t_m$ as tank volume increases, the P/V must increase with the square of the linear dimensions, which is not usually practical, again leading to $t_m$ increasing with scale.

### 1.2.2.4 Considerations for gas liquid mass transfer

Oldshue (1983) suggests that in situations where the majority of the mass transfer is in the impeller zone, the DOT may approach saturation here and will not be subject to further improvement by increased power consumption. However, the opposite is possible because more liquid will be drawn through the high mass transfer region by increased stirrer speed. Such considerations may be hypothetical because the common impeller D/T ratios used in fermenters ($D/T = 0.35\text{ to }0.45$) are a compromise between blending, shear damage and heat and mass transfer characteristics: as the optimum ratio for gas liquid mass transfer is between 0.15-0.25, most fermenters are usually under par with respect to mass transfer capability.

It is possible that sparger design becomes of increasing importance on scale-up of STRs. At small scale the stirrers usually operate at high speeds and bubble size is controlled by the stirrer, the design of the sparger being less important. As scale increases this trend may be reversed with the sparger design becoming increasingly important. Though spargers producing fine bubbles may be preferred at the larger scale
from the point of view of mass transfer, these may prove operationally troublesome, being more susceptible to blockage with the use of lower grade media (Section 1.2.1.4).

1.2.2.5 Problems with the scale-up of airlift reactors

Mixing in airlift reactors involves liquid circulation caused by density differences in the broth (buoyancy); the rising bubbles leave turbulent eddies in their wake. The tendency in these vessels is to develop axial gradients in properties as the H/T ratios encountered are large (typically 3-10:1). For example, Pollard et al. (1996) found axial DOT gradients during the fermentation of *S. cerevisiae* in a 0.25m³ pilot scale ALR. This is a relatively low volume vessel, so the extent of such gradients may be expected to increase with scale. Scale-up may additionally give radial gradients as T increases.

The flow patterns in plain airlifts (those without draft tubes: also called "bubble columns") are dependent on the sparger location. Liquid rises above the sparger and descends elsewhere, with intermixing at the boundaries of the flow zones. When internals are present (e.g. draft tubes and vertical baffles that split the vessel into half) these lead to well defined circulating flows, again fluid rising above the point of sparging and descending elsewhere.

Airlift reactors have some particular scale associated problems, for example the "wall effects" encountered in narrow laboratory columns that result in gas slugging at high gas flow rates (Q). Large diameter columns do not enter this regime and higher Q values are possible. Also for this reason, the variation in the DOT profile between draft tube and annulus positions of the sparger, noted by Pollard et al. (1996), may not be as dramatic for a large scale ALR. The difficulty in scaling up laboratory results is that Q values giving turbulence in the laboratory may only give laminar flow in the production vessel, hence reduced transport rates: liquid near the walls may be practically stationary especially if the broth has a yield stress. Though the extra sparging capacity of the larger scale appears to be advantageous, its benefits will not be realised if the plant cannot cope with higher than expected productivity.

Mixing times will also be affected by scale. Pandit and Joshi’s extensive work on mixing (1983) indicates clear scale-up problems for airlifts, as *t_m* increases with increasing H/T, and generally with T at constant H/T. Sukan and Vardar Sukan (1987)
illustrated the sensitivity of $t_{m}$ to draft tube geometry in an airlift (e.g. draft tube height, diameter, clearance from base and the height of liquid above). Rosseau and Bu'lock (1980) indicated that a draft tube improves $t_{m}$ and that minimum $t_{m}$ is obtained for equal flow areas in the draft tube and annulus. They also suggested increased liquid height to be detrimental: $t_{m}$ is approximately proportionate to $H^{1.7}$; a ten fold increase in height giving a 50 fold increase in $t_{m}$. Pollard et al. (1996) suggested an optimum liquid height for minimum $t_{m}$. Fed batch processes in airlifts may experience a considerable lengthening of $t_{m}$ by the end of the fermentation because liquid height increases, a feature which may be to a lesser extent in STRs. The reported effects of gas rate ($u_{s}$) on $t_{m}$ are variable, the consensus appears to be an optimal $u_{s}$ for minimum $t_{m}$; this probably corresponds to a transition from homogenous to turbulent flow regimes (Rosseau and Bu'lock, 1980 & Sukan and Vardar Sukan 1987). Pandit and Joshi (1983) suggest high $u_{s}$ to lengthen $t_{m}$ by virtue of an increase in dispersion height; a similar effect to increasing H/T.

Airlifts display a transition from sparger control to liquid phase hydrodynamics control of bubble size as the column height increases (Walters and Blanch, 1983); thus small scale equipment may fail to indicate the relevance of hydrodynamics within larger vessels and may lead investigations toward the control of bubble column behaviour by sparger design, the results of which are not scaleable. Thus the observations of Guy et al. (1986) of minimal dependence of holdup on viscosity with Newtonian and non-Newtonian fluids in a 0.9m high column, are likely to be applicable only to similar size equipment, as viscosity would be expected to have an impact on dispersion stability, hence interfacial area and holdup in large scale airlifts.

1.2.2.6 Considerations for cell quality

Heterogeneity within the fermenter increases with scale-up as circulation times, mixing times and the minimum eddy size increase. The maximum shear rate may also increase with scale (Oldshue, 1983). The main cell quality considerations from the momentum transfer point, are whether the cells will suffer any damage or alteration to their morphology (either wall structure or cell shape\(^1\)), due to the change in patterns of

\(^1\) In the experimental work in this thesis, morphology refers to the cell shape.
shear in the environment. The magnitude (maximum shear) and degree of exposure (circulation rate, Makagiansar et al., 1993) to shear may both be contributing factors to the possible cell quality changes mentioned. Cell damage on scale-up will mean the fermentation is not viable at the desired scale. Morphological changes could have complex consequences for the fermentation and overall process. If the cells increase their wall strength to adapt to the higher maximum shear rates (or if the environment selects for more resistant cells) then cell breakage will be harder; the benefit or otherwise of this depends on the product location. If the product is internal then harvesting it requires more energy if, for example, cell homogenisation is used during downstream processing. If the product is external then the downstream processes may benefit, as there should be less contamination of the product from cellular components. If the cell shape of a filamentous organism changes there may be various effects. The cells may become shorter and reduce productivity (Martin and Bushell, 1996) or the broth viscosity may decrease (Section 1.5.3) thereby improving mass and heat transfer. Alternatively, the cells may change growth form, from diffuse to pelleted, improving mass and heat transfer in the broth but reducing these in the region of the cells. Furthermore, fastidious cells may dislike heterogeneity and therefore alter metabolism and hence cell quality on scale-up.
1.2.3 Heat transfer

"Heat" is important at three phases during fermentations, namely during heating up for sterilisation, cooling down after sterilisation and temperature control during fermentation. Heat production during fermentation arises from the microbial metabolic activity and the dissipation of momentum introduced by agitation (in STRs) and gassing. Scale-up results in a relative increase in the former process. Heat is lost from the fermenter via the cooling surfaces (jacket and coils) and to the incoming air stream, which is a relatively minor effect (Brain and Mann, 1989). Batch and fed batch fermentations have a time dependent increase in heat transfer requirement, due to the increase in cell mass, until a stationary phase is reached.

Heat transfer for fermentations is rather distinct from chemical process industries in that the control temperature is very close to ambient conditions (25-37°C), and therefore the temperature of the cooling agent (e.g. water, chilled water and glycol); consequently the heat transfer driving force, the temperature gradient, is low. Additionally, the temperature has to be controlled within narrow limits, typically plus or minus one degree centigrade of the desired temperature. While scale-up may be expected to result in temperature gradients and difficulties in heat transfer, at least on a theoretical basis (Ashley, 1994 and Charles, 1985(b)), there have been reports that suggest the contrary. Oosterhuis et al. (1983) found heat production to be removed by heat transfer in a 25m³ vessel used for gluconic acid production; similar findings were reported by Sweere et al. (1987) for bakers yeast production in a 150m³ bubble column fermenter.

Heat transfer processes change with scale as depicted in table 1.3. Though heat transfer requirements may be difficult to predict as they depend on media composition, growth rate and agitation and aeration conditions, deficiencies are unlikely to arise and limit the process. Scale-up usually results in a shift in requirement, from one of heating to one of cooling, using a progressively cooler medium, and a need to introduce internal or external cooling elements in order to provide the necessary surface area for heat transfer. Internal cooling coils are usually employed to provide the extra heat transfer area as temperature gradients are more likely with external equipment and maintenance of sterility becomes more difficult. Thus the majority of scale-up tends to give mixing problems rather than heat transfer problems, as the internal cooling elements disrupt the
fluid flow; the exceptions are for highly viscous broths where heat transfer will be a problem (Brain and Mann, 1989).

### Table 1.3: The picture of heat transfer in relation to scale

<table>
<thead>
<tr>
<th>Feature</th>
<th>Small scale</th>
<th>Large scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilisation method</td>
<td>Batch</td>
<td>Continuous</td>
</tr>
<tr>
<td>Cooling method</td>
<td>Water: no special cooling</td>
<td>Chilled water or glycol</td>
</tr>
<tr>
<td>Heat transfer surface</td>
<td>Internal coil or cooling finger for glass vessels, or fermenter jacket</td>
<td>Fermenter jacket, internal coils and external heat exchangers</td>
</tr>
<tr>
<td>Overall</td>
<td>Heat loss&gt;heat production Heating required</td>
<td>Heat production&gt;Heat loss Cooling required</td>
</tr>
</tbody>
</table>

(Charles 1985a)

Most of the work on heat transfer in STRs has ended with correlations in the form of a conventional forced convection situations (for liquid systems):

\[
N_{NU} = \text{Constant} \times N_{RE}^a \times N_{PR}^b \times \mu_r^d \quad (\text{Radez et al., 1991})
\] (1.40)

where \( \mu_r \) represents the viscosity ratio of the bulk liquid to that at the wall, and where the constant is vessel geometry dependent. This arises from a dimensional analysis of the factors affecting the film heat transfer coefficient, \( h \) (where \( k \) is the thermal conductivity);

\[
h = f \{ \rho, \mu_{\text{bulk}}, \mu_{\text{wall}}, \rho_p, k, N, D, T, \text{other geometric dimensions & arrangements} \}
\] (1.41)

thus,

\[
N_{NU} = f \{ N_{RE}, N_{PR}, \mu_r, \text{geometric arrangements} \}
\] (1.42)

There are conflicting reports about the effects of sparging, De Maerteleire (1978) and Verma (1989) suggest that sparging initially improves the heat transfer coefficient because it improves turbulence and the erosion of the laminar sub-layer, though very high gas rates can be detrimental as the gas phase has a much lower heat transfer coefficient than the liquid. Radez et al. (1991) indicates the opposite effect except near the flooding limit of the impeller. Overall heat transfer is improved by conditions that lead to greater fluid flow (Desplanches, 1983).
As with other transport processes heat transfer difficulties are most likely to arise where non-Newtonian fluids and high viscosities are encountered, though no reported problems of temperature control in these fermentations have been found. In these cases the laminar fluid flow region may be large near the wall and other heat transfer surfaces, and the broth may even be stationary. Heat transfer through these layers will be by conduction only; according to the temperature profile illustrated in figure 1.5, a relatively large volume of liquid may be below the mainstream temperature. As the boundary layer liquid is cooler it will also have a higher viscosity and it may have a relatively low gas holdup. According to Deckwer (1980) gas liquid dispersions have better process side heat transfer coefficients than liquid systems as the gas bubbles help to erode the laminar layer; therefore the higher viscosity and lower gas movement within the boundary layer will further compound the problem of heat transfer. The cell quality consequences of this depend on the reaction of the microorganism to the low temperature. From the process point of view the problem is likely to be an economic one, the increased difficulty in heat transfer having to be addressed by a lower temperature of coolant or a higher coolant flow rate. The minimum temperature encountered by the organism will decrease with scale-up, in line with the decreasing temperature of the cooling liquid used, though for most fermentations the contact time may be too short for this to have any effect, the most likely consequences are a reduction in the rates of metabolic processes. There is also the possibility that the poorer flow at the heat transfer surfaces (higher

**COLD SIDE**

**PROCESS / BROTH SIDE**

---

**Figure 1.5**: Temperature profile during fermentation of high viscosity broth; broth cooling shown.
viscosity) may allow sufficient time for some microorganisms to attach to the surface. The attachment would make harvesting of intracellular products more difficult, and the operation of continuous cultures more problematic.

**Medium sterilisation**

Sterilisation is concerned with the destruction of microorganisms and the temperature and pressure required for this operation reflect this objective (e.g. 121°C, 1 bar pressure and 15 minutes under these conditions). The mechanisms leading to cell death also result in the destruction of biological components such as vitamins and proteins and the alteration of relatively simple molecules in the broth such as glucose and amino acids (Maillard reaction). The extent of these degradations is dictated by the sterilisation temperature and time, the result of any sterilisation regime is a particular quality of medium: a particular concentration of substrates and degradation products (Deindoerfer and Humphery, 1961).

At small scale sterilisation is usually done batchwise. As the scale increases the time required to reach sterilisation temperature, the holding period and cooling down time increase; a 10 fold increase in scale requires the sterilisation criterion to be increased by a factor of 2-3 to achieve the same degree of sterility (Deindoerfer and Humphery, 1961). For very large volumes it is necessary to operate a continuous sterilisation process, this may employ exposure to a higher temperature over a shorter period and shorter heating and cooling times to avoid excessive substrate degradation: this may even improve the nutrient quality over a batch sterilisation process. The net effect of scale on the same initial medium is a different quality at the end of sterilisation. Therefore, experiments designed to elucidate the effect of scale may be affected by variation in sterilisation result even if the same medium is used at both scales. This problem may be overcome by using industrially sterilised medium in the small scale vessels used for scale-down studies (e.g. Bosnjak et al., 1985 & Ettlter, 1990).

Deindoerfer and Humphery (1961) suggest that scale up of sterilisation should be based on an assessment of the degree of cell and nutrient destruction under different sterilisation conditions, to define limits within which changes in sterilisation time and duration will affect the outcome of the process. Their procedure could be used in reverse to estimate the sterilisation conditions required for an accurate scale-down study.
1.2.4 Physical constraints on the minimum size of experimental equipment

The two principle objectives of the scale-down approach, acquisition of accurate scale-up data on a small scale and a reduction in the cost of experimentation, are both limited by physical constraints on the minimum size of equipment that can effectively be used.

Experimentation costs can be split between capital and operating costs as illustrated in figure 1.6:

**CAPITAL COSTS**

- Vessel (shaker or fermenter)
- Instrumentation
- Assay hardware e.g. spectrophotometer / homogeniser and centrifuge

**OPERATING COSTS**

- Media
- Power
- Assay materials
- Staffing

*Figure 1.6: Simplified breakdown of experimentation costs*

Instrumentation, assay hardware and assay materials costs remain largely the same whatever the scale of operation: therefore scale reduction can only yield cost savings in the vessel construction (size) hence media and power areas and staffing (Lowenstien, 1985). The question of physical constraints is, “What is the minimum size of laboratory equipment that will provide reasonable data upon which scale-up can be based”? That is the minimum size of equipment that will give controllable and flexible behaviour whilst supplying reliable data; this depends on certain physical constraints.

The first factor that decides the minimum possible size that can be used is the degree of sampling that is required during the experiment. An experiment where only the final product titre is required can have a smaller scale than one that requires regular removal of material for assays. The fermentation volume partly depends on the volumes required to conduct the assays (though an enzyme assay may require less than one millilitre of broth, the disruption of the cells in the broth may require tens of millilitres), and the minimum necessary volume at the end of the fermentation (because scale-down models often reproduce mixing regimes the final volume at the end of the fermentation must still be sufficient to maintain these-the sampling must not alter the regime within the
apparatus). Though cost might drive experimentation size down, if the broth volume is increased the fractional loss due to sampling rapidly becomes insignificant, to a point where its effects can be disregarded: therefore sample requirement drives experimentation size up. Alternatively the volume loss due to sampling must be replaced by equal additions of liquid throughout the experiment, to maintain the liquid height to diameter ratio and therefore the hydrodynamics.

Apart from volume loss by sampling, water is also lost by evaporation. Wang (1979) found that the fractional loss of water by evaporation increased as the initial fermentation volume decreased; as much as 20% of the liquid was lost from an initial volume of 50ml in a 300ml shake flask, compared to 16% from a 200L fermenter operating at the same temperature. The loss from an actual fermentation is usually lower than this because a condenser is used to cool the exit air stream. Evaporative losses drive experimentation size up, or at least leads to much greater care in the control of evaporative loss, control of humidity in the environment of the equipment or greater cooling of the exhaust air for instance.

Large scale bubble columns are characterised by the relative independence of bubble characteristics and behaviour on the flow channel diameter, this situation is reversed if the diameter of column becomes very small. Therefore bubble column type experimental apparatus has a minimum practical diameter hence minimum volume; Schoutens et al. (1986a,b,c) settled for 10-15 L in their scale down equipment. Generally the control of hydrodynamics becomes more difficult for very small equipment, parameters such as the mixing time may become to fast to model residence and circulation time distributions.

Probes have a finite size which is not significantly different between a large or small probe, the introduction of a probe affects the hydrodynamics to a greater extent in lower volume fermentations. The influence on shear and flow patterns in the broth may be difficult to determine and include in the design of small scale equipment, which suggests operation at a scale at which the influence of probes is slight.
1.3 The Scale-Up Approach to Scale Translation

This section will summarise the different methods of process scale-up that can be used and are reported in the literature. It is intended to provide an overview as many detailed reviews on this subject are already available (e.g. Atkinson and Marituna, 1983; Charles, 1985(b); Kossen and Oosterhuis, 1985; Kossen, 1992).

1.3.1 Theoretical methods

Fundamental method

This method is a process of design from first principles and requires the micro-balances for mass, heat and momentum transfer to be set up; the difficulty in so doing are three fold. First of all these need to be three dimensional in nature and involve complicated boundary conditions. Secondly, the solutions to the balance equations are linked, for example the flow components required for the mass and heat balances are derived from the momentum balance. Finally, the momentum balance itself is difficult to generate for anything other than a single phase which is stagnant or flowing laminarly in a vessel of simple geometry. This lack of knowledge about three dimensional hydrodynamics and the impossibility of solving the micro-momentum balance, mean that the fundamental method can not be applied to bioreactor design.

Semi-fundamental method

This method overcomes the drawbacks of the fundamental method by using simplified flow models in place of a solution for the micro-momentum balance. However, the flow models are usually based on bulk conditions and ignore important regions of the bioreactor, such as near the impellers or fluid boundaries (e.g. walls or coils). The flow models fall into four categories,
- plug flow,
- plug flow with dispersion (diffusion of fluid elements),
- well mixed (single tank or tanks in series), and
- combinations of the above.

The flow models produce transport terms that can be used in micro-mass balances which can be solved numerically or sometimes analytically. Because these models are often
based on data from vessels of 10-100L their application to large vessels is risky. This is one of the most widespread methods for bioreactor design (the other being rules of thumb) but it only predicts the right order of magnitude.

**Dimensional analysis**

This method involves the identification of salient parameters governing reactor performance and then reduction of the number of individual parameters by grouping them together into ratios of forces or time constants-dimensionless numbers (DN). Essentially the important variables (e.g. power input and mass transfer coefficient) are related to the system geometry, physical properties (e.g. \( \mu, \rho \)) and operating parameters (e.g. stirrer speed). Testing the influence of a number of the parameters then only requires the value of the DN containing them to be altered rather than each parameter being altered individually.

The basic ideal of this scale-up method is similarity between scales. Scale-up involves keeping the DN constant thereby retaining the relative importance of the mechanisms involved. However, only the ratios rather than the absolute values of the mechanisms are kept constant and for processes like shear damage the latter is important.

Kossen and Oosterhuis (1985) identify four limitations to this method:

1) Usually different DN change at different rates with scale therefore it becomes necessary to select the most important parameters (Regime analysis, RA) and maintain constant values for these at the expense of others. If the regime changes on scale-up then the dimensional analysis becomes void.

2) Having identified a scale-up basis by RA it may be technically and economically impossible to maintain constant values for the chosen DN.

3) Similarity between scales is not the rule for all mechanisms involved in bioreactor performance. For example, bubble coalescence behaviour and bubble size may only be dependent on vessel dimensions at the small scale, and remain relatively constant thereafter, hence the necessity for geometric similarity is not met.

4) The selection of parameters is very critical in defining the mechanisms governing reactor performance, these may not always be obvious and can be arbitrary.

If too many variables are included in the analysis, too many DN have to be kept constant in the laboratory and plant scale. However, if an important parameter is neglected a DN will be missing from the experimentation; this could lead to the situation
where the system is only found not to work when it is actually scaled up. Fortunately excess DN can be eliminated by RA and important missing DN may be noticed because the system might not be easily described by the chosen groups during the experimentation.

Experimentally the small and large scale vessels require geometric similarity to reproduce the same flow profiles, this is often expensive and difficult to achieve; small vessels usually have flat bottoms and sometimes single impellers whereas large vessels have dished ends and most often multiple impellers and larger aspect ratios.

Dimensional analysis works reasonably well for systems only involving fluid flow, where \( N_{RE} \) and \( N_{FR} \) may be the only DN of import, but when mass and heat transfer are also involved, such as in a bioreactor, then four to five DN have to be kept constant between model and full scale, which is usually not possible.

Even if flow is the only important parameter, modelling of a viscous system may be complicated because equality of \( N_{RE} \) may only be achieved by using model fluids of different rheology in different scales of experimental vessel. Hence, a particular medium and fermentation system can not be evaluated in a scale model: i.e. only the flow properties can be tested in the model by dimensional analysis when a viscous system is studied.

**Regime analysis**

Kossen and Oosterhuis (1985) use “regime”, to describe the dominance of a particular mechanism in the performance of a system. Regime analysis is used to simplify the conditions required in order to study a system, by identifying the rate determining mechanism(s); if used in combination with DA it leads to a reduction in the number of DN that have to be kept constant between scales.

The regime of a system may be described as pure (only one mechanism dominates the performance) or mixed (two or more mechanisms have a comparable influence on the system), and can depend on kinetics (growth and product formation), transport processes or both. The process of regime analysis must determine the number of important regimes, which ones they are and whether the regime changes on scale-up. This last point is probably the main reason why laboratory equipment does not reflect large scale behaviour and the origin of many scale-up problems, because the majority of small scale apparatus have a kinetic regime, whereas production scale vessels are dominated by transport process.
Methods of regime analysis

1) Experimental methods:

The procedure involves varying the value of a parameter that has a pronounced influence on one of the mechanisms of the system. Typical parameters are the following,

i) Velocity: This is easily varied by a change of stirrer speed (STR) or gas flow rate (ALR) and influences transport processes through mixing (momentum transfer), dispersion (shear) and diffusive film coefficients. The importance of transport processes on the regime of a system are highlighted by varying this parameter.

ii) Concentration: Also easily achieved and may help to determine the importance of kinetics in the system: e.g. whether the microorganism is saturated with respect to a nutrient, and if another one is limiting.

iii) Particle size: A change in conversion rate due to a change in particle size is an indication of the influence of internal diffusion in the particle on the conversion process. Different particle sizes may be obtained from different sieve fractions or by varying fungal pellet sizes through shear, addition of viscous polymers or a change in the quantity of inoculum.

iv) Temperature: A change in temperature usually influences kinetics to a greater extent than transport processes; if a temperature change only has a small influence on the conversion rate then transport processes are probably dominating, while a large change indicates that kinetics are dominating (though here it is more difficult to distinguish a mixed regime split between kinetics and transport processes).

Unfortunately, though experimental methods may distinguish between the dominance of transport processes or kinetics they do not necessarily indicate what particular mechanism(s) is the important one. This type of regime analysis is only useful for optimisation rather than design studies because the full scale vessel is required for the regime analysis.
2) **Theoretical methods:**

i) Numerical methods: The balance equations are solved numerically and the answers compared by parameter sensitivity analysis. However, as indicated earlier these balance equations are often difficult to generate.

ii) Analytical method: A comparison of time constants (characteristic times, CT) for transfer, conversion, forces or pressures is the usual method for biological systems; dimensionless groups may also be viewed as time constants (e.g. the Peclet number which may be written as \( \frac{(\text{velocity / length})/(\text{diffusion coefficient / length}^2)}{= (\text{m}^2/\text{m})/(\text{m}^2/\text{m})} \)). The CT is the time representative of the progress of a particular mechanism, with a short time indicating a fast progress. An approximate answer can be obtained. Examples of typical CT are given in table 1.4.

<table>
<thead>
<tr>
<th>Table 1.4: Examples of characteristic times used in regime analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass transfer</td>
</tr>
<tr>
<td>Mixing time in STR</td>
</tr>
<tr>
<td>( 4x_t_c ), ( t_c ), circulation time = volume / circulating flow</td>
</tr>
<tr>
<td>Circulating flow = 2( pumping capacity of the stirrer)</td>
</tr>
<tr>
<td>Pumping capacity = 1.3N.D(^3 )</td>
</tr>
<tr>
<td>Mixing time in ALR</td>
</tr>
<tr>
<td>( \approx H^2 / D ), ( D ), dispersion coefficient = 0.35(( \nu \cdot g \cdot t^4 ))(^{1/3} )</td>
</tr>
<tr>
<td>Conversion times</td>
</tr>
<tr>
<td>Concentration / Reaction rate</td>
</tr>
<tr>
<td>Diffusion time</td>
</tr>
<tr>
<td>Length(^2 ) / Diffusion coefficient</td>
</tr>
<tr>
<td>Residence time</td>
</tr>
<tr>
<td>Volume / Volumetric flow or Length/velocity</td>
</tr>
</tbody>
</table>

The theoretical analytical method of RA can also be applied to pressure forces, stresses and tensions,

Shear stress (laminar) = Velocity / Dynamic viscosity \( N/m^2 \)

Shear stress (turbulent) = Density \( \times \) Velocity\(^2 \) \( N/m^2 \)

Buoyancy = \( \Delta \rho \cdot g \cdot \text{Length} \) \( N/m^2 \)

Yield stress = \( \tau_o \) \( N/m^2 \)

Surface tension = \( \sigma \) \( N/m \)

(Kossen and Oosterhuis, 1985)
As with $DN$, as characteristic times are ratios of properties their value indicates the importance of one mechanism over another; e.g. a low $N_{PE}$ indicates the dominance of diffusive transport over convective transport, the value of a $DN$ is in itself a regime analysis. Similarly a comparison of the laminar and turbulent stress can say which dominates. If the turbulent shear stress is greater than $\tau_o$, then $\tau_o$ is not important. The comparison of time constants is the favoured method of regime analysis in the scale-down approach to scale translation (Oosterhuis et al., 1985; Schoutens et al., 1986a; Sweere et al., 1987, 1988b, 1989; Fransden et al., 1993)

1.3.2 Empirical methods

Rules of thumb

This method is where experimental correlations have their home. Often small or intermediate scale experiments provide the correlations relating important process parameters to operating variables (e.g. the relationship of $k_a$ to $P/V$ and $\nu_*$: Equations 1.9 & 1.10). These empirical relationships are then used to predict the required operating conditions on a larger scale necessary to achieve a particular value of the process parameter that they relate to.

The procedure is to take the most important property governing the reactors performance (which can be decided upon by experience or even arbitrarily) and decide to maintain conditions which will allow a minimum or maximum condition for this property to be maintained or avoided. For example if oxygen transfer is the limiting factor the scale-up rule of thumb may be to maintain a constant value for dissolved oxygen, $k_\alpha$ or $P/V$ (as this is related to the $k_\alpha$). If shear damage is decided upon as the ruling mechanism the scale-up criterion may be to maintain a constant impeller tip speed on scale-up (which is not necessarily a sound procedure because an increase in impeller diameter results in an increase in maximum shear rate: Section 1.2.2.1).

The correlations for different process variables may be used to identify the effects of scaling up on different scale-up bases to determine the most feasible scale-up criterion, or at least the effects of a particular scale-up criterion on the fermenter performance and operating conditions. This is done by constructing a table of "scale-up criterion" verses "value of the criterion at a particular scale", (geometrically similar systems of two scales are compared). An example of such a table is given by Kossen (1992) for the scale-up
of a fermentation from 10-10,000L (Table 1.5). Such tables tend to highlight the
drawback of this method because parameters other than the chosen scale-up basis often
become adversely affected; as a consequence this scale-up procedure recommends
additional precautions at the larger scale, such as the use of multiple feed points because
scale-up at constant P/V leads to a lengthening of the mixing time.

<table>
<thead>
<tr>
<th>Scale-up criterion</th>
<th>Value or criterion at 10,000L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Equal P/V</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Equal N</td>
<td>$10^5$</td>
</tr>
<tr>
<td>Equal tip speed, ND</td>
<td>$10^2$</td>
</tr>
<tr>
<td>Equal $N_{RE}$</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Though the correct order of magnitude of a process variable can again be
predicted the pitfall of the rules of thumb method is that it is subject to the variation of
transport processes and transport related parameters with scale, as described in section
1.2 of this introduction. That is to say that the exponents in the correlations are often
scale dependent (Bartholomew, 1960 and Chandrasekharan & Calderbank, 1981). Further
problems have also been highlighted in the literature. Griot et al., (1988) found
the power input to be very sensitive to the impeller geometry in the scale-up of an
oxygen sensitive culture, therefore similarity between scales was crucial for the correct
prediction of operating conditions and performance. Fuchs et al., (1971) suggest that
the correlations used for the scale-up prediction of power input necessary to maintain
constant $k_a$, should not be derived from a vessel of less than 200L volume because the
contribution to $k_a$ from surface aeration was substantial below this volume. Mooymen
(1987) indicates that a mass transfer resistance other than the $k_a$ may be most
important to a system, especially if the critical dissolved oxygen concentration is above
zero (in which case the transport resistance labelled 4, in figure 1.2, may be the most
important). Importantly, the critical oxygen concentration may increase with the solids
content of the medium: hence scale, if a different medium quality is used at the large
scale (Section 1.2.1.4: Media components). Also the main mass transport resistance may change if scale-up results in a change in morphology of the organism (e.g. the resistance associated with diffusion through the cell aggregate, figure 1.2, may become the ruling mechanism if a filamentous microbes morphology changes from diffuse to pelleted on scale-up). Neither of the above effects could be predicted by the use of the traditional scale-up correlations. Charles (1985a) found different correlations available in the literature for the same process parameter, to give predictions that could vary by as much as 60%. In effect, the correlations can only be safely used in the equipment for which they were developed, and this method is sensitive to changes in regime that may result from scale-up.

The correlations that are used here may however, be useful for the design of scale-down equipment, which ideally is of a small scale, used in the scale-down approach to scale translation described in section 1.4.

**Trial and error**

Though this was the main method used for scale-up in the past, it has little value now, and is principally applied in process optimisation. The context in which the phrase ‘trial-and-error’ is used must be distinguished from the phrase ‘industrial trial’, while the former is a large scale fermentation in which the fermenter design and operating conditions have been “guessed”, the latter is such a fermentation in which the design and operating conditions are based on the results of a well organised scale-up strategy. The main drawback of this method are that the trials have to be performed at the production scale, which is an expensive process. The most likely scenario for a modern production process is that few trials will be performed during production, and it is more likely that an error might occur which, if noticed, could yield some useful information about the production process; generally it is unlikely to yield a process improvement. For bioreactors, trial errors are likely to result in an economic loss due to lower productivity, increase of fermentation time or production of a scrap batch.
1.4 The Scale-Down Approach to Scale Translation

The difficulty in scaling up bioreactors lies in the lack of publicly available information on transport phenomena within large scale equipment. While acquisition of further knowledge will doubtless prove useful in their scale-up, the expense and lack of suitably equipped facilities means that this will be a long time in coming. However, scale-up may be made less arduous by bearing in mind the production environment and capabilities when operating at laboratory scale. The lack of knowledge about microbial behaviour under transient conditions and large scale transport phenomena is often addressed by empiricism and extrapolation by the traditional methods of scale-up (Section 1.3); the scale-down approach attempts to bring strategy and methodology to small scale experimentation in order to rapidly obtain useful and reliable process data without the need for rigorous determination of the salient mechanisms (fundamental and semi-fundamental methods) and without the risk of applying rules-of-thumb and trial-and-error methods. The methodology may be applied to both the design of a new process (Section 1.4.2) or the modification and optimisation of an existing one (Section 1.4.3). The foregoing chapters of this introduction show that a standardly operated small vessel cannot be made to behave identically to one of a larger scale (e.g. Table 1.5). The scale-down methodology dispenses with exact similarity of scales, and instead aims to reproduce only the most important environmental conditions in the small scale model: the rate limiting mechanisms. This first requires a means of identifying the rate limiting mechanisms of the production scale (Section 1.4.1), and then the development of representative small scale models to reproduce these (Section 1.4.4). Therefore the environment of the fermenter used in the scale-up is representative of that which is expected to be encountered at the production scale.

1.4.1 Regime analysis in scale-down studies

Regime analysis based on characteristic times has been the main method for identifying the rate limiting mechanism that do, or are likely to, exist at large scale. The characteristic times are estimates of the relaxation times of the mechanisms operating within the fermenter: these need only be determined to the correct order of magnitude as
this is the level at which comparisons are made, and only those mechanisms with a similar order of magnitude will interact (Luyben, 1993). However, the order of magnitude for the liquid circulation time needs to be fairly accurate as the difference in the magnitude of mechanisms that are being compared to the mixing (circulation) is important in determining any scale-up problems. Typical characteristic times are those for circulation time and oxygen mass transfer time (1/kα). The characteristic times may either be determined from the existing equipment or from literature correlations: rules-of-thumb may also be useful. The regime analysis involves comparing the characteristic times to identify the predominant or rate limiting mechanism (pure regime) or possible interactions between different mechanisms (mixed regime). Kossen (1992) points out four factors that should be considered when deciding upon the influence of phenomena that are included in the regime analysis:

i) Whether the phenomena are “seen” by the microorganism.

ii) How the time constants of the events compare to the response times of the microorganisms.

iii) What the scales of these events are compared to the cells or cell aggregates.

iv) Whether there is a cumulative or memory effect.

The experiments that arise from the regime analysis attempt to keep the characteristic times of the ruling mechanisms constant in the scale-down equipment. There is a “problem” with the regime analysis and that is that the choice of mechanisms is critical in determining the correct regimes within the large scale vessel, and this is a matter of experience and understanding.

Two examples of regime analysis are presented from the scale-down literature. The first is that of gluconic acid production by *Glucobacter oxydans* (Oosterhuis et al., 1983 & 1985). In this investigation the characteristic times for transport and biological phenomena were determined for a 25m³ STR production vessel; these are given in table 1.6. Regime analysis based on the characteristic times indicates that:

i) Substrate consumption and growth have no effect as their order of magnitude does not match any other mechanism.

ii) Oxygen consumption and oxygen transfer time to the liquid phase have the same order of magnitude therefore limitations may occur. Additionally, te has the same order of magnitude as oxygen consumption so oxygen gradients may occur.

iii) Comparison of gas residence time and oxygen mass transfer time indicates that gas phase exhaustion will not occur.
iv) Heat production is matched by heat transfer and takes much longer than $t_e$ so there will be no temperature gradients.

\textbf{Table 1.6: Characteristic times for transport and biological phenomena in the large scale production of gluconic acid}

<table>
<thead>
<tr>
<th>Transport phenomena</th>
<th>Characteristic time, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen transfer (non-coalescing)</td>
<td>5.5</td>
</tr>
<tr>
<td>(coalescing)</td>
<td>11.2</td>
</tr>
<tr>
<td>Circulation time</td>
<td>12.3</td>
</tr>
<tr>
<td>Gas residence time</td>
<td>20.6</td>
</tr>
<tr>
<td>Oxygen transfer from gas bubble (non-coalescing)</td>
<td>290</td>
</tr>
<tr>
<td>(coalescing)</td>
<td>593</td>
</tr>
<tr>
<td>Heat transfer</td>
<td>330-650</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biological phenomena</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen consumption (zero order)</td>
<td>16</td>
</tr>
<tr>
<td>(first order)</td>
<td>0.7</td>
</tr>
<tr>
<td>Substrate consumption</td>
<td>55,000</td>
</tr>
<tr>
<td>Growth</td>
<td>12,000</td>
</tr>
<tr>
<td>Heat production</td>
<td>350</td>
</tr>
</tbody>
</table>

The scale-down step in this study, attempted to keep a constant characteristic time for $t_e$ and oxygen consumption. The second example is that of the fed batch baker's yeast production (Sweere et al., 1987a). A regime analysis conducted on a 120 m$^3$, working volume, bubble column fermenter which gave the characteristic times listed in table 1.7. The regime analysis reveals that:

i) Oxygen depletion will always occur (oxygen transfer to liquid phase is slower than oxygen consumption rate) and oxygen gradients will exist (the mixing time is large while oxygen transfer to the liquid is fast).

ii) Comparison of $t_e$ and the substrate addition rate shows that substrate gradients will occur and increase throughout the course of the fermentation.
iii) As the liquid feed is added to the vessel, the liquid height to diameter ratio increases which leads to a transition from a pure to a mixed regime (initially governed by liquid mixing then by mixing and mass transfer).

The existence of substrate gradients was deemed to be the most important factor for reduced production yield as oxygen limitation would not effect baker’s yeast because of its low respiratory capacity. The loss in yield is due to secondary metabolite production caused by high nutrient concentrations, the Crabtree effect (Section 1.5.1). The scale-down investigations were therefore based on the substrate gradients.

| Characteristic times of mechanisms relevant to the reactor performance, s |
|---------------------------------|-----------------|-----------------|
| Mixing, (liquid phase)          | 10-1000         | Oxygen transfer (liquid phase) | 1-100 |
| (gas phase)                     | 0.1-1000        | (gas phase)      | $10^3-10^6$ |
| $t_e$                           | 10-100          | Substrate consumption | 10-100 |
| Gas flow                        | 1-100           | Oxygen consumption  | 1     |
| Substrate addition              | 10-100          |                 |       |

| Characteristic times of mechanisms not relevant to the reactor performance, s |
|---------------------------------|-----------------|-----------------|
| Fed batch process               | $10^5$          | Micromixing (diffusion) | 0.01-1 |
| Growth of biomass               | $10^4$          | (turbulent erosion) | 1-100  |
| Heat transfer                   | 1000            | (laminar stretching) | 1000   |
| Heat production                 | 1000            |                 |       |

1.4.2 Theoretical procedure for the design of scale-down models

Where scale-down is to be used to aid process design, there is no existing fermenter in which a regime analysis can be conducted. In this instance a theoretical analysis is conducted based on the expected scale and type of vessel to be used. The characteristic times of the various mechanisms to be included in the regime analysis are determined from literature correlations: the correlations usually only give the correct order of magnitude for the characteristic time, but this is sufficient for the purpose of regime analysis. Sweere et al. (1987a) and Scoutens et al. (1986a) have both presented process design studies.
The procedure used by Sweere et al. (1987a) for their scale-down study is illustrated in figure 1.7. This procedure was used for designing a small scale (75L) bubble column fermenter, which was a scaled down representation of a 120m³ bubble column vessel, used for the fed batch production of baker’s yeast.

