LOSS OF YEAST QUALITY DURING MECHANICAL HANDLING IN A BREWERY: AN INVESTIGATION OF CROPPING

Lauren Basson

B.Sc. Eng. (Chemical) (University of Cape Town)

A dissertation submitted for the fulfilment of the requirements
of the degree of Master of Science in Engineering
in the Department of Chemical Engineering
University of Cape Town

Bioprocess Group
Department of Chemical Engineering
University of Cape Town

September 1996

SYNOPSIS

In large scale brewing operations, process efficiency and beer quality rely on consistent fermentations. Improper handling of yeast may result in slow or incomplete fermentations and beer of unacceptable quality. The maintenance of yeast quality during yeast handling is therefore crucial. Current guidelines for yeast handling, plant design and equipment specifications are based predominantly on empirical knowledge. The effects of different equipment, flow conditions and physical conditions to which brewery yeast is exposed are largely unknown. Hence, South African Breweries (SAB) requested a study on the possible damage to yeast (loss of yeast quality) during mechanical handling in a brewery. The investigation detailed in this thesis was limited to mechanical handling of yeast during cropping (the transfer of yeast from a fermentation vessel to a storage vessel for re-use) and had the following objectives:

- to assess the potential for a loss of yeast quality during the mechanical handling in a brewery,
- to select and validate analytical techniques for the identification, characterisation and quantification of a loss of yeast quality,
- to characterise and quantify the loss of yeast quality during yeast cropping in a brewery.

Since yeast quality is associated with the fermentative capacity of the yeast and the quality of the beer produced, assessment of yeast quality can be done by small scale fermentation. Two small scale fermentation systems (2 L EBC tubes and 500 mL measuring cylinders) were set up. The small scale fermentations were reproducible: the coefficients of variation for the attenuation data were less than 5% in both systems. More rapid analytical techniques to assess yeast quality have been developed. Nine yeast quality assays were selected for this investigation: an extracellular protease assay, methylene blue and Mg-ANS staining techniques, plate and slide counts, oxygen utilisation rate, acidification power and intracellular glycogen and intracellular trehalose contents. Reproducibility was good (coefficient of variation $< 5\%$). for the methylene blue and Mg-ANS staining techniques, the slide count technique, the acidification power test and the assay for the intracellular glycogen content.

Literature and laboratory studies indicate that mechanical and physiological stress elicit different responses from yeast. The ability of the yeast quality assays to predict the fermentation performance depends on the nature of the loss of yeast quality and the physiological basis of the assays. Since mechanical stress affects the cell envelope, the intracellular protease assay (which indicates the extent of membrane damage) and the slide count technique (which reflects replicative competence, hence functional integrity of the cell membrane) were shown to be appropriate assays for the assessment of the loss of yeast quality in response to mechanical stress. Measuring the specific oxygen utilisation rate did not reflect a loss of yeast quality in response to mechanical stress. Physiological stress results in the depletion of the intracellular reserve compound glycogen, reduced metabolic

activity, changes in the metabolic pathways expressed and loss of stress resistance. Specific oxygen utilisation rate, intracellular glycogen and intracellular trehalose contents are proposed as appropriate indicators of the response of yeast to physiological stress.

Laboratory studies are limited in their ability to simulate the brewery circuit, especially in terms of pressure drop and dissolved gases present. A pilot scale test facility was used in this study. Experiments were carried out at a local brewery (Newlands Brewery, SAB, Cape Town). Variables investigated include the effect of pump design and operating speed and the effect of different flow conditions. Ten pumps, representing six categories (peristaltic, lobe, sine, gear, diaphragm and centrifugal) were evaluated. In addition, yeast handling during routine brewery operation was assessed by sampling directly from the production facility.

The following was demonstrated (at a 95% confidence level):

- At the production scale, transfer of yeast from the base of the cone to the chiller outlet during routine brewery operation did not affect yeast quality as indicated by small scale fermentations and the selected yeast quality assays (extracellular protease activity, methylene blue staining, oxygen utilisation rate, intracellular glycogen content and intracellular trehalose content).

- Over the range of flow rates that could be achieved with the available pump and motor combinations (16 to 193 L/min $\approx$ 1 to 11.6 m^3/hr), cropping with the ten pumps under investigation had no significant effect on the quality of the cropped yeast as indicated in pilot scale experiments using the selected yeast quality assays (methylene blue staining, slide counts, oxygen utilisation rate, acidification power, intracellular glycogen content and intracellular trehalose content).

- No significant effect on the fermentation performance in small scale fermentations was confirmed for four pumps, namely the Bredel peristaltic pumps (model SP/40 and SP/50), the APV lobe pump (model CL/3/156/7) and the Maso Sine pump (model SP3"). These were operated at a range of flow rates from 16 to 162 L/min ($\pm$ 1 to 9.7 m^3/hr).

- Over a range of flowrates from 17 to 110 L/min ($\pm$ 1 to 6.6 m^3/hr), linear velocities from 0.1 to 3.7 m/s and Reynolds numbers from 86 to 1114, no difference in yeast quality (indicated by an assay for extracellular protease activity, methylene blue staining and oxygen utilisation rate) or subsequent fermentation performance in small scale fermentations could be detected.

Since pump design and operation under brewery conditions do not affect yeast quality, the selection of cropping pumps can be based on criteria such as operating characteristics, maintenance requirements, cleanability and efficiency.

This investigation was limited to laminar flow conditions representative of the brewery yeast handling circuit. Investigation of turbulent flow conditions may

identify critical flow conditions for a loss of yeast quality. A knowledge of the rheology of the various yeast suspensions (cropped, stored, re-pitched and propagated yeast) would aid in the specification of yeast handling equipment and allow the study of loss of yeast quality at a more fundamental level. Only aspects of yeast quality with a direct impact on fermentation performance were investigated. Under the conditions in the yeast handling circuit, wall damage may occur. This would result in the release of wall-associated material into the medium, reducing beer quality, and requires investigation.

The study was limited to cropping. Other areas important for mechanical stress are yeast propagation, pitching, storage with agitation and acid washing. Conditions such as growth phase and biomass concentration in these processes differ from those during cropping and may increase the susceptibility of the yeast to damage. The investigation should thus be extended to the other steps in the yeast handling process.

ACKNOWLEDGEMENTS

The following organisations are gratefully acknowledged:

The South African Breweries Beer Division for sponsoring the project and making available the opportunity to do an M.Sc. in close contact with industry. Staff of Central Office and Newlands Brewery are thanked in particular for generously giving of their time, sharing their knowledge and providing invaluable technical assistance.

Aeromix, Alfa-Laval, APV, Flo-mart, Letaba pumps, NDE, Micro Matic, Newlands Brewery (SAB) and Walter Becker for the loan of the pumps and Südmo Stainless Steel for supplying the test rig at cost.

The Microbiology Department of the University of Cape Town for the use of their facilities.

My sincere thanks to the following people:

Dr Susan Harrison, my supervisor, for her guidance and patience throughout the course of the project and for making available the opportunity to attend an international conference.

Jenny Löwenadler and Garnett Titus for their support (both technical and moral), hard work, dedication and the valuable contributions made to the project.

Linda Harrower, Maria Josias and Jacki Mshudulu for absorbing the impact of the project on the laboratory facilities and Bill Randall, Craig Balfour and Granville de la Cruz for computer-related support.

Sue Jobson for matters administrative and tying up loose ends.

Students Conrad Arendse, Rashaad Goosein and Ursula Diekman for the hours of "slave labour".

The staff and post-graduate students of the Department of Chemical Engineering at the University of Cape Town and in particular the "Biogroup" for making the Department such a pleasant and supportive environment in which to work.

My family and friends, particularly Heinrich Wolff, for support, encouragement and patience.

Finally, the Foundation for Research and Development (FRD) and the Bioprocess Research Group of the Department of Chemical Engineering are thanked for financial assistance.

TABLE OF CONTENTS

APPENDICES

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

DEFINITIONS

Attenuation	the conversion of wort sugars to ethanol, CO_2 and other fermentation products leading to a drop in the specific gravity of the fermentation medium
Attenuation rate	the rate of change in the specific gravity of the fermentation medium as a result of the conversion of wort sugars to fermentation products
Consistency	the biomass concentration of a yeast suspension expressed as the percentage wet mass
Cropping	the transfer of yeast from the fermentation vessel to the yeast collection vessel for re-inoculation into a subsequent fermentation
Fermentation performance	the fermentative efficiency of the yeast evaluated in terms of the rate and extent of attenuation and the quality of the final product
Flocculation	the agglomeration of yeast during fermentation
Generation number	a number used to indicate the number of times a batch of yeast has been cropped and re-used
Gravity	specific gravity of the wort at 20°C (expressed in °Plato) used to describe its sugar concentration
Integrity of the cell envelope	the intactness of the cell envelope
Physiological status	the position in the cell cycle (*eg.* growing phase, stationary phase, etc.)
Physiological state	yeast condition in terms of structural and functional integrity
Pitching	inoculation of a fermentation
Pitching rate	the amount of yeast inoculated into a fermentation

Propagation	the step-wise generation of biomass from laboratory to plant scale to obtain sufficient biomass for production purposes
Re-circulation	the re-use of yeast during brewery fermentation involving harvesting, storage, batching and re-inoculation
Replicative competence	the ability to reproduce
Replicative deactivation	loss of the ability to reproduce
Sedimentation	the settling of yeast during fermentation
Viability	a term to describe the ability of cells to grow and reproduce; losses of viability may occur via loss of structural integrity, cell death or replicative deactivation
Vitality	a term used within the brewing industry to describe the metabolic activity of yeast; in some cases, overall fermentative capacity may be implied
Wort	the cereal-based extract containing a range of carbohydrates which are converted to ethanol, CO_2 and other organoleptic products during the production of beer
Yeast quality	the physiological condition of the yeast in terms of its fermentative capacity and the quality of the beer produced

SYMBOLS

In equations:

S	substrate concentration
t	fermentation time
α, β	constants in exponential expression fitted to attenuation data

Statistical:

F	statistical parameter used in the analysis of variance
s	the standard deviation of a set of replicate measurements (the sample standard deviation)
s_{pooled}	a standard deviation determined by pooling data from a series of replicate measurements
t	statistical parameter, the deviation from the mean in units of the sample standard deviation, used to assess the statistical significance of the difference between means when a good estimate of the population standard deviation is not available
v	the number of degrees of freedom
z	statistical parameter, deviation from the mean in units of the population standard deviation, used to assess the statistical significance of the difference between means when a good estimate of the population standard deviation is available
σ	the population standard deviation

ABBREVIATIONS

For yeast quality assays:

AP	overall acidification power
AP(10)	spontaneous acidification power
CAP	overall cumulative acidification power
CAP(10)	cumulative spontaneous acidification power
DO	dissolved oxygen concentration
Mg-ANS	magnesium salt of 8-anilino-1-naphthalene sulfonic acid

NIR	near infrared reflectance (spectroscopy)
OUR	(specific) oxygen utilisation rate

Other:

ANOVA	analysis of variance
BICPAP	brewhouse increased capacity through parallel adjunct preparation
EBC	European Brewing Convention
CIP	cleaning in place
COP	cleaning out of place
FV	fermentation vessel
OCB	Ohlsson's Cape Brewery (Newlands Brewery)
SAB	South African Breweries
UCT	University of Cape Town
YCV	yeast collection vessel

CHAPTER

1

INTRODUCTION

1.1 BACKGROUND AND OBJECTIVES OF THE STUDY

The brewing of beer involves the use of certain yeasts, mainly species of *Saccharomyces*, to convert sugars in cereal-based extracts to ethanol, carbon dioxide and organoleptic compounds to produce a potable beverage. Beer brewing, an enormous industry in itself, is the largest biotechnological industry in the world (Oliver 1991). Developments in the brewing industry have resulted in considerable scale up and a move from traditional breweries serving local markets to large scale ventures with international markets (Renger 1991). In these large scale brewing operations, the efficient production of quality beer is imperative. Process efficiency and beer quality rely on consistent fermentations. In addition to process conditions, composition of the nutrient medium (wort), level of wort aeration and quantity of yeast inoculated (pitched), the quality of the yeast is an important determinant of the course of a fermentation (Daoud 1990). Improper handling of yeast during the brewing process may result in a loss of yeast quality resulting in slow or incomplete fermentations or the production of beer of unacceptable quality. The maintenance

of yeast quality during yeast handling is thus crucial (Stewart 1977).

The prevailing conditions in brewery yeast handling facilities have not been extensively investigated and are thus poorly characterised. Current guidelines for yeast handling, plant design and equipment specifications are based predominantly on empirical knowledge rather than on rigorous study. The effects of different equipment, flow conditions and physical conditions to which brewery yeast is exposed are largely unknown.

In the light of this, South African Breweries (SAB) approached the Department of Chemical Engineering at the University of Cape Town (UCT) to conduct a study on the possible damage to yeast (loss of yeast quality) during mechanical handling in a brewery. Within breweries, mechanical handling of yeast typically occurs when the yeast is transferred during propagation and recirculation or when the yeast is agitated during storage and acid washing procedures. The investigation detailed in this thesis was limited to mechanical handling of yeast during cropping (the transfer of yeast from a fermentation vessel to a storage vessel for re-use).

The study had the following objectives:

1. To assess the potential for a loss of yeast quality during the mechanical handling in a brewery.

2. To select and validate analytical techniques for the identification, characterisation and quantification of a loss of yeast quality.

3. To characterise and quantify the loss of yeast quality during yeast cropping in a brewery.

In addition, it would be of value to identify critical handling conditions beyond which yeast could be detrimentally affected and suggest improvements in current yeast handling techniques and plant and equipment specifications.

1.2 ORGANISATION OF THE THESIS

In Chapter 2, a literature survey is presented to assess the potential for a loss of yeast quality during the mechanical handling of the yeast in a brewery. An outline of the brewing process and a description of yeast handling during recirculation are included.

Chapter 3 deals with the identification, characterisation and quantification of a loss of yeast quality. The analytical techniques used in this investigation to identify and quantify a loss of yeast quality are presented and their selection motivated. The

application of the yeast quality assays to a loss of yeast quality in response to mechanical stress is illustrated. A proposal is made for the characterisation of a loss of yeast quality in terms of the "physiological state" of the cells and a scheme is provided for the loss of yeast quality in response to mechanical and physiological stresses.

The investigation of the potential loss of yeast quality during yeast cropping was carried out at a local brewery (Newlands Brewery, South African Breweries, Cape Town). Yeast handling during routine brewery operation was assessed by sampling directly from the production facility. A pilot scale test rig was used to study the effect of operation beyond standard conditions and to establish critical handling conditions. Variables investigated using the pilot scale rig include the effect of pump design and operating speed and the effect of different flow conditions. The equipment and experimental procedure used are described in Chapter 4, while the results obtained are presented and discussed in Chapter 5. A review of the pump designs and individual pumps evaluated during the investigation is provided in Appendix A. Features which make the different pumps suitable for applications such as yeast cropping are highlighted.

The findings of the investigation, including the identification, characterisation and quantification of losses of yeast quality and the potential loss of yeast quality during yeast cropping in a brewery, are presented in Chapter 6.

There are several additional appendices to the text. Appendix B provides a review of the statistical techniques used to analyse the experimental data. The detailed results of the statistical analyses to determine the errors associated with the analysis of yeast quality are presented in Appendix C. The results and statistical analyses for the study of cropping during routine brewery operation, the investigation of pump design and operating speed and the investigation of different flow conditions are presented in Appendix D, E and F respectively.

CHAPTER

2

THE POTENTIAL FOR THE LOSS OF YEAST QUALITY DURING MECHANICAL HANDLING IN A BREWERY

2.1 INTRODUCTION

A study of the literature illustrates that the hydrodynamic conditions to which micro-organisms are exposed may affect their activity. Biological responses to hydrodynamic stress that have been observed include a change in growth rate, nutrient uptake rate, metabolic pathways, product spectrum, morphology and the release of nucleotides (Mukataka *et al.* 1988, Logan and Dettmer 1990, Smith *et al.* 1990, Fowler and Robertson 1991, Toma *et al.* 1991, Wecker and Onken 1991). Such responses have been illustrated across a range of cell types, including bacteria, yeasts, filamentous fungi, algae, plant cells and animal cells. Examples include reduced growth and penicillin productivity as well as morphological changes in *Penicillium chrysogenum* on increasing the agitation intensity in a stirred tank reactor (Smith *et al.* 1990) and reduced rates of growth, nutrient uptake, oxygen utilisation and lysine synthesis on increased agitation of *Brevibacterium flavum* (Toma *et al.* 1991).

During a brewery fermentation, yeast reproduction results in a three to five fold increase in the biomass present (Boulton 1991). By harvesting or cropping yeast from one fermentation and re-inoculating (pitching) it into a subsequent fermentation, a brewery can sustain its yeast supply for an extended time. In addition, the efficiency of the process is optimised by reducing the requirement for laboratory yeast, yeast propagation and the management of waste yeast. Improperly handled yeast may result in problems such as hanging fermentations, poor attenuation and an altered flavour profile of the beer (McCaig and Bendiak 1985a, Pickerell *et al.* 1991), suggesting a biological response as described above. It is postulated that Brewers' yeast may exhibit biological responses as a function of possible mechanical and physiological stresses to which the yeast may be exposed during inter-stage transfers in the brewing process. Such responses are undesirable, since consistent fermentation performance is crucial for the efficient production of quality beer (Stewart 1977). An understanding of the conditions during yeast handling, the response of yeast to potential stress conditions and the concomitant loss of yeast quality is required.

In this chapter, a brief outline of the brewing process is given in order illustrate the context of yeast handling and explain the brewing terminology which will be used in the dissertation. Yeast handling during recirculation is described and aspects of yeast quality are discussed. The current knowledge on the effect of handling on the resultant quality and fermentation performance of the yeast is reviewed. The yeast handling equipment specifications of South African Breweries (SAB) are outlined. Finally, conclusions on the potential for a loss of yeast quality during mechanical handling of yeast are drawn.

2.2 OUTLINE OF THE BREWING PROCESS

In the production of beer, yeasts (mainly from the genus *Saccharomyces*) convert sugars in cereal-based extracts to ethanol, carbon dioxide and other organoleptic compounds. A schematic outline of the brewing process is given in Figure 2.1. The process can be divided into three sections: namely wort preparation, fermentation and post-fermentation treatments.

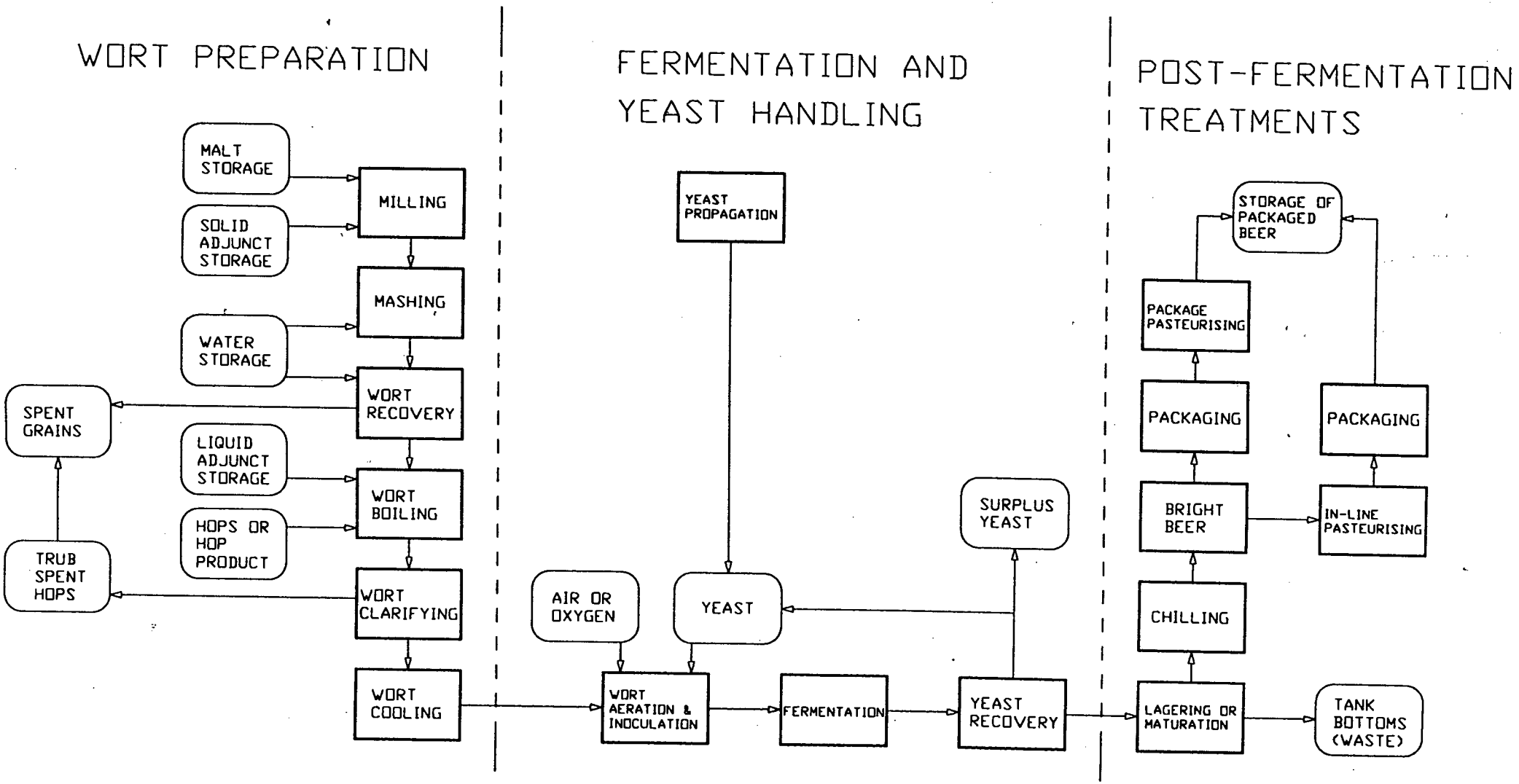

Figure 2.1 Schematic outline of brewing process (based on Oliver 1991)

2.2.1 Wort Preparation

Barley is the principle source of fermentable sugars in beers. In some African beers other cereals such as sorghum may be used. A portion of the barley may be substituted by cheaper sources of carbohydrates known as adjuncts. These include maize or rice grits (grains from which the lipid and protein-rich husks, embryos and aleurone layers have been removed) or syrups made by the hydrolysis of maize, cane sugar or invert sugar.