![Diagram](image)

**Figure 1.7:** Organisation of the theoretical analysis of fed batch bakers yeast fermentations for the purpose of scale-down. (Superscripts are detailed in the text)

The initial step \(a\) requires a determination of the characteristic times of mechanisms that are of importance to the performance of the production vessel. In this case this was done by using simple literature correlations for mixing, mass transfer, substrate consumption etc., in combination with dimensions and operating conditions for the large scale vessel. The characteristic times are then compared \(b\) in a regime analysis to determine the rate limiting mechanisms. As their process was a fed batch fermentation the volume of the broth increased with time, and their regime analysis predicted that the regime would initially be pure (one dominant mechanism) but end as mixed: the mechanisms being oxygen and then oxygen and substrate limitation, respectively. Based on the regime analysis a biomass growth rate could be proposed, that would avoid these limiting conditions. From this calculation, the substrate feed rate and cell yield could be predicted \(c\). After the theoretical stage there is experimental verification: a 75L vessel was used here. Initially the model vessel can be used to test the accuracy of the characteristic time predictions \(d\); tap water was used as the test fluid here, and the experimental characteristic times were found to be of the correct order of magnitude to the predictions. Secondly the regime analysis can be verified by fed batch fermentations \(e\). In this case they concluded that clear predictions could be made for the period of the fermentation that was a pure regime but this became more difficult for the
mixed regime, especially due to a lack of literature data regarding bubble coalescence behaviour in the medium.

The procedure used by Schoutens et al. (1986a,b,c) for their scale-down study is illustrated in figure 1.8. This procedure was used for determining the best reactor type for the large scale continuous production of isopropanol-butanol mixtures, which is an anaerobic process using immobilised Clostridium cells that yields carbon dioxide and hydrogen gas: a three phase system known as the IBE process. Having indicated a potential market for the IBE process, Schoutens et al. (1986a) carried out an analysis of reactor types to suggest a suitable vessel for the process, again the procedure involved theoretical steps and small scale investigation.

**Figure 1.8:** Organisation of the theoretical analysis of the IBE process

The initial procedure is an enhancement of that detailed in figure 1.7, in that different reactor types are compared to suggest the most suitable vessel(s) for the process. This can be done on a qualitative basis, for instance a) in figure 1.8 shows that a STR is unsuitable for the process as the immobilising matrix may be damaged: they
proposed the use of either a fluidised bed reactor with liquid recycle (FBR) or a gas lift external loop reactor with gas phase recycle (GLR). The subsequent analysis then distinguishes between the two. The vessel volumes may be determined based on an annual production target and the operating characteristics of the proposed vessels: the necessary volume for the FBR was 23 percent less than the GLR due to higher solids capacity. The vessels can then be designed b) on the basis of conventional geometries.

Again literature correlations are used to determine characteristic times of salient mechanisms, which are then compared c) to find the likely rate limiting mechanisms for the production reactors: these are then reproduced in the scale-down models. The theoretical analysis indicated that the laboratory size scale-down models should reproduce the liquid hydrodynamics (ratio between circulation time and axial dispersion time) for this study. Suitable scale-down models are then designed d). They used the scale-down vessels to develop hydrodynamic models e). These were combined with a previously developed kinetic model (describing the production of the alcohol mixtures by Clostridium spp.) to give an integrated model describing the overall reactor performances. The scale-down models were used for fermentations f), to verify the experimentally determined behaviour against the predictions g); the models could be refined until satisfactory agreement was reached. Once the validity of the models has been established the fermentation performance can be optimised in the chosen vessels h). Final comparison of the scale-down reactors indicates which is the most suitable vessel for the production scale i): in this case the FBR was chosen.

These theoretical methods indicate the power of scale-down in the fermentation development process, the only limitation is the availability of literature correlations for large scale vessels. This would lead to a process development favouring one of only a few reactor types that are well characterised, though a novel design may have proved more appropriate (Charles, 1985b). Both of these studies resulted in the design of single compartment models (Section 1.4.4.1) because they were modelling ALRs which have a fairly defined flow pattern. It should be possible to do theoretical studies on STRs to design appropriate scale-down models, by a similar process of estimating characteristic times and regime analysis. Also, where appropriate, two compartment models could be devised.
1.4.3 Experimental procedure for the design of scale-down models

These will be distinguished from theoretical studies on the basis that the scale-down work involves the analysis of an existing process to determine and compare the characteristic times. Invariably experimental studies are mostly associated with the optimisation of a process, where the scale-down is used to simulate the salient mechanisms in the laboratory. Sweere et al. (1987) adequately describe the method employed for experimental studies by the schematic presented in figure 1.9.

![Diagram](image)

**Figure 1.9**: Organisation of experimental (optimisation) scale-down studies

Regime analysis a) is again based on characteristic times but in the case of experimental studies is made easier and more accurate because the necessary data can often be directly measured from the process. Sweere et al. (1987a) indicate that the scale-down step (Figure 1.9) requires more than just regime analysis and is in fact closer to process analysis (Figure 1.10). The process analysis is a method of systematically analysing the performance of a large scale process resulting in an experimental design for small scale investigations of the larger scale. Considerable care is necessary in this phase of the study to ensure that the ruling regimes are consistent between the scales. Often the rules that are used to scale-down here will assist in the scale-up (Figure 1.9) of information to optimise the large scale process. The emphasis of experimental studies is on methods of simulation b); though workers may have used similar methods in the regime analysis and identified similar ruling mechanisms they often differ in their selection of equipment to simulate them (Section 1.4.4). Once a particular equipment has been specified it must be compared to the large scale process to determine its suitability and accuracy, and only when it adequately reproduces the
Figure 1.10: Operations required to successfully scale down an existing process

behaviour and productivity of the modelled process can it be used for further investigations. Significant mechanisms must not be ignored and new ones should not be introduced in the process of scale-down: the quality of the simulations determine the success of the application step. At this point models may be developed to describe the performance of the scale-down equipment c), with the intention of application to the existing process; usually an optimisation is also attempted. The optimisation must concentrate on parameters that can be scaled up again. For instance, increasing P/V or air flow rates at the large scale may not be possible, as the agitator motor and air compressor for this scale may have fixed capacities; in this situation the optimisation at the small scale may test the effect of multiple feed points or oxygen blending with the inlet air. If process alterations are anticipated then there is more scope for investigations at this level. Finally the models and improvements suggested by the scale-down study are applied to the original process d).

Experimental studies usually result in two compartment scale-down models because the large scale systems performance may suffer a particular nutrient distribution problem or gradient (e.g. Oosterhuis et al., 1983 & 1985 and Amanullah et al., 1994).
Additionally, the investigations are not limited by the availability of literature correlations as the information can be obtained from the original system.

1.4.4 Designs of scale-down models

Scale-up problems arise because the environment of the fermenter becomes increasingly heterogeneous with scale, as mixing becomes poorer. Whereas small fermenters are often considered well mixed with the same environmental conditions at all locations (i.e. homogeneous), large vessels may be subdivided into different zones of environment. Typically for an STR there is an impeller zone which is characterised by high shear rates, bubble breakup, high dissolved oxygen levels and good mixing, while the remainder of a large vessel may be considered to be a bubble column with bubble coalescence, lower dissolved oxygen and poorer mixing of fluid elements (Norwood and Metzner, 1960 & Andrew, 1982). The scale-down models often produces a physical representation of the different zones of a large scale vessel, though with reduced complexity of the actual mixing patterns (e.g. two compartment models: Section 1.4.4.2).

There are two main types of physical model used in the scale-down of fermentations. Where the reactor to be modelled is considered to be 'well mixed' generally a single compartment model is applied (Section 1.4.4.1). In this case the regime analysis does not indicate any rate limiting mechanisms on the large scale because the characteristic times for circulation and substrate distribution are sufficiently fast for the organism involved. For example, where the organism has a slow growth rate, but the influence of axial DOT gradients due to hydrostatic pressure require simulating. Where the reactor to be modelled is considered to be heterogeneous, a two compartment modelled is usually applied (Section 1.4.4.2). The heterogeneity can for example be the result of the circulation time being shorter than the oxygen consumption time, leading to the microorganism experiencing a gradient in oxygen concentration, due to its oxygen consumption, as it circulates throughout the vessel. Thus the scale-down model is divided into a high and low zone for the property to be simulated. As the scale-down process only concentrates on rate limiting mechanisms geometric similarity is not a pre-requisite of the small scale equipment and is more often not observed, which is self evident in the case of two compartment models.
1.4.4.1 Single compartment models

Single compartment models may either be well or 'poorly' mixed. Varader and Lilly (1982) used a 7 litre fermenter with two four bladed impellers and four baffles (well mixed), to simulate hydrostatic pressure gradients in the large scale production of *Penicillium chrysogenum* P1. They simulated hydrostatic pressure gradients by varying the fermenter over pressure in a sinusoidal cycle by controlling the exit air flow: this in turn caused the dissolved oxygen concentration to vary (Section 1.2.1.4). The DOT gradient could equally have been established by varying the inlet gas composition under constant over pressure. Gradients could also be established for feed nutrients by using timed pulses of nutrient.

As the vessel used in the above single compartment model is well mixed, the gradient is established over a period of time, rather than by position within the fermenter. Though both of the methods for establishing the DOT variation are viable the choice of the time period for the cycle is the critical factor for accurate simulation: this needs to be the axial circulation time. While Varader and Lilly (1982) used a fixed pressure cycle time it would be more appropriate to use a circulation time distribution. The magnitude and degree of variation in the circulation time would depend on the type of vessel being monitored. In airlift reactors the principle flow is axially, therefore the cycle time should be relatively short and have a small distribution; the opposite is the case for turbine stirred tank reactors, in which the fluid flow is principally in the radial direction. Varader and Lilly (1982) did not indicate the scale and type of vessel that was being modelled with a pressure cycle time of 120 seconds. Yegneswaran et al. (1991) used a 2L fermenter in which DOT gradients were produced by fluctuating the inlet air composition. The cycle time used in this study was more realistic with an average circulation taking 20 seconds (resembling a vessel of 100-120m$^3$), and was fluctuated according to a lognormal distribution. However, in the study of Varader and Lilly (1982) and Yegneswaran et al. (1991) the large scale verification was lacking, so the efficacy of the two approaches cannot be assessed. Oosterhuis et al. (1985) compared the use of a single and two compartment model for modelling DOT gradients in a 25m$^3$ STR: the DOT gradient was not due to hydrostatic pressure in this case. The single compartment model was a 2L STR with 1.5L liquid volume (well mixed), and the DOT gradient was established by varying the inlet gas composition. The two compartment model (Section 1.4.4.2) was found to be a more realistic representation of the large
scale system, however the cycle times involved in the single compartment study were again very unrealistic (an 80 second DOT cycle compared to a circulation time of 12 seconds), and these also used a fixed frequency. A single compartment model for a large scale STR in which there were DOT gradients, would require the above two models to be combined, with air composition changing on the time scale of radial circulation, and pressure changing on the time scale of axial circulation. In any case a well mixed single compartment model is not likely to reflect the actual behaviour of a large scale fermenter, as the whole broth experiences the same conditions at the same point during the fermentation. In reality the cells experience a mixture of conditions at any time, and this can only be achieved in a poorly mixed vessel or in a two compartment system. Additionally if the DOT cycle time is made very short in the single compartment, there will be no accurate DOT assessment from the probe, due to the probe response lag time. This could only be addressed in a two compartment model, where the DOT can be held relatively constant in the different compartments, and monitored by independent probes. Also two compartment models will be necessary where combined gradients are to be modelled, such as nutrient gradients with DOT gradients, and in imposing gradients in conjunction with a circulation time distribution.

‘Poorly’ mixed single compartment models have been used in the two theoretical studies described in section 1.4.2. In these studies the model airlift vessel dimensions have been calculated to maintain similar hydrodynamic characteristics between the large scale and the model; this was achieved by increasing the aspect ratios of the scale-down models compared to the large scale. This procedure for modelling would only be effective where an airlift vessel was being scaled down, while STRs would require a two compartment model in which a separate circulating pump could control the hydrodynamics (circulation time).

Kristiansen and McNeil (1987) used a poorly mixed single compartment model to scale-down an *Aureobasidium pullulans* fermentation, and study the effects of cycling on cell behaviour. They used a tubular loop fermenter to simulate a circulation loop within a large scale STR. The air was sparged at a single point and a smooth DOT gradient developed as the broth was cycled around the vessel with a peristaltic pump. They reported that the equipment worked well for the chosen system. This does appear to be a simple yet effective scale-down model, however this also appears more appropriate for the scale-down of ALRs as no turbine was included in the setup. This system is also very close to a two compartment system effectively behaving as two linked
plug flow reactors (PFR): the riser is an aerated PFR while the remainder of the tubular loop is an unaerated PFR. This system would be the only type of single compartment model to allow the use of simultaneous gradients; e.g. DOT and substrate.

1.4.4.2 Two compartment models

As the name implies these models consist of two linked 'vessels'. These may consist of a pair of linked STRs or of a STR linked to a PFR (which can be as simple as a long silicone tube); fluid exchange between the compartments is achieved by a pump. These models have been especially applied to scale-down nutrient gradients. The basic idea with respect to the nutrient gradients is that in the large scale vessel the nutrient (e.g. oxygen, substrate or pH agent) is usually added at a single location and then distributed to the remainder of the vessel by liquid circulation. The point of nutrient addition is then a region of high nutrient concentration while the remainder of the vessel is then progressively lower in the concentration of this substrate. The scale-down first requires the relative volumes of the two compartments to be determined; for example in the case of STR scale-down the ratio of compartment volumes comes from the ratio of the impeller zone to the remainder of the liquid volume in the large scale vessel. After this the nature of the two compartments should be set out. Again with the STR example, the impeller zone is regarded as a well mixed region and is consequently represented by a small STR. The remaining liquid volume may either be regarded as well mixed (for low viscosity broths, where the mean circulation time distribution has a large standard deviation) and be modelled by a second STR, or be regarded as poorly mixed (higher viscosity broth, where the mean circulation time distribution has a small standard deviation) and be modelled by a PFR (Amanullah et al., 1993). Finally the circulation rate between the two zones must be determined, and this should be based on the large scale vessel. For the case of DOT gradient modelling the radial circulation time should be used when an STR is modelled, and the axial circulation time for an ALR model. Oosterhuis et al. (1985) determined the circulation time distribution by radio pill flow follower experiments. The flow follower was a plastic sphere housing a radio transmitter, which was carried along by the fluid flow due to its neutral density. A radio detector monitored the circulation of the follower to provide accurate estimates of the circulation time distribution in the vessel.
A STR+STR combination was used by Oosterhuis et al. (1983) to model DOT gradients in a large scale *Gluconobacter oxydans* fermentation. They used two linked 2L vessels with broth volumes of 0.25 and 1.5 litres, to model different compartments volumes; the broth circulation was by a peristaltic pump giving mean circulation times in the range of 117-131 seconds. The DOT was controllable in either vessel by the sparging of air or nitrogen, though the smaller vessel was always sparged with air as this represented the impeller region of a large scale vessel. Oosterhuis et al. (1985) reported that the two compartment model was a much more realistic representation of the production scale vessel than a single compartment model in which the DOT was varied by altering the inlet air composition. A STR+STR combination was also used by Sweere et al. (1988b) to model glucose concentration gradients in a 10L STR fed batch fermentations of baker’s yeast. In this case a 5L vessel was linked to a 2L vessel with volumes of 2.2L and 1.3L, respectively. Broth was circulated with a peristaltic pump, and glucose was only added to the smaller vessel: both vessels were sparged with air. In the STR+STR systems where the DOT or nutrient is controlled at two different levels in the two fermenters, the cells experience a step change in conditions and this may not be representative of the actual large scale conditions. Therefore, in the case of DOT gradient modelling the DOT should be controlled in one vessel and left to find its own level in the other vessel; the level will depend on the circulation rate between the aerated and unaerated compartments. In the case of nutrient gradient modelling, a step change in nutrient concentration will always occur in a STR+STR combination, because the nutrient has to be added to one or other vessel and the mixing within each vessel is fast compared to the mixing of the overall system. Therefore this system may be less useful for modelling nutrient gradients, and a STR+PFR combination may be preferred.

STR+PFR combinations have been used to study ‘feed zone’ effects in a number of fermentations. In any fed fermentation (or fermentation where the pH is controlled) the nutrient is usually added at a specific point in the fermenter: often on the free liquid surface. This results in a zone in which the nutrient concentration is well above the critical level, or where the pH is far outside the limit, and this region is called the feed zone (Namdev et al., 1992). This is modelled in STR+PFR combinations by addition of the feed to the beginning of the PFR, where the residence time in the loop simulates the exposure time of the cells to the high nutrient or pH concentration: the residence time in the feed zone. The STR represents the remainder of the vessel and is well mixed. A STR+PFR combination was used by Amanullah et al. (1994) to model the effect of pH
gradients on large scale *Bacillus subtilis* fermentations. The PFR volume was estimated at 5% of the total volume, 5M Naoh was added to the beginning of the PFR via a mixing bulb, and the residence time in the PFR was varied between 30-240 seconds. Larsson and Enfors (1987 & 1988) used a STR+PFR for modelling the effect of oxygen starvation on a *Penicillium chrysogenum* fermentation: their two compartment model contained a nitrogen chamber prior to the PFR. They suggested the volume of the PFR section and the residence time within this section to be important considerations in the scale-down model. Larsson and Enfors (1993) used a STR+PFR to study the effects of oxygen limitation on *Escherichia coli* fermentations. This system allowed the natural development of DOT gradients in the PFR.

Amanullah *et al.* (1993) suggested that the choice of the two compartment model is critical to the correct modelling of nutrient gradient. They compared a STR+STR combination with a STR+PFR model for producing DOT gradients in *Bacillus subtilis* fermentations. The STR+PFR combination gave poorer performance because the exposure to anaerobic conditions was more severe in this system. If the STR+PFR model is used for DOT gradients it may only be useful for studying the effect of stagnation zones on the overall performance of the bioreactor, which may exist where the broth has a high viscosity or yield stress or where fermenter internals cause poor mixing. The STR+PFR combination appears to be more suited to the modelling of feed zone effects, and in these studies DOT limitation within the PFR section will have to be carefully controlled. George *et al.* (1993) used a STR+PFR to model the effect of glucose gradients (feed zone effects) during fed batch *S. cerevisiae* fermentation; the glucose was added to the PFR (a static mixer) but this was also aerated to avoid DOT stress. The same system and mode of operation was used by Neubauer *et al.* (1995) to study feed zone effects on *E. coli* fed batch fermentations. One final difference between STR+STR and STR+PFR models is that the fluid exchange in the STR+STR combination approximates a circulation time distribution when a fixed recirculating pump rate is used, while producing a circulation time distribution in a STR+PFR combination requires the use of a variable recirculating pump rate; therefore the former system can model a realistic system more easily.

A further two compartment model may be suggested for large scale STRs. That is to link a STR with an ALR. This is based on the view of Andrew (1982) that large STRs behave as STRs near the impeller and free bubble rise reactors elsewhere. All two compartment scale-down models inadvertently apply shear gradients to the cells, and as
yet the scale-down modelling of shear gradients in large scale vessels has not yet been attempted, though it may be important in determining the morphology of the filamentous microorganisms used in previous scale-down work. The STR+ALR system would allow shear to be isolated to the STR section, and allow the study of the effect of the magnitude of shear and the rate of circulation through the shear zone to be investigated: current STR+(STR/PFR) have too frequent an exposure to the impeller shear. The flow characteristics in the STR+ALR system would have to approximate to a lognormal circulation time distribution for accurate modelling. Furthermore, the current scale-down models in the literature do not clearly distinguish whether they are modelling large scale STRs or ALRs, and may thus introduce unrepresentative features into the scale-down model. In the case of Sweere et al. (1987b) a STR+STR combination was used to model nutrient gradients in a 120m³ bubble column fermenter. The bubble column vessel would be expected to produce different shear patterns to a STR, so a more appropriate model may have been to use a ALR+ALR or ALR+PFR combination; as yet this type of scale-down model has not been tried. Clearly a methodology needs to be bought to the selection of two compartment models: a possible procedure is given in table 1.8.

<table>
<thead>
<tr>
<th>Table 1.8: Possible procedure for two compartment model selection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Action</strong></td>
</tr>
<tr>
<td>Choose large scale reactor type</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Decide on mixing characteristics</td>
</tr>
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</table>

$^{1}$ The nature of the 2nd compartment depends on the first; if a large scale ALR is modelled then only a ALR should be chosen for the 2nd compartment when the mixing characteristics match those in $^{2}$

$^{3}$ The PFR should be selected as the 2nd compartment when a feed zone effect is to be modelled
Apart from the lack of attention to shear, there have been no attempts as yet to scale-down a system in which there are heat transfer gradients: these may be present in very fast growing cultures. The scale-down modelling could follow the same lines as that of feed zone effects. Using a STR+PFR model as the example, the cooling could be isolated to the PFR section, creating a temperature gradient between the STR and PFR.

The majority of the scale-down models produced to date only use very small volumes in each compartment. The requirement for cell quality monitoring is for frequent sampling which may lead to significant volume loss in the existing models, depending on the assays used. The hydrodynamics of the model would be seriously affected if a large sample volume was removed and not replaced by an equivalent broth volume: this could in turn affect the regime of the model. If the sample volume cannot be replaced, the overall volume of the scale-down model would have to increased to 10s of litres to reduce the significance of the sample loss. This would result in very high circulating pump flow rates (if the circulation time of a large vessel is to be accurately modelled) which could affect the cell quality. Though recirculation is not reported to affect scale-down models to date, this may not be the case when larger pumps and very high recirculation rates are used. The use of scale-down models with fast recirculation should therefore be studied further to address the effects of pumping.

1.4.4.3 Other forms of scale-down model

Some other scale-down investigations have also been attempted but these have simpler strategies. Oxytetracycline production studies carried out by Bosnjak et al. (1985) and polyene antibiotic synthesis using *Streptomyces noursei* scaled down by Ettler (1990), both involved transfer of media from the industrial scale vessel to smaller vessels of standard geometry. Using the same media would eliminate many of the problems associated with the varying quality of nutrients used during production, and the effects of sterilisation conditions. Bosnjak et al. (1985) transferred media to 1000 litre and 10 litre vessels and ran these under different conditions to the large scale vessel in order to determine if the process could be improved. The effects of temperature, agitation, aeration and medium feeding were investigated in the smaller vessels. Increasing the temperature of the process to 37°C and switching to a fed or repeated batch mode were the suggested process improvements. Ettler did similar studies.
While these two studies highlight an important consideration for scale-down experiments, the use of representative media, they ignore the idea of simulating the large scale conditions which is at the heart of the scale-down method. In such situations process improvements may come about by a change in regime due to operation at the smaller scale. For example the scale-down oxytetracycline fermentations used stirrer speeds up to 1000rpm, which makes the interpretation of results more difficult and their application more subjective.

Dahlgren et al. (1993) scaled down *E. coli* fermentations to a shake flask. They suggested that where a complex medium was used at the fermentation scale, this required the use of medium diluted to 30% of the original concentration, the addition of a buffering agent in the shake flask and the use of only 10% of the flask volume. This was necessary to avoid secondary metabolite formation because the aeration and pH control was poorer in the shake flask. This extreme level of scale-down would be undesirable from the point of controllability of the fermentation and sampling requirements, however this approach may be useful in the screening of microorganisms before the use of more complex compartment modelling.

Mannweiler and Hoare (1992) scaled down an industrial centrifuge by reducing the bowl volume of the equipment with solid metal inserts. While this approach would be acceptable for downstream process equipment (e.g. a multi-cartridge microfiltration rig could be scaled down by a similar method by reducing the rig to one cartridge) it is less suited to the scale-down of fermenters. A similar approach for the scale-down of a STR would result in the use of one mixing compartment of the large scale vessel, which could have a volume of 100s of litres, and would require a large agitator motor. Also when this approach is applied to downstream processing equipment, it requires the full scale equipment, which is naturally expensive and is what is hoped to be avoided by the application of the scale-down strategy.

1.4.5 Relevance of scale-down methods to process optimisation

Luyben (1993) points out that the most common developments in industry are concerned with alterations to existing processes or application of existing equipment for new processes, rather than the wholesale development of a new process in new equipment; this may be the use of a cheaper source of one of the fermentation substrates
or the use of a new strain of the organism for example. This situation is particularly suited to the scale-down approach. Where scale-down equipment may have already been developed to optimise the output or study the hydrodynamics within the production vessel the feasibility of proposed alterations can be very rapidly determined.

Optimisation may be achieved by increasing the final product concentration in the broth during a fixed time, improving the conversion efficiencies, increasing the production rate, reducing the foaming and product recovery cost or by recovering a better quality product for the same cost; these tests may be conducted by the process indicated for experimental studies (Section 1.4.3). Essentially cell quality profiles would have to be produced for the optimised fermentations to assess any improvements in the process.

For certain biologicals the objective may be to be the first company to get the product into the market place. Here the production may begin with a poorly developed process, however if the development used scale-down methods and equipment these may be used for optimisation during the early phases of production, thus securing a lead in the market and soon after benefiting from increased efficiencies and productivity.

1.4.6 Scope for the use of scale-down methods in process design

The scale down philosophy may be used at various stages of process design. The use of scale-down methods during process design should help identify strains of organism that will be unsuited to the production scale environment, thus avoiding the loss of time and effort that may otherwise be expended in their scale-up. Investigations may extend to determine culture stability, phage resistance, cell and product shear sensitivity, pellet formation, oxygen utilisation or uptake rate, heat releasing rate, minimum dissolved oxygen tolerance and maximum CO₂ tolerance (Jem, 1989). Scale-down can be applied in order to specify a suitable reactor type and size (Schoutens et al. 1986a,b,c). A two compartment model or tubular loop reactor could then be used to test a selected strains sensitivity to various gradients, that are predicted at the production scale. Such an application has been proposed by Larsson et al. (1993); a suitable procedure is presented in Figure 1.11. Scale down may also be used to determine the process protocols, control and emergency strategies for production. If the cell quality and fermentation profiles reflect those that will be obtained from the
production process then a great deal may be suggested about the best harvesting point, the likely recovery methods and the costs and purities that will be involved in the downstream processing.

![Diagram showing the screening method for selecting the most suitable strain of organism for production]

**Figure 1.11:** Screening method for selecting the most suitable strain of organism for production

The ultimate objective for scale-down investigations is to elucidate what the changes in the environment of the cell will be on scale-up, and how the cell quality will be affected by these (Slater, 1993). The scope for scale-down in the specification of reactor types and generally for design is limited by the quality and availability of correlations that can be used to determine the characteristic times of mechanisms that need to be included in the regime analysis. There is also a lack of large scale verification in scale-down studies. This requires different models to be compared for the same large scale fermentation, to determine the suitability of each approach, to allow more reliable experimentation in theoretical investigations. A summary of the advantages of the scale-down technique as indicated by Jem (1989), is given in table 1.9.

**Table 1.9: The advantages of the scale-down method of scale translation**

<table>
<thead>
<tr>
<th>Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>It provides more reliable scale-up data</td>
</tr>
<tr>
<td>It is useful in developing optimal strains and processes</td>
</tr>
<tr>
<td>It can solve potential operating problems</td>
</tr>
<tr>
<td>It allows more accurate cost estimates</td>
</tr>
<tr>
<td>It reduces production-to-market time, and</td>
</tr>
<tr>
<td>as a consequence it increases product competitiveness.</td>
</tr>
</tbody>
</table>
1.5 Organism Growth Characteristics

In this section the batch growth characteristics of *Saccharomyces cerevisiae* and *Saccharopolyspora erythraea* are detailed. The two organisms used in this study were selected to cover two extremes of morphological and rheological behaviour, and were expected to display different sensitivities to shear damage during growth. The product locations for these organisms were also different. *S. cerevisiae* is a eukaryotic organism that grows as discrete round or ovoid cells, with a Newtonian broth rheology. The cells are expected to be resistant to shear damage, and the products (e.g. enzymes) are internally located. *S. erythraea* is a mycelial prokaryotic organism that produces non-Newtonian broth behaviour at high cell density. The organism is more likely to suffer mechanical damage during the fermentation. In this case the main product is external to the cell, and is the antibiotic erythromycin. Both organisms were grown in batch culture.

1.5.1 Saccharomyces cerevisiae

The yeast *S. cerevisiae* has long been used in the production of bread and alcoholic beverages, and has been thoroughly researched biochemically, genetically and microbiologically. The organism is chosen for its capability to grow quickly on defined medium, both aerobically and anaerobically, and at low pH (which reduces the risk of contamination). The organism grows as discrete round or ovoid cells, with daughter cells produced by budding, and the broth has a simple Newtonian rheology. The products of its fermentation studied here are the cells and their enzymes, which in this case are glucose-6-phosphate dehydrogenase and alcohol dehydrogenase. These enzymes were chosen because they could act as markers for the cell breakage studies, as they are cytoplasmic. The cell breakage characteristics of this organism have also been studied, and it is widely used in the study of unit operations used in the production of biochemicals (e.g. filtration, centrifugation and homogenisation). It is a good test bed for eukaryotic organisms in the same way that *Escherishia coli* is for prokaryotic organisms.

The batch growth mode was chosen as the preferred method in this study, as opposed to fed-batch or continuous culture, because of the following reasons.

1) The simplicity of the equipment and techniques involved in this mode of culture.
2) The shorter duration of fermentation.
3) Lower cost of media.
4) Larger volume of fermentation broth that can be produced, satisfying the volume requirements for homogenisation and assay.
5) Easier scale-up of the process.

The batch mode of growth may be used in industry where the product is the cell biomass, which may go on to be used in baking for example.

The medium used in this study was a chemically defined medium, with glucose used as the sole carbon source. The batch growth of *S. cerevisiae* on such a medium leads to a very characteristic growth profile, which is the result of the limited respiratory capacity of the organism. In common with other non-photosynthetic organisms, *S. cerevisiae* obtains its energy, redox potential and metabolic intermediates from the catabolic pathways known as glycolysis and respiration (the tricarboxylic acid cycle and the electron transport chain). The former pathway phosphorylates the six carbon molecule glucose, and then degrades it to two three carbon molecules of pyruvate with the net production of two ATPs: this without the requirement for oxygen. Under aerobic conditions the pyruvate is converted to acetyl-CoA, and this in turn is degraded to CO₂ and H₂O by the tricarboxylic acid pathway, with the production of a further two ATPs. The reoxidation of the reduced coenzymes NADH and FADH₂ that are formed during these pathways, by the electron transport chain, yields up to 32 more ATPs. Thus, the respiratory route of glucose metabolism is the energetically favoured one (Stryer, 1981).

Under aerobic conditions and high glucose concentrations, *S. cerevisiae* is unable to metabolise all the glucose via the respiratory route because the rate of glycolysis exceeds that of respiration, with respiration effectively bottlenecked at the level of pyruvate (Sonnleitner & Kappeli, 1986). In this situation the majority of the pyruvate is first converted to acetaldehyde, with the production of CO₂, which is then converted to ethanol, by the enzyme alcohol dehydrogenase. This process is called alcoholic fermentation, and is characterised by the appearance of ethanol in the medium and a high ratio of carbon dioxide evolution to oxygen uptake (high RQ). Once the glucose level is depleted, the excreted ethanol is reassimilated and converted to acetyl-CoA, via acetaldehyde and acetate, which is then metabolised in the usual manner through the process of respiration; the metabolism of ethanol is characterised by a low RQ
(theoretically an RQ of 0.67). The growth of *S. cerevisiae* on glucose is therefore diauxic. However, the practical situation involves further complexities, because the high utilisation rate of glucose through glycolysis results in product inhibition, this can lead to the metabolism of glucose via an alternate pathway (the pentose phosphate pathway). Furthermore, other metabolites are excreted and reutilised besides ethanol. Locher *et al.* (1993) identified seven phases in the batch growth of *S. cerevisiae* on glucose, based on the principal metabolites being consumed. Figure 1.12 illustrates the diauxic growth pattern, and identifies the seven metabolic phases of Locher *et al.* (1993) which are further elaborated below; the batch profile in figure 1.12 was produced in a 20L fermentation in this study. The growth phases indicated on the RQ plot in figure 1.12 are detailed in section 3, with the results of the *S. cerevisiae* fermentations.

Phase 1: Exponential growth on glucose with ethanol production and no significant limitations. The secondary metabolites glycerol and organic acids (pyruvate, acetate and propionate) are also excreted.

Phase 2: CO₂ production ceases to be exponential.

Phase 3: Glucose fully metabolised and cells utilise the pyruvate.

Phase 4: Acetate, glycerol and smaller quantities of propionate used

Phase 5 & 6: These are characterised by ethanol consumption and excretion of acetate; there is also some reuse of glycerol.

Phase 7: Exhaustion of the ethanol and glycerol, and reuse of the acetate.
The growth phases referred to in the results and discussion are shown in relation to the metabolic phases described by Locher et al. (1993).

**Figure 1.12:** Metabolic stages in the batch culture of S. cerevisiae
1.5.2 Saccharopolyspora erythraea

*S. erythraea* is a spore forming member of the order *Actinomycetales*, which are gram-positive bacteria that form elongated cells or filaments, which usually have some degree of branching (Dietz, 1986). This organism was reclassified in 1987 to the genus *Saccharopolyspora* within the family *Streptomycetaceae*, from the genus *Streptomyces*, which also belongs to this family: hence the original name of the organism, *Streptomyces erythr(a)eus* (Labede, 1987). *S. erythraea* is aerobic and propagates via asexual thin walled conidiospores that form at the tips of the hyphae, the spores produce the vegetative mycelium, which is branched and non-septate. The spores are produced in chains and range in size from 0.7-1.3 by 0.5-0.7μm, while the mycelia have a diameter of 0.4-0.6μm and a variable length.

*Actinomycetes* are primarily soil dwelling and are widely distributed (Pelczar *et al.*, 1986). In the environment they are important for their ability to degrade a wide variety of organic matter, while industrially they are important for the antibiotics, enzymes and vitamins that they produce. The wild strain of *S. erythraea*, NRRL 2338 (Northern Utilisation Research and Development Division, U.S. Department of Agriculture), was isolated from soil samples collected in the Philippines by the Lilly Research Laboratories in 1950. The strain used in this study was CA 340 and was obtained from the Abbott Laboratories (Chicago, USA); this strain was developed for its capability to produce the antibiotic erythromycin.

*Actinomycete* morphologies vary from simple cocci to complex mycelia, which may be pelleted or diffuse. The hyphae extend only at the tips, where the cell wall is not fully matured (Bushell, 1988); branches occur at points of nutrient depletion along the hyphae, by the reversal of the wall maturation at these points. For the mycelial forms, pelleted growth and fragmentation both reduce antibiotic production, therefore branched diffuse growth is preferred, despite the increase in viscosity and complexity of the rheology that results (Bushell, 1988).

The antibiotic product of the *S. erythraea* fermentation is a secondary metabolite, that is a chemical that is not essential for the organisms growth, though it is probably important for survival in its natural habitat. The pattern of secondary metabolite synthesis can depend on the media type used. Those media supporting fast growth often lead to suppression of secondary metabolites until exponential growth is completed, so there is first a phase of growth and then a phase of product synthesis. The advantage of
this approach ought to be a higher overall production rate, as the cell initially puts all its energy into biomass production and then into product synthesis. For media that support slower growth the two phases may overlap (Bushell, 1988). There are two models for the physiological control of secondary metabolite synthesis, the first requires the depletion of an inhibitor or repressor, while the second requires the synthesis of an activator or inducer (Martin & Demain, 1980). The former model is usually associated with substrate limited media (such as nitrogen, carbon or phosphate limited media). The limiting substrate is often simple and can be quickly assimilated and metabolised, and this represses secondary metabolism, however the quantity of the substrate is small and the subsequent biomass production is limited by it. Once the substrate is depleted and biomass production ceases, or the growth rate decreases, the product synthesis begins; all other nutrients are in excess and are utilised for product synthesis and cell maintenance. The product synthesis ends if the product exerts a feedback inhibition, if the producing enzyme decays or if the basic components for its production are exhausted.

The product called erythromycin in this study, is actually a mixture of all the erythromycins produced by the organism. The erythromycins are macrolide antibiotics whose structure consists of a 14 membered lactone and two sugars (Seno & Hutchinson, 1986). There are six principal forms of erythromycin, A-F, which have slight differences in their alkyl groups but otherwise have similar properties; the major form is erythromycin A and the majority of the antibacterial activity of the mixture is due to this form (the degradation products have insignificant biological activity). The erythromycins act bacteriostatically by intracellular accumulation and by binding to the 50 S subunit of ribosomal RNA, which inhibits protein synthesis.
1.6 High Pressure Homogenisation and Wall Strength Determination

This section provides background details for the cell disruption method used in this study (Section 2.5). Cell wall rupture was necessary for the determination of the intracellular enzyme concentrations in *S. cerevisiae*, and if the cells were ruptured in a controlled manner, the process could yield an indirect determination of the cell wall strength. In the case of *S. erythraea* wall strength determinations could also be undertaken by the same method, though the antibiotic product is extracellular.

Though there are many techniques for releasing the intracellular components (Table 1.10), only mechanical methods could give a feel for the wall strength: of these high pressure homogenisation was deemed to be the most suitable (Table 1.11). Additionally, the technique is more relevant to production because it gives a useful indication of the likely damage to the organism from shear during fermentation and the handling of the broth in the initial downstream processing operations (e.g. the damage that may result during the pumping, cell concentration or separation of the broth), and in the case of *S. cerevisiae*, the requirements for the product release stage (which may actually be high pressure homogenisation).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Biological</th>
<th>Physical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-mechanical</td>
</tr>
<tr>
<td>Alkali</td>
<td>Enzymatic lysis</td>
<td>Osmotic shock</td>
</tr>
<tr>
<td>Acids</td>
<td>Phages</td>
<td>Freezing / Thawing</td>
</tr>
<tr>
<td>Solvents</td>
<td>Viruses</td>
<td>Gas decompression</td>
</tr>
<tr>
<td>Detergents</td>
<td>Inhibition of wall</td>
<td>Drying</td>
</tr>
<tr>
<td></td>
<td>synthesis</td>
<td></td>
</tr>
</tbody>
</table>

(Adapted from Schutte and Kula, 1990)
Table 1.11: Reasons for the selection of high pressure homogenisation as a cell disruption method

<table>
<thead>
<tr>
<th>Effectiveness</th>
<th>The method is effective in disrupting cells, with total breakage being obtained throughout the course of the fermentation, independent of the quality of the cells or the fermentation conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generic</td>
<td>The method is applicable to a wide range of organisms with little modification</td>
</tr>
<tr>
<td>Robustness</td>
<td>The equipment used is subject to little wear and tear</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>The results are reproducible and the operation of the equipment is reliable</td>
</tr>
<tr>
<td>Speed</td>
<td>The method is fast, allowing adequate sampling frequency to profile a fermentation.</td>
</tr>
<tr>
<td>Additional</td>
<td>The method does not contaminate the sample (c.f. chemical techniques) or denature the products (so long as the temperature is controlled)</td>
</tr>
</tbody>
</table>

(Adapted from Sayed, 1995)

1.6.1 Disruption characteristics in a high pressure homogeniser

High pressure homogenisers consist of a positive displacement pump, which forces the sample through the homogenising assembly. The assembly consists of a valve and its seat, surrounded by a close fitting impact ring. The sample is initially forced through the centre of the valve seat and is then directed at right angles against the impact ring, by the valve; the homogenised sample is then delivered to a holding tank. The discharge pressure is controlled by varying the gap between the valve and its seat.

Hetherington et al. (1971) found disruption of *S. cerevisiae* in a high pressure homogeniser, to be a first order process with respect to the number of passes through the valve (Equation 1.43), with the rate constant being a function of temperature and pressure: the pressure exponent dependent on the equipment used and the type of organism being disrupted.
\[
\log\left(\frac{Rm}{Rm - R}\right) = (k \cdot P^a)N = K \cdot N
\]  
(1.43)

Where: 
- \(R\), soluble protein release, mg/mL.
- \(Rm\), maximum soluble protein available for release, mg/mL.
- \(k\), constant which is a function of the suspension temperature.
- \(P\), the homogenisation pressure.
- \(a\), exponent which is a function of the organism, system and pressure.
- \(K\), combination of \(k \cdot P^a\) which is the dimensionless first order rate constant, the value of which is taken as the cell wall strength.
- \(N\), number of homogeniser pass.

For a particular homogenising system, temperature, pressure and organism, the rate constant (\(K\)) depends on the resistance of the cells to being ruptured. Therefore the value of \(K\) determined at a particular stage in the growth cycle of an organism, may be taken as an indication of the wall strength of the cells at that point. The value of the rate constant, is that of the slope of the line of a plot of \(\log(Rm/Rm-R)\) vs. \(N\). As \(K\) indicates the rate of disruption, the wall strength is inversely proportional to \(K\) (the larger the \(K\) value the weaker the cells).

For some organisms the pattern of protein release with pressure is more complicated. For example the release of soluble protein during the disruption of the fungal organism \(Rhizopus nigricans\), was found to be a weak function of pressure and number of passes, with \(Rm\) apparently increasing with pressure: this was put down to the further breakdown of already released soluble proteins, on continued passing through the homogeniser-micronisation (Keshavarz et al., 1990).
1.7 Rheological Behaviour of Fluids

This section provides background details to the rheological measurements used in this study (Section 2.6.9). Rheology is the study of the deformation and flow of matter. The fermentation broth rheology has an important influence on the transport processes within the fermenter (Metz et al., 1979; Olsvik and Kristiansen, 1994), and the rheology of filamentous fermentations may be especially sensitive to the environmental conditions and to scale-up (Taguchi, 1970). Therefore viscosity data was gathered for *S. erythraea* fermentations; *S. cerevisiae* fermentation broths were not considered to have a complex or sensitive rheology and were not profiled.

Rheological behaviour falls between two extremes, namely elastic and viscous behaviour. An elastic response involves the complete recovery of the energy of deformation upon removal of the deforming stress, while a viscous response involves the complete loss of the deforming energy as heat, when the body sustains flow. Elastic behaviour is described by Hooke’s Law while viscous behaviour is described by Newton’s Law; fermentation broths tend toward viscous behaviour while only solids give a purely elastic response. Mycelial fermentations usually show some degree of departure from ideal Newtonian behaviour, and a variety of models have been developed to describe non-Newtonian liquids.

The rheological properties of a fluid are determined under conditions of laminar flow and the models examine the relationship of shear stress (τ, the deforming energy which has units of force per unit area, Pa) to shear rate (γ, the velocity gradient that results from the application of a shear stress, with units of s⁻¹) (van Wazer et al., 1963). The relationships for the common rheological behaviours are displayed graphically in Figure 1.13.

**Newtonian behaviour**

For Newtonian fluids the ratio of shear stress to shear rate is constant, and is known as the coefficient of viscosity, μ (units of Pa s). The viscosity is independent of shear rate and there are no elastic properties. The viscosity is the internal friction of the fluid and is the property that resists the fluid motion. Viscosities of Newtonian fluids generally vary with composition, pH and ionic strength (Charles, 1985b).
**Pseudoplastic behaviour**

Pseudoplastic fluids display shear thinning behaviour, which is a decrease in the viscosity with increasing shear rate.

**Dilatant behaviour**

This is the opposite to pseudoplastic behaviour, with viscosity increasing with shear rate: these fluids are shear thickening.

**Bingham plastic behaviour**

These fluids do not flow until a limiting shear stress is exceeded (the yield stress). Below this stress the fluid behaves as a solid and above this stress as a liquid; the liquid behaviour may be Newtonian, pseudoplastic or dilatant.

**Time dependent flow behaviour**

Certain fluids show behaviour that depends on the length of time for which they have been flowing. The behaviour is usually the result of the flow creating more order in the liquid structure (e.g. they alignment of long polymer molecules that were initially randomly oriented). The result may be a decrease (thixotropy) or increase (rheopexy) in viscosity under steady shear conditions. The behaviour may or may not be reversible; in the latter case the flow is said to have caused "rheodestruction" of the fluid (e.g. by the breakage of long polymers).

If a filamentous broth is non-Newtonian, then it usually behaves as a pseudoplastic fluid, and may have a yield stress.
Some simple rheological models

Power Law model
This model applies to both Newtonian and non-Newtonian fluids that do not have a yield stress (Equation 1.44).

\[ \tau = K \cdot \gamma^n \]  \hspace{1cm} (1.44)

K is called the consistency coefficient (Pa.s^n) and describes the apparent viscosity of the fluid when the shear rate is unity. The Power Law / Flow Behaviour Index, n, is a dimensionless quantity, and provides a quantitative rheological characterisation of the fluid: Newtonian fluids have a n value of 1, pseudoplastic fluids have n values less than 1 and dilatant fluids have n values greater than 1. The values of K and n can be determined using the logarithmic form of equation 1.44 given below: K is given by the y intercept and n by the slope of a line of Log\(\tau\) vs. Log\(\gamma\).

\[ \text{Log} \tau = \text{Log}K + n \cdot \text{Log} \gamma \]  \hspace{1cm} (1.45)

Bingham plastic model
The model describes the behaviour of Newtonian fluids that have a yield stress, \(\tau_0\) (Equation 1.46); the fluid only flows above \(\tau_0\), so the viscosity is called \(\mu_s\), the plastic viscosity. This model can be adapted using the Power Law model to take account of non-Newtonian behaviour above the yield stress (Equation 1.47).

\[ \tau = \tau_0 + \mu_s \cdot \gamma \]  \hspace{1cm} (1.46)

\[ \tau = \tau_0 + K \cdot \gamma^n \]  \hspace{1cm} (1.47)

Casson Body model
The model describes the behaviour of pseudoplastic fluids that have a yield stress (Equation 1.48), where Kc is the Casson viscosity (Pa.s^n).

\[ \tau^{1/2} = \tau_0^{1/2} + K_c \cdot \gamma^{1/2} \]  \hspace{1cm} (1.48)
Figure illustrating the relationship of shear stress to shear strain for various rheological models.

Figure 1.13: Rheological behaviour of fluids and rheological models
1.8 Morphological Measurements by Image Analysis

This section provides background details about the image analysis technique used in this study (Section 2.6.10). The cell morphology is a key quality that may be affected by the environment that the cells are grown in. The morphology of \textit{S. erythraea} is important because it may affect the production of antibiotic (Martin and Bushell, 1996), and the rheology of the broth (Metz \textit{et al.}, 1979 & Charles, 1985b), which in turn affects the transfer processes. The principle morphological measurement related to the above concerns is the main hyphal length, with production suggested to occur only above a minimum length (Martin and Bushell, 1996), and the length of the hyphae determining the degree of entanglement that may occur between separate cells (i.e. the increase in viscosity that is likely to occur).

The detailed analysis of mycelial morphologies has only recently become possible, with the development of semiautomated (Adams and Thomas, 1988) and fully automated (Packer and Thomas, 1990) digital image analysis techniques. The system used for image analysis consists of the following components. A microscope with a motorised stage, automatic focus adjustment, controllable light source and with a close circuit television camera mounted. The camera incorporates a charge couple devise to convert the light image into one of electrical charges, the magnitude of which depends on the amount of light incident on the chip. The image is then passed through an amplifier to enhance it, and an analogue to digital transformer to make the signal resistant to external noise or signal drift. The digital data passes via a converter to an image store, which is connected to the image processor and a display. The converter may be used to ease the interpretation of the display by assigning a grey level to the individual pixels in the image. Images are displayed after transforming the digital data back to an analogue form. The microscope (stage, lamp and focus adjustment), converter, image processor and a printer are controlled by software on the hard disk of a personal computer, which is ultimately under the control of the operator via a keyboard. The image analysis process can be split into the following steps; image capture and enhancement, segmentation, object detection, measuring and analysis.
Image capture and enhancement
Conversion of the light image into a digital form for storage and processing. The image is captured by the close circuit television camera and converted into grid of picture elements (pixels). The quality of the image is enhanced by the amplifier, and then each pixel is assigned a grey level (tonal intensity) by the converter.

Segmentation
This is the process of separating the background form the regions of interest, on the captured and processed image, and is done by the process of thresholding. The user decides upon the upper and lower grey levels (the thresholds) which the organisms mycelium falls within, and then uses these levels to exclude those grey levels from the image that are above and below those of interest. False objects such as dust and media particles, are cleared from the thresholded image by using a pre-set circularity factor. The circularity is a description of the objects shape (Equation 1.49), with a circle having a value of one for this factor, and the value of the factor decreasing as the length of the object increases in relation to its width; the false objects are distinguished from the hyphae by their high circularity.

\[
Circularity = \frac{4\pi \times \text{Area}}{(\text{Perimeter})^2}
\]  

(1.49)

A skeleton of each object is then produced using the pixels that run down the centre of each hyphae, including the branches. The process of producing the skeletons occasionally results in short false branches being assigned to the main hyphae, which are the product of noise. These spikes are excluded from the analysis process by specifying a minimum branch length, this also eliminates errors that may occur from dust or media particles that may be lying adjacent to the hyphae. The image measurements (e.g. main hyphal length, number of branches and branch length) are performed on the skeletons.

Object detection
This process reduces the quantity of data for storage and analysis by producing a more compact description of the objects list of pixel co-ordinates: the objects are the regions of interest within the image.
Measurement
Quantitative data is determined for the objects. The main hyphal length is determined by finding the longest chain of unbroken pixels, and branch lengths are determined from the remaining chains of pixels. The data that can be gathered are given in table 1.12; the full distribution of measurements is produced as the output.

Table 1.12: Data available from image analysis

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main hyphal length</td>
<td>The longest unbroken length of hypha in the cells mycelium</td>
</tr>
<tr>
<td>Branch length</td>
<td>The length of a hypha attached to the main hypha</td>
</tr>
<tr>
<td>Total hyphal length</td>
<td>The combined length of all hyphae in the cells mycelium</td>
</tr>
<tr>
<td>Major axis</td>
<td>The longest chord in the mycelium</td>
</tr>
<tr>
<td>Minor axis</td>
<td>The longest chord in the mycelium, perpendicular to the major axis</td>
</tr>
<tr>
<td>Average axis</td>
<td>The average chord length from the mean major and minor axis measurements</td>
</tr>
<tr>
<td>% clump area</td>
<td>The percentage of the cells morphology that is pelleted (clumped) as compared to diffuse (mycelial)</td>
</tr>
<tr>
<td>Number of tips</td>
<td>The number of branches (growth tips) in the mycelium</td>
</tr>
<tr>
<td>Hyphal growth unit</td>
<td>The length of hyphae per growth tip</td>
</tr>
</tbody>
</table>

Analysis
Statistical analysis are made using the measurements to produce morphological descriptions of the organism.
1.9 Broth Conductivity

Cell strengths may be sensitive to the ionic strength of the surrounding medium. A typical undergraduate experiment in biochemistry is to place red blood cells in solutions of different ionic strength (salt concentrations) and observe the shape of the cells. The correct shape is observed only in isotonic solutions, while the cells become flacid in hypertonic solutions and turgid in hypotonic solutions (i.e. in solutions of low ionic strength, where the cells may even burst due to the influx of water). In batch culture the ionic strength may change as the nutrients are consumed, and this in itself may cause a trend in wall strength to be observed over the course of a batch fermentation; as the salts are consumed the solution should tend toward a lower ionic strength—if there is any associated trend in wall strength it should be for the cells to weaken as they become more turgid. Therefore the influence of the culture environment on the cell wall strength should be isolated from the influence of the engineering parameters (the shear in the environment). The broth ionic strengths were assessed by measuring the conductivity, with higher salt concentrations giving greater conductivities.

A similar effect on cell walls may be expected from the osmotic strength of the broth. A typical school biology experiment involves measuring the length of potato tuber strips in solutions of different osmotic strengths. The cell turgidity is observed to increase with decreasing osmotic strength. As the broth glucose is consumed the osmotic strength of the broth decreases therefore any associated change in wall strength should also be toward weakening. The cells of *S. cerevisiae* and *S. erythraea* should be less sensitive to the ionic and osmotic strength of the environment than red blood cells because of the greater support of the cell membranes from the rigid cell walls. Hamilton (1995) showed this strain of *S. cerevisiae* grown in batch culture in the same medium used in this study, to be resistant to cell rupture on osmotic shock.
Project aims

The basis of this investigation was that scale-up could affect the cell quality of organisms by increasing the heterogeneity in the broth and by physical differences between small and large scale vessels. Furthermore that the knowledge of cell quality was useful in the development of a process. The primary objective was to determine how the cell qualities of *S. cerevisiae* and *S. erythraea* were affected by scale-up. This would provide the necessary information for the secondary objective of scaling down the large scale fermentations to reproduce the cell quality profiles for these two organisms in laboratory scale equipment. For the primary objective fermentations were conducted at four fermentation scales, with various cell qualities (Section 3 & 4) monitored over the course of batch fermentations. The small scale vessels were considered to be 'ideal systems' for the growth of the organisms, in that the fermenters could be considered to be well mixed with a homogenous environment. The results from the small scale provided a benchmark for assessing the effects of increasing heterogeneity in the environment, as a result of scale-up. The scale-up strategies for the two organisms are elaborated at the beginning of sections 3 and 4. By assessing the effects of scale-up indications could be obtained as to which factors were the most important in influencing cell quality, and therefore which factors should be incorporated in the design of scale-down models. A well defined medium (including antifoam concentrations) was to be used throughout the inoculum development and scale-up to reduce the influence of media type (e.g. Section 1.2.1.4) on the quality; thus cell quality changes could be attributed to the particular fermenter environment.
2 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Organisms

The unicellular organism used in the experiments was *Saccharomyces cerevisiae*: strain GB4918, obtained as a freeze dried culture from Distillers Co. (Crawley, Sussex).

The filamentous organism used in this study was a near industrial strain of *Saccharopolyspora erythraea*: strain CA 340, which was kindly donated by Abbott Laboratories (Chicago, USA).

2.1.2 Chemicals

Table 2.1 lists the chemicals used in the *S. cerevisiae* fermentations. This defined medium was first devised by Feichter in 1966 and adapted at University College London by Gregory (1992), and was used to conform to departmental preference. The sterilisation Group D was dissolved in the order indicated by the superscript numbers prior to the chemical formula, and required heating and the addition of concentrated HCl to dissolve the salts. Table 2.2 lists the chemicals used in the *S. erythraea* fermentations. The medium belongs to the Shell “L” Series Media and is allledged to trigger erythromycin production by nitrogen limitation, and was used on recommendation (Bushell, 1994). The salts in sterilisation Group C in this case also required dissolving in a particular order and the addition of concentrated HCl. The salts were dissolved separately (in the Groups indicated by the superscripts) and then combined. The chemicals used in other parts of this work have been indicated at the appropriate place. The major chemical suppliers details are as follows: Aldrich Chem. Co., Gillingham, Dorset, UK; BDH Ltd., Poole, Dorset, UK; Sigma Chemical Co., Poole, Dorset, UK; Fisons, Loughborough, UK.
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Conc., g/L</th>
<th>Grade</th>
<th>Supplier</th>
<th>Product code</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sterilisation Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D+ Glucose</td>
<td>30.0</td>
<td>GPR</td>
<td>BDH</td>
<td>28450</td>
</tr>
<tr>
<td>MgSO\textsubscript{4}.7H\textsubscript{2}O</td>
<td>1.2</td>
<td>GPR</td>
<td>BDH</td>
<td>29117</td>
</tr>
<tr>
<td>meso-Inositol</td>
<td>0.16</td>
<td>98%</td>
<td>BDH</td>
<td>38044</td>
</tr>
<tr>
<td>EDTA (Na\textsuperscript{2} salt)</td>
<td>0.243</td>
<td>98%</td>
<td>Aldrich</td>
<td>10,631-3</td>
</tr>
<tr>
<td>CaCl\textsubscript{2}.2H\textsubscript{2}O</td>
<td>0.15</td>
<td>99%</td>
<td>Sigma</td>
<td>C-3881</td>
</tr>
<tr>
<td><strong>Sterilisation Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}</td>
<td>17.8</td>
<td>Grade 1</td>
<td>Sigma</td>
<td>A-5132</td>
</tr>
<tr>
<td>KH\textsubscript{2}PO\textsubscript{4}</td>
<td>5.7</td>
<td>GPR</td>
<td>BDH</td>
<td>29608</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.1</td>
<td>Analar</td>
<td>BDH</td>
<td>10241</td>
</tr>
<tr>
<td><strong>Sterilisation Group C</strong></td>
<td>Conc., mg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Biotin</td>
<td>1.0</td>
<td>99%</td>
<td>Sigma</td>
<td>B-4501</td>
</tr>
<tr>
<td>Ca.Pantothenate</td>
<td>20.0</td>
<td></td>
<td>Sigma</td>
<td>P-2250</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>15.0</td>
<td>99%</td>
<td>Aldrich</td>
<td>N785-0</td>
</tr>
<tr>
<td>Thiamine.HCl</td>
<td>4.0</td>
<td></td>
<td>Sigma</td>
<td>T-4625</td>
</tr>
<tr>
<td>Pyridoxine.HCl</td>
<td>10.0</td>
<td></td>
<td>Sigma</td>
<td>P-9755</td>
</tr>
<tr>
<td><strong>Sterilisation Group D</strong></td>
<td>Conc., mg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textsuperscript{6} FeCl\textsubscript{3}.6H\textsubscript{2}O</td>
<td>100.0</td>
<td>Analar</td>
<td>BDH</td>
<td>10110</td>
</tr>
<tr>
<td>\textsuperscript{5} ZnSO\textsubscript{4}.7H\textsubscript{2}O</td>
<td>30.0</td>
<td>SLR</td>
<td>Fisons</td>
<td>Z/1550</td>
</tr>
<tr>
<td>\textsuperscript{4} MnSO\textsubscript{4}.2H\textsubscript{2}O</td>
<td>32.0</td>
<td>98+% ACS</td>
<td>Aldrich</td>
<td>22,128-7</td>
</tr>
<tr>
<td>\textsuperscript{3} H\textsubscript{3}BO\textsubscript{4}</td>
<td>15.0</td>
<td>SLR</td>
<td>Fisons</td>
<td>B/3760</td>
</tr>
<tr>
<td>\textsuperscript{1} CoCl\textsubscript{2}.6H\textsubscript{2}O</td>
<td>5.6</td>
<td>98.3% ACS</td>
<td>Sigma</td>
<td></td>
</tr>
<tr>
<td>\textsuperscript{1} NaMoO\textsubscript{4}.2H\textsubscript{2}O</td>
<td>5.0</td>
<td>Analar</td>
<td>BDH</td>
<td>10254</td>
</tr>
<tr>
<td>\textsuperscript{2} KI</td>
<td>2.0</td>
<td>Analar</td>
<td>BDH</td>
<td></td>
</tr>
<tr>
<td>\textsuperscript{2} CuSO\textsubscript{4}.5H\textsubscript{2}O</td>
<td>0.8</td>
<td>Analar</td>
<td>BDH</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2: Defined medium composition for S. erythraea fermentations

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Conc., g/L</th>
<th>Grade</th>
<th>Supplier</th>
<th>Product code</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sterilisation Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D+ Glucose</td>
<td>35.0</td>
<td>GPR</td>
<td>BDH</td>
<td>28450</td>
</tr>
<tr>
<td><strong>Sterilisation Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>7.0</td>
<td>GPR</td>
<td>BDH</td>
<td>29619</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>3.0</td>
<td>GPR</td>
<td>BDH</td>
<td>29608</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>2.3</td>
<td>GPR</td>
<td>BDH</td>
<td>30187</td>
</tr>
<tr>
<td><strong>Sterilisation Group C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conc., mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, MgSO$_4$.7H$_2$O</td>
<td>250.0</td>
<td>GPR</td>
<td>BDH</td>
<td>29117</td>
</tr>
<tr>
<td>1, FeSO$_4$.7H$_2$O</td>
<td>25.0</td>
<td>GPR</td>
<td>BDH</td>
<td>28400</td>
</tr>
<tr>
<td>2, CuCl$_2$</td>
<td>0.53</td>
<td>GPR</td>
<td>BDH</td>
<td>27835</td>
</tr>
<tr>
<td>2, CoCl$_2$.6H$_2$O</td>
<td>1.01</td>
<td>GPR</td>
<td>BDH</td>
<td>27790</td>
</tr>
<tr>
<td>2, NaMoO$_4$.2H$_2$O</td>
<td>0.35</td>
<td>GPR</td>
<td>BDH</td>
<td>27584</td>
</tr>
<tr>
<td>3, CaCl$_2$.2H$_2$O</td>
<td>13.8</td>
<td>GPR</td>
<td>BDH</td>
<td>30605</td>
</tr>
<tr>
<td>3, ZnCl$_2$</td>
<td>10.4</td>
<td>GPR</td>
<td>BDH</td>
<td>29137</td>
</tr>
<tr>
<td>3, MnCl$_2$.4H$_2$O</td>
<td>9.75</td>
<td>GPR</td>
<td>BDH</td>
<td>30185</td>
</tr>
</tbody>
</table>
2.2 FERMENTATION EQUIPMENT

2.2.1 Fermenters and probes

A total of eight fermenters were used in this study. The fermenter, DOT probe, pH probe and control system model numbers are given in Table 2.3. Table 2.4 gives detailed dimensions of the vessels; the 42L fermenters were not accurately specified as they were only used as seed vessels.

<table>
<thead>
<tr>
<th>Fermenter ID</th>
<th>Fermenter model</th>
<th>Instrumentation</th>
<th>DOT probe</th>
<th>pH probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>7L(1)</td>
<td>LH1 2000 series I</td>
<td>TCS4 3000 series</td>
<td>327 320030/71803</td>
<td>4508824</td>
</tr>
<tr>
<td>7L(2)</td>
<td>Inceltech2</td>
<td>Inceltech</td>
<td>327 320030/71803</td>
<td>4508824</td>
</tr>
<tr>
<td>20L</td>
<td>LH 2000 series I</td>
<td>TCS 3000 series</td>
<td>322 756703/76615</td>
<td>4508824</td>
</tr>
<tr>
<td>25L</td>
<td>Inceltech</td>
<td>Inceltech</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42L(1)</td>
<td>LH 2000 series II</td>
<td>TCS 3000 series</td>
<td>322 56800/84325</td>
<td></td>
</tr>
<tr>
<td>42L(2)</td>
<td>LH 3000 series</td>
<td>TCS 3000 series</td>
<td></td>
<td></td>
</tr>
<tr>
<td>450L</td>
<td>Chemap3</td>
<td>TCS 3000 series</td>
<td>322 56800/84325</td>
<td></td>
</tr>
<tr>
<td>1500L</td>
<td>Chemap</td>
<td>TCS 3000 series</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1LH Fermentation Ltd., Reading, UK.
2Inceltech, Reading, UK.
3Chemap AG Maennedorf, Switzerland.
4Turnbull Control Systems Ltd., Worthing, UK.
5Ingold Division, Leicester, UK.
6Ingold Division. Fitted in an “Infot 764-50” housing, with “ST Triax 7” lead & AS9 socket

NB. The volume in the “Fermenter ID” in Table 2.3, refers to the total volume of the vessel: the bracketed number refers to the particular “Fermenter model”. The operating volumes used are given in Table 2.7 and 2.8.
Table 2.4: Fermenter dimensions and details

<table>
<thead>
<tr>
<th>All dimensions in metres</th>
<th>Fermenter ID</th>
<th>7L</th>
<th>7L</th>
<th>20L</th>
<th>25L</th>
<th>42L</th>
<th>450L</th>
<th>1500L</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Table 2.3)</td>
<td>(1)</td>
<td>(2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel diameter, T</td>
<td></td>
<td>0.15</td>
<td>0.15</td>
<td>0.22</td>
<td>0.21</td>
<td>0.60</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Liquid volume (L)</td>
<td></td>
<td>5</td>
<td>5</td>
<td>14</td>
<td>15</td>
<td>30</td>
<td>30</td>
<td>300</td>
</tr>
<tr>
<td>Liquid height, H</td>
<td></td>
<td>0.30</td>
<td>0.30</td>
<td>0.39</td>
<td>0.42</td>
<td>1.2</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Number of impellers</td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Impeller diameter, D</td>
<td></td>
<td>0.07</td>
<td>0.05</td>
<td>0.07</td>
<td>0.07</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Height of 1st impeller</td>
<td></td>
<td>0.07</td>
<td>0.05</td>
<td>0.07</td>
<td>0.07</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Separation of impellers</td>
<td></td>
<td>0.07</td>
<td>0.08</td>
<td>0.11</td>
<td>0.11</td>
<td>0.3</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Number of baffles</td>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

2.2.2 Off line gas analysis

Exit gas analysis was achieved by sampling the exhaust air after the condenser and filter. Exhaust gas was fed via a flow controller (RS Components Ltd., Northants, UK) to a MMG-80 mass spectrometer (VG gas analysis Ltd., Middlewich, UK) which determined the percentage composition of oxygen, carbon dioxide, nitrogen and argon. Comparison with the inlet gas was available, allowing calculation of carbon dioxide evolution rates and oxygen uptake rates.

2.2.3 Fermentation data logging

The data logging system used a program called “Real Time Data Acquisition System” supplied by Acquisition Systems Ltd (Surrey, UK). This system recorded the on-line and off-line variables shown in Table 2.5.
### Table 2.5: Variables recorded by the fermenter data logging system

<table>
<thead>
<tr>
<th>On-line variables</th>
<th>Stirrer speed</th>
<th>RPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airflow rate</td>
<td>L/min</td>
<td></td>
</tr>
<tr>
<td>Dissolved oxygen tension</td>
<td>% air saturation</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td></td>
</tr>
<tr>
<td>Fermenter over pressure</td>
<td>Bar</td>
<td></td>
</tr>
<tr>
<td>Off-line variables</td>
<td>Exit gas oxygen content</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Exit gas carbon dioxide content</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Exit gas nitrogen content</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Exit gas argon content</td>
<td>%</td>
</tr>
</tbody>
</table>

The system calculated the Carbon dioxide Evolution Rate and Oxygen Uptake Rate (moL/L.h) and the Respiratory Quotient from the above information according to the following equations,

\[
CER = Q_{\text{CO}_2} = \frac{F}{V_m \cdot V_o} \left\{ X'^{\text{CO}_2} - \frac{X'^{\text{CO}_2} \cdot \left(1 - X'^{\text{O}_2} - X'^{\text{CO}_2}\right)}{\left(1 - X'^{\text{O}_2} - X'^{\text{CO}_2}\right) - X'^{\text{CO}_2}} \right\}
\]  

(2.1)

\[
OUR = Q_{\text{O}_2} = \frac{F}{V_m \cdot V_o} \left\{ X'^{\text{O}_2} - \frac{X'^{\text{O}_2} \cdot \left(1 - X'^{\text{O}_2} - X'^{\text{CO}_2}\right)}{\left(1 - X'^{\text{O}_2} - X'^{\text{CO}_2}\right) - X'^{\text{CO}_2}} \right\}
\]  

(2.2)

\[
RQ = \frac{CER}{OUR}
\]  

(2.3)

Where: 
- \(Q_{\text{CO}_2}\), volumetric carbon dioxide flow, moL/L/h 
- \(Q_{\text{O}_2}\), volumetric oxygen flow, moL/L/h 
- \(F\), air flow rate (L/h) 
- \(V_m\), molar volume of air, taken as 22.4 L/mol 
- \(V_o\), volume of liquid in the fermenter (L) 
- \(X_i\), volume fraction (in, ' and out, ') of \(\text{O}_2\) and \(\text{CO}_2\)
2.3 Sample Analysis Equipment

2.3.1 High pressure homogeniser

Sample disruption was achieved by using a "Gaulin Micron Lab 40, High-Efficiency-Homogenizer" (APV Gaulin GmbH). The homogeniser was a hydraulic pump working batchwise at single strokes, with an operating range of 100-1600 bar. The homogeniser parts contacting the sample liquid consist of high grade stainless steel, stellite, rubber and PTFE, these parts are cooled by a glycol flow through external coils. The gap between the impact ring and the valve is 0.35mm: the gap between the valve and valve seat varies from 50-25μm, for pressures of 100-1600bar, respectively. The homogeniser requires a 40 ml sample and has a dead volume of less than 2 ml. The hydraulic mechanism is automatically controlled by a proportional electronic control system: the pressure is adjusted by a dial and displayed as a digital readout. Once an operation cycle is initiated the machine completes the cycle to give one discrete homogeniser pass, further passes require a manual transfer of homogenate.

2.3.2 Spectrophotometers

Either a Beckman DU62 or DU650 (Beckman Instruments Inc., High Wycombe, UK.) spectrophotometer was used for the absorbance measurements. The DU62 is operated by a keypad and can perform enzyme rate assays with the addition of a plug-in kinetics module. The DU650 was used for the majority of the work. This spectrophotometer is controlled by a mouse and display monitor and has rate assay capability built in.

2.3.3 Rheometer

A Rheomat 115 rotational viscometer (Contraves AG, Zurich, Switzerland) was used. The system was fitted with concentric cylinders, allowing low shear rate ranges to be measured. The cylinder arrangement consisted of a fixed inner cylindrical block and outer cylinder, with a mobile cylinder that rotated between the two. The viscosity measurements were determined from the torque on the motor, which resulted from the
liquid friction attempting to slow the rotation of the mobile cylinder, measured by a deflecting torsion bar in the rheometer. The inner block was hollow and had water inlet and outlet channels at its base, for use in temperature control.

2.3.4 Image analyser

The system consisted of a Magiscan 2A image analyser (Joyce Loebl Ltd., Gateshead, UK) with a Polyvar microscope (Reichert-Jung, Vienna, Austria). The microscope slide bench was motor driven and once the limits of a suitable field of view were established, the image analysis software was used to drive the bench and analyse all images in that field. This not only saved time but eliminated user bias toward the selection of images.
2.4 Fermentation methods

2.4.1 Inoculum development and storage.

*S. cerevisiae*

Frozen cell suspensions were used to inoculate 2L plain sided shake flasks, containing 400mL of defined medium, as the first stage of all *S. cerevisiae* fermentations. The consistency in cell concentrations at the start of each shake flask incubation, gave good reproducibility in the subsequent fermentations. The inoculum for these flasks was generated as follows,

1) 3 shake flasks of defined medium (400mL in a 2L Plain sided flask, Table 2.1) were inoculated with cells from a YDP agar slope {10g/L Yeast extract (Oxoid, Unipath Ltd., Hampshire, UK), 20g/L Glucose, 20g/L Bactopeptone (Difco Laboratories, Michigan, USA), 12g/L Agar Technical No3 (Oxoid)} and incubated until the glucose was totally consumed.

2) The cells were harvested and centrifuged under sterile conditions, using 400mL autoclavable polypropylene pots, a JA-10 rotor spinning at 5000 rpm for 15 minutes at 4°C, in a J2-M1 centrifuge (Beckman Instruments Inc., High Wycombe, UK)-average RCF equal to 2744g.

3) The supernatant was decanted and the cell pellets combined and resuspended in 250mL of sterile YDpglycerol {Twice the concentration of YDP given above, but with glycerol substituted for the agar (37.5g glycerol, Fisons, UK)}.

4) The concentration cells of the suspension was determined by cell counting under the microscope (Slide depth was 1/400mm and total area was 4×10⁶ m²).

5) The suspension was pipetted into sterile disposable bijoux bottles to give a quantity of 3.78×10⁶ cells in each bijoux.

6) One bijoux was used to inoculate each 2L shake flask.
**S. erythraea**

Frozen spore suspensions were used to inoculate 50mL Difco Nutrient Broth in 250mL baffled shake flasks, as the first stage of all *S. erythraea* fermentations. Again the consistency in spore concentrations at the start of each shake flask incubation, gave good reproducibility in the subsequent fermentations. The spore suspensions were generated as follows, observing aseptic techniques throughout.

1) *S. erythraea* was cultured for three weeks at 28°C on a tomato-oatmeal-agar sporulation medium \{ 20g/L each of Tomato Puree ( J Sainsbury plc, London ), Oat Cereal with Apple ( Milupa ), and Agar Technical No. 1 ( Oxoid )\}.

2) The petri dishes were then flooded with 4mL of Thiosulphate Ringer solution ( Oxoid ) containing 0.001% Triton X-100. The spores were suspended by abrading the mycelial mat with 1.5mm diameter sterile glass beads agitated with a glass spreader.

3) The spore suspension was transferred to a test tube, containing additional glass beads, and first vortexed and then treated ultrasonically, in order to segregate spore clumps.

4) The suspension was mixed in a 1:1 ratio with a 40% sterile glycerol solution and finally filtered through non-absorbent cotton wool to remove spore clumps, agar and mycelial fragments.

5) The spore concentration of the suspension was determined by counting under the microscope ( as before ).

6) The suspension was pipetted into sterile disposable bijou bottles to give a quantity of $10^8$ spores in each bijoux. These were stored at -20°C, where they are stable to multiple thawing and freezing ( Hopwood *et al.*, 1985 ).

7) One bijou was used to inoculate each 250mL shake flask, giving a cell concentration of $2 \times 10^6$ spores/mL which is consistent with the report of Belmar-Beiny & Thomas ( 1991 ).
### 2.4.2 Inoculum stages

The inoculum stages for the various fermentations are summarised in Table 2.6; where more than one stage was required the successive stages are indicated by a “+”. The details of each stage are given after the table with the appropriate organism.

#### Table 2.6: Summary of inoculum stages used in the fermentations

<table>
<thead>
<tr>
<th>Final fermentation volume, L</th>
<th>Inoculum Stages</th>
<th>S. cerevisiae</th>
<th>S. erythraea</th>
</tr>
</thead>
<tbody>
<tr>
<td>{vessel used }</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5, { LH7L(1) &amp; (2) }</td>
<td>1×A †</td>
<td></td>
<td>1×C + 1×D</td>
</tr>
<tr>
<td>5, { LH7L (2) }</td>
<td>2×A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14, { LH20L }</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15, { Inceltech 25L }</td>
<td></td>
<td>1×C + 3×D</td>
<td></td>
</tr>
<tr>
<td>300, { Chemap 450L }</td>
<td>6×A + 1×B ‡</td>
<td>6×C + 6×D + 1×G</td>
<td></td>
</tr>
<tr>
<td>662, { Chemap 1500L }</td>
<td>6×A + 1×B</td>
<td>1×C + 1×E + 1×F + 1×G</td>
<td></td>
</tr>
</tbody>
</table>

† indicates that the 7L vessel was inoculated with one shake flask prepared in the manner described for “Stage A”.

‡ indicates that the inoculation of the 450L vessel involved two inoculum stages, the first stage consisting of six shake flasks prepared in the manner described for “Stage A”, which were then used to inoculate a 42L fermenter prepared in the manner described for “Stage B”. The 450L vessel was then inoculated using the broth from the 42L fermentation.

The calibration procedure for probes, the heating and cooling procedures for sterilisation and the general operation of the fermenters was in accordance with the standard operating procedures in the department, and are not reported. In general, fermenters required calibration and insertion of probes before sterilisation. The DOT probe was checked for calibration after sterilisation in the vessels under 450L, by sparging oxygen free nitrogen through the air inlet filter. Ports that were to take feeds from ancillaries had blank rubber septa inserted, to maintain sterility and pressure, and could be penetrated by a needle tip attached to the ancillary by sterilisable silicon tube. The needle tips were first wrapped in non-absorbent cotton wool and then aluminium foil for autoclaving; the addition port was sprayed with industrial methylated spirits prior to penetration with the needle. Ancillaries included measuring cylinders with side arms for
the addition of acid and alkali for pH control, conical flasks with side arm for the addition of separately sterilised media components or additional antifoam. Also the 7L(1)&(2), 20L and 42L(1) required the air inlet filter to be sterilised separately. All ancillaries were sterilised in an autoclave for 20 minutes at 121°C and 1Bar. pH control during the fermentations was achieved using either NaOH or H₂SO₄.

**S. cerevisiae fermentations**

The *S. cerevisiae* defined medium listed in Table 2.1 was used throughout the inoculum and final fermentation stages for the production of this organism. The medium had to be made in four distinct sterilisation Groups (Groups A, B, C and D: Table 2.1) to avoid precipitation, interaction or denaturation of components.

**Stage A:** 400mL of defined medium within a 2L plain sided shake flask. 17 hours at 28°C in rotary incubator at 200rpm and 1” throw. Sterilisation Group B was made as a 10 times concentrated stock, 40mL of this stock was sterilised with 320mL of water purified by reverse osmosis (R.O. water) in the shake flask by autoclaving (20 minutes). Group A was made as an 8 times concentrated stock of which 30mL was sterile pipetted into the shake flask; the stock was autoclaved alongside the flask. Group C was kept as a 500 times concentrated stock kept at 4°C in a dark glass bottle and used at 2mL/L. The stock required the addition of concentrated NaOH (pH ca. 8.5) to completely dissolve the vitamins. Group D was also kept at 4°C in a darkened bottle but as a 250 times concentrate and used at 4mL/L; both concentrates were filtered through 0.2μm cellulose acetate membrane filters (Whatman Ltd., Maidstone, UK) before storage. 0.8mL and 1.6mL of Groups C and D respectively, were sterile filtered into the shake flask once it had cooled to room temperature. The filter used for this purpose was a polypropylene self assembly syringe filter housing of 25mm diameter (Sartorius AG Goettingen, Germany) fitted with a 0.2μm cellulose acetate membrane filter. The housing complete with filter was wrapped in aluminium foil and placed in a lidded polypropylene bottle, this was then autoclaved with Groups A and B. Once the housing was unwrapped, a long syringe needle was attached to the appropriate end and then stabbed through the cotton wool bung of the flask. Groups C and D were filtered through the same housing, this was then washed through with 7.4mL of R.O. water to ensure all the nutrients had been added to the flask. The flask was inoculated with the contents of one bijoux of thawed stock.
Stage B: 30L defined medium in LH42L(1) fermenter for inoculation of the Chemap 450L and LH42L(2) for the Chemap 1500L vessel.

12 hours at 28°C, 350rpm stirrer speed, 0.5vvm airflow rate, pH 4.5.

The 42L vessels represented the largest vessels available for seeding in the department, and had to be used for both 300L and 662L fermentations.

Sterilisation Group B was first made as a concentrate in 2L, which was diluted with 25.6L of R.O. water in the fermenter. Polypropylene glycol was used as antifoam for all S. cerevisiae fermentations at 1mL/L, and 30mL was added to Group B in the vessel. The mixture was sterilised in the fermenter for 30 minutes at 1bar and 200rpm stirrer speed. Group A was made as a concentrate in 1.8L and sterilised in a flask with side arm along with the other ancillaries. This was added to the fermenter after the vessel was sterilised and cooled to the required temperature, and just prior to inoculation. Groups C and D were prepared and filtered as for Stage A, in this case being filtered into the Group A flask; 60mL and 120mL of Groups C and D were used respectively. The pH of the fermenter contents was adjusted prior to inoculation with the six Stage A flasks, via the Group A flask. For inoculation of the CH1500L fermenter, the broth was transferred via a sterilised silicone tube using air over-pressure in the 42L vessel. Inoculation of the 450L fermenter required drainage of the broth into two pre-sterilised glass aspirators, followed by pumping (peristaltic pump) in to the 450L vessel; the 450L was in a different location to the seed vessel.