Barley grains are germinated under controlled conditions during a process called malting. The aim of this process is to initiate the conversion of the starch and the protein content of the endosperm of the barley grains to fermentable sugars and amino acids which may be utilised by the yeast. This germination process is arrested by increased temperature (kilning). Kilning temperatures determine the main characteristics of the malt: moderate kilning temperatures allow high residual enzyme activity and result in pale malts, while high temperatures result in low residual enzyme activities and produce dark malts (for ales and stouts). The malted barley is then milled to a coarse flour called grist. In the subsequent mashing process, the flour is steeped in water at approximately 65 °C and the hydrolysis of the starch to fermentable sugars continues due to the action of the barley α-amylase. Conversion of proteins and peptides to amino acids under the action of carboxypeptidase also occurs. Both these enzymes are relatively thermotolerant. Should solid adjuncts be added during mashing or dark malts be used, commercial enzyme preparations may be added at this stage.

The liquid extract is separated from the spent grains and other solids. The clear liquid extract is called wort or sweet wort to emphasize the high content of fermentable sugars. This wort is boiled in a wort kettle to allow inactivation of the enzymes, sterilization of the medium and concentration of the wort. Liquid adjuncts, if used, may be added to the wort kettle. Traditionally hops are added at this stage, but the high cost of hops has made post-fermentation bittering, in which the hop extracts are added as a final step before conditioning of the beer, increasingly common.

Once boiling is completed, the liquid wort is separated from the spent hops and precipitated matter (trub) either by filtration through a bed of spent hops or a stainless steel strainer or by use of a centrifuge or whirlpool tank. The clarified wort is cooled by passing it through a heat exchanger. This wort is then ready for the fermentation process.

Since the specific gravity of the wort increases as more sugars are extracted during wort preparation, the concentration of wort sugars may be related to the specific gravity of the wort. Due to the heterogeneous nature of wort, it is not possible to relate the specific gravity directly to the sugar content of the wort. However, a correlation between the specific gravity of wort (measured using a hydrometer or saccharometer) and the notional content of sucrose in the wort has been developed. In 1843, von Balling constructed tables describing this relationship.

Inaccuracies in these tables were corrected and new tables published by Plato in 1900, hence the common use of degrees Plato to describe wort concentration. Additional tables were developed by Brix and Baumé. Brix tables relate the percentage sucrose by mass to specific gravities measured at 20°C, while Baumé tables are based on salt solutions and are related to Plato tables by use of a modulus (Rose 1977). Within SA Breweries, the concentration of the wort is determined by measuring its the specific gravity at 20°C. This is referred to as "gravity" and expressed in degrees Plato which is numerically equal to the notional sucrose content on a percentage mass basis. This practice was adopted for this study.

The starting wort concentration determines the ultimate ethanol content of the beer. In order to ensure consistent fermentation, the concentration of the wort may require adjustment to a set concentration prior to fermentation. Increasingly, brewers use high starting gravities (~18 °Plato) since this allows improved utilization of vessel capacity, reduces energy requirements and provides a higher yield of ethanol per unit of fermentable extract (Knudsen 1985).

2.2.2 The Fermentation Process

The fermentation process is mainly influenced by the yeast strain used and the composition of the wort. Furthermore, environmental factors such as temperature, wort oxygen level, the type of fermentation (batch or continuous, stirred or static), the amount of yeast inoculated (pitched), the condition of this yeast (yeast quality) and the level of microbial contamination also influence the progress of the fermentation (Stewart 1977).

2.2.2.1 Yeast Strain and Propagation of the Yeast

Two kinds of yeasts are classically used in the production of beer: top fermenting yeasts which rise to the top of the fermentation vessel during fermentation and bottom fermenting yeasts which settle at the bottom of the fermentation vessel. In general, top fermenting yeasts, which were traditionally classified as *Saccharomyces cerevisiae*, produce ales at temperatures of 15 - 22°C. Bottom fermenting yeasts, which were classified as *Saccharomyces uvarum*, produce lagers at temperatures of 8 - 15°C (Oliver 1991). For diagnostic purposes *Saccharomyces cerevisiae* and *Saccharomyces uvarum* were distinguished by their metabolism of the disaccharide melibiose, which only the latter could utilise. Differentiation of yeasts based on sugar utilisation alone has been abandoned and all Brewers' yeasts have been consolidated into one species *Saccharomyces cerevisiae* (Hammond 1986). Since the yeast is responsible for the development of special flavour components in the final product, the strain employed is an important component of beer production (Knudsen 1985). Yeast may be kept as a slant culture at 2°C or, where long-term preservation is required, a vial of the pure culture may be submerged in liquid nitrogen (-196°C) (Knudsen 1985).

Propagation of yeast is done in three stages: in the laboratory, the culture or propagation plant and in fermentation vessels. Typically scale up from a single colony to 5 - 20 L occurs in the laboratory. Depending on the size of the brewery, this is increased on-site in the propagation plant to 100 - 500 hl and finally into either half- or full-size fermenters (Knudsen 1985). Several stages may be used in the laboratory and propagation plant processes (Boughton 1983).

Prevention of contamination of the yeast either by non-culture or wild yeasts or by bacteria is important since the presence of these micro-organisms reduce plant efficiency and cause undesirable flavours in the final product. Good sanitation practices are thus imperative throughout the brewing process.

2.2.2.2 Inoculation and Fermentation

The yeast is inoculated or pitched into cooled wort. The most common practice is to aerate the wort in-line to a desired level of dissolved oxygen and to pitch the yeast directly in-line before the wort reaches the fermentation vessel (Boughton 1987). Certain breweries prefer to aerate the yeast and not the wort prior to pitching (Knudsen 1985). Some breweries may use a starting tank before the fermenter in which dead cells and some particulate matter are removed over a 2 - 24 hour period.

The pitching rate or amount of yeast pitched depends on the starting concentration or starting gravity of the wort. A rule of thumb is the use of 1 million cells per millilitre per degree Plato of the wort. However, brewers may pitch up to 25 million viable cells per millilitre in 18 °Plato wort (high gravity wort) and as little as 6 million viable cells per millilitre in 9 °Plato wort (low gravity wort).

During the initial stages of fermentation, the yeast uses its internal storage reserves for the aerobic production of sterols and fatty acids required for cell growth and reproduction. This initial aerobic phase, which is limited by the amount of dissolved oxygen present (Pickerell *et al.* 1991), is followed by the anaerobic fermentation of the wort sugars, which can be represented by the following equation (Hough 1985):

$$\textit{maltose} + \textit{amino acid} \Rightarrow \textit{yeast} + \textit{ethanol} + CO_2 + \textit{heat}$$

$$100\ g + 0.5\ g \Rightarrow 5\ g + 48.8\ g + 46.8\ g + 209\ kJ$$

The conversion of the wort sugars to ethanol leads to a drop in the specific gravity of the medium. This process is called attenuation. The level at which all fermentable carbohydrates are utilised is called the attenuation limit of the wort.

In addition to ethanol, a number of higher alcohols, called fusel alcohols may be produced. These may further react to form esters and fats. Esters are import flavour compounds in beers. Other important flavour compounds include diketones,

diacetyl and sulphur compounds, such as sulphur dioxide, hydrogen sulphide and dimethyl sulphide.

2.2.2.3 Vessel Design

A variety of vessel designs and fermentation systems may be used. Batch fermentation systems are most common but some continuous fermentation processes are in operation. In modern breweries, closed fermentation vessels of cylindroconical design (Figure 2.2) are the most common (Renger 1991). These vessels are typical of large scale breweries, since the following features contribute to process efficiency: reduced process times due to efficient mixing, efficient CO_2 collection systems, use of the same vessel for primary and secondary fermentations (lagering), effective cleaning in place (CIP) and more consistent fermentations with reduced risk of microbial contamination (Renger 1991).

2.2.3 Post-Fermentation Treatments

When the fermentation has approached the attenuation limit, the yeast is removed from the fermentation vessel to separate it from the immature or "green" beer. Removal of yeast from the fermentation vessel and the recirculation of this yeast will be discussed in Section 2.3.

The green beer requires further treatment or conditioning to eliminate certain volatile fermentation products, to allow supersaturation with CO_2, to remove residual yeast cells and to remove compounds which could lead to turbidity in the beer (haze formation). In modern breweries, for example, lager beers are held at 14 - 18°C for 2 days after the completion of the primary fermentation. This process, the diacetyl rest, allows the volatilization of undesirable fermentation products. The beer may be filtered or centrifuged to remove the remaining yeast and any other solids. In high gravity brewing, the initial wort concentration is such that the concentration of ethanol at the end of fermentation is higher than that desired the final product, hence a post-fermentation dilution step is required. The beer is pasteurised and artificially carbonated before being dispatched as draught or tank beer or packaged into kegs, bottles or cans. Special treatments may be required post-fermentation to prevent the formation of beer haze. The majority of beer hazes may be related to heavy metal catalysed precipitation of polyphenols and polypeptides. Two types of haze occur: chill haze and permanent haze. Both of these appear upon chilling, but the latter does not redissolve on subsequent warming (Oliver 1991).

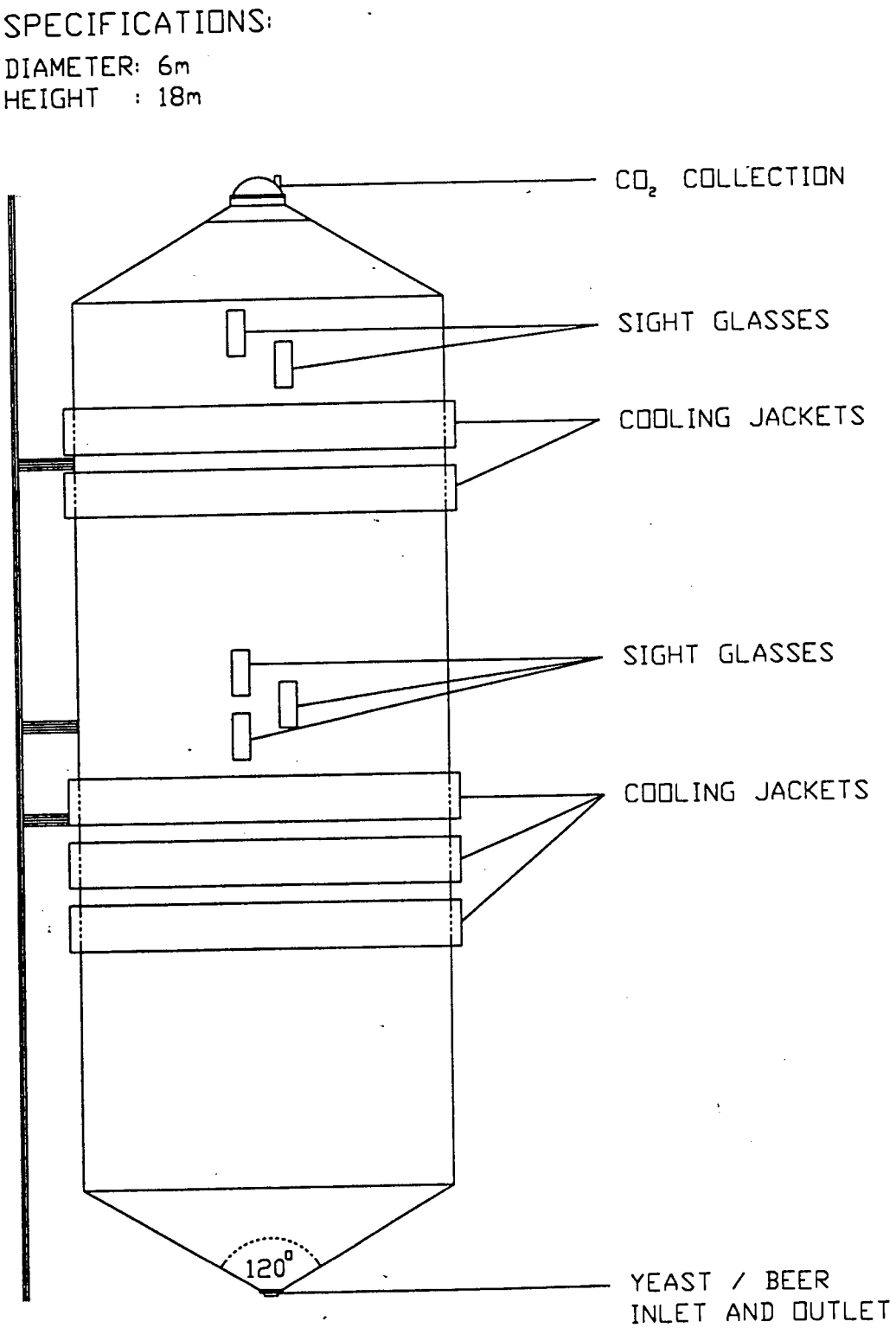

Figure 2.2 A 3000 hL cylindroconical fermentation vessel (based on Stewart 1977)

2.3 YEAST HANDLING DURING RECIRCULATION

Yeast handling circuits may differ depending on vessel geometry and whether top or bottom fermenting yeasts are used. This study was limited to cylindroconical fermentation vessels (FVs) and bottom fermenting yeasts used by SA Breweries to produce lager beers. The largest of these tanks have a capacity of 3000 hl (300 m^3) and a height of 18 m. During the fermentation process, the yeast tends to aggregate and settle in the conical base of the fermenter. On average the bases of the 300 m^3 fermenters contain 12 tons of yeast at the end of fermentation representing a three fold increase in yeast biomass during the fermentation. The settled yeast is in a nutrient-deprived state. Cooling jackets are used to maintain the temperature of the yeast within the cone at 14 to 16°C. Once the beer in the fermenter has reached the desired level of attenuation, this yeast is removed from the base of the vessel for re-use in subsequent fermentations. To ensure that yeast quality is maintained, it is important to remove the yeast from the cone as soon as the desired level of attenuation is reached (typically within 24 hours of reaching the desired level of attenuation).

A typical yeast handling circuit is depicted schematically in Figure 2.3. The yeast handling procedure comprises the following:

- transfer of the settled yeast from the cone of the fermenting vessel using a pump
- flow through pipes of specified diameter and length
- cooling to 3 ± 1 °C by passage through a heat exchanger (typically a plate and frame heat exchanger)
- holding in an uninsulated storage tank (with or without agitation) ideally for a maximum of 24 hours
- possible recirculation through the heat exchanger to maintain temperature
- determination of the amount of yeast required for re-pitching into a subsequent fermentation (based on the biomass concentration and possibly on the physiological condition of the yeast)
- transfer of the required amount of yeast from the storage tank to the pitching vessel
- re-pitching of the yeast into the aerated wort line

In this process the yeast is exposed to a variety of mechanical equipment including pumps, pipes, reductions and expansions (such as those associated with passage through the heat exchanger), valves, bends, fittings and agitators.

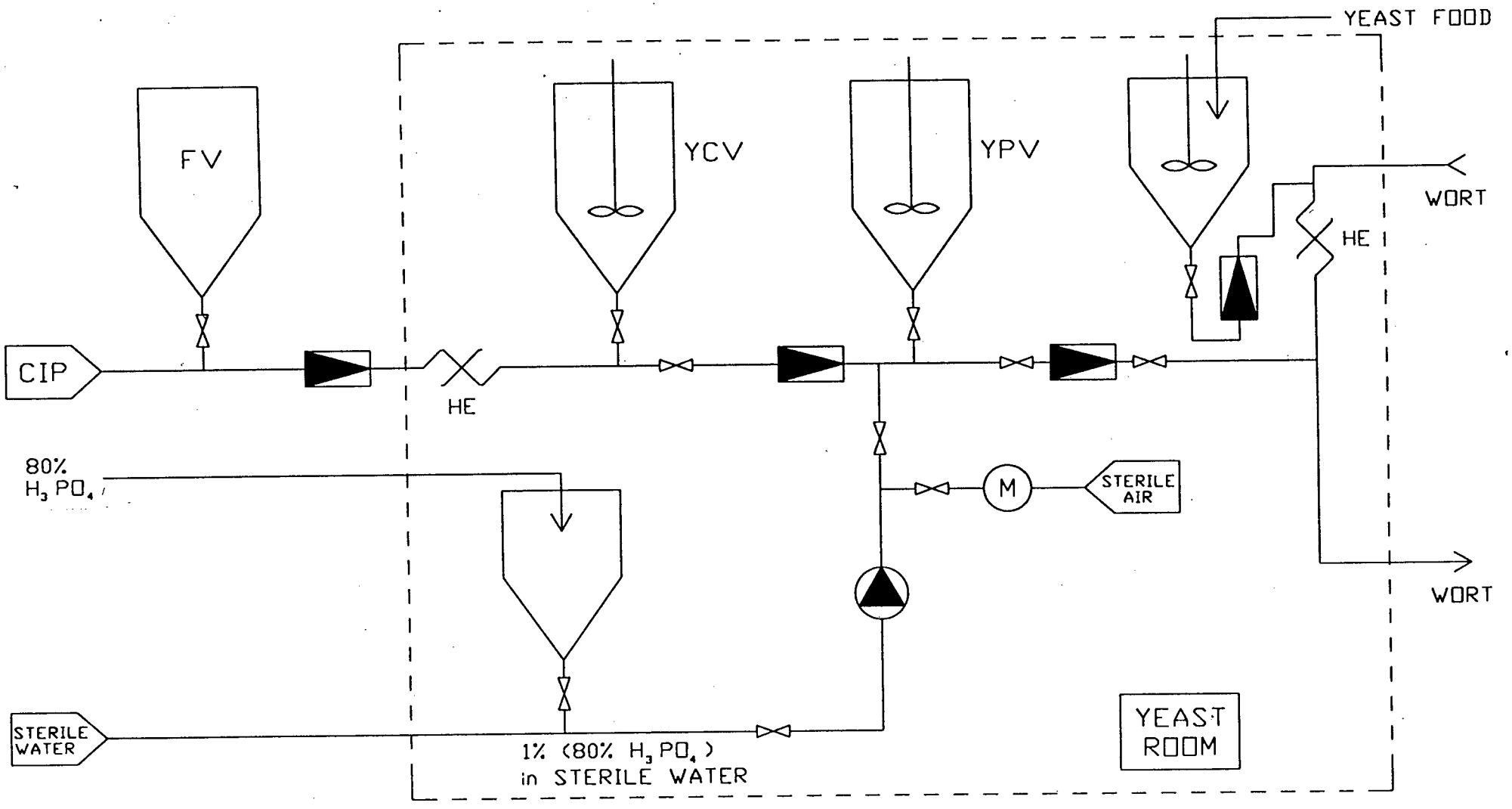

Figure 2.3 Schematic diagram of the yeast handling circuit
(FV, fermentation vessel; YCV, yeast collection vessel; YPV, yeast pitching vessel; HE, heat exchanger)

In some breweries, cooling jackets are placed on the agitated storage tanks instead of cooling the yeast in heat exchangers.

Many breweries include an acid conditioning or acid washing step where yeast is typically mixed with a dilute phosphoric or sulphuric acid solution and agitated in order to de-flocculate the yeast or kill contaminants. Acid treatment is usually conducted in the pitching vessel one to two hours before re-pitching.

Handling can be minimised by pitching the yeast directly from the FV cone into the next fermenting vessel, possibly under hydrostatic pressure. This is avoided by large breweries owing to difficulties in scheduling and the inability to verify yeast quality and calculate a suitable pitching rate prior to pitching (Boughton 1983, Avis 1990).

In some breweries, yeast is dewatered and stored in pressed form. There appears to be a detrimental effect on yeast quality (Martens *et al.* 1986).

When a batch of yeast leaves the propagation plant, it is known as "generation zero". Each time it is cropped and re-used, the generation number increases by one. Literature reports the re-use of yeast over 3 to 30 generations, however the majority of brewers discard yeast after 7 to 10 generations (Boughton 1983, Knudsen 1985). It is policy at SA Breweries to re-pitch the yeast 6 to 8 times to minimise poor yeast performance, resulting from infection, mutation or a poor physiological state (Knudsen 1985, O'Connor-Cox 1994).

2.4 YEAST QUALITY

Yeast quality can be related to the fermentative capacity of the yeast and the quality of the beer produced. Fermentation performance may be influenced by the following:

- the ability of the yeast to grow and reproduce
- the rate and extent of biomass growth
- the metabolic rate of the yeast
- the flocculation and sedimentation characteristics of the yeast
- the metabolic pathways predominating, influencing flavour compounds formed

Additional aspects of yeast quality are the integrity of the cell envelope and the ability of the yeast to withstand stress.

The above aspects of yeast quality form the basis of the analytical techniques used to identify, characterise and quantify losses in yeast quality. These aspects of yeast quality and their relation to the fermentation performance of the yeast will be

examined in more detail in Chapter 3 which deals with the identification, characterisation and quantification of yeast quality. In this section, yeast quality and the implications of loss of yeast quality are introduced.

2.4.1 Ability to Grow and Reproduce

In a fermentation, the rate of attenuation is largely dependent on the number of cells in suspension. Sufficient biomass growth after inoculation is required to effect an acceptable attenuation rate, hence the ability of the cells to grow and reproduce is an important aspect of yeast quality. Replicative ability is dependent on the presence of a functional plasma membrane (Jones 1987a). While replicative deactivation may be the first in a series of step leading to cell death, it may be reversible. Under conditions favourable to growth, the cells may return to a replicating state after a period of adaptation. An intimate relationship between the cell wall and the cell membrane is suggested, since removal of the cell wall results in irreversible alteration of the functional properties of the plasma membrane (Jones 1987a). The physical and functional integrity of the cell envelope is thus an important aspect of yeast quality.

2.4.2 Rate and Extent of Biomass Growth

In a typical lager fermentation where bottom-fermenting yeast are used, a lag period of several hours follows inoculation. Thereafter, yeast growth commences and the number of yeast cells in suspension increases. Peak cell concentrations may range from 40 million to 80 million cells/mL (Knudsen 1985). Once the level of maltose in the wort decreases, the cells tend to agglomerate (flocculation) and ultimately settle to the bottom of the fermentation vessel (sedimentation).

During the lag phase, the yeast catabolises the internal storage reserve glycogen in the presence of dissolved oxygen to produce sterols and unsaturated fatty acids (Quain 1988, Pickerell *et al.* 1991). These compounds are integral components of the yeast cell membrane and their concentration determines the extent of yeast growth and membrane integrity. Two factors may limit the extent of yeast growth: the amount of dissolved oxygen present in the wort and concentration of glycogen in the yeast (Pickerell *et al.* 1991). Several researchers (Quain *et al.* 1981, Murray *et al.* 1984) have illustrated a relationship between glycogen levels in the pitching yeast, the extent of yeast growth and fermentation performance suggesting that yeast quality is linked to the glycogen content of yeast.

2.4.3 Metabolic Rate

The metabolic rate of the cells is an important determinant of the rate of substrate utilisation and hence the rate of yeast growth and wort attenuation. The metabolic rate of the cells is dependent on temperature, wort characteristics, the

physiological status (growth phase) and the condition of the yeast.

2.4.4 Flocculation and Sedimentation Characteristics

The onset of flocculation and the rate of sedimentation is a delicate balance. On the one hand, it is desirable for the yeast to remain in suspension sufficiently long to allow efficient utilisation of the wort. On the other hand, flocculation and sedimentation are of advantage since these processes facilitate yeast removal.

The mechanism of flocculation is not fully understood. Flocculation may be attributed to the formation of salt bridges between calcium ions and the carboxyl groups on the yeast envelopes are involved (Fisher 1975, Macleod 1977). The presence of high levels of maltose in the medium prevents flocculation, hence the dispersion of the cells after inoculation and the inception of flocculation once the wort maltose has been depleted (Macleod 1977).

Flocculation and sedimentation characteristics are genetically controlled, but wort composition and the physiological state of the yeast determine the expression of these phenomena. The properties of the cell surface are important (Fisher 1975, Macleod 1977, Rose 1977, Smart *et al.* 1995). Smart *et al.* illustrated the loss of flocculating ability in starved cells of a highly flocculant yeast. The loss of flocculating ability was associated with reduction in surface charge and modification of cell surface topography. Changes in yeast quality can thus be related to changes in surface properties and associated changes in the flocculating characteristics of the cells.

2.4.5 Metabolic Pathways Predominating

During fermentation, energy for cell maintenance and biomass growth is achieved by the metabolism of wort sugars. This occurs predominantly via the Embden-Meyerhof-Parnas (E.M.P.) pathway in which the sugars are converted via pyruvic acid to ethanol and carbon dioxide. In order to utilize this glycolytic pathway, the yeast produces enzymes to break down wort sugars such as sucrose and maltose to their constituent monosaccharide units. Some yeasts are able to utilise more complex sugars, such as maltotriose and maltotetraose, which improves fermentation efficiency. Apart from ethanol and carbon dioxide, a variety of other compounds, including alcohols, aldehydes, acids, esters and sulphur-containing compounds, may be produced by yeast metabolism and by interactions between the metabolic products and wort constituents (Macleod 1977, Rose 1977). These compounds all contribute to the characteristic flavour of the beer. Deviations from characteristic flavour or the production of undesirable flavours in the final beer constitute à loss of beer quality. The complex biochemical nature of beer makes a cause and effect analysis of beer quality problematic. The production of three compounds (acetaldehyde, sulphur dioxide (SO_2) and diacetyl) and their relation to yeast quality will be discussed.

2.4.5.1 Production of Acetaldehyde and SO_2

In the penultimate step of the E.M.P. pathway, pyruvate is decarboxylated to yield acetaldehyde and carbon dioxide. The acetaldehyde is then reduced to ethanol. The reaction is reversible. The oxidation reaction is generally accompanied by the production of acetic acid and may be stimulated by the presence of oxygen or promoted by bacterial infection. Acetaldehyde and other aldehydes may also be produced by the decarboxylation of oxo acids during the formation of higher alcohols (Macleod 1977, Rose 1977).

In small quantities, acetaldehyde (the main carbonyl compound produced in beer) contributes to beer flavour. In excess, it leads to undesirable flavours. Studies of the interactions of SO_2 and acetaldehyde indicate that the stable binding of SO_2 to stale-tasting carbonyl compounds improves the stability of beer flavour. Since SO_2 and bisulphite (which may be added to improve beer stability) preferentially bind to short-chain saturated aldehydes, excess acetaldehyde may cause highly flavour-active unsaturated aldehydes to remain unbound resulting in stale beer flavours and reduced beer shelf life (Pickerell *et al.* 1991). Pickerell *et al.* suggest that high acetaldehyde levels at the end of fermentation may reflect the inability of poor quality yeast to reabsorb acetaldehyde during active fermentation. However, if high levels of SO_2 are present to stabilize the acetaldehyde, reabsorption of acetaldehyde and its subsequent conversion to ethanol may be prevented leading to high acetaldehyde levels in the final product.

SO_2 and other sulphur compounds in beer are derived from sulphates and sulphur-containing amino acids. The production of SO_2 is dependent on fermentation temperature, wort pH, wort composition, yeast pitching rates, wort oxygenation and the enzymes active in the sulphur metabolism pathway. Naturally occurring SO_2 acts as an anti-oxidant. However, in excess it may cause an undesirable flavour and may constitute a health hazard to the consumer (Pickerell *et al.* 1991).

Pickerell *et al.* (1991) indicate that end of fermentation SO_2 and acetaldehyde levels greater than 10 mg/L are unacceptable. SO_2 and acetaldehyde levels can be used as stress indicators. However, since many factors influence their production and final product levels may be influenced by complex interactions with other beer constituents, SO_2 and acetaldehyde levels are difficult to interpret, hence the use of these compounds as stress indicators is limited (O'Connor-Cox 1995).

2.4.5.2 Production of Diacetyl

Diacetyl, a vicinal diketone, is an essential flavour component of beer. It imparts a 'butterscotch' flavour to the beer and should be present in concentrations less than 0.1 ppm. Diacetyl is formed from the decarboxylation and oxidation of acetolactate excreted into the beer by the yeast during fermentation. Large quantities of diacetyl (often in excess of 0.5 ppm) are produced during the early stages of fermentation. As the fermentation progresses, the yeast assimilates the

diacetyl, converting it metabolically via acetoin to 2,3,butanediol (Siebel Institute of Technology 1978).

Diacetyl production may be influenced by yeast strain and wort composition, but more importantly by fermentation temperature, storage time and the amount of yeast in suspension. Extended warmer fermentation temperatures, prolonged storage and larger numbers of yeast in suspension favour lower diacetyl levels. Aspects of yeast quality such as the extent of biomass growth and the flocculation characteristics of yeast may thus influence diacetyl levels in the final product. High diacetyl levels may, however, also be indicative of *Pediococcus* bacterial infections (Siebel Institute of Technology 1978).

2.4.6 Integrity of the Cell Envelope

Rupture of the cell envelope (loss of cell wall integrity), which represents the most extreme degree of a loss of yeast quality, results in the release of intracellular compounds into the beer. Loss of cell wall integrity may result from autolysis or mechanical disruption of the cell envelope. The concomitant release of intracellular components into the beer affects the flavour characteristics of the beer (McCaig and Bendiak 1985b), results in the formation of beer hazes (O'Connor-Cox 1994) and causes foam instability through the release of intracellular protease enzymes (Ormrod *et al.* 1991).

Mechanical abrasion of the cell surface may cause the release of cell wall constituents such as glucan and mannoprotein as well as wall-associated enzymes such as invertase and melibiase into the beer. The formation of certain beer hazes and the concomitant reduction in beer quality have been associated with the release of glucan and mannoproteins into the beer upon agitation of the yeast slurry (Lewis and Poerwantaro 1991).

2.4.7 Ability to Withstand Stress

Trehalose is a disaccharide which accumulates within the cytosol during conditions of environmental stress such as nutrient limitation, heat shock, desiccation, freezing and exposure to toxic chemicals. It is regarded as a stress protectant (Quain 1991, Wiemken 1990). The link between stress resistance, trehalose content and yeast quality is illustrated by the superior fermentation performance of pitching yeasts with higher initial trehalose contents over those with lower initial trehalose contents (O'Connor-Cox 1995).

2.5 THE POTENTIAL FOR A LOSS OF YEAST QUALITY DURING MECHANICAL HANDLING

2.5.1 Introduction

Mechanical handling of the yeast suspension during recirculation includes:

- transfer of the yeast from the fermentation vessel to the storage vessel and from the storage vessel to the pitching vessels by pumping
- flow of the yeast through pipes and fittings such as bends, expanders, reducers and valves
- possible flow though a heat exchanger
- agitation of the yeast suspension during storage and treatments such as acid washing
- pitching of the suspension into fresh wort by pumping

Despite frequent reference to the need to transfer pitching yeast without affecting yeast quality (Boughton 1983, Knudsen 1985, Ahlquist 1986, Kronlof 1986, Boughton 1987, Schur 1990, Wheatcroft *et al.* 1993, Ball 1994), rigorous study of this is minimal. Both Wheatcroft *et al.* (1993) and Avis (1990) include the transfer of the yeast from the fermenting vessel to the storage tank in their identification of critical control points for optimising yeast quality, but do not discuss this further. To a large extent literature on mechanical handling of yeast is based on the experience of the authors, rather than on rigorous scientific study.

In this section, literature on mechanical handling of Brewers' yeast is reviewed in order to assess the potential causes of a loss of yeast quality during mechanical handling of the yeast suspension during recirculation. It is recognised that non-mechanical aspects of processes such as yeast storage and acid washing have potential to cause losses of yeast quality or may make the yeast more susceptible to loss of yeast quality during subsequent mechanical handling. However, the effects of non-mechanical aspects of storage and acid washing fall outside the scope of this study and have thus not been included in this review.

2.5.2 The Nature of the Yeast Suspension

2.5.2.1 Physical Nature

The yeast suspension is a fluid of complex nature, being comprised of three phases: liquid (beer), solid (yeast flocs) and gas (CO_2). The presence of dissolved gas increases the risk of hydrodynamic cavitation (Yan 1989) and results in varying concentrations of potentially toxic CO_2 as a function of hydrostatic pressure. The risk of hydrodynamic cavitation within the yeast handling circuit and its potential harm has not been quantified. In the absence of cavitation, the cells may interact

with bubbles disengaging from suspension as a results of pressure fluctuations.

The yeast slurry has a complex non-Newtonian rheology with viscosity being dependent on yeast concentration, floc size distribution, shear rate, duration of exposure to shear, temperature, pH, osmotic pressure and gas phase present (Reuss *et al.* 1979, Lentini *et al.* 1992). Quantification of the rheology of the yeast slurry under the physical conditions experienced in the yeast handling circuit is a complex process and has not been adequately addressed. The absence of accurate data complicates equipment selection and sizing. An understanding of the slurry rheology is required to aid in equipment selection and sizing and enable a more fundamental understanding of the fluid mechanics and hence the forces experienced by the yeast cells in the yeast handling circuit.

2.5.2.2 Biological Nature

The yeast to be cropped is in stationary phase and is nutrient depleted. An intracellular reserve material such as glycogen is required to provide energy for maintenance (Boulton 1991). Exposure of the yeast to oxygen and increased temperatures would promote metabolism. Extended metabolism of the yeast during handling may result in the early depletion of storage reserves, an increase in CO_2 and ethanol concentrations to toxic levels and the generation of metabolic heat causing potential "hotspots". These factors would contribute to a decrease in the physiological condition of the yeast and possibly increase its susceptibility to mechanical damage.

2.5.2.3 Nature of the Yeast Cell Envelope

(a) Structure of the Cell Envelope

The cell envelope of *Saccharomyces cerevisiae*, represented schematically in Figure 2.4, consists of a rigid cell wall separated from the plasma membrane by a periplasmic space.

The plasma membrane consists of a lipid bilayer made up of phospholipids, sterols and unsaturated fatty acids. Intrinsic membrane proteins are inserted into the bilayer, while extrinsic proteins cover part of the bilayer surface. Ergosterol is the main sterol component of the membrane and can not be synthesised under anaerobic conditions (Kreutzfeldt and Witt 1991, Pickerell *et al.* 1991)

For most low molecular weight substances, the plasma membrane forms a diffusion barrier between the cytoplasm and the culture medium. The plasma membrane does not have significant structural strength making cells in which the cell walls are absent or damaged susceptible to lysis when exposed to osmotic shock (Engler 1985). Several enzymes are associated with the plasma membrane. These include chitin synthetase, glucan synthetase and proton-translocating ATPase (Kreutzfeldt and Witt 1991).

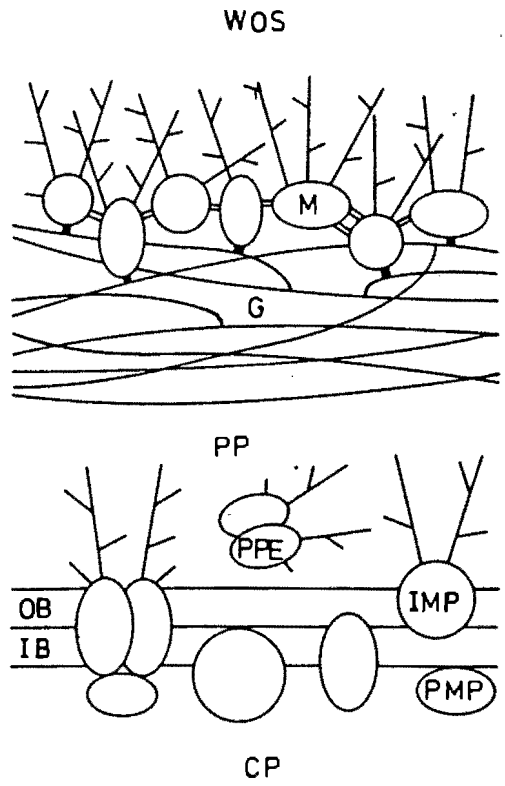

Figure 2.4 Scheme for the yeast cell envelope (Kreutzfeldt and Witt 1991)
(CP, cytoplasm; IB, OB, inner and outer halves, respectively, of the plasma membrane bilayer; PMP, peripheral membrane protein; IMP, integral membrane protein; PPE, periplasmic enzyme; PP, periplasmic space; G, glucan; M, mannoprotein; WOS, wall outer surface; (=) S-S thioester bond; (-) covalent bond)

Glucan (a polysaccharide) and mannan or mannoprotein (a glycoprotein) are the main constituents of the cell wall. These are present in approximately equal proportions. Not more than 4 % of the cell wall material consists of chitin which is mainly restricted to the bud scar areas. Lipid contents may vary from 3 to 9 % (Kreutzfeldt and Witt 1991).

Glucan is the main structural component of the cell wall. Hydrophobic interactions within the glucan molecules lead to the formation of rigid double helical structures. At least part of the cell wall glucan is arranged in a microfibrillar network which promotes wall strength (Engler 1985, Kreutzfeldt and Witt 1991). Mannoproteins appear not to form any strengthening fibrillar structures. Although the precise arrangement of the cell wall is not known, the following model is proposed: an outer layer of mannoproteins linked to each other by S-S or thioester bonds and hydrophobic interaction linked to an inner layer of glucan by bonds of unknown nature. The layers are not separate since mannoproteins may penetrate the glucan layer to an unknown extent. The porosity of the cell wall appears to be determined by the mannoprotein layer (Kreutzfeldt and Witt 1991).

Some enzymes secreted by the yeast, do not penetrate the cell wall and are trapped between the plasma membrane and the cell wall in the periplasmic space.

These are typically glycoproteins with hydrolase activities (Kreutzfeldt and Witt 1991). An example of such an enzyme is invertase, the enzyme responsible for the hydrolysis of sucrose to fructose and glucose during fermentation .

(b) Strength of the Yeast Cell Wall

To effect mechanical disruption of the cell wall, the resistance provided by the bonding within the structural network of the wall must be overcome. The ease of disruption will depend on the extent of bonding or degree of crosslinking within the wall as well as the size and shape of the cell (Engler 1985).

Genetic factors, growth conditions and the history of the biomass may affect wall strength since these affect the degree of crosslinking and thickness of the glucan layer (Engler 1985, Kula and Schütte 1987). The cell wall of *Saccharomyces cerevisiae* is approximately 70 nm thick. Wall thickness increases with age, but only a portion of the thickness may contribute significantly to the rigidity and strength of the wall (Engler 1985). The presence of bud scars in older cells interrupts the rigid glucan layer, making these cells more susceptible to mechanical damage (Vraná *et al.* 1982)

Kula and Schütte (1987) suggest that the high mechanical strength and small size of microorganism make them resistant to mechanical disruption. In high pressure homogenisers, unicellular yeast cells appear to be more difficult to disrupt than both Gram-positive and Gram-negative bacteria and filamentous fungi. A pressure of 150 MPa was required to effect 50% cell disruption of *Saccharomyces cerevisiae* in a single pass in a Stansted cell disrupter (a high pressure homogenising device), while pressures of 15 MPa, 24 MPa and 68 MPa were required to effect 50% disruption of a Gram-negative bacterium (*Escherichia coli*), a Gram-positive bacterium (*Bacillus subtilis*) and a filamentous fungus (*Aspergillus fumigatis*) respectively in a single pass (Harrison 1991).

2.5.3 Mechanical Yeast Handling

2.5.3.1 Pumping

Typically, positive displacement pumps are used in the transport of yeast slurries (Boughton 1983, Boughton 1987). The use of diaphragm pumps is also reported, although ease of cleaning must be ensured (Boughton 1983). It is suggested that low shear pumps be used (Boulton 1991) and heat generation in the pumps be minimised. Discussion of different pump designs such as peristaltic, lobe, diaphragm, screw, gear and centrifugal designs for brewery applications centres on the mechanical aspects of the pumps and not on their influence on beer or yeast quality (Schöffel 1978a, 1978b, 1979a and 1979b). A review of the mechanical aspects of the different pump designs and the suitability of different pumps for yeast cropping applications is presented as Appendix A.

During cropping, constant flow against varying line pressures (eg. on filling the

storage tanks) is required, hence positive displacement pumps should be used in preference to centrifugal pumps. Should the yeast pass through a heat exchanger prior to storage, pulsating flow is unacceptable since this jeopardises the effective cooling of the yeast. Under these circumstances, diaphragm pumps and peristaltic pumps, which produce pulsating flow, should not used for yeast cropping. Pulsation dampeners could be installed to overcome this problem, but the design should be such that cleanability can be guaranteed.

There are several rotary positive displacement pumps which produce very small flow pulsations (< 15 kPa). These include sine pumps, lobe pump and gear pumps. Apart from the performance of pumps with regard to yeast handling, the selection of specific pumps for yeast cropping should be based on a complete assessment of all the characteristics of the pumps. Such an assessment would include an evaluation of mechanical components, wear, maintenance requirements, cleanability, sanitary operation, efficiency and an overall economic evaluation of the pumps.

2.5.3.2 Fluid Flow Conditions

The fluid flow conditions within the yeast handling circuit depend on velocity, pipe diameter, biomass concentration and the rheology of the suspension. Preferred cropping rates of the order of 50 to 60 kg/min are reported (Boughton 1983, Boughton 1987). While rapid cropping is important to minimise exposure to an adverse environment, in the case of cylindroconical fermentation vessels more rapid cropping may cause channelling in the settled yeast bed (Boughton 1987) and concomitant beer losses.

A rule of thumb is to design pipe work circuits to permit a maximum linear velocity of 0.5 m/s when pumping yeast creams (Ball 1994). Typically, this will correspond to a laminar flow regime. No discussion of the effect of varying linear velocity or the effect of turbulent flow conditions have been reported in the open literature.

2.5.3.3 Flow through Heat Exchangers and Fittings

Within SA Breweries, plate heat exchangers are used to cool the yeast suspension. This involves considerable constriction and expansion of the fluid. Typical fittings in yeast handling circuits include reducers, enlargers, elbows and valves. Within SA Breweries, butterfly valves are used in the yeast handling circuits.

It is recognised that yeast handling should be minimised and yeast handling circuits kept simple to avoid detrimental effects on yeast quality (Bougton 1987, Ball 1994). To this end, it is sensible to minimise flow through constrictions and expansions. However no discussion on this has been reported with respect to Brewers' yeast.

Studies have been reported using both Bakers' yeast and Gram negative bacteria in which significant cell disruption resulted on flow through a narrow constriction or a throttled valve at which hydrodynamic cavitation was induced (Harrison and Pandit 1992). The potential for a loss of yeast quality resulting from hydrodynamic cavitation is discussed in more detail in Section 2.5.4.2.

2.5.3.4 Agitation

Agitation of the yeast slurry in a stirred tank may take place during yeast storage (to maintain suspension homogeneity), in systems which use jacketed vessels (to ensure efficient cooling) or during acid treatments (to ensure homogenous distribution of the acid solution). Agitation during storage results in a degree of decarbonation of the slurry. Numerous studies of yeast storage are reported, several of which consider agitation. McCaig and Bendiak (1985a) studied yeast storage in the presence and absence of agitation at 1 °C in 1 litre flasks (working volume: 600 mL) over a five day period. Agitation was achieved using a magnetic stirrer which was operated at the equivalent of 300 rpm in water. The results indicated that agitation over a five day period caused a 25% reduction in the number of colony forming units, an 85% reduction in the intracellular glycogen reserves and significantly poorer fermentation performance. A reduction in total cell number was also observed. This was attributed to mechanical cell disruption, but may have been the result of autolysis. A limited effect was seen on periodic agitation (2 hours per day) to maintain suspension homogeneity. The authors recognised that the observed loss of yeast quality may have been caused in part by oxygenation during agitation. No attempt to exclude oxygen is discussed and vortex formation was noted. While the results are valid in considering the extreme handling of the yeast, oxygen transfer in a small scale experiment is not representative of that found in a large scale storage tank. In addition, yeast storage on the plant seldom exceeds 72 hours. Little change in the condition of the yeast was observed over this period.

To better simulate the effect of agitation in the plant, Sall *et al.* (1988) studied the agitation of a 31800 litre tank at 400 rpm by a single blade impeller. When the headspace in the tank was minimised, no decrease in intracellular glycogen was found over 70 hours. Increase of the headspace to 34% resulted in a 40% reduction in glycogen over 70 hours. This further supports that the exclusion of oxygen is of more importance than the mechanical forces of mild agitation in fermentation performance. In addition, low temperature storage of yeast over 3 days shows a small effect on yeast quality.

It has been reported that the agitation of yeast at the end of fermentation resulted in the release of haze-forming material (Lewis and Poerwantaro 1991). The material contained glucan and mannoprotein and had a high carbohydrate to protein ratio. The presence of the wall associated enzymes invertase and melibiase suggested that the haze arose from cell wall damage, not cell disruption.