*S. erythraea* fermentations

Initial inoculum stages used a complex medium to achieve good revival of the frozen spores, and higher growth rates than achievable with the defined medium (Table 2.2). The higher growth rate was necessary to restrict the likelihood of contamination by other microbes, as *S. erythraea* is relatively slow to grow. The inoculum stage just prior to the final fermentation used the defined medium (Table 2.2) to “tune” the growth of the organism to this medium. This defined medium was made in three distinct sterilisation Groups (Groups A, B and C: Table 2.2). No antifoam was added directly to the fermenters as this generally inhibits the growth of the organism, however Berox FMT-30 (Water Management & Gamlen Ltd., Worcestershire, UK) was added selectively to suppress excess foam via a flask with side arm.
Stage C: 50mL of Difco Nutrient Broth in a 250mL baffled shake flask rotary incubation for 31-36 hours at 28°C, 200rpm, 1” throw. The nutrient broth was made according to the manufacturers’ recommendation and autoclaved in the shake flask for 20 minutes at 121°C. The flask was inoculated with one bijoux of thawed stock culture.

Stage D: 500mL of defined *S. erythraea* medium in a 2L baffled shake flask rotary incubation for 25-32 hours at 28°C, 200rpm, 1” throw.

Groups A, B and C were sterilised separately and then added aseptically to the shake flask. Sterilisation Group B was made in 415mL of R.O. water and sterilised in the shake flask for 20 minutes at 121°C. Group A was made as a concentrate (116.7g/200mL) and sterilised in a Duran bottle with the flask: 30mL of concentrate was sterile pipetted into the flask. Group C was first made as three separate parts and then combined to give a 100 times concentrate, 5mL of this was sterile filtered into the flask in the manner described for Stage A. The concentrate was stored in a dark glass bottle at 4°C. The flask was inoculated with the Stage C broth.

Stage E: 500mL of Difco Nutrient Broth in a 2L baffled shake flask rotary incubation for 30 hours at 28°C, 200rpm, 1” throw.

The nutrient broth was made according to the manufactures recommendation and autoclaved in the shake flask for 20 minutes at 121°C. The flask was inoculated with the Stage C broth.

Stage F: 5L of complex medium in a 7L(1) 24 hours at 28°C, 500rpm stirrer speed, 1vvm airflow rate, pH 7. The medium used here was suggested by Pollard in 1995 (adapted from Yamamoto, 1986). This medium was made in three parts. Part 1 consisted of 20g Bactopeptone (Difco), 30g Yeast Extract (Oxoid) and 10g Glycine (Analar, BDH) and 5mL ppg antifoam in 3.45L R.O. This was sterilised for 20 minutes at 1Bar in the fermenter. Part 2 was 150g of glucose in 0.9L and was autoclaved in a flask with side arm for 20 minutes at 121°C. Part 3 was 3.4g of KH₂PO₄ in 0.15L and was autoclaved in a Duran bottle with the side flask. Part 3 was added to the fermenter via the flask with side arm. The inoculum for this stage was the broth from stage E.
Stage G: 30L of defined *S. erythraea* medium in 42L.(2)

40 hours at 28°C, rpm, 350rpm stirrer speed, 0.5vvm airflow rate, pH 7.

Sterilisation Group B was first made as a concentrate in 2L and diluted in the vessel with 21L of R.O. water. The mixture was sterilised in the fermenter for 30 minutes at 1Bar and 200rpm stirrer speed. Group A was made as a concentrate in 1.7L and was autoclaved in a flask with side arm for 20 minutes at 121°C. 300mL of the Group C concentrate, prepared as in Stage C, was filter sterilised via the flask with side arm. The vessel was inoculated with the broth from Stage F. The broth was forced out of the base of the 7L fermenter used in Stage F, through a sterilised silicone tube, using air pressure in the headspace of the 7L vessel.

2.4.3 Fermentation stages

Tables 2.7 and 2.8 detail the methods used for sterilising the separate media Groups for *S. cerevisiae* and *S. erythraea* fermentations, respectively. Further details are given after the appropriate table. It was not possible to keep sterilisation times constant on scale-up, however the effects of the variation in time on the medium quality was minimised by separating the components into different groups.
S. cerevisiae fermentations

<table>
<thead>
<tr>
<th>Sterilisation Group</th>
<th>Fermenter ID (Table 2.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7L(1)</td>
</tr>
<tr>
<td>A</td>
<td>500mL in FSA¹</td>
</tr>
<tr>
<td>B</td>
<td>4L in Vessel</td>
</tr>
<tr>
<td>C</td>
<td>10mL FS² into FSA</td>
</tr>
<tr>
<td>D</td>
<td>20mL FS into FSA</td>
</tr>
<tr>
<td>Inoculum volume</td>
<td>400mL</td>
</tr>
<tr>
<td>Final volume</td>
<td>5L</td>
</tr>
</tbody>
</table>

¹ FSA, flask with side arm
² FS, filter sterilised through 25mm diameter syringe filter
³ autoclaved for 1.5 hours at 121°C
⁴ autoclaved for 20 minutes at 121°C
⁵ split between 2 aspirators and autoclaved for 1.5 hours at 121°C

N.B. Polypropylene glycol was used as antifoam, at 1mL/L, in Group B.

The operation of the 7L(1) and 20L fermenters was similar to that described for this size of vessel in the section 2.4.2. The larger vessels used (450L and 1500L fermenters) required the further modifications given below.

- Sterilisation Group D was autoclaved; no loss of medium quality resulted.
- Aspirators were required to hold the larger volumes of liquid. These required longer sterilisation periods in the autoclave compared to a flask with side arm. Apart from this they were treated in a similar manner to other ancillaries, and were drained into the fermenter with the use of a peristaltic pump.
- Group B (the fermenter) was sterilised at 1bar (121°C) for 30 minutes in the case of 450L vessel, and 40 minutes in the case 1500L vessel.
### S. erythraea fermentations

**Table 2.8: S. erythraea fermentation medium production method**

<table>
<thead>
<tr>
<th>Sterilisation</th>
<th>Fermentation scales (operating volume is given in Table 2.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>7L(2)</td>
</tr>
<tr>
<td>A</td>
<td>500mL in FSA&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>4L in Vessel</td>
</tr>
<tr>
<td>C</td>
<td>100mL FS&lt;sup&gt;2&lt;/sup&gt; into FSA</td>
</tr>
<tr>
<td>Inoculum volume</td>
<td>500mL</td>
</tr>
<tr>
<td>Final volume</td>
<td>5L</td>
</tr>
</tbody>
</table>

<sup>1</sup> FSA, flask with side arm

<sup>2</sup> FS, filter sterilised through 25mm diameter syringe filter

<sup>3</sup> autoclaved for 1.5 hours at 121°C

<sup>4</sup> filter sterilised through liquid filter (see text below)

<sup>5</sup> split between 2 aspirators and autoclaved for 1.5 hours at 121°C

N.B. Berox FMT-30 was used as antifoam as required during the fermentation

The modifications detailed below Table 2.7 for large scale fermentations, applied for S. erythraea fermentations also. A further modification was the method of filter sterilisation of Group C. The larger liquid volume had to be filtered via a sterilisable stainless steel housing containing a 0.2μm liquid filter. The housing was first primed with R.O. water using a peristaltic pump, the trace elements were then pumped through followed by further R.O. water to purge the housing.
2.5 Sample homogenisation method

For a particular homogenising system, temperature, pressure and organism, the rate constant \((K)\) depends on the resistance of the cells to being ruptured. Therefore the value of \(K\) determined at a particular stage in the growth cycle of an organism, may be taken as an indication of the wall strength of the cells at that point. As indicated in section 1.5.2.1, the cell wall strength is given by the value of the rate constant, \(K\) (Equation 1.4.3.), which is obtained from the slope of the line of a plot of \(\log(Rm/Rm-R)\) vs. \(N\) (Figure 2.2.). As \(K\) indicates the rate of disruption, the wall strength is inversely proportional to \(K\) (the larger the \(K\) value the weaker the cells).

Figure 2.1 illustrates the typical pattern of soluble protein release from \textit{S. cerevisiae}. The black and red squares show the protein release from two samples, taken at different fermentation times, homogenised under the same conditions (principally a low homogenisation pressure: e.g. 300bar). The triangles represent the homogenisation of the second sample at a much higher pressure (e.g. 1200bar); the three curves have the same \(Rm\) (Equation 1.43.). While the low pressure does not give complete release of the soluble proteins, it does give an incremental release with successive passes which provides more data points for the accurate determination of the release kinetics. In contrast, the higher pressure gives complete release very quickly but only few data points for the determination of the release kinetics. Therefore the wall strength determination requires the use of a low homogeniser pressure and repeated passing of the sample through the homogeniser to determine the rate kinetics, and the use of a high pressure to determine the maximum concentration of soluble protein available for release. In this study a pressure of 300bar and 100bar were found to be suitable as the lower pressure for \textit{S. cerevisiae} and \textit{S. erythraea}, respectively, while 1200bar was suitable as the upper pressure. In the figures 2.1 and 2.2 the wall strength of sample one is less than that of sample two because the rate of release of the internal soluble proteins is faster, with respect to the number of homogeniser passes.
The figure illustrates the soluble protein release profiles from a weak (sample one) and a strong (sample two) cell. Also the effect of increased homogeniser pressure on the release profile.

**Figure 2.1:** Typical profile of soluble protein release during high pressure homogenisation
The cell wall strength is determined from the slope of the lines in the above plot. The greater the slope, the weaker the cells being homogenised. The cells in sample one are weaker in this case.

**Figure 2.2:** Origin of wall strength values from homogenisation data
Homogenisation method

Each cell strength determination required the removal of approximately 80 mL of fermentation broth (40mL each for homogenisation at two pressures). A 1mL volume was removed with a teat pipette into a 1.6mL Eppendorf standing in an ice bath, this sample was used to determine the background levels of constituents in the subsequent assays. The remainder of the sample was split into two lots for homogenisation at the two different pressures. The first lot was homogenised at the higher pressure, 1200bar, because the homogeniser assembly was at its coolest at the beginning and the heating effect is greatest for the higher pressure. Three discrete passes were made through the homogeniser, and 1mL of sample was removed after each pass into a 1.6mL Eppendorf in the ice bath. After the high pressure passes the homogeniser assembly was wiped clean with a sheet of engineers blue roll paper. The assembly was not washed clean as the hold-up volume tended to dilute samples on the low pressure runs and increase the temperature of the assembly. Once dry, the remaining portion of the fermentation broth was homogenised at the lower pressure for five discrete passes. Again 1mL samples were extracted after each pass and stored on ice in Eppendorfs. After homogenisation the Eppendorfs were centrifuged for two minutes to remove debris (Anderman Eppendorf centrifuge 5414, Eppendorf Gerätbau, Netheler + Hinz GmbH, West Germany); the supernatant was assayed as required.
2.6 ASSAY METHODS

Figures 2.3 & 2.4 show the breakdown of the sampling process with details of the volumes of broth required for each assay (NB. Conductivity measurements were only taken at the large scale, to reduce the effect of sample loss on the fermentation).

---

Sample frequency is approximately every 1-4 hours over a 27 hour period

<table>
<thead>
<tr>
<th>Volume</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mL</td>
<td>DCW Pellet dried</td>
</tr>
<tr>
<td></td>
<td>Supernatant assayed for [glucose] &amp; [ethanol]</td>
</tr>
<tr>
<td>1 mL</td>
<td>Determination of background levels of protein and enzyme concentrations in broth</td>
</tr>
<tr>
<td>40 mL</td>
<td>Homogenisation at 1200 bar</td>
</tr>
</tbody>
</table>
|        | Supernatant assayed for;  
|        | i) Total soluble protein, Rm value for cell wall strength determination (Section 1.5.2.1)  
|        | ii) ADH activity  
|        | iii) G6PDH activity |
| 40 mL  | Homogenisation at 300 bar |
|        | Supernatant assayed for;  
|        | i) Total soluble protein, R value for cell wall strength determination  
|        | ii) ADH activity  
|        | iii) G6PDH activity |
| 60 mL  | Whole broth conductivity |

---

**Figure 2.3:** Illustration of the sample processing during *S. cerevisiae* fermentations

---

Sampling frequency is 2-3 samples daily over a 7-8 day period

<table>
<thead>
<tr>
<th>Volume</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 mL</td>
<td>DCW Pellet dried</td>
</tr>
<tr>
<td></td>
<td>Supernatant assayed for [erythromycin], [glucose] &amp; [nitrate]</td>
</tr>
<tr>
<td>1 mL</td>
<td>Determination of background levels of protein and enzyme concentrations in broth</td>
</tr>
<tr>
<td>40 mL</td>
<td>Homogenisation at 1200 bar</td>
</tr>
</tbody>
</table>
|        | Supernatant assayed for;  
|        | i) Total soluble protein, Rm value for cell wall strength determination  
|        | ii) G6PDH activity |
| 40 mL  | Homogenisation at 300 bar |
|        | Supernatant assayed for;  
|        | i) Total soluble protein, R value for cell wall strength determination  
|        | ii) G6PDH activity |
| 60 mL  | Whole broth conductivity |
| 20 mL  | Rheological measurements |
| 1 mL   | Morphological measurements / Image analysis |

---

**Figure 2.4:** Illustration of the sample processing during *S. erythraea* fermentations
2.6.1 Cell soluble protein measurements

The Bio-Rad protein assay (Bio-Rad laboratories, Hemel Hempstead, Herts, UK) was used to determine soluble protein concentrations in the homogenate supernatants. This method is based on the shift in absorbance maximum of the dye, Coomassie Brilliant Blue G-250, when it binds protein under acidic conditions.

Protein assay method

The standard assay method was used with the following modifications. A 1mL volume of diluted reagent was pipetted into a plastic disposable cuvette and 50μL of sample added to this. The cuvette was inverted to mix the contents, keeping the mouth covered with parafilm. The absorbance was read at 595nm between 5-30 minutes after adding the sample and at room temperature. The spectrophotometer was blanked against 1mL of dye reagent containing 50μL of R.O. water. Samples were diluted with R.O. water prior to adding them to the dye reagent such that the absorbance was kept below 0.7AU.

Calculation of soluble protein content

The soluble protein content of the samples was determined by comparison to a standard curve. The standards for this curve were prepared by making various dilutions of Bovine Serum Albumin, supplied by Bio-Rad, with R.O. water; concentrations were in the range 0.01-0.6mg/mL. These dilutions were tested for soluble protein content in the manner described. The data for BSA concentration was plotted as the dependent variable against absorbance at 595nm. A curve was fitted to this data by regression analysis, yielding an equation of the form, using the spreadsheet package “Origin” (Microcal Software Inc., Northampton, USA).

\[ y = A_0 + A_1x + A_2x^2 + A_3x^3 + \ldots \]  

Polynomial regression for the determination of soluble protein from optical density data produced by the Biorad assay.

The protein content of the homogenate samples was determined by using the derived equation. The standard curve was prepared for each batch of diluted dye reagent, which was used for a maximum of two weeks.
2.6.2 Alcohol dehydrogenase (EC 1.1.1.1), ADH

This method is based on the oxidation of ethanol to acetaldehyde by alcohol dehydrogenase in the presence of NAD, the conversion of NAD to NADH gives an increase in absorbance at 340nm (Bergmeyer, 1983): the complexing of acetaldehyde with the semicarbazide drives the reaction in the forward direction.

The following chemicals were prepared separately in R.O. water then combined, aliquoted into 40mL tubes (Falcon) and stored frozen at -70°C. Tubes were thawed and kept at 25°C when required for use. Unused mixture was discarded.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Relative volume</th>
<th>Grade / product code / supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium pyrophosphate buffer (0.1mol/L; pH 9.0 with conc. HCl; containing glycine, 1.67 mg/mL)</td>
<td>250</td>
<td>10261 / BDH</td>
</tr>
<tr>
<td>Semicarbazide hydrochloride (250mg/mL; pH ca. 6.5)</td>
<td>10</td>
<td>101196 / BDH</td>
</tr>
<tr>
<td>ethanol (96%, v/v)</td>
<td>10</td>
<td>S 2201 / Aldrich</td>
</tr>
<tr>
<td>NAD (20mg/mL)</td>
<td>20</td>
<td>N 7004 / Sigma</td>
</tr>
<tr>
<td>Glutathione reduced (90mg/mL)</td>
<td>1</td>
<td>G 4251 / Sigma</td>
</tr>
</tbody>
</table>

**Method**

1mL of the assay mixture was pipetted into a 1.6mL plastic disposable cuvette and between 200-50 μL* of homogenate supernatant was added. The sample was mixed by inverting the cuvette twice, keeping the mouth covered by parafilm. The absorbance change of the mixture was measured in the spectrophotometer over a one minute period, having blanked the spectrophotometer with a cuvette prepared as above but with an equivalent volume of R.O. water in place of the sample; the final absorbance was kept
below 1.0 AU (absorption units) by prior dilution of the samples with R.O. water. The rate of change of absorbance, $\delta$, in absorbance units per minute, was calculated by the spectrophotometer.

* The volume of sample added was reduced from 200 – 50 $\mu$L as the concentration of the ADH increased, eventually prior dilution of the samples was required, using RO water, the diluted samples were always added as 50 $\mu$L volumes.

Calculation

The enzyme activity within the sample was calculated by using the following equation, (Bergmeyer, 1983)

$$U = \left( \frac{\delta}{\varepsilon} \right) \times \left( \frac{V}{v} \right) \times D \quad (2.5)$$

Where: $U$, enzyme activity, Units/mL
   $\delta$, rate of absorbance change determined from spectrophotometer, min$^{-1}$
   $\varepsilon$, extinction coefficient of NADH, moL/L.cm
   $V$, total volume of assay mixture, mL
   $v$, volume of sample in the assay mixture, mL
   $D$, dilution factor of sample

The value of the extinction coefficient, $\varepsilon$, used was 6.22 moL/L.cm.

NB. One Unit of enzyme is equivalent to the conversion of 1mmoL of NAD in one minute under the specified assay conditions.
2.6.3 Glucose-6-phosphate dehydrogenase (EC 1.1.1.49), G6P-DH

This method is based on the oxidation of D-glucose-6-phosphate to D-gluconate-6-phosphate by G6P-DH in the presence of NADP, the conversion of NADP to NADPH gives an increase in absorbance at 340 nm (Bergmeyer, 1983).

Table 2.10: G6PDH assay Mixture

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Relative volume</th>
<th>Grade / product code / supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiethanolamine buffer (0.1mol/L; pH 7.6 with conc. HCl)</td>
<td>25</td>
<td>98% / T-1377 / Sigma</td>
</tr>
<tr>
<td>Magnesium chloride (0.1mol/L)</td>
<td>2</td>
<td>99% ACS / / Aldrich</td>
</tr>
<tr>
<td>Glucose-6-P, Na salt, (10mg/mL)</td>
<td>1</td>
<td>98% / 28,597-8 / Aldrich</td>
</tr>
<tr>
<td>NADP, Na salt, (10mg/mL)</td>
<td>1</td>
<td>98% / N-0505 / Sigma</td>
</tr>
</tbody>
</table>

Method & Calculation

The method and calculation was identical to that used for the assay of ADH except prior dilution of the sample was usually unnecessary. The value used for the extinction coefficient was 6.2 mol/L.cm.

2.6.4 Erythromycin assay

The broth supernatant from the S. erythraea fermentations was tested for the antibiotic, erythromycin, by a plate inhibition bioassay technique. The fermentation samples were decanted supernatants from the DCW determinations, which were stored frozen at -20°C. These were thawed at the end of the fermentation, diluted with RO water and then loaded on to an agar plate prepared as detailed below. The diffusion of erythromycin through the agar gel inhibited the growth of the antibiotic sensitive organism, Arthrobacter citreus, inoculated on the plate. The diameter of the zone of inhibition was related to the concentration of erythromycin in the samples loaded. Aseptic technique was required throughout the assay procedure.
Method
A 250 mL baffled shake flask containing 50mL of Nutrient Broth (1, Oxoid, Unipath Ltd., Basingstoke, Hampshire, UK.) was inoculated with a culture of *Arthrobacter citreus* from a sub-master culture on a petri dish, and incubated for 24 hours in a rotary incubator (28°C and 200rpm).

The glass petri dishes used for the assay were washed with disinfectant (Tego 2000, TH Goldschmidt Ltd., Milton Keynes, UK), sterilised with industrial methylated spirits and allowed to dry. The plates measured 260mm square and 10mm deep. For each plate used, 5mL of the overnight *A. citreus* culture was added to 300mL of sterilised nutrient agar {3.9g of Oxoid Nutrient Broth + 6.0g Bacteriological agar No. 1 (L11, Oxoid)} once the agar had cooled to 40°C. The agar was poured on to the plate and allowed to set for 2 hours. During this period the samples and standards were prepared for loading on to the plate. After this period 8mm diameter wells were cut into the agar, at 40mm centres, using a flamed metal cork borer (Pattersons, Luton, UK) attached to a vacuum suction apparatus. The wells were loaded with 100μL of appropriately diluted samples and standards using sterile pipette tips; the well layout is shown in Figure 2.5. Wells 1-8 were loaded with erythromycin dihydrate (98%; product code 85, 619-3; Aldrich) standards and A-J were loaded with fermentation samples. The standard concentrations used were 0, 5, 10, 20, 40, 60, 80 and 100 % of a 100mg/mL solution made in sterile RO water: the 100% standard was loaded in well number 1. The plate was incubated for 24 hours at 28°C and left for a further 24 hours at room temperature before reading the diameter of the zones of inhibition around the wells using a ruler.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>8</th>
<th>7</th>
<th>6</th>
</tr>
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<tbody>
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<td>C</td>
<td>D</td>
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<td>B</td>
<td>1</td>
</tr>
</tbody>
</table>

**Figure 2.5:** Well layout for erythromycin assay
Calculation of erythromycin concentration

The logarithm of the antibiotic concentration is proportional to the squared distance between the reservoir and the zone border line as described below,

\[
\ln m = \ln m_o - \frac{(d - x)^2}{4DT_o} \quad (\text{Naveke, 1988})
\]  

(2.6)

Where:
- \(m\), antibiotic concentration at the diameter, \(d\)
- \(m_o\), initial antibiotic concentration
- \((d-x)^2\), distance between reservoir and zone border
- \(D\), diffusion coefficient
- \(T_o\), absolute temperature

Standard curves were constructed on this theory in Origin (Microcal Software Inc., Northampton, USA); regression analysis gave an equation of the form of Equation 2.4, for the determination of antibiotic concentrations in the samples.

### 2.6.5 Broth glucose concentration

Broth glucose concentrations during the batch fermentations of S. cerevisiae were determined by using a glucose test kit supplied by Sigma diagnostics (Glucose test kit, 115, Sigma chemical Co. Ltd., Poole, Dorset, U.K.). The primary reaction in the test involves the conversion of glucose to glucose-6-phosphate by hexokinase. This is linked by two intermediate stages to the final step, the reduction of iodonitrotetrazolium, which gives an absorbance change at 520nm.

Approximate glucose concentrations for inoculum stages could be determined by using a cheaper glucose test (Diastix, Bayer Diagnostics, product code 2804, Ames, Basingstoke, UK).
2.6.6 Broth ethanol concentration

The concentration of ethanol formed during the batch fermentation of *S. cerevisiae* was determined by using an enzyme assay kit (Ethanol test kit, 176 290, Boehringer Mannheim GmbH, Mannheim, Germany). The method relies on the oxidation of ethanol to acetaldehyde by NAD in the presence of the enzyme alcohol dehydrogenase. The reaction requires alkaline conditions and the removal of the acetaldehyde to acetic acid by of the enzyme aldehyde dehydrogenase. The method followed was as described in the kit.

2.6.7 Broth conductivity

The broth conductivity was measured using a AGB1000 conductivity meter with temperature compensating probe (Canterbury Scientific Instruments Ltd., Canterbury, Kent). The probe was calibrated to read 1.413mS/cm at 25°C with a 0.01M KCl solution (Analar, 10198, BDH). The temperature readout was allowed to stabilise before taking the reading.

2.6.8 Dry cell weight, DCW

DCW determinations were made by centrifugation of a whole broth sample, decanting of the supernatant, washing with an equal volume of RO water, decanting of the wash water followed by overnight drying of the pellet at 105°C. The broth supernatant was kept frozen at −20°C for further assaying as necessary. In the case of *S. cerevisiae* a 20mL sample was centrifuged in a pre-weighed 50mL plastic tube for 15 minutes at 4400rpm at room temperature in a Centaur 2 MSE centrifuge (Fisons, Loughborough, UK). For *S. erythraea* 12mL samples were centrifuged in 12mL plastic tubes for 10 minutes at 10,000g and 4°C in a Beckman L7 ultracentrifuge (SW40Ti rotor). The samples were bought to room temperature in a desiccator over silica gel before weighing.
2.6.9 Rheological measurements

The rheological behaviour of *S. erythraea* broths was measured using the Rheomat 115 (Section 2.3.3). The rheometer has very wide use and an extensive shear rate range (Warren, 1992).

1) The viscometer stand was levelled with a spirit level to ensure vertical rotation of the cylinders. The cylinders were wiped dry, assembled, and screwed into the appropriate holder on the viscometer stand. The motor rested in a countersunk hole above the cylinders.

2) The temperature control system was switched on approximately 20 minutes before measurements began. The system consisted of a water bath controlled to 28°C (fermentation broth temperature), and independently checked with a mercury thermometer. Water was pumped from this bath, via silicone tubes and a peristaltic pump, through the hollow inner cylinder of measuring apparaunts.

3) The viscometer was zeroed by the following procedure. The viscometer was switched on without the cylinders coupled, and with the low pass filter set at No.3. The arbitrary reading shown on the display, was then set to ±0.00 by turning the zeroing knob to the right of the display. The zeroing was checked by switching on the motor and manually rotating the cylinder coupling to produce a reading on the display, which should go back to ±0.00 when the coupling was allowed to stop: otherwise the zeroing procedure was started again.

4) The cylinders were coupled to the motor after ensuring that the male (cylinder side) and female (motor side) parts were in correct alignment.

5) The measuring cylinder was filled to the top of the inner cylinder block with the sample (approximately 20mL of fermentation broth). The speed module was set to position ‘N’, and the speed indicator to 15, by using the speed up/down buttons; this rotated the mobile cylinder at its highest speed and ensured homogeneity of the broth. The display reading was noted when it became steady.
The speed of the motor was decreased stepwise, from step 15-step 1, at 10 second intervals; the digital reading was recorded at each stage.

6) The torque readings (Nm) and rotational speeds (rpm) were converted to shear rates (s\(^{-1}\)) and shear stresses (Pa) using conversion factors from the viscometer operating instructions booklet. Shear rates were in the range 3680-209s\(^{-1}\).

7) The rheometer was calibrated using silicone fluid, having a viscosity of 1Pa.s. This is a Newtonian fluid, and gives values of K=1 and n=1 for the Power Law model (Equation 1.44). Calibration was only required occasionally.

2.6.10 Morphological measurements

The morphology of *S. erythraea* broth samples was determined using the equipment detailed in Section 2.3.4.

1) Broth samples were diluted with RO water to the extent that each field under the microscope contained approximately 5 discrete images, typically a dilution factor of 10-40 was required. The concentration of organisms selected gave the best balance of speed of measurement (fewer fields of view to measure) and accuracy of morphological measurement (less overlap of separate mycelia and clumps).

2) Each slide was “spotted” with twelve 5μL drops of the appropriate dilution and allowed to air dry on a flat surface. The spotting method gave a more even distribution of mycelia on the dried slide compared to the drying of a large droplet or film of sample, which was probably due to surface tension forces.

3) The dried slide was stained by flooding it with a solution of 10mM methylene blue containing 1mM sodium azide, the stain was kept in a dark glass bottle under chloroform. The stain was washed off with RO water after 30 seconds and the slide allowed to dry again but now in a vertical position.
4) The microscope lamp was switched on approximately 10 minutes before analysis, to allow the system to equilibrate and produce a steady light output. The condenser was centred and focused and then the diaphragm aperture adjusted to just wider than the field of view. The light output from the lamp was adjusted to give an optimum level of illumination and contrast. The slide was focused using the 10×, and the 40× objective, and scanned for a suitable area for analysis: i.e. reasonable cell density of non-overlapping images. The total magnification was 600× (40× objective, 10× eyepiece, 1.5× camera zoom)

5) The system was instructed to measure images in 100 fields on the selected slide area, in a 10×10 grid. The microscope was first “manually” focused at the four corners of the grid: this was achieved by allowing the motorised stage to bring the corner images of the grid under the objective, and then manually controlling the focusing motor on the microscope. Once the corner focal positions had been recorded, the system could interpolate the focal positions for the remaining fields on the grid, during automatic operation.

6) The greyness levels, circularity factor (0.84, Equation 1.47) and minimum branch length (1μm) were set for the thresholding process (Section 1.5.3). The automatic analysis was begun. The data produced are given in Table 1.10

7) The calculation of the hyphal lengths used a pre-set calibration factor (pixels per micrometer) for the magnification and set-up used: this was determined by displaying the image of a graduated scale on the computer monitor and specifying the distance between two recorded positions on the scale.
2.7 Determination of Mixing Times

Bulk mixing times were determined for water in 7L(1), 20L and 1500L fermenters. Potassium permanganate was used as a dye and the mixing monitored by a spectrophotometer, linked to a computer, and fitted with a flow cell. The water was stirred at different stirrer speeds and either unaerated or aerated at 0.5 vvm according to the scheme in Table 2.11.

<table>
<thead>
<tr>
<th>Fermenter total volume, L</th>
<th>Operating volume, L</th>
<th>Stirrer speeds used, rpm</th>
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<tr>
<td></td>
<td></td>
<td>Unaerated</td>
</tr>
<tr>
<td>1500</td>
<td>662</td>
<td>200-400</td>
</tr>
<tr>
<td>20</td>
<td>14</td>
<td>100-600</td>
</tr>
<tr>
<td>7(1)</td>
<td>5</td>
<td>100-450</td>
</tr>
</tbody>
</table>

1 The maximum stirrer speeds used are lower in the case of vessels that are unaerated because these represent the limit above which surface aeration occurs.

General

Experiments were conducted by working from low to high stirrer speeds, giving sufficient time in-between each reading to allow the mixing to equilibrate to the new conditions. Once the system was ready, a constant metered flow of water was allowed to pass out to the measuring apparatus through the sampling port (1500L vessel) or drain valve (20L & 7L vessels). The response time of the system could be determined from the measured flow rates and knowing the tube lengths and diameters in the drain system. The spectrophotometer reading was allowed to steady, and then tared to zero before addition of the dye. The computer system monitoring was also begun when the system had equilibrated. The appropriate volume of dye was added to the liquid surface midway between the vessel wall and the impeller shaft. In the case of the 1500L vessel the required volume was poured onto the surface from a beaker, whereas an automatic pipette (Finnpipette, Labsystems Ltd., UK) was used for accurate dispensing of the smaller volumes required for the 7L and 20L vessels. At the moment the dye had been added, a stopwatch was started to allow back-calculation of the exact start time of the
dye addition according to the computer. The stopwatch and computer were stopped together, once the computer trace of the OD had become stable, at around 0.3 absorption units. Because the rate at which OD readings were taken by the spectrophotometer was known and each reading was numbered, the exact start time could be determined from the computer data.

**Potassium permanganate dye**

A highly concentrated (56.34g/L) solution of potassium permanganate was made for these experiments. This allowed for the addition of as discrete/small a volume of liquid as possible to the vessel, such that the volume in the fermenter was not significantly altered by the addition of the dye. The permanganate had the advantage of being able to be made into a highly concentrated solution without its viscosity increasing greatly relative to water. It could also produce a large change in absorbance on the addition of a small volume of the liquid. The particular volume that was required to be added to either of the vessels was determined such that the rise in optical density of the water in the vessel would be 0.3 absorption units at the optimum wavelength. This allowed for four mixing time determinations to be made before the water became too optically dense and had to be changed. Also by knowing the final OD on complete mixing, a check could be made of the calculated mixing times to see if they were accurate reflections of the mixing profiles. The volumes of dye added were 250mL, 5.00mL and 1.90mL for fermenter volumes of 662L, 14L and 5L, respectively. The optimum wavelength was determined as 525nm by performing a wavelength scan on a spectrophotometer, against a water blank.

**Physical set-up**

*1500 Litre experiments:*

This vessel was sampled through the sample port which was situated 0.65m above the base of the vessel and located in the upper mixing zone of the liquid volume (Table 2.4). The set-up of the system is shown in Figure 2.6; the liquid out-flow was by gravity. The water flowed through a silicone tube of 60cm length and 8mm ID to an unequal T (bubble trap), the majority of the flow passed through 90° and out to a bucket via 83cm of 8mm ID silicone tube. The remainder passed through 41cm of 1mm ID silicone tube to the flow cell. Under unaerated conditions the total flow rate out of the vessel was 202.35mL/s with a response lag of 0.39s at the flow-cell, whereas during
aeration the flow rate was 20.05mL/s with a response lag of 3.95s at the flow cell. The loss of volume from the vessel was <2%/minute.

7 & 20 Litre experiments:
These vessels were sampled via the vessel drain located in the baseplate. The system configuration is illustrated in Figure 2.7. In this case the out-flow from the vessels was controlled by a peristaltic pump (Watson Marlow, 502S operating at 70% capacity). The flow was initially via 80cm of 3mm ID silicone tube which was reduced to 41cm of 1mm ID tube; the flow rate at the cell was 2.87mL/s giving a response lag of 2.08s. Bubbles did not flow out of the baseplate of either vessel therefore a trap was not required. The loss of volume from either vessel was <3.5%/minute.

Flow-cell
This consisted of a solid block of quartz glass, the size of an ordinary cuvette with 1cm path length, except that a fine channel directed liquid flow to an optical density measuring chamber, with a volume of the order of 80µL. The light from the spectrophotometer lamp was restricted to pass through only that section of the flow path that was parallel to the light path, by blackening of all other sections of the flow-cell. Silicone tubes of 1mm ID connected to either end of the flow path where they emerged at the upper face of the flow-cell.

Electronic set-up
Spectrophotometer: A LKB Biochrom Ultrospec II (LKB Biochrom Ltd., Cambridge, UK) was used to measure absorbance changes. The spectrophotometer had an analogue, bidirectional RS232C port and LKB net digital interface. The processed photodiode signal was stored on an onboard microcomputer memory. Communication was established between the external computer via the RS232C serial link. The rate at which readings were taken was determined as 2.433/s.

Computer system: An IBM compatible PC (486sx, 33MHz, 8MB RAM, 150MB hard disk) running a software package called Lab View (National Instruments) was used for data acquisition and storage. This recorded the data from the spectrophotometer and numbered each data point. A trace of absorbance was plotted against Reading Number (effectively time) continuously and on-line.
Calculation of the mixing time

Curve fitting method/equation

The data for absorbance against elapsed time were plotted in the spreadsheet package “Origin” (Microcal Software Inc., Northampton, USA). The first order response function below, was used for curve fitting in this package. The package determined values for A, B and Xo by iteration (Habib, 1995), reporting their errors and a Chi² value for “goodness of fit”.

\[ A \left[ 1 - \exp \left( -\frac{X - X_0}{B} \right) \right] \]  

(2.7)

Where: A, total gain in absorbance, approximately 0.3 A.U.

B, speed of response, where 1*B ≈ 63% of A, 3*B ≈ 95% of A &

5*B ≈ 99% of A

Xo, initial point from where the curve was fitted

\(^1\) Due to the response lag time of the measuring system the shape of the curve of OD vs. Elapsed time was “sigmoidal” therefore the lower tail of the curve was cut off at a point Xo in order to fit the equation (2.7).

The mixing of the dye was considered to be a first order decay process, the mixing time was taken as (3*B)+Xo-Response Lag Time, or the time taken to reach 95% of A: equivalent to 95% of the total mixing time. The error in the data was typically less than 2%. The results of the mixing time experiments are given in section 4.8.
Figure 2.6: Equipment arrangement for mixing time measurements in the 1500L vessel
Figure 2.7: Equipment arrangement for mixing time measurements in the 7L & 20L vessels
3 RESULTS OF S. CEREVISIAE FERMENTATIONS

This section details the results gathered toward identifying the effects of scale-up on the cell qualities of S. cerevisiae. The cell qualities of interest were the dry cell weight, ADH and G6PDH enzymes, the cell total soluble protein, and the cell wall strength (based on cell disruption data and subsequent release of total soluble protein). While the cell biomass and enzymes are products in their own right, along with the other qualities they may be useful indicators of whether the cell is being affected by its environment: for example, greater heterogeneity in the environment may reduce the biomass yield, while greater shear may influence the cell strength. Additionally, the enzyme release profiles could be used to confirm the soluble protein release data, as these enzymes are cytoplasmically located (Follows et al., 1971). The information on the effect of scale on these cell qualities was enhanced by collecting exit gas data (Section 3.2), broth glucose and ethanol concentrations (Section 3.7) and broth conductivity data (Section 3.8).

A reference set of cell quality information was produced from small scale fermentations (7L and 20L scale), against which the large scale fermentation (450L and 1500L scale) results could be compared by statistical analysis (Section 5.2). The principal small scale vessel used was the LH 20L fermenter (Table 2.3, Section 2.2.1), which had an operating volume of 14L (Table 2.7, Section 2.4.3). This size of vessel, equipped with three turbine impellers, would be regarded as being a well mixed homogenous system, in which oxygen limitations should not occur for the operating conditions used; in which case it would be ideal as a reference base for assessing cell qualities. Though smaller vessels were available, and might be regarded as being more homogeneous, the 20L vessel was preferred because of the high volume requirements for the assays (Figure 2.3); however a limited number of smaller scale fermentations (7L scale) were conducted for comparison, though these had to be sampled less frequently. The 7L vessel was equipped with the same size impellers as the 20L vessel, and was stirred at the same rotational speed to maintain the maximum shear rates that would be encountered by the cells; however, the impeller spacing had to be reduced slightly (separation of D in the 7L compared to 1.5D for the 20L and 450L vessels, Table 2.4 Section 2.2.1), to accommodate the impellers in the reduced volume. The operating conditions were kept the same throughout the small scale fermentations.
The large scale fermentations were conducted using a fixed aeration rate of 0.5vvm, but varying impeller speeds (Table 3.1). The objective in varying the impeller speeds was to vary the shear and the circulation times that the cells would experience, and thereby determine the influence of such parameters on the cell wall strengths and the gradients in environmental parameters (e.g. DOT and shear gradients). The large scale vessels were operated at stirrer speeds that covered the range of speeds available within the scale being used, in order to cover the range of hydrodynamic behaviours and heterogeneities for that vessel. It was not considered feasible to scale-up the fermentations on the basis of power input or impeller tip speed, which are common scale-up criterion, because power measurements were not available at the small scale, and because the minimum tip speed available at the large scale was close to the upper operating limit of the small scale vessels (which may have led to more frequent breakdown of the small scale vessels). Furthermore the vessels available were not geometrically similar, so scale-up based on a fixed criterion would be an academic procedure, and would not necessarily indicate similarity of environmental conditions between scales.

Oxygen stress occurring during the 450L fermentations operated at low impeller speeds appeared to cause a lengthening of the growth cycle rather than affecting the cell qualities of interest (Section 3.2.3); thus oxygen stress was undesirable during scale-up. The possibility of oxygen stress occurring in the 1500L vessel was reduced by operating this vessel with a low volume (662L of broth). Additionally, the mixing characteristics of the vessel were simplified to that of a vessel of standard geometry with just one mixing compartment (only one impeller of D=T/3, placed at a height of D from the base of the vessel, with a further liquid height of 2D above the impeller), which would ease the design of the mixing in a scale-down model based on this vessel. Therefore, by using only one impeller the possibility of oxygen limitations could be reduced, while the maximum shear rate was increased (larger impeller diameter), thus the importance of shear effects on the cell wall strength could be investigated further.