The apparent resistance of yeast cells to complete disruption during agitation is confirmed by studies of the agitation of Bakers' yeast (*Saccharomyces cerevisiae*) in a stirred tank slurry bioreactor in the presence of inert particulates. Negligible cell disruption was illustrated in the absence of particulates at an elevated agitation speed of 770 rpm (tipspeed of 4.2 m/s) using a Rushton turbine. At low agitation rates in the presence of particulates, cell disruption was minimal, while cell wall damage (determined by invertase release) resulted. At increased agitation rates (> 300 rpm, tipspeed > 1.6 m/s) cell disruption was observed in the presence of particulates (Harrison and Pearce 1994). It has been postulated that to effect cell disruption a critical momentum is required in particle-cell collisions. Collisions at lower momentum result in cell wall damage only. This change in momentum could be effected by changing the energy input through agitation rate or changing the mass through particle size or density.

2.5.3.5 Pitching

Within SA Breweries, pitching yeasts are acid washed to effect deflocculation prior to inoculation. Acid washing results in a two-fold dilution of the suspension and may influence the physiological condition of the yeast (Simpson and Hammond 1989, Fernandez *et al.* 1993). The acid washing procedure may affect the response of the yeast to mechanical handling during pitching.

Typically, yeast is pitched directly into the oxygenated wort stream, upstream of the fermenting vessel (Boughton 1987). Discussion of pitching rate centres on biomass concentration and yeast quality. The requirement for good mixing is recognised (Ball 1994). No reference has been found to the manner of injection and resultant mixing patterns and their effect on yeast.

2.5.4 Physical Effects in the Yeast Handling Circuit

2.5.4.1 Pressure

The use of tall cylindroconical fermenters implies that the flocculated yeast settles in the fermenter cone under considerable hydrostatic pressure (150 to 200 kPa). In considering pressure effects, both the effect of hydrostatic pressure and the rate of pressure release must be considered. Pressurisation of a microbial culture alone is insufficient to disrupt micro-organisms. Some retardation of the growth of *Escherichia coli* has been reported in the pressure range 10 to 50 MPa, while a pressure of 62 MPa was required to completely suppress growth (Salle 1971). Exposure of cropped yeast to static pressures of 300 and 500 kPa for 30 minutes did not affect gross fermentation parameters or SO_2 production; however a small increase in acetaldehyde concentration in ensuing fermentation was observed (Pickerell *et al.* 1991).

Micro-organisms are able to alter their internal pressure to suit their external

environment. As this is a controlled process, a defined time scale is required. Should the rate of change of pressure in the external environment exceed this time-scale, rupture of the organism may result. Illustration of the disruption of *Escherichia coli* by a rapid change in pressure, largely in the absence of other contributory factors, has been illustrated by Fraser (1951) using explosive decompression. Edwards and Wiseman (1971) illustrated the disruption of yeast cells by inducing a pressure change over 1 millisecond.

In high gravity brewing, transfer of yeast from the depleted nutrient medium to the concentrated nutrient medium during pitching constitutes a rapid change in osmotic pressure. In responding to variations in osmotic pressure and water activity, the cell employs both a passive physical mass transfer of water to maintain a thermodynamic equilibrium and an energy dependent osmotic regulation system. Gervais *et al.* (1992) have shown that, as for pressure, the rate of change of osmotic pressure is of more importance than the magnitude of the osmotic pressure in determining its effect on the physiological condition of the cell. In addition, the physiological status of the cell affects its resistance. Early stationary phase *Saccharomyces cerevisiae* are more resilient than exponential or late stationary phase cells. McCaig and Bendiak (1985a) suggest that cells damaged by mechanical agitation or oxygen toxicity or cells with low glycogen reserves may be unable to withstand osmotic shocks.

2.5.4.2 Hydrodynamic Forces

Hydrodynamic forces may cause disruption of micro-organisms. This phenomenon is exploited in cell disruption equipment such as high pressure homogenisers, bead mills and sonication devices where forces such as shear and cavitational stresses, turbulence and impingement on stationary surfaces cause disruption of the micro-organisms (Harrison 1991). The degree of disruption may depend on several parameters. Cell disruption is more readily accomplished following growth on a defined medium or on media depletion than when grown in a complex medium (Gray *et al.* 1972). In addition, the physiological status of the cell influences its robustness. The increased sensitivity of exponentially growing cells over stationary phase bacteria has been demonstrated on disruption in a bead mill or by high pressure homogenisation, ultrasound, enzymic lysis or osmotic pressure (Harrison *et al.* 1991, Cumming *et al.* 1985, Fish and Lilly 1984). Disruption of *Saccharomyces cerevisiae* in high pressure homogenisers was independent of biomass concentration in the range of 84 to 170 kg dry mass/m^3 for a pressure range of 10 to 54 MPa and a temperature range of 5 to 40°C. A dependence may be found at higher concentrations (Harrison 1991).

Conditions in the yeast handling circuit are expected to be milder than in devices used for cell disruption. The potential effects of shear forces and hydrodynamic cavitation on yeast quality are examined.

(a) Shear Forces

Schur (1990) reports on pilot scale studies to determine the effect of shear forces during the transfer of a yeast and beer suspension on the quality of the yeast and of the beer. The suspension was transferred from one vessel to another either by using a small pressure difference between the two tanks or by pumping with an oversized centrifugal pump at 2800 rpm. The effects of biomass concentration, physiological condition of the yeast and the magnitude of the shear forces were investigated. Suspension concentrations (0.2×10^6 and 10×10^6 cells/ml beer) were obtained by adding cropped yeast to the beer. Both freshly cropped and stored yeast were used to study the effect of physiological condition. Shear forces were varied by throttling of the pump discharge. Discharge pressures of 40 kPa and 1000 kPa were used to effect "small" and "large" shear forces. The suspension was filtered after treatment. The turbidity and filterability of the unfiltered suspension and the colloidal stability, foam stability, pH and concentration of cytosolic compounds in the filtered beer were measured.

With freshly cropped yeast and young beer, no effect on the quality of both the filtered and the unfiltered beer at either yeast concentration and both throttling pressures (small and large shear forces) were observed. This suggests that the applied forces were unable to disrupt the freshly cropped yeast cells or cause any wall damage. With stored yeast suspended in matured beer, an increase in turbidity of the unfiltered beer and a decrease in filterability were observed on exposure of the suspension of lower yeast concentration (0.2×10^6) to large shear forces (throttling at 1000 kPa). At the higher biomass concentration, the decrease in filterability of the beer exposed to the larger shear forces was more marked. Turbidity was not measured. No differences in the quality of the filtered beer were observed and cytosolic compounds were not detected in any of the filtered beers after treatment. This suggests that the shear forces could not achieve measurable disruption of the stored yeast. The reduced filterability of the suspension containing stored yeast illustrates the influence of the physiological condition of the yeast on its susceptibility to mechanical damage. The apparent dependence of yeast damage on biomass concentration permits postulation that cell wall material is released in the high shear environment, however more sensitive analytical techniques would be required for its validation.

Within SA Breweries, the biomass concentration during cropping is $\pm 1000 \times 10^6$ cells/ml, while that during pitching may range from $\pm 500 \times 10^6$ (after acid washing) to $\pm 25 \times 10^6$ cells/ml (in the fermentation vessel). The potential effect of biomass concentration on the susceptibility of the yeast to shear damage requires elucidation. This may highlight the risk of shear damage in the different parts of the yeast handling circuit.

(b) Hydrodynamic Cavitation

In liquids, local increases in velocity, rapid vibration of a boundary, ultrasonic vibrations, the separation of liquid column or an overall reduction of static pressure may cause a local reduction in pressure resulting in the formation of vapour cavities. Subsequent collapse and rebound of the cavities occur until an increase

in pressure causes their destruction. This phenomenon is known as cavitation and is associated with local pressure fluctuations of the order of 1000 MPa (Harrison 1991). Large amounts of energy are released upon collapse of vapour cavities. Damage of pumps and pipe work due to hydrodynamic cavitation is a well studied phenomenon. Ultrasonic cavitation may be used to disrupt micro-organisms in the laboratory (Harrison 1991).

In the yeast handling circuit, local increase in linear velocity and concomitant reduction of the local pressure may induce hydrodynamic cavitation. This may occur, for example, during pumping or upon flow through a constriction. The potential for hydrodynamic cavitation will be aggravated by the presence of dissolved gases (Yan 1989).

Experimental studies of Bakers' yeast (*Saccharomyces cerevisiae*) and the Gram-negative bacterium *Alcaligenes eutrophus* have illustrated that the generation of hydrodynamic cavitation by flow through a constriction or a throttled valve may result in cell disruption (Harrison and Pandit 1992). The extent of disruption, measured in terms of soluble protein release, was shown to be a function of biomass concentration. Figure 2.5 shows the extent of disruption upon repeated passage through a constriction for three suspensions of *Saccharomyces cerevisiae* with different biomass concentrations (19, 80 and 140 kg/m^3 dry weight). Stationary phase yeast was used and the ratio of the diameter of the orifice to the diameter of the conduit was 0.3.

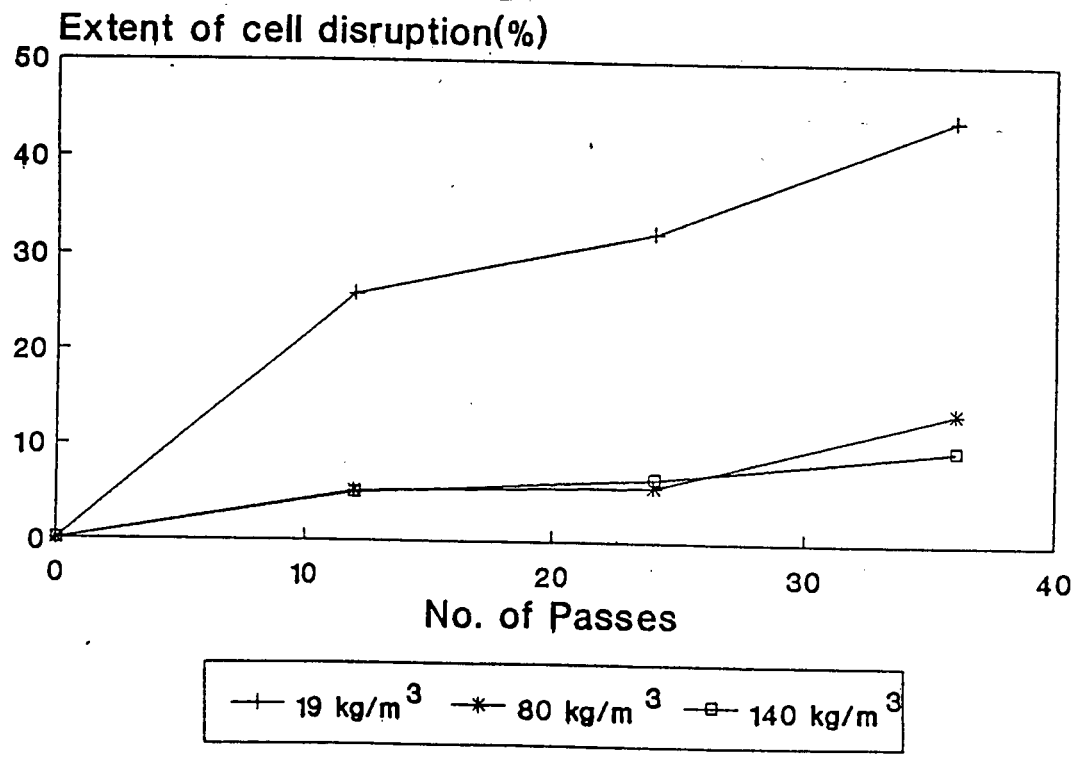

Figure 2.5 The effect of biomass concentration on cell disruption by hydrodynamic cavitation for *Saccharomyces cerevisiae* (Bakers' Yeast) (Harrison and Pandit 1992)

Hydrodynamic cavitation resulted in extensive cell disruption at the low cell concentration (19 kg/m^3 dry weight) and the extent of disruption increased with an increase in the number of passes through the orifice. At the higher cell concentrations (80 and 140 kg/m^3 dry weight), the extent of disruption was less and did not increase as significantly with an increase in the number of passes. Despite a nearly two-fold increase in cell concentration, the profiles for soluble protein release for 80 kg/m^3 and 140 kg/m^3 are nearly identical for the first 25 passes. Harrison and Pandit (1992) suggested that the higher viscosity and shear thinning behaviour of the suspension at high cell concentrations reduce the likelihood of cavitation through the damping of energy fluctuations and hence reduce the extent of cell disruption. The assessment of yeast damage by Harrison and Pandit was limited to cell disruption.

The dry biomass concentration of the yeast suspension during cropping is ± 160 kg/m^3 and is expected to be ± 80 kg/m^3 after acid washing. Cell disruption due to hydrodynamic cavitation is not expected at these high cell concentrations.

Dunlop and Namdev (1993) indicate that for plant cells the energy dissipation required for cell disruption is several orders of magnitude larger than that required for loss of replicative ability or metabolic activity. The potential for a deterioration in yeast quality via other mechanisms in response to hydrodynamic forces in the yeast handling circuit can not be discounted.

2.5.5 Simulation of the Yeast Handling Circuit in the Laboratory

Pickerell *et al.* (1991) report on a laboratory investigation of the yeast handling process. The handling circuit was simulated using a 20 L holding tank, a trilobal positive displacement pump operated at a 250 kPa back pressure, a 13-plate heat exchanger and appropriate pipe work. Yeast was recirculated in this system at a flow rate of 39 l/min and 10°C for 4 hours. A 15 to 20% increase in the number of dead cells, indicated by methylene blue staining, was observed. Intracellular glycogen levels decreased. Subsequent fermentation of the yeast in 2 L tall tube fermenters illustrated that the rate of fermentation and attenuation was reduced by subjection to the simulated handling. This was found both when a freshly cropped yeast suspension and when an aged suspension was used. Levels of SO_2 and acetaldehyde produced in the fermentation were 2 to 3 fold higher than obtained with yeast of optimal quality, suggesting yeast stress.

This study illustrated that exposure to excessive handling is detrimental to yeast quality, resulting in a product of similar quality to that obtained with yeast aged by agitation at 25°C for 24 hours. However, the laboratory circuit was limited in its ability to simulate the plant. The following deviations from the plant were apparent:

- The yeast suspension would have been largely degassed in the laboratory circuit compared to the yeast handling circuit.

- In the laboratory circuit, no mention was made of special precautions to exclude the entrainment of air and subsequent oxygenation which could lead to more rapid yeast degeneration.

- In order to simulate the 4 hour period over which cropping can occur, the yeast was recirculated in the laboratory system; hence exposure to the pump, heat exchanger and pipe work may have been considerably greater than in the yeast handling system.

Closer resemblance between the experimental conditions and the brewery yeast handling circuit are required to elucidate the mechanism of a potential loss of yeast quality during mechanical handling in the brewery.

2.6 SPECIFICATIONS FOR YEAST HANDLING EQUIPMENT

Within SA Breweries, specifications for the yeast handling equipment have been set (SAB 1992). These include:

- the use of 316 stainless steel for all product contact parts
- the use of positive displacement pumps for yeast cropping, transfer to yeast pitching vessels and pitching into the wort line
- minimal suction lengths
- maximum yeast velocity of 0.5 m/s in all yeast lines
- maximum flow rate of 50 hL/hr (83 l/min $\simeq$ 88 kg/min) during cropping
- design of lines to minimise mechanical forces on yeast in terms of shear and pressure (eg. minimum number of bends, maximum bend radii, maximum pressure drop of 250 kPa across lines, pump and chiller collectively)
- no "dead-legs"
- the use of a fully cleanable, suitably sized stainless steel agitator to homogenise the yeast in the yeast storage vessel and the yeast and acid mixture in the pitching vessel with minimum shear and no inclusion of air

The use of stainless steel, the prevention of "dead-legs" and the emphasis on cleanability of the equipment are important to ensure sanitary operation to prevent bacterial contamination which may lead to fermentation problems and beer spoilage.

The SA Breweries specifications indicate the use of positive displacement pumps for yeast transfer during recirculation, but do not specify the type of positive displacement pump to be used. However, all breweries use rotary lobe pumps for yeast transfer during recirculation. There is a general reluctance to consider other positive displacement pumps since their effect on yeast quality is not known and

the performance, durability and cleanability of alternative pump designs have not been assessed. Centrifugal pumps are used for yeast transfer during yeast propagation.

The specification of minimal suction lengths has presumably been set to prevent cavitation in the pumps. This may have arisen from consideration for operating efficiency and pump life rather than direct concern or knowledge of the effect of cavitation on yeast quality.

The flow rate and linear velocity specifications are in keeping with recommendations in the literature, but appear to have no theoretical or experimental basis. Emphasis is placed on reducing mechanical forces to which the yeast is exposed and the term "minimal shear" is used, but acceptable or critical conditions have not been quantified.

Brewery conditions may deviate from the design specifications. Furthermore, the performance claims of equipment manufacturers are not always supported by rigorous studies. Plant yeast handling circuits may thus not prevent the exposure of yeast to potentially deleterious conditions, hence an evaluation of plant performance may be of value.

2.7 CONCLUSIONS

Yeast quality can be related to fermentation performance, which is dependent on the rate and extent of biomass growth, the flocculation and sedimentation characteristics of the yeast and the metabolic pathways predominating. The rate and extent of biomass growth is dependent on the physical and functional integrity of the cell envelope, the presence of intracellular reserve compounds (especially glycogen) and the metabolic rate of the yeast, which are thus important aspects of yeast quality. Losses of yeast quality associated with the integrity of the cell envelope may also result in the release of intracellular or wall-associated material into the beer which may results in reduced beer quality. Furthermore, yeast quality may also associated with the ability of the yeast to withstand stress.

Although the physical conditions in the yeast handling circuit require characterisation, it is expected that mechanical disruption of the yeast during cropping is not likely. Loss of yeast quality by other mechanisms may occur. These could include wall damage, replicative deactivation and metabolic changes which could result in altered fermentation performance and a reduction in beer quality.

An investigation of yeast transfer during pitching and propagation falls outside the scope of this thesis. However, it is recognised that mechanical disruption (and loss of yeast quality by other mechanisms) may occur more readily during pitching and propagation due to the lower biomass concentrations. During propagation, the cells

are in a growing state and may be particularly susceptible to mechanical damage.

In order to study the effect of brewery yeast handling on yeast quality, it is important that the experimental conditions resemble the brewery situation. Laboratory studies may be limited in their ability to simulate the brewery circuit, especially in terms of pressure drop and dissolved gases present.

Specifications for yeast handling equipment are based on tradition and experience rather than rigorous scientific study. Verification of the need for certain design constraints such as pump design and flow specifications, the evaluation of existing plant operation and the establishment of the critical conditions beyond which yeast is detrimentally affected may be of considerable value to improve current yeast handling techniques and plant specifications. At the same time an improved understanding of the physiological response of yeast to physical condition may be achieved.

CHAPTER

3

THE IDENTIFICATION, CHARACTERISATION AND QUANTIFICATION OF A LOSS OF YEAST QUALITY

3.1 INTRODUCTION

A loss of yeast quality can be identified by a reduction in the fermentative capacity of the yeast and the quality of the final product. In a brewery, the selection of a batch of yeast for re-use is generally based on an assessment of the quality of the yeast prior to re-inoculation. To be of practical value, the method(s) used to assess the quality of the yeast should be rapid to perform and the results should be predictive of the fermentation performance of the yeast in a subsequent fermentation. Various rapid methods for assessing yeast quality have been developed. These have been reviewed by Jones (1987a and 1987b), Henschke and Eglinton (1991), Iserentant (1993) and Lentini (1993). As rapid assessment of yeast quality was not essential in this investigation, yeast quality could be assessed by small scale fermentations. The aim of this chapter is not to provide a review of all techniques available for the assessment of yeast quality, but rather to present those that were selected for this investigation and to evaluate whether these yeast quality assays are accurate predictors of yeast quality and subsequent fermentation performance.

The characterisation of a loss of yeast quality requires a knowledge of how the cells have been damaged. Within the brewing industry, cell disruption, cell death and the loss of the ability of the cells to grow and reproduce (replicative deactivation) may all be said to constitute a loss of "viability" (Jones 1987a, Lentini 1993). Detrimental changes to the physiological condition or metabolic activity of the yeast may be described as losses of "vitality" (Lentini 1993), but this definition may be extended to include a loss of the ability of the yeast to withstand stress (O'Connor-Cox 1995). The response of yeast to stress conditions and the concomitant loss of yeast quality is expected to be a function of the nature, the duration and the intensity of the stress conditions. To allow more accurate characterisation of the nature of the loss of yeast quality over the broad definitions given above, certain physiological cell states have been defined and a scheme indicating the transition between these different physiological states in response to mechanical and physiological stresses is proposed.

In this chapter, a review of the different analytical techniques used to identify and quantify a loss of yeast quality, a rationale for the choice of each method for this investigation and an evaluation of its ability to quantify the desired parameter is provided in Section 3.2. The application of these yeast quality assays to a loss of yeast quality in response to mechanical stress is illustrated and their ability to predict the subsequent fermentation performance of the yeast is examined in Section 3.3. Finally, in Section 3.4, the physiological state approach to the characterisation of a loss of yeast quality as outlined above is presented.

3.2 THE IDENTIFICATION AND QUANTIFICATION OF A LOSS OF YEAST QUALITY

The analytical techniques or yeast quality assays selected for the identification and quantification of yeast quality for this investigation include an analysis of the integrity of the cell envelope, the ability of the yeast to grow and reproduce, the metabolic rate of the yeast and the presence of intracellular reserve compounds and a stress resistance indicator. The selected analytical techniques are listed in Table 3.1. and discussed in turn in this section. An understanding of their mechanism, the nature of damage identified and their experimental limitations is sought. Small scale fermentations were used to provide an overall assessment of the loss of yeast quality.

The review of the literature to assess the potential loss of yeast quality during mechanical handling in a brewery (Chapter 2) indicated that, under the conditions in the yeast handling circuit, cell wall damage would more likely result from mechanical handling of the yeast than cell disruption. The release of wall-associated material accompanying wall damage would cause the formation of beer haze. This investigation was limited to factors with a direct impact on the fermentation performance of the yeast, hence wall damage was not investigated.

The reproducibility of these yeast quality indicators was determined by calculating the pooled standard deviation (s_{pooled}) for replicate measurements as outlined in Appendix B. The calculations to determine the pooled standard deviation for each technique are presented in detail in Appendix C.