The results of the various fermentation experiments are reported in this section, while the statistical comparison and effect of scale-up on the cell qualities is reserved for Section 5.2. Apart from the use of the cell qualities to assess the effects of the cell environment on the products of the fermentation, the quality profiles have some value in the determination of the best point for harvesting the broth, to maximise the
concentration of a particular product, or ease the processing of the cells. The uses of the quality profiles to this end have been discussed in Section 5.3.

**Batch growth profiles**

In this study a simplified division of the batch growth profile is made (compared to the description of Locher et al., (1993) presented in Section 1.5.1), based on the biomass production profiles (Figures 3.10 and 3.11) which appear to be diauxic. Essentially the first growth phase in the diauxic profile, corresponding to phases 1 and 2 described by Locher et al. (1993) (Figure 1.12), involves the metabolism of glucose, and is referred to as the "**glucose growth phase**" in this study. The remainder of the diauxic profile (phases 3 to 7 in Figure 1.12) is referred to as the "**ethanol growth phase**" in this study, as the ethanol produced during the glucose growth phase is consumed throughout this period (Figure 3.31). The ethanol growth phase is further subdivided into an "**early**" and "**late**" phase, on the basis of the distinct trends in the growth profile and respiratory quotient of these two phases: the dividing point between the early and late ethanol phase, is called the "**RQ step**" (Figure 1.12). The early ethanol phase correspond with the diauxic lag, and has a gradually increasing RQ; though there is minimal biomass increase in this period, there is ethanol consumption. The late ethanol phase is the second period of biomass production, and has a lower RQ than the early ethanol phase: the RQ gradually declines over this growth period. The division of the growth profile into the glucose, early ethanol and late ethanol phases is convenient for the discussion of the cell quality profiles for this organism and has a fair connection to the underlying metabolism; these subdivisions of the growth profile are also illustrated in figure 1.12. The fermentation ends when the RQ rises above 0.67, following the second peak in carbon dioxide evolution rate: the value of 0.67 is the theoretical RQ for the aerobic consumption of ethanol.
3.1 CONSISTENCY OF FERMENTATION METHOD

The initial protocol for inoculum preparation was designed to allow transfer of cells to the study fermenter while still in the glucose growth phase. For the 7 and 20L scale, this required inoculum transfer after 12 hours growth in each inoculum stage, using the same medium as the fermentation throughout (Section 2.4.2). The presence of glucose was used as an indication of growth on glucose, and was checked by using the 'Diastix' glucose test (Section 2.6.5). It was reasoned that the enzyme content of the cells (a part of the cells quality) would change in response to the metabolites being consumed, so this protocol would eliminate any adaptive response of the cells (i.e. a change in enzyme content) due to a change in primary nutrient in the environment, and thus maintain the balance of cell qualities. This philosophy was applied to the initial fermentations: Fermentation ID\(^1\) 7L(1)C, 20L, A, B, E, F, G, H, I, J and 450LB (Table 3.1). This protocol was altered for the remainder of the fermentation program to one using an inoculum containing early ethanol phase cells. The absence of glucose indicated a shift to the early ethanol phase (Diastix test, Section 2.6.5). Firstly this was necessary to reduce the lag period in the study fermenter, and reduce the overall fermentation time to a period that could be reasonably followed in a single day-night period. Secondly, the stability of ADH and soluble protein assayed in the glucose and early ethanol phase was greatly enhanced by this protocol (Section 3.5 & 3.4). Fermentation characteristics such as exit gas compositions, dry cell weight and cell wall strength (Section 5.2.1) appear unaffected by the growth phase of the inoculum. Reasons for the variation in inoculum generation time have been given in section 3.2.1.

\(^1\) Fermentation ID: The initial part of the fermentation identity refers to the particular vessel used (Table 2.3), while the letter refers to a particular batch in that vessel, the details of which are given in Table 3.1. For example, 7L(1)C refers to the batch “C” (Table 3.1) carried out in the LH 2000 series I fermenter (Table 2.3), of 7L volume.
<table>
<thead>
<tr>
<th>Fermentation ID (in the order of experimentation)</th>
<th>Operating volume, L</th>
<th>Stirrer speed, RPM: (Tip speed, m/s)</th>
<th>Total inoculum generation time, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>7L(1)C 1</td>
<td>5</td>
<td>700 : (2.53)</td>
<td>12.00</td>
</tr>
<tr>
<td>20LE</td>
<td>14</td>
<td>700 : (2.53)</td>
<td>12.00</td>
</tr>
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<td>300</td>
<td>100 : (1.05)</td>
<td>26.20</td>
</tr>
<tr>
<td>1500LB</td>
<td>662</td>
<td>350 : (5.50)</td>
<td>26.15</td>
</tr>
<tr>
<td>1500LA</td>
<td>662</td>
<td>200 : (3.14)</td>
<td>20.30</td>
</tr>
</tbody>
</table>

The airflow rate was 0.5vvm throughout

1 Fermentation ID: The initial part of the fermentation identity refers to the particular vessel used (Table 2.3), while the letter refers to a particular batch in that vessel, the details of which are given in Table 3.1. For example, 7L(1)C refers to the batch "C" (Table 3.1) carried out in the LH 2000 series I fermenter (Table 2.3).

NB the fermentations inoculated with glucose phase cultures are above the dotted line.
3.2 EXIT GAS ANALYSIS RESULTS

The gas analysis results within each scale are described in section 3.2.3; figures 3.2 to 3.9 within this section, show the exit gas analysis data for the *S. cerevisiae* fermentations. The data shown include the dissolved oxygen tension (DOT, % air saturation), oxygen uptake rate and carbon dioxide evolution rate (OUR and CER, mmol/L.H: Equation 2.1 and 2.2, respectively), and the respiratory quotient (RQ, dimensionless: Equation 2.3). The manner of presentation and meaning of the relative fermentation time, are described first in section 3.2.1. Section 3.2.2 details the parameters that have been used to make interscale comparisons of the gas analysis data (Section 5.2), and are given in the tables associated with Figures 3.2 to 3.9.

3.2.1 Method used for the graphical presentation of the gas analysis data

Within any particular scale of operation there is some variation in the fermenter lag period, due to differences in the inoculum generation time (Table 3.1), which were usually the result of unanticipated events. Examples of these events are the poor operation of an acid or alkali pump, necessitating replacement, or the loss of mass spectrometer monitoring, requiring the attention of a technician. Between scales the lag time was affected by the difference in the number of inoculum stages (Table 2.6) and the volume fraction of inoculum used (Table 2.7). Additionally, the inoculum generation time was altered to reduce the lag period (Section 3.1). Owing to the diauxic growth profile (Section 1.5.1) it was more convenient to plot the results so that the growth phases could be superimposed, which could not be achieved without adjusting for the differences in the lag period. The fermentation elapsed times were adjusted to a common reference point, in order to superimpose the growth phases on the graphs in this results section; the reference point chosen was the end of the glucose growth phase. The end of the glucose growth phase was easily identifiable from the exit gas analysis data, and was taken as the point at which the RQ fell below 1.0, following the first peak in CER (Figure 1.12). Glucose depletion was confirmed to be at this point by independent testing (Section 3.8). The fermentation elapsed times were adjusted to the common reference point by subtracting the time taken to get to the end of
the glucose growth phase. The resulting “Relative fermentation time” used as the x-axis on the graphs in this results section, has the end of the glucose growth phase at zero, the ethanol growth phase in positive hours and the glucose growth phase in negative hours; the more negative the hour the closer it is to the point of inoculation. The scale of the x-axis however, remains in hours.

Figure 3.1 illustrates how Figures 3.2 to 3.9 have been derived form the original mass spectrometer output and data logger recording: Figures 3.2 to 3.9 are plotted in the manner of Figure 3.1(II), and are the raw trends for the various fermentations. The data logging computer recorded the fermentation variables against “Elapsed fermentation time” as in Figure 3.1(I) with the top x-axis. The x-axis is adjusted to one of “Relative fermentation time” as described above and illustrated in Figure 3.1(II). To improve the clarity of the graphs the glucose growth phase is cut off at -8 hours relative fermentation time as no sampling was performed earlier than this. The RQ varies sharply about the peak in CER at the end of the glucose growth phase (Figure 3.1(I)). For the purpose of clarity here, the RQ has been plotted on a Log₁₀ scale in figures 3.2 to 3.9.

### 3.2.2 Parameters used for the comparison of the gas analysis data

The tables presented below the gas analysis figures (Figures 3.2-3.9) summarise the values of the parameters used for the comparison of the gas analysis data. The RQ step, duration of fermentation, total growth time, peak CER and peak OUR are derived from the points illustrated by the capital letters A to E, respectively, in Figure 3.1. The CO₂ evolution, O₂ uptake and mean RQ during the ethanol growth phase are derived as below. The data in the tables is also summarised in Figures 5.1 to 5.3, for the purpose of interscale comparison of the gas analysis results.

The **RQ step** splits the ethanol growth phase between an early and late phase and is determined by the point at which the RQ falls below 0.6; this is an arbitrary selection with regard to being based on any known change in respiratory metabolite, however it does correspond to a neat and consistent division between the two trends in RQ during the ethanol growth phase. The position of the RQ step is also seen as an inflection in the DOT profile. Along with the **duration of fermentation** (in effect the duration of the ethanol growth phase in hours) in relative hours these parameters allow comparison of the similarity of the growth phases of the various fermentations within any particular
(Initial 12 hours are for the inoculum stage and have been excluded)

Figure 3.1: Derivation of parameter values for gas analysis figures
scale. The duration of the fermentation is measured to the end of the ethanol growth phase, this is taken as the point at which the RQ rises above 0.67: the theoretical RQ for the respiration of ethanol; the depletion of ethanol ties in with this point (Figure 3.31). The total growth time from inoculation is the full duration of cell growth from the beginning of the first inoculum stage to the end of the fermentation; Figure 3.1(I) shows this scale as the bottom x-axis; the first 12 hours, corresponding to the growth of the inoculum in the shake flask, have been omitted from the figure. This parameter allows an assessment of the overall reproducibility of the fermentation experiments.

Peak CER at the end of the growth phases allows comparison of the growth rates and quantities of biomass at these points. This is because the magnitude and rate of CO₂ production depends on the amount of biomass present and the its growth (metabolic) rate. CER rather than OUR is the chosen parameter as less signal error will be associated with it; this is because the difference between the inlet and outlet concentrations of CO₂ can be 40 to 160 times that of O₂ at the peaks (the pH was controlled throughout the fermentations).

CO₂ evolution and O₂ uptake during the ethanol growth phase, and their mean ratio, are indicated at the bottom of the associated table. These allow a more quantitative comparison of the various fermentations. These also allow a comparison of normal batches to those where there is DOT limitation, and no ethanol phase CER peak occurs. The CO₂ evolution and O₂ uptake (mmol/L) were determined from the area under the respective curves during the ethanol growth phase. The calculation of these parameters for 1500LB (Figure 3.9) was adjusted for the missing data by taking the area of a rhombus for these sections; the data loss was the result of a computer system failure at the data logger. Whereas the overall CO₂ evolution and O₂ uptake would have been more useful the evolution and uptake during the glucose growth phase could not be determined due to variations in inoculum times and may account for some of the differences between the values obtained for the ethanol growth phases. Despite this, these parameters remain useful in assessing the effects of DOT limitation, which only occurs in the ethanol growth phase. The mean ratio is useful as it summarises and combines the CER and OUR data.
3.2.3 Gas analysis results

This section describes the exit gas analysis data produced at each scale of fermentation. Where average results are given, these have been derived from the data in the tables associated with the appropriate figures. For the small scale (7L and 20L vessels), the average results are an indication of the reproducibility of the batch fermentation, as the fermentation conditions were identical in each batch; the consistency of fermentations is discussed in section 5.1. Interscale comparisons are made in section 5.2.

The DOT profiles for certain fermentations show a probe drift from the calibration set point, following sterilisation. The probes were all calibrated between 99.9-5% DOT (100% and 0% dissolved oxygen, respectively) according to the manufacturers recommendation. However, the DOT probe for the 7L vessel and 20LD gave a 10% drift above the high set point; for 20LE this was 20%. The probes for 20LA, 450LB and 450LC gave a drift below the lower set point, and for these fermentations the actual point of DOT stress is only identifiable from the CER/OUR profiles: DOT stress occurring when these profiles depart from the exponential pattern. The DOT profile for 450LA is within calibration, and where the reading is approximately 5% DOT, this corresponds to zero dissolved oxygen according to the calibration.

7 Litre fermentations (Figures 3.2 to 3.3)

The DOT profiles of these fermentations show the level of dissolved oxygen remaining above 10% air saturation, though the probe is a little unstable as the DOT rises above the initial set point of 100%. There does not appear to be any oxygen stress at a DOT of 10% because the CER and OUR profiles remain exponential and rise to a sharp peak at the end of the ethanol growth phase.

The mean duration of the ethanol growth phase is 15.0h with a standard deviation as a percentage of the mean (Sdp) of 2.6%. The RQ step occurs on average after 8.9h into the ethanol growth phase, with a Sdp of 3.4%. The average total growth time is 42.9h with a Sdp of 7.4%.

The mean peak CER at the end of the glucose growth phase is 64.7mmoL/L.h with a Sdp 6.1%. The mean peak CER at the end of the ethanol growth phase is 23.5mmoL/L.h with a Sdp 16.5%.
The mean CO₂ evolution over the ethanol growth phase is 135mmol/L with a Sdp 15.1%. The mean O₂ uptake over this phase is 249mmol/L with a Sdp of 10.6%; this gives a mean RQ of 0.54 and a Sdp of 4.4%.

20 Litre fermentations (Figures 3.4 to 3.6)

The DOT in 20LA & B falls to zero toward the end of the fermentation, according to the probe reading; however, the DOT in 20LC to F remains above 10%. The probe does show some instability in the profiles for 20LD & E. The CER and OUR profiles for 20LC to F suggest some oxygen limitation toward the end of the fermentations, as the profiles tend to plateau (changing from exponential to linear rates). As the DOT probe is located in the bottom mixing compartment adjacent to the sparger, it may not indicate the average broth DOT and an axial DOT gradient may exist.

The mean duration of the ethanol growth phase is 19.0h with a Sdp of 7.6%. The RQ step occurs on average after 12.4h into the ethanol growth phase, with a Sdp of 18.4%; this step occurs earlier for fermentations inoculated with early ethanol phase cells. The average total growth time is 45.5h with a Sdp of 3.3%.

The mean peak CER at the end of the glucose growth phase is 98.90mmol/L.h with a Sdp 4.2%. The mean peak CER at the end of the ethanol growth phase is 31.5mmol/L.h with a Sdp 8.7%.

The mean CO₂ evolution over the ethanol growth phase is 241mmol/L with a Sdp 7.1%. The mean O₂ uptake over this phase is 429mmol/L with a Sdp of 13.3%; this gives a mean RQ of 0.57 and a Sdp of 7.0%.

450 Litre fermentations (Figures 3.7 to 3.8)

The DOT at this scale falls to zero in 450LA to C during the ethanol growth phase, the decline being fastest at the lower stirrer speed. The point at which an oxygen stress develops in the system corresponds to the point where the CER and OUR profiles change from an exponential to a linear trend, resulting in an increase in the duration of the fermentation from that point onward. 450LD shows no sign of oxygen stress, and the CER and OUR profiles display sharp peaks at the end of the fermentation. At 10 hours in the profile of 450LB there is a sharp rise in the DOT, this corresponds to the stirrer speed being increased from 200 to 225rpm. This was the first fermentation carried out where the DOT appeared to become limiting, and the stirrer speed was
increased slightly to prevent this, though in hindsight it should have been left at the original value to maintain the shear characteristics of the system.

The mean duration of the ethanol growth phase excluding 450LA is 17.3h with a Sdp 4.3%. The RQ step occurs on average after 10.4h into the ethanol growth phase, with a Sdp of 13.6%. The average total growth time is 51.6h with a Sdp of 1.0%.

The mean peak CER at the end of the glucose growth phase is 86.2mmol/L.h with a Sdp 3.5%. The mean peak CER at the end of the ethanol growth phase is an inappropriate parameter for comparison because only 450LD has a peak at this stage of growth.

The mean CO\textsubscript{2} evolution over the ethanol growth phase is 233mmol/L with a Sdp 7.5%. The mean O\textsubscript{2} uptake over this phase is 392mmol/L with a Sdp of 7.7%; this gives a mean RQ of 0.56 and a Sdp of 4.7%.

**1500 Litre fermentations (Figure 3.9)**

The DOT falls to zero in both these fermentations. The air flow was increased to attempt to compensate for the fall, giving the sharp rises in the DOT profiles, though in hindsight the air flow should have been left unchanged as it could influence the pattern of shear seen by the organism by altering the circulation patterns of the broth. The gap in the data of 1500LB was the result of a computer system failure in the data logging equipment.

The mean duration of the ethanol growth phase is 16.4h with a range of 0.7h, the range in the value being a more appropriate measure of variability than the standard deviation, as there are only two fermentations to compare. The RQ step occurs on average after 10.4h into the ethanol growth phase, with a range of 0.06h. The average total growth time is 47.5h with a range of 4.4h.

The mean peak CER at the end of the glucose growth phase is 98.8mmol/L.h with a range of 6.5mmol/L.h. The mean peak CER at the end of the ethanol growth phase is again an inappropriate measure because neither of the fermentations at this scale shows a peak; both fermentations have plateauing CER and OUR profiles at the end of the fermentation.

The mean CO\textsubscript{2} evolution over the ethanol growth phase is 204mmol/L with a range of 96mmol/L. The mean O\textsubscript{2} uptake over this phase is 380mmol/L with a range of 178mmol/L; this gives a mean RQ of 0.54 and a range of 0.008.
Figure 3.2: Exit gas analysis for 7L(1)A & B S. cerevisiae fermentations
**Figure 3.3:** Exit gas analysis for 7L(1)C *S. cerevisiae* fermentation

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>7L(1)C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impeller tip speed, m/s</td>
<td>2.53</td>
</tr>
<tr>
<td>RQ step, h relative time</td>
<td>9.0</td>
</tr>
<tr>
<td>Duration of fermentation, h relative time</td>
<td>14.6</td>
</tr>
<tr>
<td>Total growth time from inoculation, h</td>
<td>46.6</td>
</tr>
<tr>
<td>Peak CER (during glucose growth phase), mmol/L.h</td>
<td>69.0</td>
</tr>
<tr>
<td>Peak CER (during ethanol growth phase), mmol/L.h</td>
<td>28.0</td>
</tr>
<tr>
<td>CO₂ evolution during ethanol growth phase, mmol/L</td>
<td>158</td>
</tr>
<tr>
<td>O₂ uptake during ethanol growth phase, mmol/L</td>
<td>279</td>
</tr>
<tr>
<td>Mean RQ during ethanol growth phase</td>
<td>0.566</td>
</tr>
</tbody>
</table>
**Figure 3.4:** Exit gas analysis for 20LA & B *S. cerevisiae* fermentations
**Figure 3.5:** Exit gas analysis for 20LC & D. *cerevisiae* fermentations
DOT % air saturation, CER & OUR mmol/L.h

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>20LE</th>
<th>20LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impeller tip speed, m/s</td>
<td>2.53</td>
<td>2.53</td>
</tr>
<tr>
<td>RQ step, h relative time</td>
<td>13.0</td>
<td>13.3</td>
</tr>
<tr>
<td>Duration of fermentation, h relative time</td>
<td>19.7</td>
<td>19.8</td>
</tr>
<tr>
<td>Total growth time from inoculation, h</td>
<td>46.0</td>
<td>48.0</td>
</tr>
<tr>
<td>Peak CER (during glucose growth phase), mmol/L.h</td>
<td>102.0</td>
<td>97.1</td>
</tr>
<tr>
<td>Peak CER (during ethanol growth phase), mmol/L.h</td>
<td>33.9</td>
<td>31.4</td>
</tr>
<tr>
<td>CO₂ evolution during ethanol growth phase, mmol/L</td>
<td>249</td>
<td>246</td>
</tr>
<tr>
<td>O₂ uptake during ethanol growth phase, mmol/L</td>
<td>434</td>
<td>453</td>
</tr>
<tr>
<td>Mean RQ during ethanol growth phase</td>
<td>0.574</td>
<td>0.543</td>
</tr>
</tbody>
</table>

**Figure 3.6:** Exit gas analysis for 20LE & F S. cerevisiae fermentations
DOT % air saturation, CER & OUR mmoL/L.h

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>450LA</th>
<th>450LB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impeller tip speed, m/s</td>
<td>1.05</td>
<td>2.09</td>
</tr>
<tr>
<td>RQ step, h relative time</td>
<td>15.4</td>
<td>8.8</td>
</tr>
<tr>
<td>Duration of fermentation, h relative time</td>
<td>48.3</td>
<td>16.7</td>
</tr>
<tr>
<td>Total growth time from inoculation, h</td>
<td>83.4</td>
<td>52.2</td>
</tr>
<tr>
<td>Peak CER (during glucose growth phase), mmoL/L.h</td>
<td>85.3</td>
<td>85.0</td>
</tr>
<tr>
<td>Peak CER (during ethanol growth phase), mmoL/L.h</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CO₂ evolution during ethanol growth phase, mmoL/L</td>
<td>233</td>
<td>198</td>
</tr>
<tr>
<td>O₂ uptake during ethanol growth phase, mmoL/L</td>
<td>392</td>
<td>359</td>
</tr>
<tr>
<td>Mean RQ during ethanol growth phase</td>
<td>0.595</td>
<td>0.551</td>
</tr>
</tbody>
</table>

Figure 3.7: Exit gas analysis for 450LA & B. cerevisiae fermentations
**Figure 3.8:** Exit gas analysis for 450LC & *D. cerevisiae* fermentations
DOT % air saturation, CER & OUR mmol/L.h

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>1500LA</th>
<th>1500LB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impeller tip speed, m/s</td>
<td>3.14</td>
<td>5.50</td>
</tr>
<tr>
<td>RQ step, h relative time</td>
<td>10.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Duration of fermentation, h relative</td>
<td>16.7</td>
<td>16.0</td>
</tr>
<tr>
<td>Total growth time from inoculation, h</td>
<td>45.4</td>
<td>49.7</td>
</tr>
<tr>
<td>Peak CER (during glucose growth phase), mmol/L.h</td>
<td>95.5</td>
<td>102.1</td>
</tr>
<tr>
<td>Peak CER (during ethanol growth phase), mmol/L.h</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CO₂ evolution during ethanol growth phase, mmol/L</td>
<td>158</td>
<td>250</td>
</tr>
<tr>
<td>O₂ uptake during ethanol growth phase, mmol/L</td>
<td>291</td>
<td>469</td>
</tr>
<tr>
<td>Mean RQ during ethanol growth phase</td>
<td>0.543</td>
<td>0.534</td>
</tr>
</tbody>
</table>

**Figure 3.9:** Exit gas analysis for 1500LA & B. cerevisiae fermentations
3.3 DRY CELL WEIGHT MEASUREMENTS

3.3.1 Dry cell weight results

The dry cell weight results for 7 and 20L fermentations are given in Figure 3.10 and those for 450 and 1500L fermentations are given in Figure 3.11. Generally, the glucose growth phase ends with 5-6 g/L in all fermentations and scales. This is followed by minimal growth during the early ethanol phase, which typically provides only a further 1 g/L of DCW. A final yield of 11-12.25 g/L DCW was achieved at the end of the late ethanol phase (the end of the fermentation). The biomass productivity was consistent over the scale range studied. The specific growth rates during these three phases are given in the tables associated with the figures, and are summarised in Section 3.3.2.

The following points should be noted. The gap in the data for 7L(1)C between 5 and 15 hours corresponds to an overnight period that was not monitored. The apparently low biomass levels of 450L, A and B are because the final dry cell weight measured were not at the end of the fermentation. The erratic readings of 1500LB were the result of confusion over the method of dry cell weight determination whilst being aided by students during a pilot plant training week, otherwise the errors applicable to the results are those described in Section 3.7.1.

In the case of 450LA & B where the DOT falls to zero (Figure 3.7) and the oxygen supply has begun to limit the growth, seen as constant values of CER and OUR from the point of limitation. Though the growth rate was reduced, the yield of DCW by the RQ step was unaffected. The biomass at the end of monitoring was approximately 7g/L for these fermentations. However, this does not correspond with the end of growth cycle, the 450LA fermentation finished after a total of 48 hours relative fermentation time, and 450LB after a further 5 hours. The final biomass may have reached a similar level to those fermentations with no DOT stress (e.g. the last 5 hours of growth in 450LC yielded approximately 5.5g/L).
<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Specific growth rate, $h^{-1}$ (1 l/D, Insufficient data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Tip speed, m/s)</td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>7LA (2.53)</td>
<td>0.35</td>
</tr>
<tr>
<td>7LB (2.53)</td>
<td>0.3</td>
</tr>
<tr>
<td>7LC (2.53)</td>
<td>0.3401</td>
</tr>
<tr>
<td>20LA (2.53)</td>
<td>0.11</td>
</tr>
<tr>
<td>20LB (2.53)</td>
<td>I/D¹</td>
</tr>
<tr>
<td>20LC (2.53)</td>
<td>0.27</td>
</tr>
<tr>
<td>20LD (2.53)</td>
<td>0.24</td>
</tr>
<tr>
<td>20LE (2.53)</td>
<td>I/D</td>
</tr>
<tr>
<td>20LF (2.53)</td>
<td>0.16</td>
</tr>
<tr>
<td>20LG (2.53)</td>
<td>0.15</td>
</tr>
<tr>
<td>20LI (2.53)</td>
<td>0.15</td>
</tr>
<tr>
<td>20LJ (2.53)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Figure 3.10: Dry cell weights for 7L and 20L S. cerevisiae fermentations.
### Fermentation ID

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Specific growth rate, h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>450LA (1.05)</td>
<td>0.20</td>
</tr>
<tr>
<td>450LB (2.09)</td>
<td>0.31</td>
</tr>
<tr>
<td>450LC (3.14)</td>
<td>0.38</td>
</tr>
<tr>
<td>450LD (4.19)</td>
<td>0.16</td>
</tr>
<tr>
<td>1500LA (3.14)</td>
<td>0.17</td>
</tr>
<tr>
<td>1500LB (5.50)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

¹ I/D, Insufficient data

**Figure 3.11:** Dry cell weights for 450L and 1500L *S. cerevisiae* fermentations
3.3.2 Specific growth rates

The specific growth rates were determined by calculating the gradients of straight lines fitted to plots of LnDCW vs. Time, assuming growth to be exponential. Where,

\[ \text{LnDCW} = \text{LnDCW}_{t=0} + \mu \cdot t \quad \text{is of the form} \quad y = C + mx \quad (3.1) \]

and; \( \text{LnDCW} \) = Natural logarithm of the DCW = y-axis
\( \mu = ( \text{Ln2} / \text{doubling time} ) \), the specific growth rate ( \( \text{h}^{-1} \)) = m, the gradient
\( \text{LnDCW}_{t=0} \) = the initial DCW = C, the y intercept
\( t \) = time ( h ) = x-axis

The gradients were calculated over the three phases of the growth cycle ( Section 3, batch growth profiles ) for each fermentation, though in some cases the rate was ‘not determinable’ due to a lack of sufficient data for a particular growth phase. The specific growth rates are illustrated in Figure 3.12; the range of growth rates for the different growth phases at the various scales are summarised in Table 3.2 below. The average Sdp during the glucose growth phase ranges from 4-19\%, during the early ethanol phase from 10-31\% and from 11-18\% during the late ethanol phase. The mean specific growth rates over all the scales are 0.230, 0.017 and 0.091\( \text{h}^{-1} \) during the glucose, early ethanol and late ethanol growth phases, respectively. The respective Sdp for these means are 38, 72 and 20\%.

<table>
<thead>
<tr>
<th>Fermentation scale</th>
<th>Range of specific growth rates according to growth phase, ( \text{h}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose phase</td>
</tr>
<tr>
<td>7 Litre</td>
<td>0.31-0.36</td>
</tr>
<tr>
<td>20 Litre</td>
<td>0.11-0.34</td>
</tr>
<tr>
<td>450 Litre</td>
<td>0.14-0.41</td>
</tr>
<tr>
<td>1500 Litre</td>
<td>0.14-0.29</td>
</tr>
</tbody>
</table>

Table 3.2: Summary of specific growth rates during \textit{S. cerevisiae} fermentations
Figure 3.12: Comparison of specific growth rates in *S. cerevisiae* fermentation

7L scale (■), 20L scale (▲), 450L scale (▲), 1500L scale (▲)
3.4 SOLUBLE PROTEIN ASSAY RESULTS

Homogenisation of the cells at 1200bar for three discrete passes was sufficient to release all the cells soluble protein into the homogenate. The total soluble protein content (Rm, Equation 1.43, Section 1.6) was determined by assay (Section 2.6.1). The Rm value was useful in the determination of cell wall strength (Section 1.6), and as a simple measure of the cells productivity of enzymes. The volumetric protein content (mg/mL) of the broth throughout the fermentation is shown in figures 3.13 and 3.14, and the specific protein content (mg/gDCW) is illustrated in figures 3.15 and 3.16, and detailed in section 3.4.1. The volumetric productivity increases in all case with biomass production (i.e. during the glucose growth phase and late ethanol phase), and is approximately constant during the early ethanol phase. The release of soluble protein into the broth supernatant within the fermenter, the background release (Ro, mg/gDCW), has been detailed in section 3.4.2; this parameter may be an indicator of cell damage in the fermenter environment.

3.4.1 Rm, Maximum soluble protein released by homogenisation

Two patterns of specific soluble protein content exist, which appear to be related to the growth phase of the inoculum; these are shown in Figures 3.15 & 3.16. In all cases the soluble protein content rises during the glucose growth phase, the level at the end of the glucose growth phase is either higher than or close to the actual level that is maintained during the early ethanol growth phase. For fermentations inoculated with glucose phase cells (Section 3.1) the level here is 100mg/gDCW, whereas it is 200-250mg/gDCW for those inoculated with early ethanol phase cells. 7L(1)C and 20LD show the opposite to expected trend during the early ethanol phase, in the case of 20LD the low specific protein content is due to higher than average DCW in this period. The respective levels during the late ethanol phase are 200-250 mg/gDCW and 250-300mg/gDCW; the fermenters inoculated with glucose phase cells show a step change in soluble protein content at the RQ step. The differences in the level of soluble protein appear to be due to differences in the stability of certain proteins, for example ADH (Section 3.5.1).

The calculation of the soluble protein levels for 450LC, used an average calibration curve, because the particular curve for this data gave lower than expected optical density readings, which was probably the result of denatured protein standard: this is likely to account for the deviation from the average specific protein levels.
**Figure 3.13:** Maximum soluble protein release for 7L and 20L fermentations

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Approximate range of Rm, mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>7LA (2.53)</td>
<td>0-1.20</td>
</tr>
<tr>
<td>7LB (2.53)</td>
<td>0.25-1.30</td>
</tr>
<tr>
<td>7LC (2.53)</td>
<td>0.13-1.38</td>
</tr>
<tr>
<td>20LA (2.53)</td>
<td>I/D</td>
</tr>
<tr>
<td>20LB (2.53)</td>
<td>I/D</td>
</tr>
<tr>
<td>20LC (2.53)</td>
<td>0-1.38</td>
</tr>
<tr>
<td>20LD (2.53)</td>
<td>0-0.63</td>
</tr>
<tr>
<td>20LE (2.53)</td>
<td>0-0.63</td>
</tr>
<tr>
<td>20LG (2.53)</td>
<td>0.13-1.00</td>
</tr>
<tr>
<td>20LH (2.53)</td>
<td>I/D&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> I/D, Insufficient data
### Figure 3.14: Maximum soluble protein release for 450L and 1500L fermentations

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Approximate range of Rm, mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>450LA (1.05)</td>
<td>0.25-1.13</td>
</tr>
<tr>
<td>450LB (2.09)</td>
<td>0.13-0.25</td>
</tr>
<tr>
<td>450LC (3.14)</td>
<td>0.50-1.38</td>
</tr>
<tr>
<td>450LD (4.19)</td>
<td>0.25-1.38</td>
</tr>
<tr>
<td>1500LA (3.14)</td>
<td>0-1.25</td>
</tr>
<tr>
<td>1500LB (5.50)</td>
<td>0.38-1.38</td>
</tr>
</tbody>
</table>

<sup>1</sup> I/D, Insufficient data
<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Glucose growth phase</th>
<th>Early ethanol phase</th>
<th>Late ethanol phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>7LA (2.53)</td>
<td>50-100</td>
<td>150-200</td>
<td>≈200</td>
</tr>
<tr>
<td>7LB (2.53)</td>
<td>175-250</td>
<td>≈225</td>
<td>≈250</td>
</tr>
<tr>
<td>7LC (2.53)</td>
<td>175-325</td>
<td>I/D</td>
<td>I/D</td>
</tr>
<tr>
<td>20LA (2.53)</td>
<td>25-75</td>
<td>≈75</td>
<td>150-250</td>
</tr>
<tr>
<td>20LB (2.53)</td>
<td>I/D¹</td>
<td>≈75</td>
<td>150-250</td>
</tr>
<tr>
<td>20LC (2.53)</td>
<td>50-275</td>
<td>≈225</td>
<td>225-275</td>
</tr>
<tr>
<td>20LD (2.53)</td>
<td>25-100</td>
<td>≈50</td>
<td>≈225</td>
</tr>
<tr>
<td>20LE (2.53)</td>
<td>25-125</td>
<td>75-125</td>
<td>I/D</td>
</tr>
<tr>
<td>20LG (2.53)</td>
<td>50-200</td>
<td>≈125</td>
<td>I/D</td>
</tr>
<tr>
<td>20LH (2.53)</td>
<td>I/D</td>
<td>I/D</td>
<td>250-275</td>
</tr>
</tbody>
</table>

¹ I/D, Insufficient data

NB. *italic ID numbers indicate a glucose phase inoculum*

**Figure 3.15:** Maximum specific soluble protein release for 7L and 20L fermentations
<table>
<thead>
<tr>
<th>Fermentation ID (Tip speed, m/s)</th>
<th>Approximate range of Rm, mg/gDCW</th>
<th>Glucose growth phase</th>
<th>Early ethanol phase</th>
<th>Late ethanol phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>450LA (1.05)</td>
<td>125-225</td>
<td>≈225</td>
<td>I/D</td>
<td></td>
</tr>
<tr>
<td>450LB (2.09)</td>
<td>≈50</td>
<td>50-75</td>
<td>I/D</td>
<td></td>
</tr>
<tr>
<td>450LC (3.14)</td>
<td>225-350</td>
<td>300-350</td>
<td>I/D</td>
<td></td>
</tr>
<tr>
<td>450LD (4.19)</td>
<td>100-250</td>
<td>≈250</td>
<td>225-300</td>
<td></td>
</tr>
<tr>
<td>1500LA (3.14)</td>
<td>25-200</td>
<td>200-250</td>
<td>≈225</td>
<td></td>
</tr>
<tr>
<td>1500LB (5.50)</td>
<td>150-375</td>
<td>200-225</td>
<td>225-275</td>
<td></td>
</tr>
</tbody>
</table>

1 I/D, Insufficient data

NB. *italic ID numbers indicate a glucose phase inoculum*

**Figure 3.16:** Maximum specific soluble protein release for 450L and 1500L scale
3.4.2 Background protein release, Ro

The background soluble protein release (the protein released into the broth supernatant, within the fermenter) was assessed by measuring the protein level of the supernatant prior to homogenisation of the sample, to determine the effect of scale on cell rupture within the vessel. The background levels of soluble protein are given in Figures 3.17 & 3.18, and the Fractional release (Equation 3.2) is given in Figures 3.19 & 3.20: where the “Total available soluble protein” is given by \(( \text{Ro} + \text{Rm} )\).

\[
\text{Fractional protein release} = \frac{\text{Background release}}{\text{Total available soluble protein}}
\]  

(3.2)

For the majority of the fermentations Ro stays below 10mg/gDCW, and apart from 450LC, is approximately 20mg/gDCW or less: the unusual result for 450LC has been discussed in Section 3.4.1. This represents less than 5% of the Total available soluble protein being found in the broth supernatant for most fermentations, and apart from 20LF, it is less than 10% for the remainder. The background release does not appear to be influenced by the growth phase of the inoculum.
<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Approximate range of Ro values, mg/gDCW</th>
</tr>
</thead>
<tbody>
<tr>
<td>7LA (2.53)</td>
<td>0-8</td>
</tr>
<tr>
<td>7LB (2.53)</td>
<td>3-23</td>
</tr>
<tr>
<td>20LA (2.53)</td>
<td>1-5</td>
</tr>
<tr>
<td>20LB (2.53)</td>
<td>0-10</td>
</tr>
<tr>
<td>20LC (2.53)</td>
<td>1-15</td>
</tr>
<tr>
<td>20LD (2.53)</td>
<td>1-7</td>
</tr>
<tr>
<td>20LF (2.53)</td>
<td>2-17</td>
</tr>
<tr>
<td>20LG (2.53)</td>
<td>0-8</td>
</tr>
<tr>
<td>20LH (2.53)</td>
<td>0-5</td>
</tr>
</tbody>
</table>

1 Impeller tip speed, m/s

**Figure 3.17:** Background protein levels for 7L and 20L fermentations
<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Approximate range of Ro values, mg/gDCW</th>
</tr>
</thead>
<tbody>
<tr>
<td>450LA (1.05)¹</td>
<td>1-9</td>
</tr>
<tr>
<td>450LB (2.09)</td>
<td>2-8</td>
</tr>
<tr>
<td>450LC (3.14)</td>
<td>10-40</td>
</tr>
<tr>
<td>450LD (4.19)</td>
<td>0-12</td>
</tr>
<tr>
<td>1500LA (3.14)</td>
<td>≈2</td>
</tr>
<tr>
<td>1500LB (5.50)</td>
<td>1-8</td>
</tr>
</tbody>
</table>

¹ Impeller tip speed, m/s

**Figure 3.18:** Background protein levels for 450L and 1500L fermentations
### Fermentation ID

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Maximum fractional protein release</th>
</tr>
</thead>
<tbody>
<tr>
<td>7L (2.53) ( ^1 )</td>
<td>0.025</td>
</tr>
<tr>
<td>7LB (2.53) ( ^1 )</td>
<td>0.100</td>
</tr>
<tr>
<td>20LA (2.53)</td>
<td>0.050</td>
</tr>
<tr>
<td>20LB (2.53)</td>
<td>0.050</td>
</tr>
<tr>
<td>20LC (2.53)</td>
<td>0.050</td>
</tr>
<tr>
<td>20LD (2.53)</td>
<td>0.100</td>
</tr>
<tr>
<td>20LF (2.53)</td>
<td>0.175</td>
</tr>
<tr>
<td>20LG (2.53)</td>
<td>0.060</td>
</tr>
<tr>
<td>20LH (2.53)</td>
<td>0.025</td>
</tr>
</tbody>
</table>

\( ^1 \) Impeller tip speed, m/s

**Figure 3.19:** Fractional protein release for 7L and 20L fermentations
**Figure 3.20:** Fractional protein release for 450L and 1500L fermentations

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Maximum fractional protein release</th>
</tr>
</thead>
<tbody>
<tr>
<td>450LA (1.05)</td>
<td>0.030</td>
</tr>
<tr>
<td>450LB (2.09)</td>
<td>0.125</td>
</tr>
<tr>
<td>450LC (3.14)</td>
<td>0.100</td>
</tr>
<tr>
<td>450LD (4.19)</td>
<td>0.050</td>
</tr>
<tr>
<td>1500LA (3.14)</td>
<td>0.025</td>
</tr>
<tr>
<td>1500LB (5.50)</td>
<td>0.050</td>
</tr>
</tbody>
</table>

1 Impeller tip speed, m/s
3.5 ADH Activity Results

Figures 3.21 and 3.22 show the volumetric ADH activities (Units/mL) for the *S. cerevisiae* fermentations. In most cases the ADH activity rises with the biomass production (i.e. during the glucose growth phase and late ethanol phase), and is approximately constant during the early ethanol phase. The level of activity measured during the early ethanol phases is affected by the growth phase of the inoculum (Section 3.1).

Figures 3.23 & 3.24 show the trends in specific activities. When a fermenter is inoculated with a glucose growth phase culture, the specific activity of ADH appears to rise from 0 to ≤600 Units/gDCW over the glucose growth phase in the fermenter (20LG), and maintain this level over the early ethanol phase, until the RQ step. The activity then rises to a level of approximately 500-1500 Units/gDCW over the step; 7L(1)C maintains a level of approximately 1000 Units/gDCW, throughout the fermentation though there is a large data gap. However, for fermenters inoculated with early ethanol phase cells, the activity rises from 0 to ≥2000 Units/gDCW over the fermenter glucose growth phase, maintains a level of 1900-2500 Units/gDCW over the early ethanol phase, and between 1800-3000 Units/gDCW over the late ethanol phase. The activity for 450LC is slightly higher during the early ethanol phase at between 3000-4000 Units/gDCW. The differences in activity appear to be the result of differences in the stability of ADH, rather than the result of differences in the productivity of ADH, and these differences seem to be the result of the different inoculum protocols (Section 3.5.1) as all other operational procedures were the same. Also worthy of note is the fact that the proportion of soluble protein that is ADH, is increasing over the EEP (for some fermentations at least) as the specific protein content of the cells is remaining relatively constant.
Figure 3.21: ADH activity for 7L and 20L *S. cerevisiae* fermentations
Figure 3.22: ADH activity for 450L and 1500L *S. cerevisiae* fermentations
**Fermentation ID**

(Tip speed, m/s)

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Range of specific ADH activity, Units/gDCW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>7LA (2.53)</td>
<td>0-2200</td>
</tr>
<tr>
<td>7LB (2.53)</td>
<td>1000-2250</td>
</tr>
<tr>
<td>7LC (2.53)</td>
<td>1400-500</td>
</tr>
<tr>
<td>20LA (2.53)</td>
<td>I/D ²</td>
</tr>
<tr>
<td>20LB (2.53)</td>
<td>I/D</td>
</tr>
<tr>
<td>20LC (2.53)</td>
<td>0-3000</td>
</tr>
<tr>
<td>20LD (2.53)</td>
<td>I/D</td>
</tr>
<tr>
<td>20LE (2.53)</td>
<td>I/D</td>
</tr>
<tr>
<td>20LG (2.53)</td>
<td>100-300</td>
</tr>
<tr>
<td>20LH (2.53)</td>
<td>I/D</td>
</tr>
</tbody>
</table>

² I/D, Insufficient data

**NB. italic ID numbers indicate a glucose phase inoculum**

**Figure 3.23:** Specific ADH activity for 7L and 20L *S. cerevisiae* fermentations
Fermentation ID | Range of specific ADH activity, Units/gDCW
---|---
( Tip speed, m/s ) | Glucose growth phase | Early ethanol phase | Late ethanol phase
450LA (1.05 ) | 300-2700 | 2500 | 3000
450LB (2.09 ) | I/D<sup>1</sup> | 0 | 500-1500
450LC (3.14 ) | 2000-2750 | 3000-4000 | I/D
450LD (4.19 ) | 300-2200 | 1800-2400 | 2000-2500
1500LA (3.14 ) | 0-2000 | 1500-2000 | 1800
1500LB (5.50 ) | 500-2700 | 1500-2200 | 2000-3000

<sup>1</sup> I/D, Insufficient data  
NB. italic ID numbers indicate a glucose phase inoculum

Figure 3.24: Specific ADH activity for 450L and 1500L *S. cerevisiae* fermentations
3.5.1 Decay of ADH activity

The rate of decay of ADH activity in the homogenate of an *S. cerevisiae* culture inoculated with glucose growth phase cells was investigated (20LB). The homogenate was assayed immediately following homogenisation with no pre-centrifugation (Section 2.6.2), in-order to minimise the time between the breaking of cells and the assay for the enzyme. The homogenate was assayed again twice, at one minute intervals. These assays were then compared to an ADH determination conducted under the usual experimental conditions, where the "normal time after homogenisation" for the assay is between 10-30 minutes after homogenisation. The results of this trial are shown in Figure 3.25, and compared with some fermentations where the inoculum had been grown into the early ethanol phase (20LC, 450LA & D and 1500LA).

During the initial part of the early ethanol phase of 20LB the loss in activity over the first 2.5 minutes following homogenisation ranges from 60-70% of the initial activity (initial activity being measured 30 seconds after homogenisation. The loss reduces to 20% by the RQ step, and further reduces to 10% by the end of the late ethanol phase. This pattern is reflected in the ADH activity profile determined by the normal assaying procedure.

The ADH activity profiles for fermentations inoculated with early ethanol phase cells and assayed under normal procedures, shows ADH levels in the ethanol growth phases comparable to those determined for 20LB 30 seconds after homogenisation. A test for the decay of ADH activity for fermentations inoculated with early ethanol phase cells was not conducted, and the activities of such cultures has been assumed to be stable.
Figure 3.25: Decay of ADH activity in fermentation inoculated with glucose phase cells
3.6 G6PDH Activity Results

Figures 3.26 and 3.27 show the volumetric G6PDH activities (Units/mL) for the *S. cerevisiae* fermentations. In all cases the G6PDH activity rises over the glucose growth phase. For the large scale and 7L fermentations the trend is toward steadily increasing activity during the ethanol growth phase. The ethanol growth phase trend for the 20L fermentations is not very clear, thought the tendency appears to be for steady activity over the early ethanol phase, and higher activity over the late ethanol phase.

The trends in specific activity are given in Figures 3.28 & 3.29. Generally, the fermentations show an increase in activity over the glucose growth phase and a fairly consistent activity over the ethanol growth period; the peak in activity being reached following the miniature CER/OUR peak at the beginning of the early ethanol phase. The activity then declines toward the end of the late ethanol phase. The 20LA & B fermentations show a peak in activity at the RQ step, this is not reflected in any of the other fermentations, though the levels either side of this peak are more consistent for this scale at 50 Units/gDCW. The average ethanol phase activity at 450 litre scale is higher than the other scales at 80 Units/gDCW, though there is no trend with stirrer speed. At 1500 Litre scale the activity is greater throughout the fermentation for the lower stirrer speed. Also, there does not appear to be any connection of G6PDH activity with the growth phase of the inoculum. The range of activities are summarised in the table below; though the level of activity is variable, the upper level of activity is similar for certain scales and growth phases.

*Table 3.3: Range of G6PDH activities during S. cerevisiae fermentation*

<table>
<thead>
<tr>
<th>Fermentation scale</th>
<th>Range of G6PDH activities according to growth phase, U/gDCW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>7 Litre</td>
<td>30-85</td>
</tr>
<tr>
<td>20 Litre</td>
<td>15-80</td>
</tr>
<tr>
<td>450 Litre</td>
<td>20-80</td>
</tr>
<tr>
<td>1500 Litre</td>
<td>0-55</td>
</tr>
</tbody>
</table>
**Figure 3.26:** G6PDH activity for 7L and 20L *S. cerevisiae* fermentations
**Figure 3.27:** G6PDH activity for 450L and 1500L *S. cerevisiae* fermentations

<table>
<thead>
<tr>
<th>Fermentation ID (Tip speed, m/s)</th>
<th>Approximate G6PDH Activity, Units/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>450LA (1.05)</td>
<td>0.05-0.38</td>
</tr>
<tr>
<td>450LB (2.09)</td>
<td>I/D&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>450LC (3.14)</td>
<td>0.05-0.40</td>
</tr>
<tr>
<td>450LD (4.19)</td>
<td>0.05-0.40</td>
</tr>
<tr>
<td>1500LA (3.14)</td>
<td>0.0-0.30</td>
</tr>
<tr>
<td>1500LB (5.50)</td>
<td>0.08-0.30</td>
</tr>
</tbody>
</table>

<sup>1</sup> I/D, Insufficient data
<table>
<thead>
<tr>
<th>Fermentation ID (Tip speed, m/s)</th>
<th>Specific G6PDH activity, Units/gDCW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>7LA (2.53)</td>
<td>30</td>
</tr>
<tr>
<td>7LB (2.53)</td>
<td>30-60</td>
</tr>
<tr>
<td>7LC (2.53)</td>
<td>70-85</td>
</tr>
<tr>
<td>20LA (2.53)</td>
<td>I/D&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>20LB (2.53)</td>
<td>I/D</td>
</tr>
<tr>
<td>20LC (2.53)</td>
<td>15-80</td>
</tr>
<tr>
<td>20LD (2.53)</td>
<td>I/D</td>
</tr>
<tr>
<td>20LG (2.53)</td>
<td>50-60</td>
</tr>
<tr>
<td>20LH (2.53)</td>
<td>I/D</td>
</tr>
</tbody>
</table>

<sup>1</sup> I/D, Insufficient data

**Figure 3.28:** Specific G6PDH activity for 7L and 20L *S. cerevisiae* fermentations
<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Specific G6PDH activity, Units/gDCW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>450LA (1.05)</td>
<td>30-75</td>
</tr>
<tr>
<td>450LB (2.09)</td>
<td>I/D¹</td>
</tr>
<tr>
<td>450LC (3.14)</td>
<td>40-70</td>
</tr>
<tr>
<td>450LD (4.19)</td>
<td>20-80</td>
</tr>
<tr>
<td>1500LA (3.14)</td>
<td>15-30</td>
</tr>
<tr>
<td>1500LB (5.50)</td>
<td>0-55</td>
</tr>
</tbody>
</table>

¹ I/D, Insufficient data

**Figure 3.29:** Specific G6PDH activity for 450L and 1500L *S. cerevisiae* fermentations
3.7 ERRORS IN THE PRINCIPLE ASSAYS

Estimates of assay errors allowed rational decisions to be made, regarding whether observed effects were due to scale-up or whether they were in the limits of error.

3.7.1 Errors in the measurement of DCW

The original method consisted of centrifuging 1mL samples of broth in dried and pre-weighted 1.6mL Eppendorfs, for 10 minutes in a micro-centrifuge, taking four repeats for each sample. The error for this method was highly dependant on the actual dry cell weight being measured (Figure 3.30), especially at very low dry cell weights, which were in the lower measurement range of the balance. By switching to a measurement based on a 20mL sample the error in DCW measurement was greatly reduced; for instance a measured DCW of 0.001g in an Eppendorf has a standard deviation of 50%, however this becomes a weight of 0.02g for a 20mL sample and has an error of the order of 5% (confirmed by repeat measurements). Typically weights of between 0.025-0.25g were measured when taking 20mL samples, therefore single samples were taken for DCW determinations, and considered to have an error of 5% or less. Additional reasons for switching from the Eppendorfs was that they were prone to disturbance in the drying oven and taking many repeats was time consuming, especially when the sampling frequency was high.

![Figure 3.30: Variation of error in DCW measurement with measured sample weight](image-url)
3.7.2 Errors in the measurement of soluble protein, ADH and G6PDH activities

Assays were generally not repeated during routine sampling of fermentations, unless an obvious discrepancy appeared in the results, because speed of assaying was necessary to obtain sufficient number of samples in each growth phase to build a useful profile. Also, the reproducibility of the assays as tested on packed yeast, was sufficiently good to negate the need for extensive repeating of assays (Sayed, 1995). Packed yeast was resuspended in R.O water at a wet cell weight of 18g/L (approximately 6g/L dry cell weight) to assess the reproducibility of the assays, by repeating the homogenisation (at 1200 bar) and the assays in triplicate. Packed yeast was deemed more suitable than an actual fermentation broth because of its stability and lack of growth. The standard deviations in the results of the assays over the 0-3rd pass, are given in the table below as percentages of the mean.

For the purposes of the graphs, which use the maximum release at 1200 bar, the largest error between 1st and 3rd pass has been used, in the absence of repeated assaying: this gives standard deviations in the region of 11%, 12% and 6% of the actual value, for soluble protein, ADH and G6PDH, respectively. Though the error in the background values of the enzyme assays is high, the actual activity in the broth before homogenisation is close to zero, so the error is of little significance. Also noteworthy is the error in the soluble protein assay reduces as the sample becomes more evenly homogenised, and that of the ADH assay variability stabilises with increasing homogenisation: ADH is released more slowly than the average soluble protein. The error of the G6PDH assay is relatively constant as it is quickly released in relation to the average soluble protein (Follows et al. 1971).

<table>
<thead>
<tr>
<th>Assay</th>
<th>Standard deviation as a percentage of the mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 pass</td>
</tr>
<tr>
<td>Soluble protein</td>
<td>5.7</td>
</tr>
<tr>
<td>ADH</td>
<td>69.0</td>
</tr>
<tr>
<td>G6PDH</td>
<td>141.0</td>
</tr>
</tbody>
</table>
Broth glucose and ethanol concentrations were measured in order to confirm the primary metabolites during the growth phases of the batch fermentation. Two fermentations were monitored at large scale for broth glucose and ethanol concentrations: the results are given in Figure 3.31. Both fermentations show glucose depletion, with simultaneous ethanol production, before zero hours relative fermentation time: i.e. during the “glucose growth phase”. This is followed by a period of ethanol consumption, from zero hours relative fermentation time onwards: i.e. during the ethanol growth phase. Ethanol depletion ties in fairly closely with the CER/OUR peak at the end of the late ethanol phase (Figures 3.8 & 3.9). Ethanol production is of the order of 15-18g/L from an initial 30g/L of glucose. The ethanol growth phase RQ is never 0.67 (Figures 3.2 to 3.9), the expected theoretical RQ for the aerobic consumption of ethanol, even though there is ethanol consumption over this period.

Figure 3.31: Broth glucose and ethanol concentrations for *S. cerevisiae* fermentation
3.9 Broth Conductivity Analysis

Whole broth conductivity was measured to assess whether any trends in the cell wall strength profiles could be related to changes in the broth osmotic characteristics. Measurements were made at 1500L scale, the results of which are given in Figure 3.32. For 1500LA the conductivity only varies between 25.4-25.7 mS/cm, whereas the range of conductivities for 1500LB is from 21.8-26.5 mS/cm, with an average of 24.1 mS/cm. In neither case is there any strong linkage or trend of conductivity with growth phase. Evidently the salt concentrations used in this medium are well in excess of the requirements for growth with the quantity of glucose used (Table 2.1).

Figure 3.32: Whole broth conductivity for *S. cerevisiae* fermentation
3.10 DERIVED RESULTS: CELL WALL STRENGTH, K

Cell wall strengths were derived from the soluble protein release data gathered during the homogenisation of the cells. The cell wall strength is taken as the dimensionless rate constant from the Hetherington equation (Equation 1.43). The means of calculating the K value, and a further explanation of its meaning are given in section 2.5. The cell wall strength profiles are given in figures 3.33 & 3.34, and the ranges of "wall strength according to growth phase" given in the associated tables, are summarised in figure 3.35; the range of wall strength is taken between the maximum and minimum K defined by the limits of the error bars.

Recalling that the cell wall strength is inversely proportional to the value of K, at the 7L scale, 7L(1)A initially shows cell wall strengthening with K ranging from 0.12-0.06, then slight weakening over the RQ step, with a final K of 0.10. Alternatively, 7L(1)B shows wall strength increasing slightly over the whole fermentation, from a K value of 0.15-0.10.

For the 20L fermentations, those inoculated with glucose phase cells (20LA & B) show lower wall strength over the glucose and early ethanol phases, than those inoculated with early ethanol phase cells (20LC & D). Again all the fermentations show wall strengthening over the ethanol growth period, with final K values in the range of 0.10-0.04.

The fermentations at 450L also display cell strengthening over the ethanol growth period, and possible weakening of cells during the glucose growth phase. There is no noticeable trend with stirrer speed. Again 450LB, which was inoculated with glucose phase cells, shows lower glucose phase wall strength compared to the remainder of the 450L fermentations, which were inoculated with early ethanol phase cells. For the lower stirrer speed at the largest scale, 1500LA shows slight wall strength decrease before the glucose peak and then consistent wall strength over the ethanol growth period, with a K value around 0.10. After initially constant wall strength for 1500LB, the pattern of strength reverts to an increasing strength trend over the ethanol growth phase.
Figure 3.33: Cell wall strength for 7L and 20L S. cerevisiae fermentations

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Range of K value according to growth phase, Dimensionless</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>7LA (2.53)</td>
<td>I/D(^1)</td>
</tr>
<tr>
<td>7LB (2.53)</td>
<td>0.19-0.11</td>
</tr>
<tr>
<td>20LA (2.53)</td>
<td>I/D</td>
</tr>
<tr>
<td>20LB (2.53)</td>
<td>I/D</td>
</tr>
<tr>
<td>20LC (2.53)</td>
<td>0.15-0.05</td>
</tr>
<tr>
<td>20LD (2.53)</td>
<td>0.20-0.08</td>
</tr>
</tbody>
</table>

\(^1\) I/D, Insufficient data
<table>
<thead>
<tr>
<th>Fermentation ID (Tip speed, m/s)</th>
<th>Range of K value according to growth phase, Dimensionless</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose growth phase</td>
</tr>
<tr>
<td>450LA (1.05)</td>
<td>0.250-0.063</td>
</tr>
<tr>
<td>450LB (2.09)</td>
<td>0.225-0.088</td>
</tr>
<tr>
<td>450LC (3.14)</td>
<td>0.263-0.138</td>
</tr>
<tr>
<td>450LD (4.19)</td>
<td>0.188-0.075</td>
</tr>
<tr>
<td>1500LA (3.14)</td>
<td>0.150-0.050</td>
</tr>
<tr>
<td>1500LB (5.50)</td>
<td>0.200-0.138</td>
</tr>
</tbody>
</table>

1 I/D, Insufficient data

**Figure 3.34:** Cell wall strength for 450L and 1500L *S. cerevisiae* fermentations
**Figure 3.35:** Summary of cell wall strength ranges for *S. cerevisiae* fermentation
4 RESULTS OF S. ERYTHRAEA FERMENTATIONS

This section details the results gathered toward identifying the effects of scale-up on the cell qualities of S. erythraea. As for S. cerevisiae, the small scale (7L and 20L scale) fermentation results were to form a reference set of cell quality information, against which the large scale fermentation (450L and 1500L scale) results could be compared. The key issue of interest in the case of S. erythraea, was the sensitivity of this filamentous organism to shear damage on scale-up. From this viewpoint, the impeller speed was varied at all scales of fermentation, to cover the range of tip speeds available in each vessel, and thereby cover the range of shear rates and shear gradients available (the shear gradients being affected by the varying circulation times). Varying the impeller speed was a viable option for this organism, as low tip speeds did not cause oxygen limitations; this was because the organism was relatively slow growing (Table 4.2), and the maximum biomass production was low (Section 4.1). The ranges of tip speeds studied at the various scales, had some degree of overlap (Table 4.1), though the fermentations were not scaled on the basis of tip speed for reasons given in section 3.

The mixing systems in the three smaller vessels had a degree of similarity in their geometries; the 7, 25 and 450L vessels used for the S. erythraea fermentations (Table 2.6), had three turbine impellers with a diameter T/3: the lower impeller was one impeller diameter from the base of the vessel, and the separation of the impellers was 1.5D (Table 2.4). The 1500L vessel had the same configuration as that used for the S. cerevisiae fermentations (Section 3). Apart from the varying stirrer speed, the remainder of the operating conditions were kept the same between all the fermentations.

The cell qualities of interest were the dry cell weight, broth erythromycin concentration, the cell morphology, the broth rheology, the cell total soluble protein, and the cell wall strength (which is derived for the total soluble protein data). The principle product in this fermentation is the erythromycin antibiotic. The cell biomass should not only influence the rate at which the antibiotic is produced, but along with the cell morphology (Section 1.5.3), it may influence the broth rheology: the rheology of the broth is an important factor in the operation of the fermentation, and may also influence the subsequent processing of the broth (Section 1.5.4). The cell wall strength may be an important consideration for the downstream processing of this organism because a
cell separation phase may be used as an initial purification step, as the product is extracellular, and premature cell breakage would be undesirable. Apart from being used in the determination of the cell wall strength (Section 1.5.2), the cell soluble protein content may be a simple indicator of the metabolic activity of the cell. Broth conductivity data was also gathered to assess the influence of the osmotic environment on the cell wall strength (Section 3.8). No exit gas analysis data is shown because the CER and OUR were low and subject to considerable noise in the signal. However, exit gas monitoring was useful because contamination was easily identified by the occurrence of a very high CER and OUR, with associated low DOT.

Table 4.1 summarises the program of successful *S. erythraea* fermentations. Attempts were made to expand the range of stirrer speeds investigated at 25L, 450L and 1500L scale, however contamination problems resulting from the integrity of the seed vessels prevented this. *S. erythraea* fermentations are susceptible to contamination because of the slow growth rate of the organism and neutral pH of the medium. The inoculum was transferred from one stage to another at maximum dry cell weight concentration. For the final transfer stage this was at approximately 5g/L for the defined medium used. The generation time required to get the inoculum to the required concentration for transfer (Table 4.1), was more variable than for *S. cerevisiae*.

The results of the cell quality determinations for the various fermentation experiments are reported in this section. In the case of *S. erythraea*, determining the effect of scale-up on the cell qualities, by a rigorous statistical comparison of the small and large scale results, was not possible because of the lack of data at the large scale. Instead, a qualitative comparison of small and large scale results is made in Section 5.2, to illuminate the effects of scale on *S. erythraea* cell qualities. Apart form the use of the cell qualities to assess the effects of the cell environment on the products of the fermentation, the quality profiles have some value in the determination of the best point for harvesting the broth, to maximise the concentration of a particular product, or ease the processing of the cells. The uses of the quality profiles to this end has been discussed in Section 5.3.
### Table 4.1: *S. erythrea* fermentation program

<table>
<thead>
<tr>
<th>Fermentation ID (in the order of experimentation)</th>
<th>Operating volume, L</th>
<th>Stirrer speed, RPM: (Tip speed, m/s)</th>
<th>Total inoculum generation time, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>7L(2)(^1)A</td>
<td>5</td>
<td>250: (0.66)</td>
<td>67.0</td>
</tr>
<tr>
<td>7L(2)B</td>
<td>5</td>
<td>500: (1.31)</td>
<td>71.0</td>
</tr>
<tr>
<td>7L(2)C</td>
<td>5</td>
<td>750: (1.96)</td>
<td>55.0</td>
</tr>
<tr>
<td>7L(2)D</td>
<td>5</td>
<td>1000: (2.62)</td>
<td>66.0</td>
</tr>
<tr>
<td>7L(2)E</td>
<td>5</td>
<td>500: (1.31)</td>
<td>71.0</td>
</tr>
<tr>
<td>25LA</td>
<td>15</td>
<td>500: (1.83)</td>
<td>66.0</td>
</tr>
<tr>
<td>25LB</td>
<td>15</td>
<td>750: (2.75)</td>
<td>55.0</td>
</tr>
<tr>
<td>25LC</td>
<td>15</td>
<td>1000: (3.67)</td>
<td>67.0</td>
</tr>
<tr>
<td>450LA</td>
<td>300</td>
<td>250: (2.62)</td>
<td>118.0</td>
</tr>
<tr>
<td>1500LA</td>
<td>662</td>
<td>200: (3.14)</td>
<td>142.0</td>
</tr>
</tbody>
</table>

*The airflow rate was 0.5vvm throughout*

\(^1\) Fermenter IDs are given in table 2.3.
4.1 DRY CELL WEIGHT RESULTS

The results for the determination of dry cell weight are shown in figure 4.1; in all
cases the initial dry cell weight is of the order of 0.5g/L. Table 4.2 summarises the dry
cell weight data; the specific growth rates were calculated over the exponential growth
phase as described in Section 3.3.2 (NB though the dry cell weight profiles for 25LA
and 25LB closely match, the calculated specific growth rates are markedly different; this
is because there are only a few data points for determining the specific growth for 25LA,
and this is reflected in the higher error for the specific growth rate for this fermentation).
At the small scale the dry cell weight is only affected at the highest stirrer speed used; for
the 7L and 25L vessels the maximum dry cell weight is reduced by 50% at the highest
stirrer speed compared with the lower speeds. The maximum dry cell weight at the 450L
and 1500L scales is reasonably close to that achieved for low stirrer speeds at the smaller
scale, despite tip speeds at the higher end of the range. The error in the determination of
dry cell weights is less than 10%, as the actual measured weights range from
0.006-0.072g (Figure 3.30).

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Tip speed, m/s</th>
<th>Maximum DCW, g/L</th>
<th>Specific growth rate, h(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>7L(2)A</td>
<td>0.66</td>
<td>4.9</td>
<td>0.058 (0.013)(^1)</td>
</tr>
<tr>
<td>7L(2)B</td>
<td>1.31</td>
<td>5.8</td>
<td>0.020 (0.004)</td>
</tr>
<tr>
<td>7L(2)C</td>
<td>1.96</td>
<td>5.7</td>
<td>0.039 (0.007)</td>
</tr>
<tr>
<td>7L(2)D</td>
<td>2.62</td>
<td>2.4</td>
<td>0.009 (0.005)</td>
</tr>
<tr>
<td>25LA</td>
<td>1.83</td>
<td>5.2</td>
<td>0.033 (0.005)</td>
</tr>
<tr>
<td>25LB</td>
<td>2.75</td>
<td>5.5</td>
<td>0.023 (0.001)</td>
</tr>
<tr>
<td>25LC</td>
<td>3.67</td>
<td>2.5</td>
<td>0.079 (0.001)</td>
</tr>
<tr>
<td>450LA</td>
<td>2.62</td>
<td>4.5</td>
<td>0.033 (0.002)</td>
</tr>
<tr>
<td>1500LA</td>
<td>3.14</td>
<td>4.1</td>
<td>I/D(^2)</td>
</tr>
</tbody>
</table>

\(^1\) (Standard deviation), \(^2\) Insufficient data for determination.
Figure 4.1: Dry cell weight profiles for *S. erythraea* fermentations

( Impeller tip speed given in legend, m/s )
4.2 ERYTHROMYCIN PRODUCTION RESULTS

The results for the determination of broth erythromycin concentration are plotted in figure 4.2 and specific concentration are plotted in figure 4.3; the productivities are summarised in table 4.3. Erythromycin production appears either to overlap the biomass growth curve or occur toward the middle of the exponential growth phase. For example at the 7L scale production in batch 7L(2)A begins at mid exponential phase (at approximately 25h elapsed time) while in batch 7L(2)C the production begins at the start of fermentation (Figure 4.2); there does not appear to be a clear trend with impeller tip speed. Production of erythromycin appears to near the maximum by the end of the exponential growth phase, and actually peaks just after the end of the exponential growth phase. The maximum broth concentration for the 7L vessel at tip speeds between 0.66-1.96m/s is approximately 42mg/gDCW. For the highest tip speed at this scale, initial production is very rapid but then continues at a slower rate to finish at a higher specific activity of 91.5mg/gDCW (7L(2)D). Production of erythromycin in the 25L vessel is lower at 13.1-31.6mg/gDCW for tip speeds of 1.83-2.75m/s, respectively. Production in the 450L vessel was high with a maximum of 85mg/gDCW, and low in the 1500L vessel with a maximum of 18.8mg/gDCW. The error in the determination of erythromycin concentrations are discussed in section 5.2.2.

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Impeller tip speed, m/s</th>
<th>Maximum broth [Erythromycin], mg/gDCW</th>
</tr>
</thead>
<tbody>
<tr>
<td>7L(2)A</td>
<td>0.66</td>
<td>41.3</td>
</tr>
<tr>
<td>7L(2)B</td>
<td>1.31</td>
<td>43.2</td>
</tr>
<tr>
<td>7L(2)C</td>
<td>1.96</td>
<td>42.1</td>
</tr>
<tr>
<td>7L(2)D</td>
<td>2.62</td>
<td>91.5</td>
</tr>
<tr>
<td>25LA</td>
<td>1.83</td>
<td>13.1</td>
</tr>
<tr>
<td>25LB</td>
<td>2.75</td>
<td>31.6</td>
</tr>
<tr>
<td>25LC</td>
<td>3.67</td>
<td>I/D1</td>
</tr>
<tr>
<td>450LA</td>
<td>2.62</td>
<td>85.0</td>
</tr>
<tr>
<td>1500LA</td>
<td>3.14</td>
<td>18.8</td>
</tr>
</tbody>
</table>

1 Insufficient data for determination.

Table 4.3: Summary of broth erythromycin concentration results
Figure 4.2: Broth erythromycin content during *S. erythraea* fermentations
Figure 4.3: Specific erythromycin content during *S. erythraea* fermentations
4.3 Broth Rheology Results

The viscosity ($\mu$) of the broth was determined by taking the slope of a straight line passing through the origin, fitted to the data of shear stress ($\tau$) vs. shear rate ($\gamma$), using a Newtonian model (Equation 4.1): an example is given in Figure 4.4(A).

$$\tau = \mu \cdot \gamma$$

Where, $\tau = \mu \cdot \gamma$ is of the form, $y = m \cdot x$ \hspace{1cm} (4.1)

The Newtonian model was compared to a Power Law model (Equation 1.44, Section 1.7) and found to give a better fit to the data. Though the Power Law model may be used to describe the rheology of Newtonian fluids, the extra mathematical steps required in the determination of the consistency coefficient and flow behaviour index (Equation 1.44, Section 1.7) resulted in a relatively poor fit to the data. Figure 4.4 was generated from the 150 hour broth sample of fermentation 1500LA; figure 4.4(A) shows the raw data for this sample with a Newtonian model fit, figure 4.4(B) illustrates the determination of the consistency coefficient and flow behaviour index for a power law model fit, and figure 4.5(C) shows how the back-calculation of the shear stresses from the $K$ and $n$ values has resulted in the alteration of the rheological description from a dilatant to pseudoplastic one (Section 1.7) using the Power Law model.

Results for the measurements of viscosity made on $S. erythraea$ broths are given in Figure 4.5. Though the viscosity increases with dry cell weight, the actual change is very small. For the 7L vessel the viscosity at the highest tip speed (2.62m/s) is below that of the other speeds, however this is not reflected in the results of the 25L vessel, with comparable viscosities at all speeds. The viscosity in all cases is of the order of 2mPaS, with a slight trend toward decreasing viscosity as the scale increases. The viscosity tends to decrease during the erythromycin production phase (Section 4.2) in-line with the decreasing dry cell weight (Section 4.1).
Figure 4.4: Determination of the rheology of *S. erythraea* broths
Figure 4.5: Broth viscosity during *S. erythraea* fermentation
4.4 Cell morphology results

The results of the determination of the main hyphal length are presented in figure 4.6, this was the morphological parameter of interest, in connection with the rheology and erythromycin production (Section 1.8). The morphology of all inocula based on the main hyphal length, was similar (30-40μm). At an impeller tip speed of 0.66m/s in the 7L vessel, the main hyphal length increases to a maximum of 80-90μm, only decreasing at the end of the fermentation, when the hyphae began to disintegrate, as observed under the microscope. The main hyphal length at tip speeds between 1.31-2.62m/s, is either less than or equal to 40μm, and tends to decrease progressively with increasing tip speed and over the course of the fermentation. The variation of main hyphal length shows a similar pattern in the 25L vessel, with the lower tip speed (1.83m/s) allowing main hyphal length to increase to 67μm, while the higher tip speeds (2.75-3.67m/s) reduced the main hyphal length over the period of fermentation. The 450L and 1500L vessel, which operated at tip speeds of 2.62 and 3.14m/s respectively, show a decreasing main hyphal length over time, with the major length remaining lower in the 450L vessel.
(Impeller tip speed is given in legend, m/s)

**Figure 4.6:** Hyphae major length during *S. erythraea* fermentation
4.5 SOLUBLE PROTEIN CONTENT RESULTS

4.5.1 Effect of pressure on the release of soluble protein

A sample of *S. erythraea* fermentation broth was used for this analysis (fermentation 7L(2)E, with a tip speed of 1.96m/s). Aliquots of this broth were homogenised at nine different pressures between 100-1500bar, and for 5 to 9 discrete passes. The results are plotted in Figure 4.7: the curves were fit using the Hetherington equation (Equation 1.43, section 1.6.1). At each pressure the majority of the release is obtained within the first three passes, and the maximum obtainable release increases with pressure; hence RM is dependant on pressure (Figure 4.8).

![Figure 4.7: Effect of pressure on the release of soluble protein from S. erythraea](image-url)
Figure 4.8: Variation of Rm with pressure for *S. erythraea* homogenisation
4.5.2 Maximum soluble protein content

In the case of *S. erythraea* the maximum soluble protein content has been taken as the Rm value at 100bar homogenisation pressure. The maximum release at 1200bar could not be used, as in the case of *S. cerevisiae*, because the high pressure caused micronisation of the cell debris to release extra protein (Section 1.6.1). Conversely, the pressure of 100bar is more suited for the determination of the Rm value because it is the lowest available pressure in the homogeniser (Section 2.3.1), and should give the lowest micronising effect.

The volumetric soluble protein contents (mg/mL) are shown in figure 4.9. The majority of fermentations show soluble protein content increase to a maximum and then decline. The actual maximal value is variable: at the 7L scale this varies from 0.2 to 0.6mg/mL, it is approximately 0.4mg/mL at the 25L scale, below 0.1mg/mL at the 450L scale and 0.2mg/mL for the 1500L fermentation. The specific protein content (mg/gDCW) of the cells is given in Figure 4.10, with the maximum content summarised in Table 4.4. At the 7L scale the point of maximum soluble protein content appears to have been missed in 7L(2), A, C and D, though the level is in the region of 65-220mg/gDCW (Table 4.4). For the 25L scale the soluble protein content increases over the exponential growth phase and decreases following the maximum level, which is in the range of 90-130mg/gDCW. For the 450L vessel the soluble protein content increases over the exponential growth phase but remains low (under 25mg/gDCW); the soluble protein level decreases after the maximum is reached. The pattern of soluble protein content is the same in the 1500L vessel but the maximum level is greater (71mg/gDCW): the soluble protein content at the large scale is lower than that obtained at the 25L scale.
### Table 4.4: Summary of soluble protein results

<table>
<thead>
<tr>
<th>Fermentation ID</th>
<th>Impeller tip speed, m/s</th>
<th>Maximum soluble protein released, mg/gDCW</th>
</tr>
</thead>
<tbody>
<tr>
<td>7L(2)A</td>
<td>0.66</td>
<td>65 (± 4.0 )</td>
</tr>
<tr>
<td>7L(2)B</td>
<td>1.31</td>
<td>84 (± 3.4 )</td>
</tr>
<tr>
<td>7L(2)C</td>
<td>1.96</td>
<td>220 (± 10.8 )</td>
</tr>
<tr>
<td>7L(2)D</td>
<td>2.62</td>
<td>40 (± 7.7 )</td>
</tr>
<tr>
<td>25LA</td>
<td>1.83</td>
<td>106 (± 6.2 )</td>
</tr>
<tr>
<td>25LB</td>
<td>2.75</td>
<td>91 (± 1.3 )</td>
</tr>
<tr>
<td>25LC</td>
<td>3.67</td>
<td>129 (± 12.3 )</td>
</tr>
<tr>
<td>450LA</td>
<td>2.62</td>
<td>28 (± 2.0 )</td>
</tr>
<tr>
<td>1500LA</td>
<td>3.14</td>
<td>71 (± 4.8 )</td>
</tr>
</tbody>
</table>
Figure 4.9: Maximum soluble protein content for *S. erythraea*
Figure 4.10: Maximum specific soluble protein content for *S. erythraea*
4.5.3 Background protein release, Ro

The background release (mg/gDCW) was assessed in the manner described under section 3.4.2, and the fractional release was determined using equation 3.2: the results are given in figures 4.11 and 4.12, respectively. The important periods for comparison of the background protein levels, are during the exponential growth and erythromycin production phases: these periods mostly fall within the first 75 hours of fermentation (Figures 4.1 and 4.2, respectively). During these phases the cells are metabolically active, and may be supposed to be maintaining their structural integrity; high levels of soluble protein in the broth supernatant may indicate cell damage of the filamentous organism, caused by shear forces in the fermenter environment. The levels of soluble protein in the broth supernatant during the erythromycin production period, may influence the ease of purification of the antibiotic product. Toward the end of the fermentation, the background release is expected to increase due to cell lysis when the cells fragment: cell fragmentation at the end of the fermentation, was observed under the microscope.

During the first 100 hours fermentation at the 25L scale Ro is below 20mg/gDCW for all the tip speeds used; this corresponds to a fractional release below 20%. The protein release during the first 75 hours of fermentation at the 7L scale is also below 20mg/gDCW for all batches, however the fractional release at the end of 75 hours is between 20-35%, increasing with tip speed. The release of soluble protein from the large scale vessels increases from 0 to 50 hours elapsed time, with Ro of the order of 20-25mg/gDCW. The pattern of fractional release for these vessels, however, differs from the small scale in that soluble protein is increasingly found in the broth supernatant; the Ro steadily increases from 20 to 30% over the first 75 hours of fermentation.

After 75 hours fermentation time the 7L fermentations show the fractional protein release increasing; in the case of 7L(2)B, this occurs after 100 hours elapsed time. The fractional protein release in the 25L fermentations is similar to the 7L scale, but occurs after 100 hours elapsed time. For the large scale fermentations, the pattern of fractional protein release continues from the pattern observed for before 75 hours elapsed time. The final level of release is of the order of 70 to 80% of the total system soluble protein.
Figure 4.11: Background soluble protein release for *S. erythraea*
Figure 4.12: Fractional soluble protein release for *S. erythraea*
4.5.4 Soluble protein degradation

An assessment of the degradation of soluble protein was made under two conditions. Firstly, to assess the degradation during the homogenisation and analysis. Here the fermentation broth was homogenised, centrifuged and then kept on ice as described under Section 2.5. The homogenate supernatant was then periodically sampled for soluble protein over 50 minutes. Secondly, an assessment was made of the degradation of soluble protein released into the broth within the fermenter. This was done by homogenising the broth and keeping the homogenate, un-centrifuged, in a sterile universal standing in a water bath at 28°C (fermentation temperature).

Figure 4.13 shows the results of the first trial, with the degradation of soluble protein assessed under a variety of protein concentrations. Under all the soluble protein concentrations observed, there is no indication of significant degradation. The important range for consideration is from 10 to 15 minutes following homogenisation, which is the usual period within which the soluble protein assay is conducted.

Figure 4.14 shows the results of the second trial, again for a range of soluble protein concentrations within the homogenate. Though there is little indication of decay under these conditions over the first few hours following homogenisation, there is significant decay over a 24 hour period.

4.5.5 Error in the assessment of soluble protein concentration for S. erythraea

The level of error in the Biorad assay was determined as for S. cerevisiae (Section 3.7.2), except that S. erythraea fermentation broth from 7L(2)E was used, and the error was assessed for over 200 samples repeated in triplicate. On average the standard deviation was 8.003% of the mean soluble protein concentration.
Figure 4.13: Soluble protein degradation in homogenate kept on ice
Figure 4.14: Soluble protein degradation in homogenate kept at 28°C
4.6 Whole Broth Conductivity

The conductivity of the fermentation broth from 1500LA was measured and found to increase by 50%, from 11.37-22.4mS/cm, over the course of the culture period: illustrated in Figure 4.15. This may have been caused by a faulty probe, however this could not be verified as the probe failed on the next use ( the conductivity would have been expected to fall as the ionic substrates were consumed over the course of the fermentation ).