Table 3.1 Analytical methods to selected for the identification and quantification of a loss of yeast quality

Type of Assay	Selected Assay
Assays for the release of intracellular compounds	(1) protease assay
Staining techniques	(1) methylene blue, (2) magnesium salt of 8-anilino-1-naphthalene sulfonic acid (Mg-ANS)
Assays based on cell replication	(1) plate counts (2) slide counts
Metabolic activity indicators	(1) oxygen utilisation rate (2) acidification power
Intracellular reserve indicators	(1) glycogen content
Stress resistance indicators	(1) trehalose content

3.2.1 Assays for the Release of Intracellular Compounds

3.2.1.1 The Protease Assay

(a) The significance of extracellular protease activity

Brewers' yeast contains several intracellular proteolytic enzymes (proteases). The main function of these is the degradation of proteins within the cell and in the surrounding medium and the activation or inactivation of certain enzymes as part of metabolic control (Ormrod *et al.* 1991). Proteases are released during fermentation. The level of protease activity in the medium was found to be dependent on yeast strain and process parameters (Ormrod *et al.* 1991). Yeast propagated under "ideal" laboratory conditions showed lower initial levels of protease activity than yeast propagated by large scale brewing (Mochaba *et al.* 1993). The high protease activity of the brewery yeast was found to persist during fermentations under brewery conditions, while the laboratory propagated yeast continued to show low levels of extracellular protease activity over a seven day fermentation period. An increase in extracellular protease activity was observed when cell growth declined. Extracellular protease activity was found to be significant during post-fermentation and storage phases (Slaughter and Nomura 1992a). It is suggested that the level of external protease activity may be related to the extent of cell death and autolysis that has occurred during post fermentation

storage (Slaughter and Nomura 1992, Mochaba *et al.* 1993). The presence of protease in beer is undesirable since it leads to foam instability (Ormrod *et al.* 1991) and the persistence of protease activity during fermentation suggests that yeasts with high protease activity should not be used for fermentations. It is suggested that a protease assay may be used as an indicator of the quality of pitching yeasts (Mochaba *et al.* 1993). However, correlation between protease activity in pitching yeasts and aspects of fermentation performance such as rate and extent of attenuation or the presence of off-flavours in the final beer, has not been reported.

The suggested relationship between external protease activity and the extent of autolysis suggests that the level of external protease activity may give an indication of the extent of disruption or loss of the integrity of yeast cell envelopes during mechanical handling of the yeast. Since partial disruption of the cell envelope is expected to result in a release of protease into the surrounding medium, it is suggested that a measure of external protease activity be used to detect the loss of yeast quality through at least partial disruption yeast cell envelopes. In an experimental set-up, total cell counts could be used to determine to portion of cells that have been disrupted completely.

(b) Equipment and procedure

Several protease assays have been proposed. Yokosawa *et al.* (1983, cited by Ormrod *et al.* 1991) use a synthetic protein substrate dimethylcasein and quantify the newly formed amino groups and fluorescamine by fluorescent intensity. Reicheneder and Narziss (1987, cited by Ormrod *et al.* 1991) developed a method using azocasein - a dye bound protein. Mochaba *et al.* (1993) report a method using resorufin-labelled casein as substrate. The resorufin-labelled casein is broken up into resorufin-labelled peptides by proteases in the supernatant of a centrifuged yeast sample. These peptides are not precipitated by trichloroacetic acid hence their concentration, measured spectrophotometrically or fluorometrically, gives an indication of the proteolytic activity in the supernatant. Mochaba *et al.* (1993) claim that the method is highly sensitive, reproducible and can be performed rapidly. This makes it suitable for use as a routine assay in production facilities.

The method of Mochaba *et al.* (1993) was used in this investigation.

For the protease assay (Mochaba *et al.* 1993), the following reagents were prepared using distilled, de-ionised water:

(i) Incubation Buffer:	Tris-HCl Buffer (0.2 mol/L Tris-HCl, pH 7.8, 0.02 mol/L $CaCl_2$)
(ii) Stop Reagent:	Trichloroacetic acid (5% w/v)
(iii) Assay Buffer:	Tris-HCl (0.5 mol/L, pH 8.8)
(iv) Substrate Solution:	Casein, resorufin-labelled (Boehringer Mannheim Biochemica) (0.4% w/v). Aliquots of 50 µL were transferred to Eppendorf tubes which were stored in a deep freeze until required.

Yeast slurries to be analysed were transferred into Eppendorf tubes using pipettes and centrifuged for 5 minutes (centrifuge: Omega). The supernatants were decanted and centrifuged again for 5 minutes to remove any remaining yeast. The supernatants were transferred into new Eppendorf tubes. 50 μL of incubation buffer was added to the pre-prepared Eppendorf tubes containing 50μl of the casein substrate. For the samples from the yeast slurries, 100 μL supernatant was added to the tubes and for the blank, 100 μL deionised, distilled water was added. The contents of the tubes were mixed by tapping the tubes gently on the laboratory bench. The tubes were incubated overnight (14 hours) at 37°C. 480 μL of the stop reagent was added. Incubation at 37°C continued for 10 minutes, whereafter the tubes were centrifuged for 5 minutes and the supernatants decanted into new Eppendorf tubes. 400 μL of the supernatants were pipetted into 1 mL cuvettes (Biorad). 600 μL of the assay buffer was added and the contents mixed by tapping the cuvettes gently on the laboratory bench. The absorbances of the cuvette contents were measured immediately at 574 nm using a spectrophotometer (Varian Cary UV-Visible 1E).

The protease activity was expressed as

$$\Delta A_{sample} = Absorbance_{sample} - Absorbance_{blank}$$

(c) Reproducibility of the assay

A pooled standard deviation (s_{pooled}) of 0.02 was calculated for the protease assay (Appendix C, page C1). Since this value was calculated for 22 degrees of freedom, it may be regarded as a good estimate of the population standard deviation (σ) (Appendix B). The coefficient of variation for this assay was determined to be 21%. This suggests that the method is not very precise and is thus not expected to be very sensitive to changes in external protease activity.

Lodolo *et al.* (1996) also report a standard deviation (s) of 0.02 for this assay. The method is recommended as an indicator of yeast quality for the evaluation of pitching yeasts during routine brewery operation. It is suggested that yeast slurries with protease activities exceeding $\Delta A = 0.05$ should not be used re-inoculation (Lodolo *et al.* 1996). The large standard deviation of the method, however, makes the use of the protease assay as a single criterion for the selection of pitching yeasts questionable.

3.2.2 Staining Techniques

Within the brewing industry, certain stains are commonly used to detect and quantify yeast "viability" (Lentini 1993). The stains operate by one of two mechanisms: the ability of the intact plasma membrane to prevent the stains from entering the cytoplasm or the ability of living cells to chemically modify the stains within the cytoplasm. The ability of a stain to penetrate the plasma membrane depends on the lipid solubility of the stain and on the physical state of the membrane (Jones 1987a). In addition, the success of the different stains to identify "viable" and "non-viable" cells may depend on yeast strain, the physiological status (stage in the cell cycle), the pH or ionic strength of the staining solution and the contact time (Jones 1987a, Jones 1987b, Lentini 1993).

Stains used are classified either as brightfield (examined using a light microscope) or fluorochrome stains (examined with UV illumination to cause fluorescence of the stain). Examples of brightfield stains that have been successfully used to monitor the "viability" of yeast include methylene blue, rhodamine B, crystal violet and eosin Y. Examples of fluorochrome stains include acridine orange, fluorescein isothiocynate (FITC), janus green, magnesium salt of 1-anilino-8-naphthalene sulphonic acid (Mg-ANS) and fluorescein diacetate (FDA) (Lentini 1993). A brightfield stain (methylene blue) and a fluorochrome stain (the magnesium salt of 1-anilino-8-naphthalene sulphonic acid (Mg-ANS)) were used in this investigation.

3.2.2.1 The Methylene Blue Staining Technique

Methylene blue is the most commonly used staining technique within the brewing industry (Lentini 1993). It is recommended as a "viability" assay by the European Brewing Convention (EBC) (1962), the Institute of Brewing (IOB) (1970) and the American Society of Brewing Chemists (ASBC) (1980).

(a) The mechanism of the methylene blue staining technique

The mechanism by which methylene blue produces differential staining of "viable" and "non-viable" cells is a matter of debate. The European Brewing Convention (1962), the American Society of Brewing Chemists (1976), Parkkinen *et al.* (1976), Chilver *et al.* (1978) and McCaig (1990) support the idea that living cells which have intact cell membranes exclude the dye and remain colourless, while the permeability of the membranes of non-living cells allows the entry of the dye into the cytoplasm and hence the staining of non-living cells. Lentini (1993) suggests that the stain penetrates both living and non-living cells and that it is reduced to a colourless compound in living cells, while dead cells are unable to reduce the stain and remain coloured. Jones (1987a) suggests that entry of the dye into the cytoplasm of living cells results in its auto-oxidation to a colourless compound. Failure of a cell to stain results from the balance between the rate of influx of the stain through the membrane and the rate of oxidation within the cytoplasm. Vogel (1989) indicates that the oxidised form of the stain is blue while the reduced form is colourless. This suggests that any decolorisation of the dye within living cells would be reductive in nature and not oxidative as indicated by Jones (1987a).

Chilver *et al.* (1987) measured the absorbance (660 nm) of identical concentrations of living and heat-killed yeast cells suspended in phosphate-buffered saline to which methylene blue (100 mg/L) had been added. More stain was removed from the suspension of dead cells, than from that of living cells. The dead cells were also visibly blue. The absorbance for the living cells remained unaltered over a period of two hours. It is argued that should an enzymatic reaction have occurred, it occurred before the measurements were made and it occurred to a fixed extent (unlike bacterial systems where complete decolorisation would occur). Living cells which had been in contact with methylene blue did not turn blue upon subsequent killing with heat. Chilver *et al.* (1978) conclude that the action of methylene blue is one of permeability.

To determine the "viability" of stored Baker's yeast, Parkkinen *et al.* (1976) compared the use of methylene blue and three fluorochromes (acridine orange, acriflavine and primuline) to the classical plate count method which relies on cell growth and reproduction for the enumeration of "viable" cells. Suspensions of fresh Baker's yeast and heat killed yeast cells were mixed to give a range of suspensions containing 10 to 90% heat-killed cells. All the staining techniques and the plate counts accurately reflected the apparent viabilities of the suspensions and gave essentially the same results over the entire range of apparent viabilities. When assessing the "viability" of a yeast suspension which was stored at 35°C over a period of 16 days, the fluorochromes showed similar results and appeared sensitive to the increase in the proportion of "non-viable" cells in the population from 0% to 100%. Methylene blue staining showed corresponding results until the proportion of "non-viable" reached 50%. Thereafter, methylene staining under-estimated the number of "non-viable" cells relative to the other stains which showed similar results. Parkkinen *et al.* (1976) note that methylene blue gave different results depending on whether the cells had died naturally during prolonged storage or had been killed by heat, but do not reflect further on the implication of this observation on the mechanism of the action of the stain.

When the condition of the yeast deteriorates as a result of processes such as yeast storage, it has been noted that methylene blue staining increasingly under-estimates the percentage of "non-viable" cells relative to enumerations based on the ability of the cells to grow and reproduce (mainly the slide count technique) (Gilliland 1959, European Brewing Convention 1962, Institute of Brewing 1970, American Society of Brewing Chemists 1976, American Society of Brewing Chemists 1980, Chilver *et al.* 1978, King *et al.* 1981, McCaig 1990). When no replication occurs, 30 to 40% of the cells may be unstained by methylene blue (Chilver *et al.* 1978).

Methylene blue may act by both proposed mechanisms. Primarily, differential staining may be dependent on the intactness of the cell membrane: intact membranes would exclude the stain, hence cells with intact membranes would remain colourless while membrane damaged cells would stain. The results of Parkkinen *et al.* (1976) showed that heat-killed cells stained blue, hence methylene blue staining provided an accurate reflection of the apparent "viability" of the suspensions of fresh and heat-killed cells. Chilver *et al.* (1978) also showed that heat-killed cells stained blue. This suggests that heat-killing may result in membrane damage in addition to enzyme deactivation. However, Chilver *et al.* showed that living cells that had been exposed to methylene blue failed to stain after exposure to heat. The heat treatment employed in this case may not have damaged the cell membrane, hence the failure of the cells to stain.

It is suggested that degradation of the yeast during storage results in deterioration of the cell membranes. Cells with damaged membranes would allow the penetration of the stain into the cytoplasm. A portion of the cells may have damaged membranes, but may be still be metabolically active. Should the stain be decolorised in metabolically active cells as proposed by Jones (1987a) and Lentini

(1993), these cells would appear unstained and would be counted as "viable". Jones (1987b) suggests that replicative ability is linked to the state of the cell envelope. The unstained metabolically active cells with damaged membranes would not reproduce and would be counted as "non-viable" using the enumerations based on cell growth and reproduction. It follows that in the presence of metabolically active membrane damaged cells, the reduction of methylene blue within these cells, would result in higher apparent "viabilities" than indicated by cell growth and replication. This would explain the apparent over-estimation of yeast "viability" relative to methods measuring replicative competence indicated by Gilliland (1959), the European Brewing Convention (1962), the Institute of Brewing (1970), the American Society of Brewing Chemists (1976 and 1980), Chilver *et al.* (1978), King *et al.* (1981) and McCaig (1990).

The above discussion illustrates that cell death and replicative deactivation represent two different types of loss of yeast quality. The use of the term "viability" to describe both mechanisms is inaccurate. Furthermore, the assumption that metabolically active cells ("living" cells) have intact membranes and metabolically inactive cells ("dead" cells) have damaged membranes to explain the mechanism of methylene blue staining (European Brewing Convention 1962, American Society of Brewing Chemists 1976, Parkkinen *et al.* 1976, Chilver *et al.* 1978 and McCaig 1990) is incorrect. Depending on the mechanism of the damage, cells with membrane damage may display metabolic activity and metabolically inactive cells may have intact membranes. The results of methylene blue staining should be interpreted within this context and treated with caution.

(b) Application of the methylene blue staining technique

In brewing applications, a knowledge of the replicative ability of the population is required. Methods based on the ability of the cells to grow and reproduce, such as the slide count technique, are more appropriate than methylene blue staining to assess the quality of the yeast. At higher "viabilities", there is a degree of correspondence between methylene blue and slide count results (European Brewing Convention 1962, Chilver *et al.* 1978, King *et al.* 1981, McCaig 1990). Methylene blue staining is regarded as an acceptable indicator of "viability" at "viabilities" greater than 80% (European Brewing Convention 1962, McCaig 1990) or 90% (King *et al.* 1981). During routine brewery operation, where yeast is generally in a good condition, methylene blue staining may be more practical to use since it is simpler and less time consuming. Where significant loss of yeast quality may result, methylene blue "viability" requires careful interpretation.

The difference in the "viability" results obtained with the methylene blue staining technique (indicating cell death associated with concomitant membrane damage and enzyme deactivation) and those obtained with the slide count technique (indicating the total number of metabolically inactive and replicatively deactivated cells), may be used to indicate the extent of replicative deactivation resulting from mechanical damage to the cells, relative to cell death.

(c) Equipment and procedure

Methylene blue staining is rapid and requires only simple equipment such as a light microscope and a haemocytometer or normal microscope slide (Lentini 1993). A stain solution is mixed with the yeast suspension and the percentage of stained and hence "non-viable" cells in the suspension is determined microscopic counting. Table 3.2 provides a summary of variations of the methylene blue staining technique. Differences arise in methylene blue concentration, the ionic composition and strength of the staining solutions and the contact time between the staining solution and the yeast suspension before the counting commences.

Lee *et al.* (1981) suggest that a wide range of methylene blue concentrations from 0.003 - 0.2% (w/v) can be applied and recommend an initial concentration of 0.025% (w/v). In accordance with the method recommended by the EBC, IOB and ASBC, a 0.01% (w/v) solution of methylene blue (May and Baker Chemicals) was used in the SAB method, initially selected for this investigation (referred to as the UCT method). The ionic composition and ionic strength of the diluent are expected to influence the uptake of the dye (Jones 1987a). Calcium or other divalent cations should be provided. It is suggested that the dye penetrates dead cells in a few seconds in solutions of low osmotic pressure, but requires longer where the osmotic pressure is greater (European Brewery Convention 1962). The SAB and UCT methods have a lower initial ionic strength than the standard method of the EBC, IOB and ASBC. However, the contact time of the methods is at least one minute which should allow sufficient time for dye penetration. The method of Lee *et al.* (1981) is recommended on a basis of accuracy, primarily because of the ionic composition and ionic strength of the diluent (full strength Ringer's solution) (Jones 1987a). It was adopted in preference to the SAB method and is referred to as the "modified" methylene blue method. Lee *et al.* (1981) claim that the ratio of stained to unstained cells remains constant for at least two days.

The accuracy of all staining techniques is dependent on the number of cells per field of view and the assessment of sufficient fields of view (Jones 1987a). For the methylene blue staining technique, it is recommended that 40 to 60 cells are present per microscope field using medium magnification (600x) (EBC Analytica Microbiologica 1977) and a total of 1000 cells are usually counted (Institute of Brewing 1970, King *et al.* 1981, McCaig 1990). The dilution ratios of the methods used in this investigation were adjusted according this recommendation and a total of approximately 500 cells were counted 400x magnification.

(d) Reproducibility of the methods used in this investigation

Pooled standard deviations (s_{pooled}) of 2% were calculated using 68 and 22 degrees of freedom for the SAB/UCT method and the "modified" methods respectively (Appendix C, page C2). Hence both may be regarded as good estimates of the population standard deviations (σ). The coefficient of variation was calculated to be 2%, hence the methods are reasonably precise.

Table 3.2 Variations of the methylene blue staining technique

Staining Preparation	Stain/Yeast Ratio	Contact Time	References
Dissolve methylene blue (0.01 g) in distilled water (10 mL). Add sodium citrate dihydrate (2 g) and stir until dissolved. Filter through filter paper and make volume of filtrate to 100 mL with distilled water to give a final methylene blue concentration of 0.01% (w/v).	Mix dye solution with equal volume of a suspension of yeast sample. Cell concentration should be such that 40-60 cells are present per microscope field at medium magnification (600x) after mixing with the dye solution.	(1),(2),(3),(5),(6) Not indicated (4) 1 - 5 min	(1) Gilliland (1959) (2) European Brewing Convention (1962) (3) Institute of Brewing (1970) (4) American Society of Brewing Chemists (1976) (5) EBC Analytica Microbiologica (1977) (6) Pauls' Malt (1989)
Solution A: methylene blue (0.1 g) in distilled water (500 mL) Solution B: KH_2PO_4 (13.6 g) in distilled water (500 mL) Solution C: $Na_2HPO_4.12H_2O$ (2.4 g) in distilled water (100 mL) Solution D: 498.75 mL solution B + 1.25 mL solution C Solution E: Mix the 500 mL of solution D with 500 mL solution A to give final buffered methylene blue solution (0.01% (w/v)) with pH of approximately 4.6.	(1) Dilute yeast suspension with methylene blue solution so that 100 cells are visible per microscopic field at 540 - 750x magnification. (2) Add 2 mL of 1% yeast suspension to 1 mL methylene blue solution. (3) Mix methylene blue and cell suspension to give 1 x 10^7 cells/mL (4) Mix methylene blue and cell suspension to give 1 x 10^6 cells/mL	(1) 1 - 5 minutes (2) 15 minutes (with vigorous shaking) (3),(4) 5 minutes	(1) American Society of Brewing Chemists (1976) (2) Parkkinen *et al.* (1976) (3) King *et al.* (1981) (4) McCaig (1990)
Dilute methylene blue in phosphate-buffered saline (PBS) to give a final concentration of 50 mg/L (0.005% (w/v)). PBS: $NaH_2PO_4.H_2O$ (0.22 g), Na_2HPO_4 (1.2 g) and NaCl (8.5 g) in 1 L distilled water. Filter (0.22 μm) and autoclave at 121°C for 10 minutes. Nominal pH = 7.4.	Not indicated.	2 minutes (minimum)	(1) Chilver *et al.* (1978)

Table 3.2 (continued)

Staining Preparation	Stain/Yeast Ratio	Contact Time	References
Dissolve methylene blue (0.0001% (w/v)) in distilled water and filter (0.45 μm)	Add 0.2 mL of methylene blue solution to 0.2 mL of cell suspension.	1 hr at 25°C	(1) Trevors *et al.* (1983)
Dissolve methylene blue (0.1 g) in distilled water (100 mL). Add sodium citrate dihydrate (0.2 g) to 10 mL of the 0.1% methylene blue solution. Dilute to 100 mL with distilled water to give a final methylene blue concentration of 0.01% (w/v).	(1) Transfer enough yeast slurry into a test tube of saline (0.85%) to give a suspension such that the markings of a pipette are only just visible. Mix two drops of this suspension with two drops of the methylene blue solution on a spot plate. (2) Dilute yeast slurry 100x in 0.9% sterile saline solution to give a final cell concentration ~ 10^6 cells/mL. Mix 1 mL of this suspension with an equal volume of the methylene blue solution.	1 minute	(1) SAB (1993) (2) UCT method
Dissolve methylene blue (0.025 g), NaCl (0.9 g), KCl (0.042 g), $CaCl_2.6H_2O$ (0.048 g), $NaHCO_3$ (0.02g) and glucose (1 g) in 100ml in distilled water (100 mL) to give a final methylene blue concentration of 0.025% (w/v).	(1) Dilute cell suspensions down to ~ 2-4 x 10^8 cells/mL with Ringer salt solution. Mix 0.1 mL of this suspension with 0.9 mL of the methylene blue solution. (2) Dilute yeast slurry 100x in 0.9% sterile saline solution to give a final cell concentration ~ 10^6 cells/mL. Mix 0.1 mL of this suspension with 0.9 mL of the methylene blue solution.	(1) 10 minutes (maximum) (2) 1 minute	(1) Lee *et al.* (1981) (2) UCT "modified" method

3.2.2.2 The Mg-ANS Staining Technique

The magnesium salt of 8-anilino-1-naphthalene sulfonic acid (Mg-ANS) has been recommended as an alternative to the methylene blue staining technique (Jones 1987a, American Society of Brewing Chemists 1981, King *et al.* 1981, McCaig 1990). It is quick and simple relative to the slide count technique to which it corresponds well. Its application may be limited by the need for a fluorescent microscope.

(a) The mechanism of the Mg-ANS staining technique

Mg-ANS staining is expected to differentiate between "viable" and "non-viable" cells based on the ability of the dye to penetrate cells with damaged membranes. The dye stains cytoplasmic proteins which produce green fluorescence when illuminated with ultraviolet light. Dead and membrane damaged cells thus fluoresce green while living cells with intact membranes remain unstained (King *et al.* 1981, McCaig 1990). This mechanism is supported by the correlation between Mg-ANS staining and slide counts over the entire range of "viability" from 0 to 100% (King *et al.* 1981, McCaig 1990). The latter depends on the presence of well functioning membranes for the enumeration of cells with the ability to grow and reproduce (Jones 1987a).

(b) Equipment and procedure

Use of a 0.3% (w/v) Mg-ANS solution is reported (American Society of Brewing Chemists 1981, King *et al.* 1981, Trevors *et al.* 1983, McCaig 1990). Diluents used include sterile, distilled water (American Society of Brewing Chemists 1981, King *et al.* 1981, Trevors *et al.* 1983) and a 2% (v/v) solution of ethanol in sterile, distilled water (McCaig 1990).

An equal volume of the 0.3% (w/v) Mg-ANS (Sigma) solution of McCaig (1990) and the test suspension containing about 10^6 cells/mL were mixed and incubated at room temperature for 5 minutes. A drop of the mixture was placed on a slide and covered with a coverslip. The slides were examined at 400x magnification under a fluorescent microscope (Nikon). At least 500 cells were counted. The number of non-fluorescing cells, divided by the total number of fluorescing and non-fluorescing cells represented the percentage of "viable" cells in the population.

(c) Reproducibility of the method used in this investigation

The standard deviation was estimated as 4%. This exceeded the value for the methylene blue staining techniques (s_{pooled} = 2%). The green fluorescence was pale which made the interpretation of the staining difficult. The situation could be improved by limiting the time interval between mixing of the dye and the counting of the fluorescing cells.

3.2.3 Assays Based on Cell Replication

Fermentation performance depends largely on the ability of cells to grow and reproduce. Hence a knowledge of the percentage of replicatively competent cells in the yeast population is of prime importance in the selection of yeast for re-use and the determination of the amount of yeast required for repitching. Since mechanical stress may result in membrane damage which has been linked to replicative deactivation, a measure of replicative deactivation was required for this study. Two technique were considered: the plate count technique and the slide count technique.

3.2.3.1 The Plate Count Technique

(a) Background

In the plate count technique, a suitable dilution of cells is grown on nutrient agar plates. The number of replicatively active cells is determined by the number of visible colonies (colony forming units). This is expressed as a percentage of the total number of cells in the original suspension, determined by a total microscopic count. It indicates the portion of the population that was able to replicate.

The type of medium used for the plates, the incubation temperature and the incubation time may all affect the plate counts (Jones 1987a). A wort-based medium is commonly used for Brewers' yeast to resemble the production medium (European Brewery Convention 1962). Incubation temperatures vary from 15 to 37°C (Jones 1987a). Incubation times are generally 2 to 3 days, hence the method is too slow for use in routine brewery operation (Jones 1987a).

Plate counts normally predict lower viabilities than methods such as methylene blue and slide counts (European Brewing Convention 1962, American Society of Brewing Chemists 1980). It has been suggested that some processes other than the inability to reproduce are being measured (Jones 1987). The method is also prone to large errors (European Brewery Convention 1962, King *et al.* 1981, Jones 1987a) and the reproducibility of the method was found to be very low (American Society of Brewing Chemists 1980).

(b) Equipment and procedure

Two different media were used for the plate counts: 50 g/L wort agar (Merck) and MYPG agar containing 3 g malt extract, 3 g yeast extract, 5 g peptone, 10 g glucose and 15 g agar per litre. The MYPG agar was preferred to the wort agar as the wort agar is of an uneven quality (plates were often soft despite the addition of bacto-agar).

Initial yeast suspensions of about 10^6 cells/mL were used. The total number of cells in the initial suspension was counted by haemocytometer under 400x magnification (microscope: Nikon). At least 500 cells were counted.

The total cell number was calculated as follows:

$$\text{total cell concentration} = Y/Z \times 25 \times 10^4 \times D \text{ (cells/mL)}$$

where Y was the number of cells counted, Z the number of squares counted, D the dilution factor and 25×10^4 the volume of the chamber.

Step-wise dilutions (1:10) of the sample solutions were made in 0.9% NaCl. Spreadplates were prepared using 100 μL aliquots of the 10^{-3}, 10^{-4} and 10^{-5} dilutions. These were incubated at 30°C for three days. Plates with 30 to 300 colonies were counted to determine the number of colony forming units. The "viability" of the population was expressed as the number of colony forming units as a percentage of the total initial number of cells.

(c) Reproducibility of the method used in this investigation

The method as a whole showed poor reproducibility: a pooled standard deviation of 17% was calculated for replicate measurements and 27 degrees of freedom (Appendix C, page C3). This corresponded to a coefficient of variation of 53%. The low reproducibility of the method is confirmed by the American Society of Brewing Chemists (1980). Owing to the low reproducibility and the long incubation period required, the method was not expected to be of use for the quantification of replicative competence.

3.2.3.2 The Slide Count Technique

(a) Background

In the slide count technique, an appropriate dilution of a suspension of cells is placed onto a layer of nutrient agar on the surface of a slide and incubated for a period of time (usually 16 - 18 hours). The formation of micro-colonies is evaluated microscopically to determine the portion of replicatively competent cells present. The technique was developed to overcome the source of errors, low reproducibility and long incubation period of the plate count technique (Gilliland 1959, Jones 1987a). The slide culture technique is recommended as a "viability" assay by the European Brewery Convention (1962), the Institute of Brewing (1970) and the American Society of Brewing Chemists (1981).

As indicated (Section 3.2.2), the slide count technique was used as a reference method when assessing different "viability" stains, since the results are a direct measurement of the replicative ability of cells and have been found to be accurate over a broad range of "viability" (Chilver *et al.* 1978, American Society of Brewing Chemists 1981, King *et al.* 1981, McCaig 1990). The reproducibility of the method is lower than the methylene blue and Mg-ANS staining techniques (American Society of Brewing Chemists 1981). The Mg-ANS staining technique and the slide count technique have been reported to give equivalent information over a range of viabilities (American Society of Brewing Chemists 1981, King *et al.* 1981, McCaig 1990).

Since replicatively competent cells are expected to form micro-colonies after a short lag time and damaged cells may recover their replicative ability after a longer lag

period, the incubation temperature and time are important aspects of the technique. The composition of the diluent and the agar base placed onto the slide are also important (Jones 1987a). The technique thus indicates which cells are replicatively competent under the test conditions. Investigations correlating the results of the slide culture technique with the subsequent fermentation performance of the yeast have not been reported in the literature.

(b) Equipment and procedure

Table 3.3 presents the different protocols of the slide count technique reported in the literature. The technique recommended by the American Society of Brewing Chemists (1981, 1984) was selected. This technique was preferred, since it was simpler to perform and the use of ordinary microscope slides instead of expensive haemocytometers allowed the simultaneous processing of more samples. Samples were incubated at room temperature. At least 500 micro-colonies and cells were counted at 400x magnification (microscope: Nikon). Single cells which had not produced colonies were regarded as dead or replicatively deactivated.

(c) Reproducibility of the method used in this investigation

The pooled standard deviation (s_{pooled}) was 3%. This was determined for 24 degrees of freedom hence it may be regarded as a good estimate of the population standard deviation (σ) (Appendix C, page C3). The coefficient of variation was 3%, which indicated that the method is reasonably precise and can be used to detect loss of replicative competence in response to stress.

Table 3.3 Variations of the slide count technique

Procedure	Medium	Diluent	Incubation Time	Incubation Temperature	Reference
Suspend the yeast sample in the diluent to give a concentration in the range of 5-10 x 10^6 cells/mL. Mix the suspension with an equal volume of melted medium, place two drops of the mixture onto a haemocytometer slide and gently place a flame-sterilised coverslip over the mixture. Place the slide in an incubator at 37°C and allow the cells to fall to the base of the counting chamber (± 5 minutes). Seal the edges of the coverslip with melted petroleum jelly to minimise evaporation. Transfer the slides to an incubator to allow the formation of micro-colonies. Using a microscope, count the proportion of cells which have developed into micro-colonies.	(1) Wort, gelatin (6%) (2),(3),(4),(6),(7) MYPG medium*, gelatin (12%) (5) Wallerstein Laboratory Nutrient WLN) agar	(1) Not indicated (2),(3),(4) Saline (0.85%) (4),(6),(7) Ringer's solution** (1/4 strength) (5) P8S***	(1) Overnight (2),(4) 16 hrs (3),(5) Not indicated (6),(7) 18 hrs	(1),(2) 18°C (3),(6),(7) 15-20°C (4) 15-25°C (5) 28°C	(1) Gilliland (1959) (2) European Brewery Convention (1962) (3) Institute of Brewing (1970) (4) EBC Analytica Microbiologica (1977) (5) Chilver *et al.* (1978) (6) Pauls' Malt (1989) (7) McCaig (1990)
With a forceps, hold a 7.6 x 2.5 cm slide and flame over a burner. Pipet ± 1 mL of molten medium over the slide. After the medium has solidified, pipet two drops of the test suspension (± 1 x 10^6 cells/mL) onto the medium. Cover lightly with flame-sterilised cover slip. Place the slide in a petri dish, cover and incubate. Using a microscope, count the proportion of cells which have developed into micro-colonies.	(1) Wort, 1.5% $Zn.SO_4.7H_2O$ solution (1%), agar (1.5%) (2),(4),(5) MYPG medium*, 1.5% $Zn.SO_4.7H_2O$ solution (1%), maltose (6%), agar (1.5%) (3) Wort, agar (1.5%)	(1),(2),(3),(4),(5) Saline (0.85%)	(1),(2),(4),(5) 18 hrs (3) 17 hrs	(1) Room temperature (2),(4),(5) 20-22°C (3) 18°C	(1) American Society of Brewing Chemists (1980) (2) American Society of Brewing Chemists (1981) (3) King *et al.* (1981) (4) Trevors *et al.* (1983) (5) American Society of Brewing Chemists (1984)

* MYGP medium: malt extract (3 g), yeast extract (3 g), peptone (5 g), glucose 10 g, distilled water (1 L)

** Ringer's solution (1/4 strength): NaCl (2.25 g), KCl (0.105 g), $CaCl_2$ (0.16 g), Na_2CO (0.05 g), distilled water (1 L)

*** Phosphate buffered saline: $NaH_2PO_4.H_2O$ (0.22 g), Na_2HPO_4 (1.2 g), NaCl (8.5 g), distilled water (1 L)

3.2.4 Metabolic Activity Indicators

Metabolic activity, which is referred to as yeast "vitality" in the brewing industry, is expected be a significant determinant of the fermentation performance of the yeast since it affects the rate of substrate utilisation and hence the rate of yeast growth and wort attenuation. Measures of metabolic activity are thus expected to be predictive of the fermentation performance of the yeast. However, since metabolic activity is dependent on environmental conditions, cells may respond differently to the assay and the fermentation conditions (Jones 1987a), limiting the predictive ability of the tests.

Potential physiological and mechanical stresses during yeast handling were expected to influence the metabolic activity of yeast, hence two indicators of metabolic activity were selected for this study: oxygen utilisation rate and acidification power.

3.2.4.1 Oxygen Utilisation Rate

(a) The significance of the rate of oxygen utilisation

During aerobic microbial growth, the rate of growth can be correlated to the rate of substrate utilisation and the specific oxygen utilisation rate. In beer production, the initial period of fermentation is aerobic. Oxygen is used mainly for the oxidation of glucose and glycogen for energy generation and the formation of biomass and for the oxidation reactions involved in lipid biosynthesis (Henschke and Eglinton 1991). The lipids produced include sterols and fatty acids. These are essential for yeast membrane function and may limit growth (Boulton and Quain 1987).

Daoud and Searle (1986) observed a correlation between the physiological state of yeast and the rate at which the yeast takes up oxygen from an oxygen saturated medium. Yeast which had been given sufficient oxygen to maintain their physiological condition showed higher oxygen utilisation rates than yeast in presumably poorer physiological condition which had been deprived of oxygen during successive fermentations. In the BRF Yeast Vitality Test developed by Kara *et al.* (1987), the "vitality" of a yeast sample is expressed in terms of the oxygen utilisation rate (OUR) of the "viable" portion of the cells. The measure of yeast "vitality" or metabolic activity is thus dependent on the method used to predict yeast "viability". Kara *et al.* (1987) determined "viability" by methylene blue staining. For yeast populations with more than 90% living cells, this correlates to replicative competence (European Brewing Convention 1962, King *et al.* 1981, McCaig 1990). In this region, "vitality" based on oxygen utilisation rate correlates well with fermentation performance (Kara *et al.* 1987).

In a study of the influence of storage time and temperature on yeast stored under beer or in pressed form, Martens *et al.* (1986) showed good correlation between OUR, glycogen content and "viability". A similar study was conducted by Wheatcroft *et al.* (1988). The oxygen utilisation rates, glycogen contents and

"viabilities" (indicated by methylene blue staining) of stored and acid washed yeast were compared to fermentation performance. The changes in the physiological condition of yeast brought on by the different storage conditions were detected as changes in OUR and glycogen content, but not as a significant increase in the "non-viable" cell population. Acid washing reduced oxygen utilisation rates by variable amounts, but acid washed yeast showed better fermentation performance than non-acid washed yeast. In the laboratory scale experiments to establish whether a correlation exists between yeast "vitality" in terms of OUR and fermentation performance, yeast samples, stored at different temperatures to effect differences in physiological condition, were acid washed before inoculation. Wheatcroft *et al.* (1988) could not find a relationship between OUR and fermentation performance in laboratory or production scale fermenters. Given the variable effect of acid washing on oxygen utilisation rates, this is not conclusive evidence against a correlation between OUR and fermentation performance prior to acid washing.

In all the above studies fermentation performance was assessed by monitoring the rate of substrate utilisation or the rate of carbon dioxide evolution. Concomitant cell growth was not indicated nor was beer quality considered.

Boulton and Quain (1987) observed a correspondence between sterol synthesis upon exposure to oxygen and the rate at which oxygen is consumed. Iserentant (1993) suggests a relationship between OUR and sterol content. This is supported by Daoud and Searle (1986). Iserentant (1993) notes that high oxygen utilisation rates are usually coupled with high ATP production rates, suggesting that oxygen utilisation rate would be a better predictor of initial yeast growth than of overall fermentation performance.

(b) Equipment and Procedure

Daoud and Searle (1986) developed equipment to measure the oxygen utilisation rate (OUR) of a sample of yeast. Kara *et al.* (1987) extended this work and developed the BRF Yeast Vitality Test. Equipment for this test is now manufactured commercially (Crisp Instrumentation, UK). Wheatcroft *et al.* (1988) used a smaller scale more rapid method which gives similar results to the BRF method.

The apparatus used to measure oxygen utilisation rates generally consists of a stirred vessel containing an air-saturated medium to which a sample of yeast is added. The decrease in the dissolved oxygen concentration is measured over a period of time. For comparative purposes the specific oxygen utilisation rate is determined, hence the biomass present is determined. The results may be corrected for the "viability" of the cells. Table 3.4 summarises the systems used by different researchers in comparison with the systems used in this study (UCT Method A and UCT Method B). In the UCT methods, a dissolved oxygen electrode from Yellow Springs Instruments was used. The corrections for "viability" were based on methylene blue staining.

Table 3.4 Systems used to measure oxygen utilisation rates

Researchers	Vessel	Agitation (1) Aeration (2) and Temperature Control (3)	Suspension Preparation	Medium	Pitching Rate	Duration (1) and Temperature (2)	Cell Assay
Daoud and Searle (1986)	2 L Quickfit vessel (1.5 L medium)	(1) circulation loop (2) air sparging (3) vessel in waterbath	centrifugation and resuspension of cells in sterile water (ratio 1:1)	wort, yeast maltose medium	1 g dry wt/L	(1) 1 hr (2) 20 °C	dry weight
Kara *et al.* (1987)	500 mL Quickfit vessel	(1) magnetic stirrer (100 rpm) (2) air sparging (3) jacketed vessel	(a) centrifugation and resuspension of cells in sterile water (ratio 1:1) (b) determine number of cells/gram, mass of slurry suspended in 25 mL wort to give 10^6 cells/mL	wort	(a) 1 g dry wt/L (b) 0.55 g dry wt/L	(1) not indicated (2) 20 °C	dry weight
Wheatcroft *et al.* (1988)	2.5 mL teflon cell	(1) magnetic stirrer (2) medium pre-aerated (3) jacketed vessel	centrifugation and resuspension of cells in N_2 sparged, ice cold Ringer's solution containing EDTA (ratio 1:3)	yeast maltose medium	1% wet weight/vol	(1) 20 min (2) 25 °C	cell count
UCT Method A and UCT Method B	glass vessels (75, 125 and 147 mm)	(1) magnetic stirrer (Heidolph MR 2002) (250 rpm) (2) surface aeration (3) (A) prior adjustment of medium (B) jacketed vessel	sample taken as is	(A) distilled, deionised water (B) distilled, deionised water with EDTA (1.9 g/L)	1 - 3 g dry wt/L	(A) (1) 10 min (2) 25 °C (B) (1) 5 min (2) 20 °C	(A) dry weight (B) cell count

Experimental parameters of importance in OUR measurement include dissolved oxygen concentration, temperature, test medium, pitching rate, duration of the test, method of conducting cell assay and handling of the yeast samples. These are discussed below. In addition, the range of oxygen utilisation rates obtained is strain dependent (Daoud and Searle 1986).

(i) Dissolved oxygen concentration (DO)
Above a certain dissolved oxygen concentration (DO), the oxygen utilisation rate is independent of the dissolved oxygen concentration of the test medium. For unicellular microorganisms, such as yeast, this critical dissolved oxygen concentration is in the range of 0.3 - 0.7 ppm (Daoud and Searle 1986). Peddie *et al.* (1991) confirmed that OUR is independent of DO over the range of 0.5 to 8 ppm. Both UCT methods rely on surface aeration on agitating prior to inoculation. The resultant initial and final DO values were approximately 7 ppm and 4 ppm respectively, so the systems operated well within the range in which OUR values are independent of DO concentration.

(ii) Temperature
As OUR is a function of growth rate, it is recognised that the oxygen utilisation rate increases with increasing temperature. For example, at 20°C the OUR of a sample of yeast is 0.22 mg O_2/min/g dry yeast, while the OUR of an identical yeast sample is 0.55 mg O_2/min/g dry yeast at 30°C (Daoud and Searle 1986). Higher test temperatures allow for more rapid evaluation of the OUR of a yeast sample (Wheatcroft *et al.* 1988). The dissolved oxygen concentration of the medium is also dependent on the temperature. Maintenance of constant temperature is important to ensure accurate and reproducible results. Jacketed vessels are generally used. For UCT Method A, the medium was pre-warmed to 25°C prior to inoculation with the yeast suspension. This temperature was generally constant for the duration of the test (10 min), but changes of up to 0.5°C have been observed. For UCT Method B the vessel was jacketed and connected to a circulating constant temperature waterbath (20°C).

(iii) Test medium
There is no significant difference in the OUR measured in different media (yeast maltose broth, MRS broth, wort, water and Ringer's solution), provided that the different saturation concentrations of the media are taken into account (Daoud and Searle 1986, Wheatcroft *et al.* 1988). This is consistent with the metabolism of intracellular glycogen during the initial 2 hours after pitching when the available extracellular carbohydrates are not metabolised (Quain *et al.* 1981). Peddie (1988) stressed the advantages of using water as a suspending medium. Of particular convenience is the fact that clean conditions, rather that sterile conditions are adequate. However, yeast tends to flocculate in water. Agitation of the medium during the test generally overcomes the flocculation of the yeast, but the extent of deflocculation influences the oxygen utilisation rate measured. To allow complete deflocculation, a deflocculant such as EDTA could be added to the test medium. For UCT method A, distilled, deionised water was used, while for UCT Method B, an EDTA solution (1.9 g/L) was used.

(iv) Pitching rate

Specific oxygen utilisation rate is independent of pitching rate (Daoud and Searle 1986). For comparative purposes, OUR values are thus always expressed as specific oxygen utilisation rates. Increased pitching rates can be used to speed up the test. Furthermore, high pitching rates (1 - 2 g dry weight/L) have been recommended to reduce the likelihood of changes in the oxygen utilisation rate with time (Peddie *et al.* 1991). The UCT methods used 1 to 3 g dry weight/L.

(v) Determination of biomass present

To report a specific oxygen utilisation rate, the quantity of yeast present in the test suspension is determined. Dry weights or cell counts are generally used. Dry weights may reduce the accuracy of the test, owing to the variability in the glycogen content of different samples of yeast. Peddie *et al.* (1991) suggested that cell counts provide greater accuracy, but make the assay less precise. For UCT Method A, dry weights were used to determine the cell concentration in the testing chamber, while cell counts were used for UCT Method B.

(vi) Sample handling

Peddie (1988) stressed the importance of limiting the exposure of the yeast sample to oxygen during sample handling and storage and the need to assess the OUR of the sample as soon as is practicable. Exposure to oxygen leads to variability in the results. Peddie *et al.* (1991) showed that prior to storage the oxygen utilisation rate may be variable, but after storage the OUR value is more constant. For comparative work it is thus important to standardise the procedure. In this study, yeast samples were stored under beer, thus limiting exposure to oxygen. Directly before inoculation into the test medium the samples were shaken briefly and a fixed volume is pipetted into the test medium.

(c) Reproducibility of the methods used in this investigation

Pooled standard deviations (s_{pooled}) of 0.0009 mg O_2/min g viable cells (dry weight) and 0.002 mg O_2/min/10^8 viable cells were calculated for Method A and Method B respectively (Appendix C, page C4). These standard deviations were calculated for 55 and 22 degrees of freedom respectively, hence both standard deviations (s) may be regarded as a good estimates of the population standard deviations (σ). The coefficient of variation for Method A was calculated to be 8%, while that for Method B was 18%. The larger standard deviation for the method using cell counts (Method B) is consistent with Peddie *et al.* (1991). However, both methods are not very precise and are thus not expected to be sensitive to small changes in the metabolic activity of the cells.

3.2.4.2 Acidification Power

(a) The significance of acidification power

Yeast cells attempt to keep a balance between the intracellular and extracelluar hydrogen ion (H^+) concentrations (Kara *et al.* 1988). Protons (H^+) are excreted spontaneously on transfer to an unbuffered medium or after the addition of a suitable substrate. Spontaneous H^+-efflux is sustained solely by endogenous H^+ reserves and energy sources such as glycogen. The energy for substrate-induced H^+-efflux is obtained from endogenous and fermentable exogenous sources (Opekarová and Sigler 1982, Kara *et al.* 1988). These observations form the basis of the Acidification Power Test (APT) developed by Opekarová and Sigler (1982), which measures both spontaneous H^+-efflux (spontaneous acidification power) and substrate-induced efflux of H^+ (substrate-induced acidification power).