![Whole broth conductivity for 1500LA S. erythraea fermentation](image)

**Figure 4.15:** Whole broth conductivity for 1500LA *S. erythraea* fermentation
4.7 Derived Results: Cell Wall Strength, K

Values for K, the dimensionless coefficient in the Hetherington equation (Equation 1.43, Section 1.6.1), were determined using the protein release data, from the homogenisation of *S. erythraea* broth. The value of K was estimated by trial-and-error fitting of equation 4.2 (the modified form of equation 1.43), to protein release data obtained at a homogeniser pressure of 100bar. The pressure of 100bar was the lowest that could be used on the equipment (Section 2.3.1), and was selected so as to give incremental release of soluble protein on successive passes through the homogeniser, by disruption of intact cells, and to limit micronisation of the already released proteins (Section 1.6.1). Whereas in the case of *S. cerevisiae* the Rm value could be determined by the maximum release obtained at a homogenisation pressure of 1200bar, this was not the case for *S. erythraea* (Section 4.5.1). Though a curve of the form described by the equation 1.43 could be fitted to the release data at 100bar, the Rm at this pressure had to be estimated along with K, by trial-and-error: this gave a larger degree of error in the estimates of K. Equation 4.2 was used as the curve fitting function for a plot of R vs. N. The results of the cell wall strength determinations are given in Figure 4.16.

At the 7L scale the wall strength is initially high at the lowest stirrer speed with a K value of 0.5. The cells appear weaker as the stirrer speed increases to the next level, with the K value at approximately 1.5, and then strengthen to the initial level with further increases in the stirrer speed. 25LB shows a distinct trend in wall strength from low to high over the course of the fermentation, with K falling from 1.5 to 0.5. The wall strength of 25LA matches that of 25LB at a similar elapsed time, while that of 25LC is higher until an elapsed time of 150 hours. The K value for cells in 450LA ranges from 1.0-0.5, and in 1500LA is weaker, with K ranging from 1.5-1.0. In all cases, where there is a trend in the wall strength during the fermentation, it is from weaker to stronger cells over the course of the fermentation.

\[
R = Rm_{100} \times (1 - \exp(-K \times N)) \tag{4.2}
\]

Where R is the protein concentration released at homogeniser pass number N

- \( Rm_{100} \) is the Rm at a pressure of 100bar (estimated by trial-and-error)
- K is the dimensionless coefficient (estimated by trial-and-error)
Figure 4.16: Cell wall strength profiles for *S. erythraea* fermentations

( Impeller tip speed is given in legend, m/s )
4.8 FURTHER RESULTS: FERMENTER MIXING TIMES

In order to design a scale-down model based on the largest fermenter, the 1500L vessel, information was required regarding the circulation time of broth within the vessel. With the available equipment it was not possible to measure the circulation time directly, instead this had to be inferred from the bulk mixing time. The mixing time measurements were conducted as described in section 2.7. Apart from the circulation time within the 1500L vessel, the mixing times within the small scale vessels (the 7 and 20L fermenters, table 2.3) were also determined. The mixing time within these vessels was useful in the interpretation of the S. erythraea fermentation results, and for comparison to the 1500L vessel. The mixing times plotted against stirrer and tip speed are given in figures 4.17 & 4.18, respectively. The range of mixing times observed are summarised in table 4.5.

<table>
<thead>
<tr>
<th>Vessel ID</th>
<th>Range of mixing times (95% of total mixing time)(^1), Seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unaerated</td>
</tr>
<tr>
<td>7L(1)</td>
<td>21.1-64.4</td>
</tr>
<tr>
<td>20L</td>
<td>21.8-107.9</td>
</tr>
<tr>
<td>1500L</td>
<td>22.5-43.6</td>
</tr>
</tbody>
</table>

\(^1\) Section 2.7, 'Calculation of mixing time'

In the unaerated condition, the mixing times do not decrease uniformly with increased stirrer speed for the 7 & 20L vessels. In the case of the 20L vessel there is a slight plateau between 300 and 500rpm, whereas the 7L vessel shows a slight increase in mixing time during this range of speeds. This appears to be the result of increased segregation of the mixing zones at these speeds, the segregation being greater for the smaller vessel as it has the same size of impeller as the 20L (Table 2.4). The difference in the impeller to vessel diameter ratio between these vessels is reflected by surface aeration beginning earlier in the 7L vessel at 500rpm, compared to the 20L vessel where
it occurs at 650rpm. In the aerated condition there appears to be an interplay of impeller and aeration dominated mixing. At the lowest stirrer speed of 100rpm, both vessels show very fast mixing, indeed the 20L vessels shows the fastest mixing time for this vessel at this speed. At this impeller speed the mixing is aeration dominated, and the vessels behave as bubble columns, with axial circulation loops. As the stirrer speed increases to 300rpm, so does the mixing time; in this case the mixing becomes dominated by the impeller as the radial circulation loops disrupt the influence of aeration on the mixing. The mixing time in the 20L vessel reaches a second minimum by 500rpm, and in the 7L vessel by 700rpm, again showing the greater segregation of the mixing compartments in the smaller vessel. Beyond the second minimum there is a slight increase in mixing time with stirrer speed for these two vessels.

The 1500L vessel shows little difference between the aerated and unaerated mixing times. The mixing time increases by 20% over a tip speed range of 3.1-4.7m/s, and by a further 40% from a tip speed of 4.7-6.3m/s; it appears that the upper and lower mixing compartments in these vessels become more segregated over the range of speeds available. However, at equivalent tip speeds, between 2.5-4m/s, the mixing times between this and the small vessels is also approximately equivalent.
Figure 4.17: Fermenter mixing times against impeller speed
Figure 4.18: Fermenter mixing times against impeller tip speed
5 DISCUSSION

Cell quality profiles have been generated for batch fermentations of *S. cerevisiae* (Section 3) and *S. erythraea* (Section 4), at four scales of fermentation (7, 20/25, 450 and 1500L: Tables 2.7 and 2.8), and at various impeller tip speeds (Tables 3.1 and 4.1). This section is split into four subsections to discuss various aspects of these results.

The further use of cell quality profiles depends on the reproducibility of the fermentations and the reliability of the assay methods used. Section 5.1 discusses the consistency of the *S. cerevisiae* fermentation and assay techniques. The majority of the cell quality information gathered in this study, was for *S. cerevisiae* fermentations, with a number of repeated fermentations at the 20L scale. The 20L fermentations can therefore be compared to assess their reproducibility. Similar means of comparison as used for *S. cerevisiae* could have been used to assess the reproducibility of *S. erythraea* fermentations if a number of repeat batches were conducted, however due to a shortage of time *S. erythraea* fermentations did not involve repeat batches (Section 4), so no such comparison was made in this case.

The primary project objective was to determine whether the cell qualities of the two organisms used would be affected by scale-up, due to differences in the engineering variables operating in the environment of the cells. The secondary objective of the project was to use the cell quality information gathered from the scale-up process, to design a scale-down model of the largest scale fermenter used (the 1500L vessel). The model was to reproduce the salient environmental conditions causing cell qualities to vary in the large scale vessel, compared to the small scale vessels; thus producing cellular material in a small vessel, with cell qualities similar to those produced from a large scale fermentation. Section 5.2 examines the effect of scale-up on the cell qualities of the two organisms. The salient environmental conditions required to be reproduced in the scale-down model would depend on the cell qualities affected by the scale-up process; assuming scale-up had a noticeable effect on the cell qualities.

The cell quality profiles have a further use in their own right, and that is in determining the best harvesting point of the fermentation, with respect to having an optimal mix of cell qualities. The optimum mix of qualities partly depends on the product location, which determines the further processing that will be necessary to purify the product. These concepts are further elaborated for the two organisms in section 5.3.
The small scale fermentation profiles have been used to illustrate the principles in this section of the discussion, as the majority of the cell quality profiles are from the small scale. The same principles may be used for determining the best harvesting point in the large scale fermentations, making any necessary adjustment for the to the harvest point based on the appropriate cell quality profiles.

Section 5.4, on future studies, indicates the applicability of scale-down methods to the modelling of cell quality profiles for the organisms used in this study. The nature of scale-down systems that may be used for the study of engineering variables, chiefly shear and circulation, that may influence the quality of cells on scale-up in this study, have been detailed.
5.1 Reproducibility of S. cerevisiae fermentations and the reliability of the assay methods

The cell quality profiles need to be reproducible for the generated data to be useful in assessing the effect of scale-up, and predicting the best point of harvest. The variability between fermentations comes partly from the differences in the operation of one fermentation and another and partly from the difference in the behaviour of the cells. Considerable effort was made to limit operational differences by attempting to keep all conditions the same in the hope that the cells behaviour would also be the same. However, minor differences in operation may occur upon interchange of a similar probe or air exit pump following damage or failure of the original, or due to the maintenance of a probe or other fermenter part. Variations can also result if the inoculum generation time or quality is allowed to vary. The later cause of variation appears to be the key influence on cell quality in the fermentation of S. cerevisiae, as the growth phase of the inoculum affects the stability of the cells soluble protein (Section 3.51). This cause of variations has been minimised by a strict protocol of inoculum generation (Section 2.4.1), which gave consistent cell concentrations, in a particular growth phase, at the start of each experiment. Operational variations were assessed by comparing exit gas data from like fermentations that were conducted at the small scale. Table 5.1 compares a range of parameters from 20LB, E & F, which are repeat fermentations at the 20L scale having very similar inoculum protocols (Table 3.1).

The exit gas profile may be used to predict the course of the fermentation and hence the point at which a desired cell quality will be achieved. In order to do this the duration of the growth phases and the quantities and rates of gas consumption and production must be reproducible. Table 5.1 compares the timing of recognisable events in the exit gas profile, for example the end of the glucose growth phase, the RQ step during the ethanol growth phase and the end of the ethanol growth phase (Section 3, ‘Batch growth profiles’). The timing of these events has a maximum standard deviation of 5% from the mean, when compared using total growth times (Section 3.2.2), to compensate for the slight variation in the generation time of the inoculum. The variation in the CER at the end of the glucose and ethanol growth phases are also assessed, and here the maximum standard deviation is 8% of the mean. The mean RQ for the ethanol growth phase, which summarises all the exit gas data for this period, has a standard deviation of just 4.7% of the mean. An overall mean RQ is not available because some
fermentations lack mass spectrometer data from the beginning of the glucose growth phase. The reproducibility of the fermentations is high, considering that the fermentations being compared are separated by 9 months and use different batches of glycerol stocks to inoculate the shake flasks.

<table>
<thead>
<tr>
<th>Fermentation parameter compared</th>
<th>Standard deviation of the average as %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variation of the length of the glucose growth phase</td>
<td>4.9%</td>
</tr>
<tr>
<td>Variation of the timing of the RQ step</td>
<td>3.1%</td>
</tr>
<tr>
<td>Variation of the length of the ethanol growth phase</td>
<td>2.9%</td>
</tr>
<tr>
<td>Variation of the peak CER value at the end of the glucose growth phase</td>
<td>3.0%</td>
</tr>
<tr>
<td>Variation of the peak CER value at the end of the ethanol growth phase</td>
<td>8.0%</td>
</tr>
<tr>
<td>Variation of the mean RQ during the ethanol growth phase</td>
<td>4.7%</td>
</tr>
</tbody>
</table>

The standard deviations taken as a percentage of the means for the various assays are, less than 5% for dry cell weight, 10.6% for soluble protein, 11.7% for ADH activity and 6.1% for G6PDH activity (Section 3.7). The errors in the soluble protein and enzyme activity determinations are the combined errors of homogenisation, sample dilution and assay: these results are comparable to those of Sayed (1996) who indicated the combined error to be less than 7% for these assays when using the same homogenisation equipment and similar assay techniques, but a larger sample population. It was therefore concluded that the reproducibility of the assays was good enough, and the error low enough for detecting variations in the cell quality between fermentations.
5.2 EFFECTS OF SCALE-UP ON CELL QUALITY

5.2.1 S. cerevisiae

The *S. cerevisiae* experiments were conducted on the basis that the 20L vessel was an ‘ideal system’ for the growth of the organism, in that the fermenter could be considered a well mixed system providing a homogenous environment for the cells. The results for the cell quality profiles obtained from this vessel could then be used as a standard, against which the effects of heterogeneity in the environment of the cells in the large scale fermenters could be assessed: the large scale fermenters being considered as more heterogeneous systems. A limited number of experiments were conducted in the smaller 7L vessel to confirm the homogenous nature of the 20L vessel. Though the 7L vessel would be considered a more homogenous system than the 20L vessel due to the faster bulk mixing (Section 4.8), and thus would be preferred for generating standard cell quality profiles, the maximum volume of the vessel was insufficient for the level and volume of sampling required (Figure 2.3). Using this scheme of experimentation the results of the cell quality profiles can be compared using the statistical test known as “Analysis Of Variance (ANOVA)”. This test is used for hypothesis testing, and in this case could establish the validity of the null hypothesis, that scale-up has no effect on cell quality of *S. cerevisiae*. Initially the test was applied to the full set of data, by considering the 7, 20, 450 & 1500L results to be groups of data belonging to the same population; the resulting probability (p) values showing to what extent this was true. Secondly, the results of the 7, 450 & 1500L results were compared individually to the 20L results, assuming the 20L vessel to be a standard for comparison; the results indicating whether the cell quality at any particular scale of fermentation were different from those of the 20L scale. The results and the method for using this test are given in appendix 1.

**Comparison of exit gas analysis data**

A number of parameters may be compared from the gas analysis results. Fermentation event times have been compared in figure 5.1, the time taken to reach noticeable events in the fermentation profile, using total fermentation times (Section 3.22) to eliminate variability in inoculum generation times. Peak CER values have been
compared in figure 5.2, and ethanol phase exit gas analysis data have been compared in figure 5.3.

Fermentation event times (Figure 5.1)

The occurrence of the glucose peak, RQ step and end of the fermentations (Section 3, ‘Batch growth profiles’), in terms of Total fermentation time, can be compared for similarity between the scales of operation. On this time frame the question of how closely matched the growth phases are, can be answered.

The glucose growth phase peak in CER occurs on average after 29.5h with the standard deviation taken as a percentage of the mean (Sdp) of 13.2%. Excluding 450LA (which had oxygen limitation: section 3.2.3), the RQ step during the ethanol growth phase occurs on average after 40.1h with a Sdp of 9.0%, whereas the total duration of fermentation averages 46.5h with a Sdp of 7.7%. The 450L fermentations times are above average due to a lengthier transfer procedure between the seed and main vessel (Section 2.4.2, S. cerevisiae fermentations, Stage B).

**Figure 5.1:** Comparison of event times during *S. cerevisiae* fermentations
Peak CER values (Figure 5.2)

All fermentations display a glucose growth phase CER peak as there is no oxygen stress. The average peak height is 89.8mmol/L.h with a Sdp of 17.7%, however the average for the 7L fermentations is just 64.7mmol/L.h with a Sdp of 6.1% and that for the remainder is higher at 96.1mmol/L.h with a lower Sdp of 10.5%. Only the 7 & 20L fermentations and 450L C and D give a peak in CER at the end of the fermentation, the remainder of fermentations showing CER to be constrained by oxygen limitation (Section 3.2.3). The average peak height at the end of the fermentation is 30.3mmol/L.h with a Sdp of 18.9%. Again the average for the 7L fermentations is lower than the general mean at 23.5mmol/L.h with a Sdp of 16.5%. The CER and OUR trends for the 7L vessel are low, and would be expected to be equivalent between scales when identical media and airflow conditions are used. The low values are probably the result of the airflow rate actually delivered at the vessel, being less than that indicated on the control unit (therefore CER and OUR are low according to equations 2.1 and 2.2), the raw data was reanalysed and found to have used the correct values for the batch volume and airflow rate, so these are not the cause of the low values. Unfortunately the airflow can not now be checked by independent means as the airflow configuration of this vessel was subsequently altered.

Figure 5.2: Comparison of Peak CER values during S. cerevisiae fermentations
Ethanol phase CO\textsubscript{2} evolution, O\textsubscript{2} uptake and mean RQ (Figure 5.3)

Due to oxygen limitations constraining the peak CER values at the end of the some fermentations (450L A and B also 1500L A and B, Section 3.2.3), the gas analysis data during the ethanol growth phase can be compared using the total CO\textsubscript{2} evolution and O\textsubscript{2} uptake during this period: the ratio of the two, the mean RQ, may also be compared (Section 3.2.2). The exit gas analysis data during the glucose growth phase can not be compared, due variability in the inoculum generation time (reasons for which are given in section 3.1).

Excluding the 7L fermentations the averages for the parameters are 228mmol/L CO\textsubscript{2} evolved with a Sdp of 12.4% and 410mmol/L O\textsubscript{2} consumed with a Sdp of 14.6%. For the 7L fermentations the values are 134mmol/L (Sdp of 15.1%) and 249mmol/L (Sdp of 10.6%) for CO\textsubscript{2} and O\textsubscript{2} respectively. However, the average for the mean RQ for all the fermentations is 0.55 with a Sdp of 5.5% only.

Figure 5.3: Comparison of CO\textsubscript{2} evolution and O\textsubscript{2} uptake in \textit{S. cerevisiae} fermentation
Comparison of the exit gas analysis data by use of the ANOVA test (Appendix table 1) shows that the fermentation event times for the 7L and 1500L fermentations illustrated in figure 5.1, have good agreement with those of the 20L scale; the poor agreement of the 450L results is caused by the increased inoculation time for this vessel (Section 2.4.2, inoculum stage B). The glucose and ethanol growth phase peak CER values (Figure 5.2) show little agreement between scales, except for the glucose phase peak values of the 20L and 1500L scales. The ethanol growth period CO2 evolution, O2 uptake and mean RQ (Figure 5.3), have better agreement between the 20, 450 & 1500L scales than between the 20 & 7L scales; however all scales have similar metabolic behaviour when compared by using the mean RQ during the ethanol growth phase.

**Comparison of specific growth rates (Figure 3.12 & Appendix table 2)**

The specific growth rates were compared between the glucose, early ethanol and late ethanol growth phases, using the data given in the tables from Figures 3.11 & 3.12. The probability of the growth rates during the early and late ethanol phases belonging to the same population of data is above 60%, however the growth rates are more variable for the glucose growth phase. The individual comparisons reveal that the 1500L results have a high probability of belonging to the same group of data as the 20L results (‘p’ = 50%), while the 7 & 450L results are unlikely to (‘p’ equals 2.5% and 10%, respectively). The average specific growth rates during this growth phase for the 7 & 450L fermentations, are higher than those of the 20L and 1500L fermentations (Figure 3.12). The specific growth rates should be similar for identical media and airflow rates, so it is difficult to draw any conclusion here. The majority of results indicate that fermentation scale does not influence this parameter.

The following parameters (soluble protein content, ADH & G6PDH activities and cell wall strength as represented by the K value: equation 1.43) are compared between the glucose, early ethanol and late ethanol phases of the various fermentations, using the values of the maximum and minimum levels that the parameters takes during these phases. The maximum and minimum levels are given in the tables associated with the appropriate figures: for example the maximum and minimum levels of specific soluble protein content for the fermentation 20LC, are 275 & 225mg/gDCW, respectively—while for 20LD the maximum and minimum level are both 225mg/gDCW (Figure 3.15). Though the comparison is most effective when the parameter profiles are flat during a
particular growth phase (e.g. specific soluble protein content during the early and late ethanol phases in Figure 3.16), they may also be useful in assessing whether a parameter is within a particular range, for profiles that are changing during a particular growth phase (e.g. specific soluble protein content during the glucose growth phase in Figure 3.16).

**Comparison of soluble protein release data (Appendix tables 3 & 4)**

The comparison of soluble protein data excludes those fermentations inoculated with glucose phase cells (Section 3.1), as the stability of cell soluble proteins (e.g. ADH) is low for these batches (Section 3.4.1 & 3.5.1). In virtually all cases the probability that the various sets of data belong to the same population, is sufficiently high (‘p’ value above 15%: Appendix 1) to be able to accept that fermentation scale has no influence on this parameter.

**Comparison of ADH activity data (Appendix tables 5 & 6)**

The comparison of ADH data is also only between those fermentations inoculated with early ethanol phase cells, for the same reasons as given for the soluble protein results. Comparison of the data as a whole indicates a high degree of similarity in the results of all scales, except for the maximum level of volumetric ADH activity during the glucose growth phase (‘p’=10%: Appendix table 5), and the minimum level of specific ADH activity during the late ethanol phase (‘p’=12%: Appendix table 6). Though the source of the variation between these comparisons, and the other variations in ADH activity that are suggested (e.g. the difference in the maximum level of specific ADH activity during the glucose growth phase of 7 & 20L fermentations: Appendix table 6), can be attributed to the results of a particular fermentation scale, the main problem in comparing ADH activities is the need for more data at the 20L scale. Thus, the majority of comparisons show no effect of fermentation scale, with the differences requiring more data to clarify the issue.

**Comparison of G6PDH activity data (Appendix tables 7 & 8)**

All the data in the tables associated with Figures 3.26-3.29 were used for the ANOVA tests because G6PDH activity was not suspected to decay after homogenisation. The ‘p’ values for the tests are mostly in excess of 25%, indicating G6PDH activity does not vary on scale-up. The discrepancy between the volumetric
activities obtained for the maximum level during the glucose growth phase (‘p’=6%: Appendix table 7), is not apparent when comparing the specific activities (Appendix table 8). The only major difference is in the minimum level of G6PDH activity, of the 450 & 1500L fermentations, during the early ethanol phase.

**Comparison of cell wall strength data (Appendix table 9)**

The ANOVA test results indicate cell wall strengths (K values) to be in good agreement, between the maximum and minimum levels, and within the three growth phases of *S. cerevisiae*; the results only show variation between the 450L results, at the maximum level of K during the glucose growth phase, and the minimum level of K during the early ethanol phases, and for the minimum level of K during the early and late ethanol phase in the 7L fermentations. However, the shape of the profiles for the cell wall strengths of the 7 & 1500L fermentations (Figures 3.33 & 3.34), suggest more variation from the results of the 20L scale, than is indicated by the statistical test. At the 7L scale the cell wall strength profile shows less variation over the whole growth cycle, than is observed for the 20L scale (Figure 3.33). The wall strength profile for the lower stirrer speed at the 1500L scale (fermentation 1500LA), is similar to the 7L scale, while the higher stirrer speed at the 1500L scale (fermentation 1500LB) gives a profile matching the 20 & 450L scales. Therefore, the effect of scale on this parameter is not completely explained by the ANOVA test.

The shape of the wall strength profiles may be explained to some extent by the differences in the mixing times between the vessels. The mixing time results (Section 4.8 & Figure 4.18) indicate the mixing time is lower in the 7L vessel compared to the 20L vessel at the same operating conditions (e.g. 16.1s compared to 20.6s, respectively: at 0.5vvm & a tip speed of 2.53m/s), while the mixing time is higher in 1500LA (22.5 seconds) compared to the 20L vessel, though the tip speed is also greater for 1500LA (3.14m/s); the mixing time was found to be higher still for 1500LB, at approximately 35 seconds. Though the circulation times could not be determined directly from the mixing experiments (Section 4.8), the circulation times are likely to increase from the 7L to 1500L scale, if the linear dimensions of circulation paths are taken into consideration (Joshi et al., 1982: Section 1.2.2.3). Simplified calculations of the circulation times are given in appendix 2. Ayazi Shamlou et al. (1994) suggested that the rate constant for hyphal breakage (filamentous form of *Penicillium chrysogenum*) in a stirred tank fermenter had a moderate dependence on the inverse of the circulation time, which may
explain the increased strength observed for the 7L fermentations: the cells adapting to
the greater shear, or the greater shear selecting for cells of higher wall strength. As the
mixing time, hence circulation time, for 1500LA is longer than that of the 20L scale, the
increased wall strength observed here may have some connection to the greater
dimensions or power input of the larger vessel: Makagainsar et al. (1993) showed
hyphal breakage to depend on a “Power dissipation function” \( P_D D_t \). Simplified
calculations for the power input are given in appendix 2. The observed wall strength is
likely to be the result of the balance between the disruptive forces of the environment and
the regenerative forces of the cell (van Suijdam & Metz, 1981a), with disruptive forces
active near the impeller and regeneration occurring in the remainder of the vessel. Thus,
higher recirculation rate and greater tip speed, may both be responsible for reducing the
maximum K values observed in the 7L and 1500LA fermentations, that is for increasing
the cell wall strength. Again the mechanism may either be for cells to increase their wall
strength to avoid disruption, or for cells of a higher wall strength to be selected by the
environment. The cells of \( S.\) \textit{cerevisiae} do not actually appear to be damaged by the
shear in their environment (as is the case for the filamentous organism in the studies of
Makagainsar et al., 1993 & Ayazi Shamlou et al., 1994), as the background levels of
soluble protein do not appear to vary between the various fermentations (Section
3.4.2).

**Summary**

The statistical analysis has shown ethanol phase specific growth rates, soluble
protein content, ADH and G6PDH activities to be unaffected by fermentation scale. The
gas analysis data indicated that event times were comparable between scales, the peak
CER values bared little resemblance between scales, exit gas compositions during the
ethanol growth phase of the 7L fermentations varied from other scales but the mean ratio
of CER to OUR to be similar between scales. The cell wall strength profiles were not
usefully compared by the ANOVA test. However, the indication is that wall strength is
unaffected by scale-up from 20L to 450L scale, though the effects of scale-up to the
1500L vessel and down to the 7L vessel are not conclusive. Overall \( S.\) \textit{cerevisiae}
appears to be scale insensitive between 7 & 1500L, therefore an accurate prediction of
the cell quality from a fermentation at the 1500L scale may be obtained from the broth of
a 7-20L vessel, so long as the inoculum quality and medium composition are the same
between the scales and no oxygen limitation occurs.
5.2.2 S. erythraea

This section covers the effects of fermentation scale on the cell quality of S. erythraea. The results of the S. erythraea fermentations can not be compared by rigorous statistical means because the necessary quantity of data regarding large scale cell qualities, could not be acquired within the duration of the experimental program. Also, the S. erythraea results have the dual consideration of the effect of increasing impeller tip speed on the cell quality to account for. For the comparison of S. erythraea results, a qualitative comparison is therefore undertaken. The effects of scale on the individual cell quality are described in turn.

Comparison of dry cell weights

Figure 5.4 shows the maximum biomass production for the impeller tip speeds and fermentation scales used. Scale-up from 7 to 25L may produce an increase in the maximum tip speed that the cell culture can withstand, before a reduction in the biomass production occurs, from approximately 2-2.75m/s. However, the actual maximum biomass value overall, may be slightly lower in the 25L vessel compared to the 7L, at 5.5g/L compared to 5.8g/L. For the 450L scale, the dry cell weight is lower than the 25L vessel at approximately the same tip speed (2.62m/s), at 4.5g/L; the dry cell weight in the 1500L vessel is reduced below that of the 450L vessel, at the slightly higher tip speed of 3.14m/s. Scale-up with an equivalent tip speed and vessel geometry should increase the circulation time within the vessel (Appendix 2), thus scale-up may increase the critical tip speed above which damage occurs, by lengthening the time the cell spends in the parts of the vessel where it can regenerate itself. However, under these equivalent conditions the larger vessel has a greater maximum shear rate (Section 1.2.2.1), so scale-up may also reduce the maximum biomass attainable within a particular scale.

Comparison of broth rheology

The broth viscosity shows no linkage to impeller tip speed at the 7 & 25L scale, with the maximum viscosity in the range of 2-2.35mPas (Figure 5.5). However, the trend is toward decreasing viscosity with scale-up, the maximum viscosity in 1500LA being 1.92mPas (Figure 5.5). The maximum viscosity may be reducing because the maximum biomass is also decreasing with scale-up.
Figure 5.4: Effect of fermentation scale and impeller tip speed on the maximum biomass and erythromycin production of *S. erythraea*
Figure 5.5: Effect of fermentation scale and impeller tip speed on the maximum main hyphal length, broth viscosity and erythromycin production of *S. erythraea*
**Comparison of cell morphology**

As the impeller tip speed increases at the small scale, the maximum main hyphal length is reduced (Figure 5.5); the drop in hyphal length is very marked for the 7L vessel when the tip speed is increased above 0.7 m/s. At the highest tip speeds within the 7 & 25L vessels the main hyphal length does not increase beyond that of the inoculated cells, instead it declines over the whole growth period; this is also the case for the fermentations at the 450 & 1500L scale (Figure 4.6). For a tip speed of approximately 2 m/s, the maximum main hyphal length is greater in the 25L vessel compared to the 7L vessel; this could be the result of the longer circulation time that is presumed to exist in the larger vessel (Appendix 2), based on the greater linear dimensions (Joshi *et al.*, 1982: Section 1.2.2.3). The dominant effect appears to be the result of the circulation time rather than the tip speed, because the main hyphal length in the 1500L vessel remains greater than in the 450L vessel (Figure 4.6), even though the tip speed is greater for the 1500L vessel by 20% (Table 4.1): again the circulation time is presumed to be greater in the larger vessel, especially when the larger vessel has only one impeller compared to three for the 450L vessel (Table 2.4).

**Comparison of erythromycin production**

For the 7L scale, the tip speed does not influence the erythromycin production, whereas the opposite appears to be the case for the 25L vessel, with greater production for increased tip speed (Figure 5.5). The production is independent of the biomass for the two smaller scales, and scale-up between 7 & 450L does not alter the maximum erythromycin concentration (Figure 5.4); however, the maximum concentration is lower in the 25L vessel and in the larger 1500L vessel. The main hyphal length appears to be above any minimum requirement for erythromycin production in all scales (Martin & Bushell, 1996), seeing as production still occurs for those fermentations where the main hyphal length has dropped to as low as 10 μm (Figures 4.6 & 4.2). The effects of tip speed and scale-up on erythromycin production, require further attention, preferably using chromatographic methods for the measurement of erythromycin for improved sensitivity. The errors in the determination of the erythromycin concentration by the plate bioassay method, may be as great as 20%. The errors can arise from pipetting and the control of the thickness of the agar layer on the bioassay plate (Section 2.6.4); the errors increase with clearance diameter due to the logarithmic association (Equation 2.6).
Comparison of cell soluble protein content

As for *S. cerevisiae*, the cells soluble protein content may be a simple indicator of its metabolic activity, with high soluble protein contents resulting from an increase in the cells inducible enzymes. However, with *S. erythraea* fermentations, a large and varying proportion of the soluble protein may be found in the broth (Section 4.5.3); therefore a comparison of soluble protein levels between scales must account for the internal and external concentrations of soluble protein. Additionally, only the maximum levels of soluble protein may be compared, as the cells soluble protein content does not maintain any constant level during the fermentation (Section 4.52).

The maximum total soluble protein content of the *S. erythraea* fermentations is given in Figure 5.6, this includes the cells content plus the content of the broth; the maximum Rm is also given in Figure 5.6 for comparison. Apart form the lowest tip speed at the 7L scale, the maximum total soluble protein content of the small scale fermentations is above 100mg/gDCW. The low protein content at the highest and lowest tip speeds used at the 7L scale, are likely to be due to missing the sampling point for peak soluble protein (Figure 4.9 & 4.10). The small scale results do not show any strong trend with tip speed. The total soluble protein content of the 450L fermentation is only 40mg/gDCW, while that of the 1500L fermentation is in the lower range of the small scale fermentations, at 100mg/gDCW (Figure 5.6). The cells soluble protein content, Rm, of the 450L & 1500L fermentations are in the lower range of measured Rms (Figure 5.6). The scale-up effect appears to be a reduction in the Rm, bearing in mind that the maximum total soluble protein contents observed for the small scale may not be the peak values obtainable; however, this requires clarification by more intensive sampling. The reduction in protein content on scale-up crudely suggests reduced metabolic activity, which may lead to reduced product formation.

Comparison of cell wall strengths

Where there is a trend in cell wall strength during the course of a fermentation, it was observed to be a steady strengthening over the course of the fermentation (Section 4.7). This pattern of the profile allows a comparison to be made between the upper and lower limits of K between the fermentations (the maximum and minimum levels of the wall strength, respectively); the limits of K are compared in Figure 5.7. As with the soluble protein release data, some further clarification of the wall strength data is necessary, by more intensive sampling of the 7L and 25L scales; thus the effect of
Figure 5.6: Effect of fermentation scale and impeller tip speed on the maximum total soluble protein content and Rm of *S. erythraea*
The limits of the K value were determined from the results presented in Figure 4.16.

**Figure 5.7:** Effect of fermentation scale and impeller tip speed on the upper and lower limits of cell wall strength of *S. erythraea*
impeller tip speed on the wall strength is not clear. However, the scale-up from 7 to 1500L appears to increase the minimum level of the K value, that is to make the cells remain weaker.

Considering the background release of soluble protein data (Section 4.5.3), increasing the tip speed at the 7L scale during the exponential growth phase results in an increase in Ro. Increasing stirrer speed corresponds to increasing power input and reducing circulation time (Appendix 2), which are the same parameters implicated in the increased cell rupture observed by Makagaysing et al. (1993) for filamentous fermentations of *Penicillium chrysogenum*. The disruption (Ro) at the 25L scale at equivalent tip speeds to the 7L scale is lower, presumably because the circulation time is longer. The increase in Ro with a greater degree of scale-up implies the maximum shear in the fermentor to affect the cell rupture in addition to the power input and circulation time (the maximum shear increases with scale, Section 1.2.2.1). Unlike the case of *S. cerevisiae* where the increased shear in the environment of the cells resulted in the cells strengthening their cell walls, or stronger cells being selected for, the increased shear in the environment of the *S. erythraea* fermentations results in an increase in the cell rupture.

**Summary**

The scale-up effects on *S. erythraea* are as follows. The maximum biomass attainable within a particular scale may be reduced on scale-up; however, the critical tip speed beyond which biomass production is affected within a certain scale, may increase with scale-up. It is possible that the biomass production is reduced at a certain circulation rate, and the tip speed required to maintain this circulation rate increase with scale. The morphology may also be connected to scale and tip speed in a similar manner, because the main hyphal length increases with scale, at an equivalent tip speed: the circulation time is also increasing with scale at an equivalent tip speed. The maximum broth viscosity also reduces with scale-up, which may be the result of decreasing maximum biomass. The effect of scale-up appears to be a reduction in the soluble protein production, however, this, and the effect of scale on the erythromycin production, requires further evaluation. The indications are that cell wall strength remains lower and cell rupture increases on scale-up. The effect may depend on the power input, circulation rate and the maximum level of shear.
5.3 OPTIONS FOR CELL HARVESTING BASED ON CELL QUALITY PROFILES

One of the reasons for gathering cell quality data from the manufacturing point of view, is to be able to make rational decisions regarding the harvesting point of a fermentation. Charles (1985b) indicates that the point of maximum production does not necessarily equate to the point of lowest production cost. The profiles of cell quality gathered for the two organisms allow the determination of the point of optimum cell quality from the cost point and for subsequent processing. The cell quality data are discussed firstly for *S. cerevisiae* and then for *S. erythraea*, with suggestions made regarding the point of optimum cell quality for harvesting.

The need for volumetric productivity (product concentration per volume of broth) must be weighed against the needs for specific productivity (product concentration per weight of cells). A high volumetric productivity means that fewer batches, or a lower batch volume, are required to reach a yearly production target; the volumetric productivity is usually greatest at the end of the fermentation, when the dry cell weight is greatest (for example figures 3.13, 3.14, 3.21, 3.22, 3.26 and 3.27). However, many processes involve some form of volume reduction prior to the downstream processing operations, particularly when the product is internal and the cells are to be homogenised (Rossen, 1987). Where the product is internal, the efficient use of the homogeniser and purification equipment depends on a concentrated feed stream and here the specific productivity of the batch is paramount. Though a high volumetric productivity is desirable to the economics of the process, the specific productivity has overriding importance as the majority of the process cost lies in downstream processing, particularly when high purity is required.

As indicated in the introductory section to this discussion, the results of the small scale fermentations have been taken as the main examples to illustrate how cell quality profiles can be used in determining the best point to harvest a fermentation.
5.3.1 *S. cerevisiae*

*Fermentation profiles*

The CER profiles for the batch cultures of *S. cerevisiae* show the 7 phases identified by Locher *et al.* (1993) and described in section 1.5.1. Taking batch 20LC (Figure 3.5) as an example fermentation for comparison with the findings of Locher *et al.* (1993), phases 1 is from -8 to -1 hours “Relative fermentation time”, phase 2 from -1 to 0 hours, phase 3 from 0 to 1.5 hours, phase 4 from 1.5 to 11 hours, phase 5 from 11 to 12.5 hours, phase 6 from 12.5 to 17.5 hours and phase 7 from 17.5 to 18 hours. The results indicate that the glucose in the medium is consumed during the metabolic phases before zero hours relative fermentation time, and the maximum broth ethanol concentration is at the point of glucose depletion, subsequently the ethanol is consumed from zero hours to the end of phase 6 (Figure 3.31). Though Locher *et al.* (1993) suggest phase 4 to be due to the consumption of previously excreted acetic acid, glycerol and propionic acid, the results here and those of Dehghani (1996), using a similar fermentation protocol at the 7L scale and high pressure liquid chromatography to measure broth ethanol concentrations, show consumption of the produced ethanol in this phase.

5.3.1.1 *Dry cell weight*

Though Locher *et al.* (1993) suggest the batch growth of *S. cerevisiae* on glucose to involve the metabolism of at least six compounds, the pattern of biomass production is diauxic (Figures 3.10 & 3.11) as indicated by Sonnleitner and Kappeli (1986) and Feichter *et al.* (1987). The specific growth rates (Table 3.2) show biomass increasing over the glucose growth phases, little to no biomass production over the early ethanol phase and then further increase in biomass over the late ethanol phase: the early ethanol phase has been indicated to be equivalent to the diauxic lag (Section 1.5.1). Considering biomass as the product, in terms of volumetric productivity the options for cell harvesting are either at the end of the glucose growth phase, or toward the end of the late ethanol phase. In the former instance the volumetric productivity is low, however the operational costs of the fermentation, in terms of power input to agitation, compression cost for sparging etc., are also low. Over the early ethanol phase
the operational costs increase without any increase in biomass productivity. Finally, both the volumetric productivity and operational costs increase over the late ethanol phase. Though the best point for harvesting biomass is at the end of the late ethanol growth phase where the biomass concentration is maximal, the particular point of cell harvesting has now to be decided based on the other cell qualities (e.g. ADH and G6PDH enzymes and cell wall strength) that have been determined, to obtain the desired mix of qualities.

5.3.1.2 Soluble protein content

The soluble protein as a whole is unlikely to be considered a product of yeast fermentation. However, it may be regarded as a contaminant during the purification of products like ADH and G6PDH. Dehghani (1996) demonstrated specific ADH activity (Units/mg soluble protein) to be highest at the end of the glucose growth phase, while specific G6PDH activity (Units/mg soluble protein) was greatest at the beginning of the late ethanol growth phase, in fermentations of this strain of *S. cerevisiae* using a similar protocol in a 7L vessel. If either of these enzymes is the product, then the respective points of maximal activity should be chosen as the harvesting points, to minimise the level of contaminating proteins during the purification process. This is especially important where the product is a protein because a common method of purification involves selective precipitation, with the desired protein isolated in a particular fraction of protein precipitate; the importance being that greater specific activity of the product protein in relation to the total protein content gives a greater yield of the product in the desired fraction. The importance of harvesting at these points could outweigh the need for volumetric activity because a high proportion of the production cost is in purification, especially if a high degree of purity is desired, and increasing the product proteins concentration in relation to the most likely contaminating compounds (other proteins) would simplify and reduce the cost of its purification.