The involvement of plasma membrane APT-ase in the extrusion of protons, suggests that the acidification power (AP) is dependent on membrane function as well as the glycolytic potential of the yeast cell (Henschke and Eglinton, 1991). Acidification power may thus be a good indicator of the physiological state of yeast cells. Opekarová and Sigler (1982) suggest that cells with acidification power values (defined in (b) below) of 2.5 to 1.0 are in good physiological condition, those with AP values of 1.0 to 0 are partially damaged and cells with AP less than 0 are considerably damaged.

It is expected that spontaneous AP values could be correlated to the endogenous energy reserves (glycogen) present in the cell, the initial rate of oxygen utilisation and the rate and extent of cell growth during fermentation. The substrate-induced AP may be correlate to the rate of substrate utilisation during fermentation. Since the success of a brewery fermentation depends on both the rate and extent of yeast growth and the rate of substrate utilisation, the overall AP (spontaneous AP plus substrate-induced AP) should give an indication of overall fermentation performance.

Manson and Slaughter (1986) showed a moderate correlation between fermentation time under brewery conditions and spontaneous AP. Kara *et al.* (1988) produced a range of yeast in different physiological conditions by fermenting yeast in worts with different initial oxygen concentration and by storing yeast for times at 4°C and 12°C. Fermentation performance was assessed as the time taken to ferment wort from 10 °Plato to 5 °Plato in laboratory fermentations. Good correlation between overall AP and fermentation performance was observed. The correlation coefficient was 0.942 for one yeast strain at 18°C and 0.990 at 12°C for another strain. Other strains showed similar results suggesting that the use of the APT as a predictor of fermentation performance has wide applicability. Kara *et al.* (1988) also showed correlation between AP and OUR (measured as described by Kara *et al.* (1987)). The correlation coefficient was 0.809.

Fernandez *et al.* (1991) conducted an investigation of the effect of different storage temperatures on the fermentation performance of yeast using the APT. Despite the

different AP values for 30 days of storage at 10°C, 15°C and 25°C (0.8 - 1.7), the fermentation performance of these yeasts was almost identical. These results suggest that AP values in isolation may not be sufficient to predict fermentation performance. Fernandez *et al.* (1993) demonstrated that the rate of acidification of the medium, the OUR and the rate of CO_2 evolution showed some correlation.

Mathieu *et al.* (1991) investigated the acidification power of yeast during fermentation. The spontaneous AP showed trends corresponding to typical glycogen profiles during fermentation and could also be correlated to the glycogen content of yeast cells during storage. Both these observations suggest that spontaneous AP can be used as an indicator of glycogen content.

When assessing AP during the course of fermentation, a minimum in the overall AP was observed after 1 day of fermentation (Mathieu *et al.* 1991). Yeast with similar initial AP values showed different minimum AP values. The minimum AP value could be correlated to fermentation performance (decrease in specific gravity) with a correlation coefficient of 0.84. Mathieu *et al.* (1991) developed a method to predict fermentation performance involving pretreatment of the yeast. Yeast samples were suspended in sterilized wort at the standard pitching conditions. After a 15 minute contact time, cells were centrifuged and subjected to the Acidification Power Test. The contact time was not regarded as significant, but a minimum of 15 minutes was recommended. Good correlation was observed between overall acidification power after pretreatment and fermentation performance (r^2 = 0.89).

The results of Kara *et al.* (1988) and Fernandez *et al.* (1993) caution against the use of the APT to predict the fermentation performance of acid washed yeast. Correlation was displayed in some strains, but not in others. The absence of correlation was noted for *Saccharomyces uvarum* J-2036, the main production strain of SA Breweries and confirmed by SA Breweries (O'Connor-Cox 1994). However, the potential of the APT to give an indication of the metabolic response of yeast to mechanical handling of yeast required investigation, hence its inclusion in this study.

(b) Equipment and procedure

For the Acidification Power Test developed by Opekarová and Sigler (1982), yeast cells were washed three times and suspended in sterile distilled water (pH = 6.3) at 15 to 30 g dry mass/L). After incubation at 30°C for 10 minutes, the suspension pH was measured and glucose added to a concentration of 45 mM. After another 10 minutes the pH was measured again. The acidification power (AP) was defined to be the difference between the initial pH (6.3) and the final pH (after 20 minutes).

Modifications of this are reported by Manson and Slaughter (1986), Kara *et al.* (1988), Mathieu *et al.* (1991), Fernandez *et al.* (1991 and 1993) and Patino *et al.* (1993). The method of Patino *et al.* (1993) (described overleaf) was used in this investigation.

A pre-weighed 50 mL plastic centrifuge tube was filled with 30 mL of yeast slurry and centrifuged (Beckman TJ-6) for 5 min at 3000 rpm. The supernatant was discarded and surplus yeast removed from the tube leaving 4.5 g of yeast. Washing of the yeast was conducted as follows: the yeast was resuspended in 25 mL of cold distilled water (2 - 4°C). Care was taken to ensure that no clumps remained and a homogenous suspension obtained. The suspension was centrifuged again and the liquid decanted. This washing procedure was repeated twice. After decanting the last supernatant, the yeast pellet was resuspended in 25 mL of cold distilled water. The suspension was poured into a beaker which has been placed on a magnetic stirrer. A second 25 mL aliquot of cold, distilled water was used to rinse the centrifuge tube and the rinse water added to the suspension in the beaker. The stirrer (Heidolph MR 2002), which was pre-set at 250 rpm, and a timer were started simultaneously. A clean pH probe (Hanna Instruments) was placed into the yeast suspension. Care was taken not to knock the pH probe against the stirrer, since this affected the calibration of the probe. The probe also had to be submerged sufficiently to obtain a stable pH reading. After 60 s the first pH reading ("time zero") was taken. pH readings were taken every minute for the first 10 minutes, whereafter 2.5 mL of a 20% (w/v) maltose solution was added. pH readings at 1 minute intervals continued for another 10 minutes. The temperature was recorded at 5 minute intervals starting at "time zero".

The spontaneous acidification power was taken to be the difference between pH 6.3 and the pH reading at 10 minutes (pH(10)). The substrate-induced acidification power was the difference between pH(10) and pH(20), the final pH reading. The overall AP was the difference between 6.3 and pH(20). In addition, as suggested by Patino *et al.* (1993), the pH values recorded at one minute intervals were converted to proton concentrations. The absolute difference in H^+ concentration between each successive minute was calculated and added together to give the Cumulative Acidification Power (CAP).

Several factors determine the accuracy and reproducibility of the Acidification Power Test. These are discussed below.

(i) The concentration of yeast in the final yeast suspension

Acidification power appears to be unaffected by large variations (up to approximately 10%) in the amount of yeast in the final suspension, provided a critical yeast concentration is exceeded (Henschke and Eglinton 1991). Opekarová and Sigler (1982) demonstrated that this critical concentration is 10 mg dry weight/mL (approximately 2 - 3 g wet weight/50 mL). The concentration of yeast in the method used in this investigation (4.5 ± 0.01 g wet weight/50 mL) and the methods of all other researchers mentioned in this report, exceed this limit.

(ii) Washing the yeast pellet

Repeated washing of the yeast pellet is time consuming. Kara *et al.* (1988) insisted that washing is necessary to remove material bound to the cell surface which could interfere with the results of the test. Attempts made to reduce the number of washes affected reproducibility. Hence three washes, as prescribed in all APT methods, were used. Physiological saline or a buffered medium could be used for washing, but the effect of this on the test requires investigation.

(iii) Calibration of the pH probe

Kara *et al.* (1988) stress the importance of the accuracy of the pH probe and suggest the use of all glass probes. The UCT method uses an all glass probe (Hanna Instruments) and a Hanna pH meter. The probe was calibrated using standard buffers. The calibration of the probe was checked at the end of each test

to see if any probe drift occurred. Generally the probe was stable, but probe drift of up to 0.05 pH units was observed in cases where the probe was knocked against the sides of the beaker or against the stirrer bar. Care was taken to prevent this.

(iv) Temperature control

Kara *et al.* (1988) centrifuged at 4°C to limit metabolic activity of the yeast. Washing and resuspension was done with water at 25°C. Presumably in the absence of a refrigerated centrifuge, Patino *et al.* (1993) used water at 4°C for washing and resuspension. The UCT method followed that of Patino *et al.* (1993). The temperature of the medium was observed to increase from 4°C to approximately room temperature during the 20 minutes of the AP test. The pH meter was fitted with a temperature probe for temperature correction. The metabolic rate of the yeast, however, is dependent on temperature. Since the temperature rise during the test appeared to be nearly identical for all samples tested and reproducibility was good, the rise in temperature was not expected to be of significance during the comparative work conducted. While maintenance of a constant temperature is desirable, resuspending yeast samples that are stored at 4°C in water at 25°C may constitute a heat shock. This may affect the physiological state of the yeast and the AP results. If the Cumulative Acidification Power is used to indicate the acidification power of the yeast sample, constant temperature during the test may be desirable. It is interesting to note that Patino *et al.* (1993), who define the Cumulative Acidification Power (CAP), use 4°C water in the test and have no means of temperature control. The effect of temperature in the Acidification Power Test requires further investigation.

(v) The pH of the suspending medium

The pH of the distilled water used by Opekarová and Sigler (1982) was 6.3. This differs from the pH of the distilled water used by other researchers and in this investigation. Since the acidification power value is always determined as 6.3 - pH(20), it would be advisable to adjust the pH of the suspending water to 6.3 in order to compare results obtained on different days and by different researchers.

(vi) Fermentable substrate added

Patino *et al.* (1993) showed that in order for the APT to have accurate predictive ability, it is important to use an exogenous energy source similar to that which the yeast will encounter during the fermentation and to which the yeast was exposed prior to the test. The AP values of identical samples of yeast were slightly lower when maltose was used instead of glucose. The UCT method used maltose in preference to glucose, since the brewery wort predominantly contained maltose.

(vii) Sample handling

Kara *et al.* (1988) report that AP values and fermentation performance decrease with increasing storage times (10 - 21 days). During an investigation of the effect of brewery storage on AP, Mathieu *et al.* (1991) observed no significant influence on AP results following 3 days storage (conditions not indicated). In this investigation, samples were stored in ice water at 4°C and analysed within 48 to

72 hours after sampling. No significant influence on AP was observed over this period.

(viii) Duration of the test
Opekarová and Sigler (1982) suggest that within the 10 minutes all pH changes are complete for both spontaneous and substrate induced acidification. In this investigation, the pH became constant within a few minutes during spontaneous acidification. During substrate-induced acidification, 10 minutes was insufficient for all changes in pH to occur. Although the rate of change of pH decreased towards the end of the test, the pH continued to drop after 20 minutes and a stable value was not necessarily reached. The evaluation of the results in terms of the Cumulative Acidification Power overcomes this problem to some extent. It may, however, be of use to continue the test to a stable pH value to gain additional information about the physiological state of the yeast. In the UCT method, the absence of stability may be attributed to the temperature increase in the yeast suspension during the course of the test.

(ix) Interpretation of the results
APT results were generally interpreted as suggested by Kara *et al.* (1988):

(i) Spontaneous acidification power	6.3 - pH(10)
(ii) Substrate-induced acidification power	pH(10)-pH(20)
(iii) Overall acidification power	6.3 - pH(20)

The pH readings may rise and fall quite considerably during the course of the test. This is not reflected if only the pH(10) (the pH reading 10 minutes after "time zero") and pH(20) (the final pH value) are interpreted. Although correlation between spontaneous AP and fermentation performance (Manson and Slaughter 1986) and between overall acidification power and fermentation performance (Kara *et al.* 1988) has been demonstrated, other methods of interpreting the results of the APT have been proposed to overcome the limitations of the standard interpretation of the APT results.

Fernandez *et al.* (1993) expressed the acidification rate as the change in pH between time zero and the end of the test over the duration of the test. This method still does not reflect the behaviour of the system during the test and a true indication of the acidification rate of the yeast is not obtained. In addition, owing to variations in the starting pH, rates should rather be expressed as a change in H^+ concentration.

Patino *et al.* (1993) observe that the Acidification Power Test reflects the energy change within a cell in terms of H^+ ions, measured on a logarithmic scale. Furthermore, pH increases caused by H^+ uptake by the cells, followed by the typical decline in pH have been observed. To represent the energy changes within the cells, Patino *et al.* (1993) expressed the results of the Acidification Power Test as Cumulative Acidification Power (CAP). This eliminates the logarithmic nature of the pH scale and takes into account all the pH changes that occur during the test. The CAP has been found to be more sensitive than the AP to changes in the

physiological condition of yeast and the subsequent fermentation performance (Patino *et al.* 1993).

(c) Reproducibility of the method used in this investigation
Pooled standard deviation (s_{pooled}) of 0.09 and 0.06 pH units were calculated for the spontaneous (AP(10)) and overall acidification power (AP). The coefficient of variation for the spontaneous and overall acidification power was 4% and 2% respectively (Appendix C, page C5). The pooled standard deviation (s_{pooled}) for the cumulative acidification power calculated for the spontaneous acidification period (CAP(10) was 1×10^{-5} and that for the acidification over the whole test (CAP) was 2×10^{-5}. The respective coefficients of variation were 32% and 15% (Appendix C, page C6). The values were all calculated for 43 degrees of freedom so the standard deviations (s) may be regarded as a good estimates of the population standard deviations (σ). The coefficients of variation suggest that the cumulative acidification power values (CAP(10) and CAP) would not be sensitive to changes in acidification power, while the acidification power values (AP(10) and AP) will be reasonably sensitive to acidification power changes resulting from stress conditions.

3.2.5 Intracellular Reserve Indicators

3.2.5.1 Glycogen content

(a) The significance of glycogen
Glucan, mannan, trehalose and glycogen occur as intracellular carbohydrates in brewing yeast. Of these, the acid-soluble glycogen fraction has been identified as a crucial internal reserve compound. Glycogen is a polymer of glucose in which subunits of 10 to 15 glucose units linked via α-1,4 glycosidic bonds are joined to each other by α-1,6 glycosidic bonds. It forms a readily available carbon and energy storage compound in yeast which can account for up to 40 % of the cell dry mass (Patel and Ingledew 1973, Quain *et al.* 1981, Quain and Tubb 1982, Murray *et al.* 1984). The accumulation of glycogen in brewing yeast shows 3 distinct phases: rapid depletion during the initial hours following pitching, accumulation during the bulk of the fermentation and gradual depletion towards the end of fermentation (Quain and Tubb 1982, Murray *et al.* 1984). The onset of glycogen accumulation appears to result from the onset of a nutrient limiting condition causing a decreased growth rate (Patel and Ingledew 1973, Quain 1981). This could be brought about by nitrogen limitation (Patel and Ingledew 1973) or possibly oxygen limitation. Decline in glycogen concentration at the end of fermentation is attributed to the provision of maintenance energy under starvation conditions (Quain 1988).

On pitching, a period of aerobic cell growth is required prior to the onset of fermentation. Studies of the relative rates of oxygen uptake and uptake of carbohydrates from the medium illustrate that oxygen uptake occurs in the first 4 hours. Uptake of glucose is only observed after 4 to 6 hours (Quain *et al.* 1981, Murray *et al.* 1984). Uptake of maltose and maltotriose both require induction and

undergo catabolite repression (Quain *et al.* 1981). Hence it is only the intracellular glycogen that is available as carbon and energy source initially. Metabolism of glycogen results in the generation of ATP, reducing power (NADPH) and the synthesis of sterols and fatty acids. These compounds are critical in maintaining the structure and function of the cell membrane and thus regulating transport across the cell wall and determining the extent of multiplication of cells (Quain *et al.* 1982, Murray *et al.* 1984, Quain 1988).

Quain *et al.* (1982) and Murray *et al.* (1984) have illustrated that fermentation performance is influenced by the intracellular glycogen levels on pitching. Reduced glycogen levels correlate with a reduced rate of attenuation and yeast growth. Quain *et al.* (1982) determined a stoichiometric ratio between glycogen consumption and sterol synthesis in the initial hours following pitching, in which 69 mg sterol are formed per gram of glycogen catabolised. A minimum requirement of 7 mg sterol per litre was suggested to ensure satisfactory fermentation of 10 °Plato wort. This correlates with a minimum glycogen concentration of 160 - 200 mg/L. Hence, pitching based on glycogen content in place of biomass may enhance fermentation predictability. The ability to overcome the negative effects of low glycogen content by overpitching has been shown by both Quain *et al.* (1982) and Murray *et al.* (1984) in standard and high gravity fermentations respectively. Pitching on a basis of biomass in place of cell number provides a partial correction for glycogen content.

Cantrell and Anderson (1983, cited by Quain 1988) determined a higher stoichiometric ratio between sterol synthesis and glycogen catabolism in large scale fermentations than observed in the laboratory by Quain *et al.* (1982). As oxygen is supplied over a longer period in the large scale system, the use of wort carbohydrates for partial sterol synthesis may occur. In addition, sample handling prior to glycogen analysis is of utmost importance owing to its rapid catabolism in the presence of oxygen. The study of Cantrell and Anderson as well as that of Sall *et al.* (1986) have resulted in the debate over the role of glycogen in predicting large scale fermentation performance remaining open.

(b) Equipment and procedure

Procedures reported for the quantification of glycogen in yeast can be divided into four groups:

1. Extraction and fractionation of internal carbohydrates based on the method of Trevelyan and Harrison (1956), followed by quantification using anthrone (Patel and Ingledew 1973, Quain *et al.* 1981, Quain 1988, Slaughter and Nomura 1992a)

2. Extraction and fractionation of intracellular glycogen, followed by enzymatic hydrolysis of glycogen and quantification based on glucose (Quain 1981, 1988, Murray *et al.* 1984, Sall *et al.* 1988)

3. Staining of the intracellular glycogen with iodine (Quain and Tubb 1983, Quain 1988)

4. Determination of glycogen by analysis of near infrared reflectance (NIR) spectroscopy (SA Breweries, personal communication 1994)

In the first method, glycogen is extracted into acid and alkali. The amount of hexose in each fraction is determined non-specifically by the anthrone reagent. Comparison of this to other methods has suggested substantial overestimation of glycogen at low concentrations (1.5 - 10 fold) owing to partial extraction of β-1,6-glucan. However, at high concentrations of glycogen (greater than 30%), underestimation occurs due to inefficient extraction (Quain 1981, 1988).

In order to improve the analysis, improvement of the extraction of glycogen and of its quantification can be considered separately. To enhance specificity for glycogen in the quantification step, an enzymatic method is used in which glycogen is selectively hydrolysed by amyloglucosidase and the released glucose determined enzymatically by a system such as the hexokinase/glucose 6-phosphate dehydrogenase system (Becker 1978, Quain *et al.* 1981). Interference from mannan and glucan found in the alkali and acid extracts respectively is prevented.

Several methods of extraction have been compared by Quain (1981). The basis of these and their relative shortfalls and advantages are given in Table 3.5.

Table 3.5 Methods of extracting glycogen

Method	**Recorded shortcomings and advantages**
Digestion in hot KOH. Precipitation of glycogen by adding ethanol.	Oligosaccharides do not precipitate. Excessive precipitation times of alkaline extracts.
Digestion in sodium carbonate (0.25 M, boil, 45 min). Precipitation of mannan from alkali extract. Digestion in perchloric acid (0.5 M, boil, 30 min) to form the acid-soluble fraction.	Acid-soluble fraction includes β-1,6-glucan. Hydrolysis of carbohydrates occurs during prolonged digestion at high temperature
Digestion in sodium carbonate (0.25 M, boil, 90 min)	Hydrolysis of cell wall to enable the *in situ* enzymatic digestion of glycogen. Hydrolysis of carbohydrates occurs during prolonged digestion at high temperature.
Digestion in sodium carbonate (0.25 M, 100°C, 15 min), resulting in alkali-soluble fraction. Digestion in perchloric acid (0.5 M, boil, 10 min) resulting in acid-soluble fraction. Adjustment of pH.	Chemical hydrolysis of carbohydrates minimised (~3%). Extraction times minimised by reduction of the yeast:extractant ratio. Improved extraction efficiency.

Combination of the last extraction method indicated in Table 3.5 and the enzymic determination of glycogen is the recommended procedure in this category. However, a linear relationship between the other combinations and this method has been shown (r^2 = 0.89 - 0.92). While 20 to 30 samples can be processed in a working day, this method remains applicable in the research environment, rather than as a routine technique to determine pitching rates (Quain 1981, 1988).

The advantage of a rapid method of glycogen determination has been illustrated. A procedure based on the staining of intracellular glycogen reserves with an aqueous solution of iodine and potassium iodide has been proposed (Quain and Tubb 1983). The intensity of staining can be determined by absorbance and is proportional to the amount of glycogen determined enzymatically (r^2 = 0.95), provided the final yeast concentration does not exceed 4 g dry mass per litre. The following relationship between absorbance at 660 nm and glycogen concentration (determined enzymatically) was determined across 42 experimental data points using *Saccharomyces cerevisiae* NCYC1026:

$$[\text{glycogen}] = (A_{660} - 0.26) / 1.48$$

Criticism of this method includes poor resolution at low concentration (O'Connor-Cox, 1994), problems with flocculent yeast and unsuspecting extrapolation beyond the demarcated range (Quain 1988). A qualitative determination of glycogen content can be made by staining of the cells with an I_2/KI solution and viewing microscopically. A dark brown coloration corresponds to a high glycogen concentration whereas a yellow coloration corresponds to the absence of glycogen.

SA Breweries have adopted the use of near infrared reflectance (NIR) spectroscopy to quantify intracellular glycogen. In mid-infrared spectra, peaks in the region 1000 to 700 cm^{-1} correspond to carbohydrate compounds. Differences in the second derivatives of such spectra at 995, 965, 850 and 813 cm^{-1} have been attributed to variations in the mannan and glycogen content of yeast. Correlation between the peak height ratio at 994/965 cm^{-1} and 850/813 cm^{-1} and enzymatically determined glycogen concentrations have been shown (r^2 = 0.81-0.83). This correlation was used to establish a calibration curve for the readings of the infrared spectrometer (Perkin-Elmer 1750). The use of dried samples in place of fresh yeast was investigated. Drying yeast in a microwave did not have a significant effect on the catabolism of glycogen hence the technique was developed for dried yeast (Moonsamy *et al.* 1995).

In this investigation, the glycogen content of cells was quantified by the NIR technique of SA Breweries.