5.3.1.3 ADH activity

As mentioned in section 1.5.1, this enzyme catalyses the interconversion of acetaldehyde and ethanol at the junction of the glycolytic and TCA metabolic pathways. As expected the specific level of this enzyme increases over the glucose growth phase (Figures 3.23 & 3.24), while the cells are under stress from their limited respiratory
capacity and converting pyruvate to ethanol. Where cultures were inoculated with early ethanol phase cells, which was the preferred protocol for maintaining ADH stability (Section 3.5.1), the specific activity of this enzyme increases very slowly from the end of the glucose growth phase onward. The specific ADH activity is relatively constant over the whole of the ethanol growth phase, so there is scope for harvesting for ADH at any point during this growth phase and obtaining maximal specific productivity.

Dehghani (1996) found ADH activity for this organism to peak at the end of the glucose growth phase and maintain a lower level over the ethanol growth phase, suggesting the end of the glucose growth phase to be the optimum point for harvesting for ADH. Fermentations 20LC and 1500LB show evidence of this behaviour (Figures 3.23 & 3.24) and the lack of ADH peaks at this point in the profiles of other fermentations, appears to be the result of the differences in the timing of samples. With the range of parameters being monitored (Figure 2.1) the minimum turnaround between samples was one hour. As the batch growth of *S. cerevisiae* on glucose involves many changes in metabolite, with some metabolic phases lasting only for a short duration, it is easy to miss a particular peak in enzyme activity, and this appears to be the explanation of the discrepancies. Further investigations could resolve the differences if the number of monitored parameters is reduced, to improve the resolution of the profiles. This would apply to the profiles of all the parameters studied.

### 5.3.1.4 G6PDH activity

The patterns of specific activity for G6PDH (Figures 3.28 & 3.29) are in reasonable agreement with published data (Dehghani, 1996), with increase in the specific activity over the glucose and beginning of the early ethanol phase, followed by constant activity and then decline toward the end of the late ethanol phase. Dehghani (1996) did however report the activity to be constant over the early ethanol phase, then to peak at the beginning of the late ethanol phase before declining over all of the late ethanol phase: 20LA & B show activity profiles of this pattern. Based on these results the best specific productivity is toward the end of the early ethanol phase and over the RQ step. The preferred harvesting point is before the RQ step for this enzyme to avoid the risk of losing activity if the process is delayed into the late ethanol phase. G6PDH activity profiles would benefit from further resolution, by using a higher sampling frequency, to clarify the best harvest point for this enzyme.
5.3.1.5 Cell wall strength

The conductivity of whole broth samples was measured in order to assess any linkage of the trends in cell wall strength. The results in section 3.9 show that broth conductivity remains constant over the course of the fermentation, thus precluding any possibility of influencing the cell wall strength (Section 1.9).

Considering the wall strength profiles for the fermentations that were inoculated with glucose growth phase cells (Figures 3.33 & 3.34: 20LA, 20LB & 450LC), there appears to be wall strengthening over the course of the fermentation (the derivation of wall strength data is detailed in section 2.5). For these cases the concentrating of the cells in the initial downstream processing steps, becomes easier as the fermentation progresses (because the likely methods of concentrating are filtration or centrifugation), while the harvesting of internal products such as ADH, becomes harder (if homogenisation is the chosen cell breakage method). However the above profiles of wall strength are only likely to be relevant where the biomass is the product, as inoculating with glucose phase cells results in rapid soluble protein and ADH decay in the homogenates (Sections 3.4.1 & 3.5.1). Thus, for fermentations using glucose growth phase cells in the inoculum and where the biomass is the product, the best harvesting point is the end of the ethanol growth phase because the cells are easiest to concentrate (because they are less likely to break during processing), and the volumetric productivity is greatest (highest biomass concentration, Section 5.3.1.1).

The wall strength profiles for the glucose growth phase, in the fermentations inoculated with early ethanol phase cells, show mixed results. Some fermentations show weak cells initially becoming stronger and then weakening again before the end of the glucose growth phase (Figures 3.33 & 3.34, 20LC, 450LD, 1500LA), 450LA & 450LB show strong cells weakening over this phase, and 1500LB shows constant strength over this phase and the beginning of the early ethanol phase. The expected profile of wall strength, would have been for cells to weaken over this phase, because the cells in the inoculum were from the early ethanol phase and are stronger than those from the glucose growth phase according to the results of fermentations inoculated with glucose phase cells (e.g. the results of 20LA, Figure 3.33). Apart from the initial strengthening of the cells seen in 20LC, 450LD and 1500LA, which may have been an adaptive response of the cells to the change in environment on inoculation, these fermentations and 450LA and 450LB follow the expected profile of cell wall weakening over the course of the glucose growth phase.
The interpretation of the profiles in the glucose growth phase may have become further complicated by metabolic events in the latter part of exponential growth (end of phase 1 according to Locher et al. 1993: Figure 1.12, Section 1.5.1). Stryer (1981) and Zubay (1983) indicate a shift in glucose metabolism occurs from the glycolytic pathway to the pentose phosphate pathway, because of product inhibition of the enzyme phosphofructokinase. Dehghani (1996) reported a drop in hexokinase and ADH activity at the point of the metabolic shift. At this point the supply of intermediates for cell wall synthesis may increase, leading to peaks in the cell wall strength profile (low K values); this may account for the increase in wall strength seen in 20LD & 450LA, at -1 & -2 hours relative fermentation time, respectively (Figures 3.33 & 3.34). Possible links of glycolytic and pentose phosphate pathway intermediates to cell wall synthesis, are via fructose-6-phosphate conversion to N-acetylglicosamine, and mannose-6-phosphate, which are crosslinking and primary components of the wall material mannan (Thorpe 1984). Therefore, the observed cell wall strength for this growth phase may be highly dependent on the underlying metabolism of cell, and though the trend is for weakening as the cell biomass increases (for fermentations inoculated with early ethanol phase cells), a metabolic shift may lead to strengthening.

The trend in cell wall strength during the early ethanol phase for the majority of fermentations is for strengthening (Section 3.10). As the specific growth rate is close to zero during this phase (Section 3.3.2), the cell metabolism can be directed toward secondary strengthening of the cell wall. The late ethanol phase is characterised by cells of constant wall strength, with the actual strength at its highest level. Unlike the glucose growth phase, the wall strength is independent of the increase in biomass.

A variety of harvesting options exist based on the observed wall strength profiles and the cell concentrating operation chosen in down-stream processing; the downstream processing equipment may already exist, in the case where a new processes is being designed to fit existing equipment. In a low shear process, such as microfiltration, for concentrating cells in downstream processing, the best harvest point is at the end of the glucose growth phase: the cells being weakest at this point and consequently easier to homogenise. The precise strength can be ‘tuned’ by delaying the harvest into the early ethanol phase, as the wall strength increases during this period, to ensure that there is no premature release of cell internal products during the concentrating phase, due to excessive weakness of the cells: the wall strength being minimal at the end of the glucose growth phase. Where a high shear method like centrifugation is used for the initial
concentrating step in downstream processing, the best harvest point is during the beginning of the late ethanol growth phase (phases 5 & 6 according to Locher et al., 1993: Figure 1.12, Section 1.5.1). The wall strength at this point is maximal but there is no prospect for ‘tuning’ it, as the wall strength profile here is flat. The cost of homogenising the cells is also greatest at this point, however this is offset by higher specific productivity of ADH and G6PDH (Sections 5.3.1.3 & 5.3.1.4, respectively), as well as higher volumetric productivity. Where new equipment is to be specified this can be done on the best balance of costs and productivities.

Depending on the desired product one or other growth stage can be selected for optimal wall strength. If the biomass is the product, the end of the fermentation is the desired harvest point, giving maximal wall strength. Where the internal constituents of the cell are the products, the cells may be harvested following the glucose growth phase up to the beginning of the late ethanol growth phase, to optimise both wall strength and productivity.
5.3.2 S. erythraea

5.3.2.1 Dry cell weight

The dry cell weight of S. erythraea fermentations is unlikely to be considered a product; the main product of the S. erythraea fermentations is the excreted antibiotic erythromycin. Neither the specific productivity of erythromycin or the point of maximum biomass are important as the product is located outside the cell.

5.3.2.2 Broth erythromycin content

For this product a high volumetric productivity is desired, as the product is extracellular. Broth erythromycin concentration was observed to peak shortly after the end of the exponential growth phase (Figure 4.2). This pattern of productivity is confirmed by McDermott et al. (1993) for batch cultures of this organism using the same medium. Though growth and production are not expected to overlap, as the medium is nitrogen limited (Martin & Demain, 1980), an overlap is actually observed; this may be due to the slow growth rate of the organism on the fairly minimal medium that was used. This puts a very tight constraint on the harvesting point, as biomass increase does not yield further productivity gains.

5.3.2.3 Broth rheology

The broth viscosity and rheological behaviour would be important if they changed significantly during the production phase (Section 1.7); the results (Figure 4.5) show that broth viscosity does not change by more than 15%, and the rheology remains Newtonian (Section 4.3), so these parameters have little influence here. These fermentations had very low biomass and specific growth rates (Section 4.1) due to the minimal nutrient content of the medium (Table 2.2), if these were altered to improve the growth rate and biomass of the process, it would be more likely that the rheological profile would become complex. However, excluding the cell disintegration phase toward the end of the fermentation, the greatest change in rheology is likely to occur during the exponential growth phase, which overlaps the production phase. This may shift the harvest to a point before the maximum erythromycin production, if the viscosity is
appreciably greater with a higher biomass, to reduce the problems of mixing, oxygenation and mass transfer (e.g. equation 1.7 in section 1.2.1.2 and equation 1.13 in section 1.2.1.3).

5.3.2.4 Cell morphology

There may be two reasons for monitoring the morphology. Firstly, the cell size may influence the ease of separation of the cells from the broth. The majority of fermentations show the cells getting smaller with elapsed time (i.e. main hyphal length decreasing: Section 4.4), so the process of cell separation from the broth may become increasingly difficult (depending on the separation process), therefore the emphasis is on earlier harvesting in the production phase. Secondly, the cell morphology is suggested to have some intrinsic importance to the production of erythromycin, with production only occurring above a minimal hyphal size, so this constraint has to be satisfied (Martin and Bushell, 1996); however, no constraint was observed in this study (Section 5.2.2). The cytoplasmic volume may be more important than the cell lengths, in determining the likelihood of the cells producing erythromycin as this is a better indication of the cells capacity for metabolic activity. If the cells maintained their volume by increasing their branching or the hyphal diameter, they may still produce erythromycin despite the main hyphal length decreasing. This cannot however be deduced from the present study, as the hyphal diameter and cell volume were not monitored. If some connection were found between another morphological parameter and the erythromycin production, morphology would also be useful to monitor for the latter purpose.

5.3.2.5 Soluble protein content of the cell

The soluble protein may be regarded as a contaminant, and should become separated from the product efficiently by filtration or centrifugation of the cells. However, harvesting must be completed before significant cell disintegration begins and the proteins become released (Section 4.5.3). The background release of soluble protein appears to be a useful indicator of the viability of the cells and the point of disintegration. Cell disintegration is undesirable, as the released proteins and cell components are contaminants; therefore harvesting must not be delayed to this point.
However, in an industrial process the disintegration of the cells (probably caused by nutrient depletion) would be prevented by nutrient feeding.

5.3.2.6 Cell wall strength

The fermentation product (erythromycin) is excreted into the fermentation broth, and the most likely initial downstream processing step is to separate the cells from the broth supernatant by filtration or centrifugation. The cell wall strength profile during the production phase could have an impact on the selection of the cell harvesting point and method, if it shows a change or particular weakness at some point of the growth cycle. However, where there is a trend in wall strength during the production phase, it is toward strengthening of the cells (Section 4.7), thus cell harvesting becomes easier as the production phase progresses. This drives the best harvest point to a later stage of the production phase and allows the use of more vigorous cell separation techniques.

From the limited large scale experiments, background release of soluble proteins appears to be a larger problem, with broth soluble protein concentrations continually increasing with elapsed time (Section 4.5.3): this drives the harvest to an earlier point in the production phase, in order to reduce the level of contaminants during downstream processing. Though this point is valid for the batch process and media used in this study, it is not likely to be of such importance in an industrial process, where a richer medium and feeding strategy should limit cell disintegration. Under these circumstances the fermentation would be harvested at maximum productivity.
5.4 Future studies

The overall objective of this study was to recreate the cell quality profiles observed in the largest scale of vessel used in this study, the 1500L vessel, in some smaller scale equipment; that is to carry out a scale-down study on the basis of cell quality. The results (Sections 3 and 4) suggest that the effects of scale on some of the cell qualities (e.g. wall strengths of the two organisms and \textit{S. erythraea} biomass production: Section 5.2) may be connected to the rate at which cells circulate through the high shear zone of the impeller as well as the magnitude of shear. This would be an interesting basis for future investigations. In the introduction it was described how scale-down models could be successfully used to model the behaviour of cells experiencing concentration gradients in large scale fermenters (Section 1.4.4.2). The design of such systems could also be adapted for modelling the effects of shear on the cells, by considering the cells to be in a momentum gradient as they flow around circulation loops within the vessel. Existing scale-down studies based on stirred tank reactors with external recirculating flows, have already inadvertently subjected cells to momentum gradients in addition to the studied concentration gradients; however, the challenge in this case is to ensure that there are no concentration gradients in the scale-down model, or at least the extent of the gradients is small enough not to influence the cells quality. Unlike previous scale-down studies which use a true gradient, with the concentration of a parameter changing gradually as the fluid elements flow along the circulation path, the shear pattern that needs to be reproduced here, is more of a step change from a high to low shear zone. This is because the indications are that shear damage only occurs in the vicinity of the impeller (e.g. by high shear in the trailing vortices behind the blades of a disc turbine) for stirred tank fermenters (Ayazi Shamlou \textit{et al.}, 1994).

The scale-down model would be a representation of the circulation path of a fluid element in a stirred tank fermenter (Figure 5.8). The model would simplify the complex mixing patterns of the large scale vessel to be modelled, by reproducing the characteristics of a single mixing compartment from the larger vessel. The types of scale-down equipment used for studies to date include stirred tank reactors with external circulation loops, airlift loop reactors and conventional airlifts; though, the physical nature of any model attempting to reproduce the shear effects of this study must include a turbine agitator in the high shear zone (the ‘box’ in Figure 5.8).
Multi-turbine fermenter

circulation paths for fluid elements are shown

Basic plan of a scale-down model

The fluid cycles between the 'BOX', which imparts the high shear to the cells, and the 'circulation loop', which represents the low shear zone.

Figure 5.8: General plan for a scale-down model
The scale-down model must circulate fluid between the high and low shear zones ('box' and 'recirculation loop') at the same rate as in the large scale vessel, and in a plug flow manner (i.e. no backmixing as the broth is circulated). The mixing time determinations undertaken in this study (Section 4.8), indicate that the cell qualities may be affected even though the difference between the mixing times of the small (7 & 20L) and large scale (1500L) can be minimal, therefore the value used for the circulation times is very critical to the design of the scale-down model. Previous studies have used values for the mean circulation time, whilst the current opinion favours the use of a circulation time distribution, to vary the circulation rate and accurately model the mixing characteristics of a large vessel. For either case, more accurate determinations of the mixing behaviour are required for the vessels used in this study. This study attempted to determine the circulation time of a single mixing compartment of a large vessel, by simplifying the large vessel to just one mixing zone by the use of one impeller; this was necessary because a relatively simple means of mixing time measurement was used. However, even in this simplified system it proved to be beyond the scope of the equipment to determine the circulation time. Thus, future studies should use more accurate means to determine the circulation time directly: e.g. flow follower methods (Bonakdarpour et al., 1994). The mixing time of any scale-down model could eventually be compared to the original vessel by the method used here (Section 2.7).

Apart from the measurement of the circulation time, a measurement of the shear (e.g. maximum and average shear rates) of the 1500L vessel would be required, and these reproduced in the scale-down system for accurate reproduction of the conditions. This might require the use of greater tip speeds or impeller diameters than would normally be used for a small scale vessel.

Figure 5.9 illustrates a possible scale-down model, consisting of a stirred tank with external circulation loop, for the study of the effects of circulation through a high shear zone on the cell qualities. The model uses a single turbine in the high shear zone, to simplify the overall mixing. The vessel may be 'squat' to allow for large diameters of impeller to be used. The aeration devise should be below the impeller, to create the trailing vortex system found in a conventional stirred tank (Middleton, 1985), and also in the circulation loop to stop DOT gradients becoming severe. All scale-down models should set the feeding times for the pH adjusting solution or nutrients, longer than the circulation time, to avoid concentration gradients. The return flow would need to be directed toward the impeller, to stop cells from bypassing the high shear of the impeller.
The system consists of a single turbine STR with external circulation loop.

The circulation time is controlled by the peristaltic pump.

Air is sparged to the impeller and to the circulation loop to stop DOT gradients becoming severe.

Geometric similarity is not a prerequisite, therefore large diameter turbines may be used to reproduce the maximum shear of a the large scale system.

**Figure 5.9:** Scale-down model using a peristaltic pump to create a circulating flow
vortices while circulating in the system. The circulating flow in the loop can be close to plug flow for this system as the tube diameter is small (of the order of 1cm internal diameter) and the peristaltic pump stops backflow. The total volume for any scale-down system should not be less than 15 litres, to cater for the volumes of sample required to perform cell strength determinations and other assays. The actual circulation times required are considerably faster than have been used in some studies to date. The mixing time results indicate bulk mixing to be complete within 20 to 40 seconds at the larger scale, suggesting a circulation time of the order of 5 to 10 seconds if complete mixing is considered to take 4 circulations (Pandit and Joshi, 1983). At this rate the pump used in the circulation loop may impart considerable shear effects on the cells in addition to the impeller (the pump flow rates being of the order of 90-225L/min, for a 15L broth volume taking 4 circulations for complete mixing in 40 to 20 seconds, respectively). Therefore it may be better to eliminate a pump from the set-up and produce the circulation flow by using either an airlift arrangement (Figure 5.10) or the pumping action of the turbine to create the circulating flow (Figure 5.11).

The airlift systems (Figure 5.10) would incorporate a turbine impeller above the air sparge point. Airlift reactors are able to produce high circulation rates without introducing high shear, the measured mixing times in the small scale vessels used in this study, when air was sparged in at low stirrer speeds (i.e. when the vessels behaved as airlifts), were indeed fast (Figure 4.17). If a different circulation time was required for a fixed airflow rate, this could be achieved by varying the height of the vessel, with the volume maintained by the use of 'solid' draft tubes of a compensating volume. However, the main drawback of this system is the dependence of the circulation time on the airflow rates, and the inability of the system to produce a circulation time distribution at a fixed airflow rate. This may only be overcome by the introduction of an independently driven axial impeller (low shear/high flow) above the turbine (Figure 5.11). This impeller would control the liquid circulation throughout the vessel, and could introduce a circulation time distribution by having a variable operating speed. The axial impeller would discharge downward in order to give a controllable liquid surface, and this would require the air supply to the turbine to be from above. The air supply could in fact be split to multiple points to stop DOT stress. The flow can at best be approximated to plug flow, by making the diameter of the draft tube and the gap between the draft tube and wall, as low as possible by using a large aspect ratio.
An airlift pattern of scale-down model eliminates the need for a separate pump to circulate the broth. However, circulation times and DOT gradients in the downcomer are more difficult to control.

**Figure 5.10:** Scale-down models using the airflow from the sparger to create a circulating flow
Incorporating an axial impeller into an airlift design allows better control over the circulation time. The liquid flows down in the draft tube to give better control, this allows air to be sparged in the downcomer and riser to stop DOT gradients.

No circulating pump is required, therefore cell damage should be minimal during circulation at high rates.

**Figure 5.11:** Scale-down models using an axial impeller to create a circulating flow
A turbine impeller acts as a centrifugal pump, with a suction at the centre of the disc created by a radial discharge flow. The head pressure in the discharge flow stream could be used to create the circulating flow for a scale-down model; the design of a model based on this principle is given in Figure 5.12. An aerated reservoir holds the majority of broth above the impeller housing. The broth from the reservoir is directed toward the centre of the turbine disc, with further air supplied in the impeller housing. The broth is pumped out of the housing at its circumference, and back to the top of the reservoir, but above the liquid to aid degassing. The plug flow is aided by making the reservoir aspect ratio large. The system could use different size impellers to perform the pumping action and provide different quantities and patterns of shear, though it would require individually matched impeller housings to maintain an efficient pumping action. The circulation times could be controlled both by the stirrer speed used and by varying the length of the circulation loop; the difference in the volume of the impeller housing and circulation loop would be taken up by the liquid reservoir. However, this system would be governed by the minimum head pressure required to create the circulating flow, which would determine the lowest stirrer speed that could be used (hence the minimum shear that could be studied).

Overall the airlift system with axial flow impeller (Figure 5.11) might be the favoured design because it is part stirred tank and part free bubble rise reactor, which is a close physical representation of a large scale STR (Andrew, 1982). The system would have independent control of the circulation time (axial impeller), turbine impeller speed and air flow rate. This system could be modified for the study of other gradients in the environment of the cell; for example the effect of heat transfer gradients could be modelled by replacing the turbine with a cooling/heating source, or concentration gradients could be introduced by making fast additions of concentrated chemicals at a single point in the vessel. Eventually the scale-down system could be used to predict the effects of greater degrees of scale-up on the cell qualities by using increased circulation times and tip speeds in the model, this may then lead to a change in the cell qualities of *S. cerevisiae*. 

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Figure 5.12: Scale-down model using the impeller pumping capacity to create a circulating flow

N.B. The impeller housing has to be close to the size of the impeller
6 CONCLUSION

1) The use of cell quality profiles in the evaluation of scale-up effects depends on the reproducibility of the data. In the case of *S. cerevisiae* the fermentation profiles and assays have been shown to be sufficiently reproducible, to be of value in further investigations. For *S. erythraea* the assay reproducibility is also good, and the fermentation variability, though not rigorously tested, has been limited by the use of strict inoculum generation procedures.

2) Where there are many metabolic changes during the growth of an organism, such as in the case of *S. cerevisiae*, a peak in the activity of an enzyme or cell wall strength may be easily missed. Therefore, such fermentations require intensive sampling and it may be better to reduce the number of monitored variables to improve the resolution of the profiles. Sampling may also have to be timed to coincide at key points in the fermentation profile.

3) The cell quality of the inoculum can have a considerable effect on the cell quality observed in the fermenter. For *S. cerevisiae* the inoculum quality appears to influence the stability of the cell soluble proteins (e.g. the enzyme ADH: Section 3.5.1), when released from the cell by homogenisation. Transfer of inocula growing in the glucose growth phase lead to low stability of soluble proteins, while transfer of cells growing in the early ethanol phase lead to improved stability. The decay of soluble protein was particularly evident during the early ethanol phase within the fermenter.

4) For *S. cerevisiae*, statistical analysis has shown that many cell qualities (e.g. specific growth rates, soluble protein content, ADH and G6PDH activities) were insensitive to fermentation scale in the range of 5 to 662L operating volume. Though the cell wall strengths (as determined from homogenisation data) were difficult to compare by statistical analysis, the indications were that this quality was also unaffected by scale-up in the range of 20 to 450L, with the effect of further scale-up to 1500L and scale-down to 7L requiring further investigation. Cell disruption in the fermenter did not appear to be significant. The gas analysis data indicated that event times and metabolic behaviour in the ethanol growth
phase (as indicated by the respiratory quotient) were comparable between scales, thought the magnitude of the carbon dioxide evolution and oxygen uptake bared little resemblance between scales. Thus, a 20L vessel is a good scale model of a 1500L vessel, so long as the inoculum quality and medium composition are the same between the scales and no oxygen limitation occurs.

5) The release of soluble protein from *S. erythraea* during homogenisation was found to be dependent on the homogenisation pressure.

6) *S. erythraea* was more sensitive to the environment (impeller tip speed) and to fermentation scale, with cell qualities possibly being affected by shear and circulation rates. The maximum biomass attainable within a particular scale may decrease on scale-up, though the critical tip speed at which biomass production is affected, may increase with scale-up, suggesting a possible relation to liquid circulation rate. The reduction in maximum biomass with scale may have been responsible for the reduction in apparent broth viscosity with scale-up. The cell morphology appeared to be principally affected by the circulation rate, with main hyphal length increasing with scale for an equivalent tip speed. The cells soluble protein content and wall strength were reduced on scale-up (the soluble protein content did not show any connection to impeller speed at the small scale, while the reduction in wall strength appeared to be related to increasing shear with scale-up); the profiles of these two cell qualities would benefit from further enhancement by more intensive sampling. The background release of soluble protein increased with stirrer speed (increasing power input and reducing circulation time) and scale-up (increasing the maximum shear), suggesting increasing cell disruption in the fermenter under these conditions. Some further investigation of the effect of broth ionic and osmotic strength on the cell wall integrity of this organism is required. The effects of impeller speed and fermentation scale on the erythromycin production were not conclusive, and further investigation of this should use more sensitive chromatographic techniques to measure erythromycin concentration.
7) Background soluble protein release was a useful indicator of cell disintegration, and hence viability of cultures, in *S. erythraea* fermentations. Dry cell weight measurements did not usefully indicate viability after the exponential growth phase because empty cell husks could not be distinguished from intact cells.

8) The cell quality profiles for the two organisms were shown to be useful in the determination of the best harvesting point for the fermentation, from the productivity and processing viewpoints. The cell wall strength information is particularly useful from the processing point, as it can help decide which equipment would be most suited to cell separation and concentration to avoid premature release of cell internal components; where equipment already exists the wall strength information can help decide which growth phase is the most appropriate for harvesting.

9) The investigation of cell qualities that are affected by shear, may be possible using scale-down models based on the recirculation of cells through a high shear zone. This requires improved estimates of the circulation time in the vessel to be modelled and an estimate of the shear that is to be reproduced.
APPENDIX 1

Method for using the statistical test called “Analysis of variance” (ANOVA)

This statistical test is used for testing hypotheses. Roughly phrased the hypothesis in this case is that “there is no difference in the cell qualities between any of the scales of fermentation or stirrer speeds used”: this is the null hypothesis.

The data from the different fermentation scales are first considered to belong to different groups of samples from the same population of data. The statistical tests then compares the variance of the sample means of the different groups with the variance within the samples that the means came from, to give an indication of whether the different groups of samples belong to the same (accept the null hypothesis) or separate (reject the null hypothesis) populations of data. The “F-test” is the customary form in which the test is conducted (Wonnacott & Wonnacott, 1985), and is given in equation A.1. The value of the ‘F’ ratio that is calculated is then used to determine a ‘p’ value from a table of ‘F-critical points’. The ‘p’ value is the probability of getting sample means that vary by as much as observed. ‘p’ has values ≤1. A ‘p’ value of 1 indicating that there is a 100% chance that the sample means belong to the same population of data, (i.e. the null hypothesis is true), and the observed variations between the groups of samples is due to chance (e.g. experimental fluctuations). Decreasing values of ‘p’ indicating a reducing chance that the data from the different groups of samples belong to the same population of data. The ‘p’ value chosen for acceptance/rejection of the null hypothesis is arbitrary, with a reasonable level of ‘p’ in the range of 0.15-0.25 (Wonnacott & Wonnacott, 1985). The ‘p’ values for the various ANOVA tests given in Tables A.1-A.9, were determined by using the built in statistical function in the computer package “Excel” (Microsoft Corporation, US). The ‘p’ values in the Tables A.1-A.9 are given as percentages rather than fractions.

\[ F = \frac{n s^2_x}{s^2_p} \]  

(A.1)

Where, \( s^2_x \) is the variance between samples \( s^2_p \) is the variance within samples \( n \) is the number of samples
ANOVA results for the comparison of the effect of fermentation scale on the cell qualities of *S. cerevisiae*

As explained in the introduction to section 5, the 20L results are taken as the standard to which the rest of the data are compared. The ANOVA test is first applied to “All” of the data, and then the 7, 450 & 1500L results are individually compared to the “standard” results obtained at the 20L scale.

**Test results for the gas analysis data**

The fermentation event time data for these tests was taken from Figure 5.1, and the exit gas data from the tables associated with Figures 3.2-3.9.

---

**Appendix table 1: ANOVA results for the gas analysis data for *S. cerevisiae* fermentation**

<table>
<thead>
<tr>
<th>Cell quality compared</th>
<th>P value for comparison between, %</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of the end of the glucose growth phase, Total fermentation time (h)</td>
<td>0.06</td>
<td>38</td>
</tr>
<tr>
<td>Location of the RQ step, Total fermentation time (h)</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>End of fermentation, Total fermentation time (h)</td>
<td>0.2</td>
<td>13</td>
</tr>
<tr>
<td>Peak CER at the end of the glucose growth phase, mmol/L.h</td>
<td>3e-3</td>
<td>7e-4</td>
</tr>
<tr>
<td>Peak CER at the end of the ethanol growth phase, mmol/L.h</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>CO₂ evolved during the ethanol growth phase, mmol/L</td>
<td>0.1</td>
<td>7.2e-3</td>
</tr>
<tr>
<td>O₂ consumed during the glucose growth phase, mmol/L</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Mean RQ during the glucose growth phase</td>
<td>59</td>
<td>33</td>
</tr>
</tbody>
</table>

ND, No data for this scale
Test results for the specific growth rates

The data for these tests was taken from the tables associated with Figures 3.10-3.11.

Appendix table 2: ANOVA results for the specific growth rates during the three growth phase of S. cerevisiae fermentation

<table>
<thead>
<tr>
<th>Growth phase compared</th>
<th>P value for comparison between, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td>Glucose growth phase</td>
<td>2.5</td>
</tr>
<tr>
<td>Early ethanol phase</td>
<td>95</td>
</tr>
<tr>
<td>Late ethanol phase</td>
<td>65</td>
</tr>
</tbody>
</table>
**Test results for the soluble protein release data**

The data for these tests was taken from the tables associated with Figures 3.13-3.14. The data were compared during the three growth phases of *S. cerevisiae*, between the upper and lower limits of soluble protein indicated in the figure tables.

**Appendix table 3: ANOVA results for the volumetric soluble protein release data during the three growth phase of *S. cerevisiae* fermentation**

<table>
<thead>
<tr>
<th>Growth phase compared</th>
<th>P value for comparison between, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td>Glucose growth phase</td>
<td></td>
</tr>
<tr>
<td>Maximum protein level</td>
<td>83</td>
</tr>
<tr>
<td>Minimum protein level</td>
<td>57</td>
</tr>
<tr>
<td>Early ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum protein level</td>
<td>20</td>
</tr>
<tr>
<td>Minimum protein level</td>
<td>47</td>
</tr>
<tr>
<td>Late ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum protein level</td>
<td>50</td>
</tr>
<tr>
<td>Minimum protein level</td>
<td>18</td>
</tr>
</tbody>
</table>

NV, no variance in one of the data sets
No df, zero degrees of freedom in one of the results, too few data for test
The data for these tests was taken from the tables associated with Figures 3.15-3.16. The data were compared during the three growth phases of *S. cerevisiae*, between the upper and lower limits of specific soluble protein indicated in the figure tables.

**Appendix table 4: ANOVA results for the specific soluble protein release data during the three growth phase of *S. cerevisiae* fermentation**

<table>
<thead>
<tr>
<th>Growth phase compared</th>
<th>P value for comparison between, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td>Glucose growth phase</td>
<td></td>
</tr>
<tr>
<td>Maximum protein level</td>
<td>64</td>
</tr>
<tr>
<td>Minimum protein level</td>
<td>70</td>
</tr>
<tr>
<td>Early ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum protein level</td>
<td>57</td>
</tr>
<tr>
<td>Minimum protein level</td>
<td>30</td>
</tr>
<tr>
<td>Late ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum protein level</td>
<td>50</td>
</tr>
<tr>
<td>Minimum protein level</td>
<td>100</td>
</tr>
</tbody>
</table>

* a, data have the same or very similar value

No df, zero degrees of freedom in one of the results, too few data for test
Test results for the ADH activity data

The data for these tests was taken from the tables associated with Figures 3.21-3.22. The data were compared during the three growth phases of *S. cerevisiae*, between the upper and lower limits of ADH activity indicated in the figure tables.

Appendix table 5: ANOVA results for the volumetric ADH activity data during the three growth phase of *S. cerevisiae* fermentation

<table>
<thead>
<tr>
<th>Growth phase compared</th>
<th>P value for comparison between, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td>Glucose growth phase</td>
<td></td>
</tr>
<tr>
<td>Maximum ADH level</td>
<td>10</td>
</tr>
<tr>
<td>Minimum ADH level</td>
<td></td>
</tr>
<tr>
<td>Early ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum ADH level</td>
<td>88</td>
</tr>
<tr>
<td>Minimum ADH level</td>
<td>29</td>
</tr>
<tr>
<td>Late ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum ADH level</td>
<td>87</td>
</tr>
<tr>
<td>Minimum ADH level</td>
<td>62</td>
</tr>
</tbody>
</table>

NV, no variance in one of the data sets

1, minimal activity at the lower limit in all the fermentations
The data for these tests was taken from the tables associated with Figures 3.23-3.24. The data were compared during the three growth phases of *S. cerevisiae*, between the upper and lower limits of specific ADH activity indicated in the figure tables.

**Appendix table 6: ANOVA results for the specific ADH data during the three growth phase of S. cerevisiae fermentation**

<table>
<thead>
<tr>
<th>Growth phase compared</th>
<th>P value for comparison between, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td>Glucose growth phase</td>
<td></td>
</tr>
<tr>
<td>Maximum ADH level</td>
<td>37</td>
</tr>
<tr>
<td>Minimum ADH level</td>
<td>33</td>
</tr>
<tr>
<td>Early ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum ADH level</td>
<td>56</td>
</tr>
<tr>
<td>Minimum ADH level</td>
<td>33</td>
</tr>
<tr>
<td>Late ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum ADH level</td>
<td>79</td>
</tr>
<tr>
<td>Minimum ADH level</td>
<td>12</td>
</tr>
</tbody>
</table>

No df, zero degrees of freedom in one of the results, too few data for test
NV, no variance in one of the data sets
1, zero activity in the 20L results therefore no data to compare to
Test results for the G6PDH activity data

The data for these tests was taken from the tables associated with Figures 3.26-3.27. The data were compared during the three growth phases of S. cerevisiae, between the upper and lower limits of G6PDH activity indicated in the figure tables.

Appendix table 7: ANOVA results for the volumetric G6PDH activity data during the three growth phase of S. cerevisiae fermentation

<table>
<thead>
<tr>
<th>Growth phase compared</th>
<th>P value for comparison between, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td>Glucose growth phase</td>
<td></td>
</tr>
<tr>
<td>Maximum G6PDH level</td>
<td>6</td>
</tr>
<tr>
<td>Minimum G6PDH level</td>
<td>99</td>
</tr>
<tr>
<td>Early ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum G6PDH level</td>
<td>97</td>
</tr>
<tr>
<td>Minimum G6PDH level</td>
<td>29</td>
</tr>
<tr>
<td>Late ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum G6PDH level</td>
<td>95</td>
</tr>
<tr>
<td>Minimum G6PDH level</td>
<td>74</td>
</tr>
</tbody>
</table>
The data for these tests was taken from the tables associated with Figures 3.28-3.29. The data were compared during the three growth phases of *S. cerevisiae*, between the upper and lower limits of specific G6PDH activity indicated in the figure tables.

**Appendix table 8:** ANOVA results for the specific G6PDH data during the three growth phase of *S. cerevisiae* fermentation

<table>
<thead>
<tr>
<th>Growth phase compared</th>
<th>P value for comparison between, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td>Glucose growth phase</td>
<td></td>
</tr>
<tr>
<td>Maximum G6PDH level</td>
<td>34</td>
</tr>
<tr>
<td>Minimum G6PDH level</td>
<td>29</td>
</tr>
<tr>
<td>Early ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum G6PDH level</td>
<td>50</td>
</tr>
<tr>
<td>Minimum G6PDH level</td>
<td>24</td>
</tr>
<tr>
<td>Late ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum G6PDH level</td>
<td>78</td>
</tr>
<tr>
<td>Minimum G6PDH level</td>
<td>39</td>
</tr>
</tbody>
</table>
Test results for the cell wall strength data

The data for these tests was taken from the tables associated with Figures 3.33-3.34. The data were compared during the three growth phases of *S. cerevisiae*, between the upper and lower limits of K value indicated in the figure tables, and graphically displayed in Figure 3.35.

---

**Appendix table 9: ANOVA results for the cell wall strength data during the three growth phase of *S. cerevisiae* fermentation**

<table>
<thead>
<tr>
<th>Growth phase compared</th>
<th>P value for comparison between, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td>Glucose growth phase</td>
<td></td>
</tr>
<tr>
<td>Maximum K level</td>
<td>25</td>
</tr>
<tr>
<td>Minimum K level</td>
<td>73</td>
</tr>
<tr>
<td>Early ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum K level</td>
<td>45</td>
</tr>
<tr>
<td>Minimum K level</td>
<td>22</td>
</tr>
<tr>
<td>Late ethanol phase</td>
<td></td>
</tr>
<tr>
<td>Maximum K level</td>
<td>48</td>
</tr>
<tr>
<td>Minimum K level</td>
<td>42</td>
</tr>
</tbody>
</table>
APPENDIX 2

Predicted circulation times, power inputs and dissipation values for different impeller tip speeds, at various scales of fermentation.

Liquid circulation times have been predicted for the various operating conditions used in the fermenters in this study (Figure 1A2). The calculations have used the approximate relation of Holmes et al. (1964) (Equation 1.36, Section 1.2.2.3), with the dimensions given in table 2.4 and the operating speeds given in tables 3.1 and 4.1. The effects of aeration have been excluded for simplicity. The experimental mixing times are given in figure 4.18 (Section 4.8), and were determined for the aeration conditions used.

Circulation times are predicted to decrease with increasing tip speed, but increase with scale at a fixed tip speed (Figure 1A2). An increase in mixing time was obtained with scale-up from 7 to 662L for the mixing experiments in this study (Section 4.8). However, the predicted trend in circulation time for the 1500L vessel does not agree with the mixing time data obtained in this study, which is probably the result of assuming no aeration: the circulation time should increase over the range of tip speeds used in the 1500L vessel under the operating conditions used (Section 4.8).

The power input calculations used equation 1.31 (Section 1.2.2.2), again excluding the effects of aeration for simplicity; the actual power input was determined by multiplying the power input determined from equation 1.3.1 by the number of impellers in the particular vessel (Table 2.4). The vessel dimensions and stirrer speeds used were the same as for the circulation time calculations. The liquid density was assumed to be that of water, and a Power number of 5.5 was used (Middleton, 1985). The power dissipation values were obtained by dividing the power input by the broth weight, using the broth volumes given in tables 2.4.

Power input (Watts) is calculated to increase with scale-up at a constant tip speed (Figure 2A2), though power input is lower in the 1500L vessel compared to the 450L vessel at an equivalent tip speed, because the larger vessel only has one impeller against three impellers in the 450L vessel. However, power dissipation (W/kg) is calculated to fall with scale-up at a constant tip speed.
Figure 1A2: Predicted \( t_c \) (and measured \( t_m \)) for various impeller tip speeds

Figure 2A2: Calculated power inputs and dissipation values at various impeller tip speeds
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