Samples were stored under beer in the absence of oxygen at 2 to 4°C. Yeast was collected by centrifugation (Beckmann J2-2/M/E, GSA rotor) at 4°C. Centrifugation was used in place of filtration owing to better temperature control, lower exposure to oxygen and greater efficiency. The pellet was transferred to a ceramic plate and microwaved (Sharp Carousel) at medium-high for 20 minutes. The dried cells were ground in a coffee grinder (Krups). The yeast powder analysed by NIR spectrometry and glycogen concentrations determined according to the calibration curve established by SA Breweries.

Advantages of the SA Breweries technique include the simplicity of the procedure, the ability to perform the analysis on dried yeast samples which can be stored indefinitely and the rapidity with which results can be generated. However, the low correlation coefficient between peak ratios and enzymatically determined glycogen concentration used in establishing the calibration curve is expected to translate into a relatively high determinate error. This was not of great consequence in this investigation which relied on comparisons between different samples analysed in the same way. Owing to the rapid catabolism of glycogen, the handling of yeast prior to and during drying could result in large indeterminate errors. Care was taken to ensure uniform sample preparation.

(c) Reproducibility of the method used in this investigation
The pooled standard deviation (s_{pooled}) of NIR glycogen assay was calculated to be 1% (w/w) for 49 degrees of freedom, hence it may be regarded as a good estimate of the population standard deviation (σ) (Appendix C, page C7). The coefficient of variation was 4%, which indicates that the test would be reasonable sensitive to differences in glycogen content.

3.2.6 Stress Resistance Indicators

3.2.6.1 Trehalose content

(a) The significance of trehalose
Trehalose, 1-α-D-glucopyranosyl-α-D-glucopyranoside, is a non-reducing disaccharide which consists of two glucose molecules linked together by an α-1,1-glucosidic bond. Traditionally trehalose was regarded as a reserve carbohydrate. Trehalose accumulates under conditions of nutrient (carbon, nitrogen or phosphate) limitation (Quain 1991). Yeast habitually accumulates soluble reserve compounds, such as amino acids and polyphosphates, within vacuoles. However, trehalose is a purely cytosolic compound (Wiemken 1990). Wiemken (1990) argues that large quantities of trehalose in the cytoplasm should affect the state of the cytosol, particularly with respect to water activity. This may result in the slowing down of the metabolism, promoting a transition to a resting state. Under conditions of nutrient limitation, trehalose may thus act as a protecting agent which inhibits metabolic activity to prevent function and preserve the structural integrity of the cytoplasm. Other environmental stresses such as heat shock, desiccation, freezing and exposure to toxic chemicals (eg ethanol, cadmium, copper sulphate and hydrogen peroxide) also induce the accumulation of trehalose (Wiemken 1990, Quain 1991). This further substantiates the hypothesis that trehalose is a stress protectant rather than a reserve carbohydrate.

It has been proposed that cells with high trehalose contents are more resistant to environmental stresses than cells with low trehalose contents. In an investigation of the osmotolerance of twelve different yeast strains, D'Amore *et al.* (1991) noted that osmotolerant yeast strains had high trehalose contents. Strains with high trehalose contents were also more resistant to long term storage (freezing at -20°C

and storage under beer at 4°C). Van Dijck *et al.* (1995) confirm the correlation between trehalose content and stress resistance for stationary phase cells and cells grown on non-fermentable carbon sources. The correlation does not hold for fermenting cells where the initiation of fermentation (by the addition of glucose) results in the mobilisation of trehalose and subsequent loss of stress resistance. In the complete absence of trehalose, non-fermenting cells are more stress resistant than fermenting cells. This suggests that there are factors other than trehalose which contribute to stress resistance in non-fermenting cells. One of these factors could be heat shock proteins, which are known to be induced by environmental stress (Van Dijck *et al.* 1995, Wiemken 1990). For stationary phase cells and cells growing on non-fermentable carbon sources, Van Dijck *et al.* (1995) suggest that specific factors which are responsible for high stress resistance appear to be present. Only when these factors are present is trehalose able to give a further improvement in stress resistance.

Yeast handling subsequent to fermentation involves stationary phase cells in a nutrient deprived state. Mechanical handling, cooling, storage, acid washing and other handling procedures may expose these cells to additional environmental stress. The trehalose content of these cells may affect their ability to survive these stresses. In an investigation of the changes in brewing yeast during storage under industrial conditions, Sall *et al.* (1988) could not establish a correlation between trehalose content and fermentation performance. The investigations did not take into account the absolute levels of trehalose present in the cells under investigation. D'Amore *et al.* (1991) attempted to correlate the initial trehalose content with subsequent fermentation performance after storage. Using several different strains of yeast, D'Amore *et al.* (1991) conducted a laboratory study to determine the role of trehalose in the maintenance of physiological condition during brewery storage conditions (4°C, 30 days). For each strain of yeast investigated, cells with higher initial trehalose contents displayed higher cell "viabilities" (as indicated by plate counts) after storage under beer for 30 days than cells with lower initial trehalose content. The relationship between the amount of trehalose present and the ability to remain replicatively competent during storage appeared to be strain dependent. In some strains the effect of higher trehalose contents was quite dramatic with "viabilities" of up to 99% after 30 days of storage. D'Amore *et al.* (1991) did not extend their work to investigate the relationship between trehalose content and subsequent fermentation performance.

The correlation between trehalose content and the maintenance of cell "viability" during storage appear to be well established. O'Connor-Cox (1995) mentions the superior fermentation performance of pitching yeasts with higher initial trehalose contents over those with lower initial trehalose contents and suggests that trehalose content be used as a predictor of fermentation performance. The influence of trehalose on the response to yeast handling procedures (eg. pumping, transport through lines, cooling in a heat exchanger, acid washing) is not known. This forms part of the current investigation on yeast handling.

(b) Equipment and procedure

Several methods that have been developed to determine the intracellular trehalose contents of cells. These include thin layer chromatography, high pressure liquid chromatography (HPLC), the anthrone method, enzymatic methods and near infrared reflectance (NIR) spectroscopy (Kienle *et al.* 1993, SA Breweries: personal communication 1995). Two types of enzymatic method are used, namely kinetic and end-point methods (Kienle *et al.* 1993).

Thin layer chromatography is highly specific, but very time consuming, while the HPLC method is not as specific or as rapid as any of the other methods (Kienle *et al.* 1993). In the anthrone method, trehalose is assayed according to the method of Lillie and Pringle (1980). After washing out glucose from intact cells, anthrone is used to determine the amount of sugar remaining in the cells. This method is not specific since anthrone reacts with different sugars. However, the results obtained are similar to those obtained by the end-point enzymatic methods, since trehalose is essentially the only remaining sugar. This method was used in the studies by D'Amore *et al.* (1991), Slaughter and Nomura (1992a) and Sall *et al.* (1988).

Both kinetic and end-point enzymatic methods use trehalase, the enzyme which breaks down trehalose. The rate of the trehalase reaction varies with the concentration of trehalose and can thus be used as an indicator of the trehalose content of the cells. This is the basis of the kinetic method. The method is sensitive to interferences from the sample to be assayed and is strongly dependent on the purity of the enzyme used for the assay (Kienle *et al.* 1993).

In the end-point enzymatic method of Kienle *et al.* (1993), acid trehalase is purified from a *suc2* mutant of *Saccharomyces cerevisiae*. The enzyme catalyses the breakdown of trehalose into its constitutive glucose monomers. Cells are washed to remove glucose prior to extraction of trehalose. The extracted trehalose solution is treated with the acid trehalase, whereafter a commercial glucose oxidase/peroxidase system (Boehringer Mannheim Biochemica) is used to determine the glucose content. The assay is highly specific since the acid trehalase used was found to hydrolyse no other disaccharide than trehalose.

The end-point enzymatic method described by Kienle *et al.* (1993) is essentially the same method used by Van Dijck *et al.* (1995) and by SA Breweries except that Van Dijck *et al.* (1995) use trehalase extracted from *Humicola* and the SA Breweries method use trehalase extracted from porcine kidney (Sigma). Kienle *et al.* (1993) caution against the use of these enzymes since they lack the specificity of acid trehalase which reduces the accuracy of the method.

As for glycogen, SA Breweries use NIR spectrometry for trehalose determination. This method was used in this investigation and samples for trehalose analysis were prepared and analysed as described for the glycogen assay (Section 3.2.5.1 (b)).

The calibration curve for the NIR spectrometry was based on the enzymatic method described above hence the accuracy of the trehalose method is dependent on both

the accuracy of the enzymatic method and the degree of correlation between the spectrometer and enzymatic results. Furthermore, microwave drying results in a significant increase in the temperature of the yeast sample. Since temperature and heat shock are known to induce the formation of trehalose (Quain 1991, Wiemken 1990), the results obtained using the NIR spectrometer may not be representative of the original samples. Since relative trehalose contents are of importance in this study, the accuracy of the values was not of prime concern. Provided the induction of trehalose is constant, this should be eliminated through calibration with the enzymatic method. Care was taken to standardise the drying procedure in order to avoid large indeterminate errors.

(c) Reproducibility of the method used in this investigation

The pooled standard deviation (s_{pooled}) for replicate measurements was calculated to be 1% (w/w) for 49 degrees of freedom (Appendix C, page C7). This standard deviation may be regarded as a good estimate of the population standard deviation (σ). Although the standard deviation of the assay is the same as that of the glycogen assay, the lower trehalose content of the yeast cells ($\pm$ 4%), results in a large coefficient of variation of 26%. Although the sensitivity of the NIR technique to changes in glycogen content may be reasonable, the technique will not be sensitive to changes in trehalose content.

3.2.7 Small Scale Fermentations

(a) Background

Yeast quality can be assessed most comprehensively by conducting small scale fermentations in which fermentation occurs under similar conditions to the brewery fermentations. Aspects of yeast quality that can be determined include fermentation rate, cell growth, the production of secondary metabolites and undesirable compounds (Henschke and Eglinton 1991). Small scale fermentations, however, take several days to complete and are thus not of use to predict the fermentation performance of pitching yeasts in commercial brewing situations. Several rapid small scale fermentation techniques have been developed (D'Amore *et al.* 1991, King *et al.* 1981, Manson and Slaughter 1986). These are generally conducted with agitation and at higher temperatures in order to accelerate the fermentation process. Approximately 24 hours may still be required before reliable and reproducible results can be obtained (Lentini 1993), hence this technique is also of limited use in commercial brewing applications.

While small scale fermentations may not truly reflect the industrial situation (Henschke and Eglinton 1991), they remain a useful research tool. Where rapid results are not essential for decision-making, the standard, non-accelerated small scale fermentations can be used. Where a large number of experiments need to be conducted, the more rapid fermentations may be more appropriate.

(b) Equipment and procedure

Several small scale fermentation techniques have been reported. The standard, non-accelerated systems are summarised in Table 3.6. These are generally not agitated and are terminated at different stages depending on the information required from the experiment.

For this investigation, two sets of standard small scale fermentations were set up. One (the SAB/UCT system) consisted of 2 L EBC tubes (EBC Analytica Microbiologica 1977) and the other (the UCT system) of standard 500 mL measuring cylinders.

The wort for the fermentations was filtered to remove any particulates (Whatman no. 1) and appropriate volumes dispensed into the shaking vessels: 2 L wort into 5 L round bottomed flasks (2 L system) and 500 mL wort into 1 L screw cap bottles (500 mL system). The shaking vessels were steam sterilised for 30 minutes in an open autoclave. Prior to inoculation of the wort at room temperature, 0.08 g/L of a yeast food (Nutromix), which had been autoclaved separately, was added. The pitching rate and aeration procedure are described in Table 3.6. After aeration, the yeast/wort mixture was transferred to the fermentation vessels and placed in a temperature controlled (11°C) water bath.

The fermentations were terminated when no change in specific gravity occurred over a period of 24 hours (usually after 12 days of fermentation). The small scale fermentations were monitored as follows:

(1)	Yeast growth	Cell counts were performed daily by Coulter Counter (Industrial D model). Total biomass production was estimated by determining the wet mass of the settled yeast bed at the end of fermentation and expressing this relative to the initial wet mass of yeast pitched.
(2)	Wort attenuation	The specific gravity of the medium was determined daily using a densitometer (Anton Paar DMA 55).
(3)	pH, SO_2, acetaldehyde and diacetyl	Samples were taken after 8 and 12 days of fermentation for the 2 L EBC system and at the end of fermentation for the 500 mL system (due to limited volume available for sampling). The analyses were done according to the standard procedures used by SA Breweries (Pickerell *et al.* 1991).

Table 3.6 Summary of small scale fermentation systems

Researchers	Yeast Strains	Vessels	Medium (1) type (2) volume	Pitching Rate	Temperature	Oxygenation of Medium
Ahvehainen and Mäkinen (1981)	*S. cerevisiae* VTT-A-63015 VTT-A-66024	12 L tube (height 1 m)	(1) a. all-malt wort (original extract 10.2-10.5 w/v) b. high gravity wort (original extract 16.0-16.5 w/v) (2) (not indicated)	2.5 g/L (centrifuged yeast)	10°C	pre-aerated to 8 mg/L dissolved O_2
Fernandez *et al.* (1991) Fernandez *et al.* (1993)	*S. uvarum* CC-0101, CC-0103 CC-0104, CC-0111 *S. uvarum* J 3015, J 2036	2 L Erlenmeyer flasks	(1) wort (15 °Plato) (2) 1.4-1.6 l	18 ± 2 x 10^6 cells/mL	Day 0 - 3: 10°C Day 3 - end: 15°C	pre-aerated to saturation
Kara *et al.* (1988)	*S.cerevisiae* NCYC 1681 *S. uvarum* NCYC 1324 3 Commercial Strains	1.5 L tall tube fermenters	(1) all-malt wort (10 °Plato) (2) (not indicated)	2.5 g/L (centrifuged yeast)	12°C 18°C	pre-aerated to saturation
Quain *et al.* (1981) Quain and Tubb (1982)	*S.cerevisiae* NCYC 240 NCYC 1062	(not indicated)	(1) a. all-malt wort (10 °Plato) b. yeast extract, peptone, salt medium with glucose or glucose and maltose (2) 1.5 l	5 x 10^6 cells/mL 10 x 10^5 cells/mL	20°C	pre-aerated to saturation
Sall *et al.* (1988)	*S. uvarum* (strain not specified)	2 L Pyrex cylinder	(1) production wort (15 °Balling) (2) 2 l	20 x 10^5 cells/mL	14°C	pre-aerated to 8 mg/L dissolved O_2
Slaughter and Nomura (1992a)	*S.cerevisiae* NCYC 1108	1 L bottle	(1) malt extract medium (10° Plato) (2) 700 ml	1 x 10^7 cells/mL	20°C 30°C	(not indicated)

Table 3.6 (continued)

Researchers	Yeast Strains	Vessels	Medium (1) type (2) volume	Pitching Rate	Temperature	Oxygenation of Medium
UCT/SAB (2 L system) Kruger *et al.* (1982) Pickerell *et al.* (1991)	*S. uvarum* AJL 2036	2 L EBC fermentation tubes	(1) production wort (±16 °Plato) (2) 2 l	14 g/L yeast slurry (at 60% consistency (wet weight) and 100% "viability" as indicated by methylene blue staining) (± 25 x 10^6 cells/mL)	11°C	After inoculation (2 L wort in 5 L round bottomed flasks) shaken 35x through two foot arc. Shaking (35x) repeated after 4 hrs, evolved gas released, flasks reshaken (35x) before pitching in fermenters.
UCT (500 mL system)	*S. uvarum* AJL 2036	500 mL standard measuring cylinders	(1) production wort (±16 °Plato) (2) 500 ml	As above	As above	As above, with 500 mL wort in 1 L screw cap bottles (Schott)

To allow comparison between the rate and extent of wort attenuation, attenuation profiles of substrate utilisation (expressed as °Plato) were established for the fermentations. Exponential curves of the form

$$dS/dt = \alpha e^{\beta t}$$

where S is the substrate concentration, t is fermentation time and α and β are constants, were fitted to data generated over the first 150 hrs of fermentation. In the above expression, the α-constant corresponds to the initial substrate concentration, while the β-constant corresponds to the rate of substrate utilisation. Figure 3.1 is an example of typical experimental data obtained and the exponential fit. The correlation coefficient is 0.97, which indicates that an exponential expression provides a good fit to the data over the initial fermentation period.

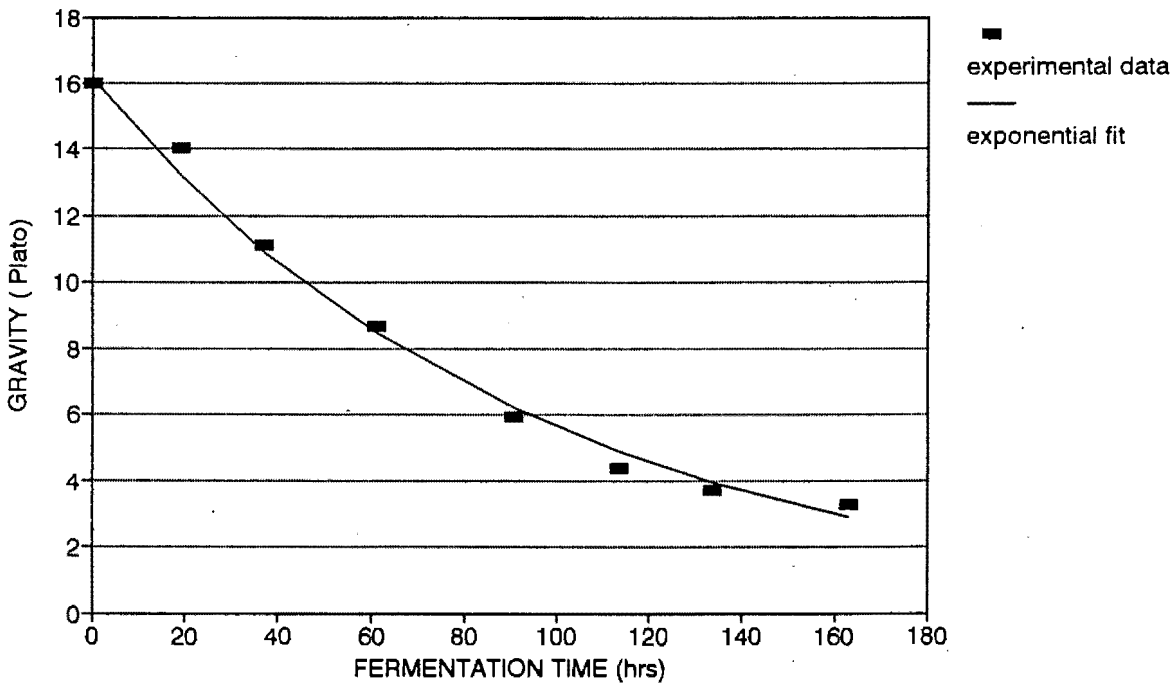

Figure 3.1 Example of typical attenuation data over the initial fermentation period and the exponential curve fitted to the data

Several parameters are important to ensure the accuracy and reproducibility of the fermentations.

(i) The method of pitching
Yeast can either be pitched directly as slurry or as an amount of centrifuged yeast. The pitching rate can be defined in terms of mass or cell number.

In the method of SA Breweries adopted for this investigation (Kruger *et al.* 1982, Pickerell *et al.* 1991), the pitching rate is adjusted to correct for differences in physiological condition of the yeast based on methylene blue staining and

differences in the biomass concentration of the slurry. The biomass concentration or "consistency" of the slurry is expressed in terms of the wet mass of yeast in the slurry determined by centrifugation (10 minutes, 1600 g). The required mass of yeast is then pitched directly as a slurry. If the slurry is inhomogeneous, this may lead to inaccurate pitching. The same problem would occur if the slurry were to be pitched volumetrically based on cell counts. Pitching with centrifuged yeast could eliminate this inaccuracy provided the centrifugation is performed reproducibly.

Expression of the pitching rate in terms of mass rather than cell number may have a compensatory effect. The mass per cell is a function of its glycogen content. Cells with a low glycogen content do not ferment as well as cells with high glycogen contents (Quain *et al.* 1982, Murray *et al.* 1984), yet if the pitching rate were defined in terms of mass, more cells with lower glycogen content would be pitched and the fermentation may not differ significantly from that of less yeast with a higher glycogen content. In brewery applications this compensatory effect is beneficial, however, when small scale fermentation is used to assess the physiological condition of the yeast, it reduces the sensitivity of the small scale fermentations to detect subtle differences in the physiological condition of the pitching yeast.

(ii) Wort oxygenation

For accuracy and reproducibility, the standardisation of wort oxygenation is essential. This can best be achieved by the injection of oxygen prior to inoculation through a glass sinter equipped with a flow meter. The dissolved oxygen concentration can be measured using an electrode. Less accurate methods include shaking the wort in half-filled flasks for 30 minutes on an orbital shaker at 120-140 rpm or in the case of 2 L EBC fermentation tubes by inverting the fermentation tubes 20 times after pitching (EBC Analytica Microbiologica 1977).

In all methods reported by researchers other than those associated with SA Breweries, the fermentation medium is aerated to saturation prior to pitching. In the UCT/SAB method wort oxygenation occurs through shaking as described in Table 3.6. While the method will provide equivalent oxygen transfer if conducted correctly, it is subject to operator variability, inaccuracy and reduced control. However, it does minimise the rate of change of dissolved oxygen concentration by providing oxygen transfer in the presence of the yeast. The SAB method was adhered to for comparative purposes. The reproducibility of the fermentations will be discussed in (c) below.

(iii) Temperature control

The maintenance of the desired fermentation temperature is essential. Temperature control of both the 2 L EBC tubes and the 500 mL measuring cylinders was achieved by placing these in waterbaths, designed to ensure a uniform temperature distribution.

(c) Reproducibility of the 2 L and 500 mL fermentations

Figure 3.2 and 3.3 provide cell count and attenuation profiles respectively for 5 replicate fermentations in the 2 L EBC tube system. Figure 3.4 and 3.5 are cell count and attenuation profiles respectively for 4 replicate fermentations in the 500 mL measuring tube system (using a different yeast sample at twice the standard pitching rate). The pooled standard deviations of the fermentation and beer quality indicators for all the fermentations done during the investigation were calculated. The detailed calculations are given in Appendix C (pages C8 - C15) and the statistical parameters are summarised in Table 3.7 (2 L system) and Table 3.8 (500 mL system).

The pooled standard deviations for the 2 L system (Table 3.7) were calculated for 15 - 17 degrees of freedom. While this is less than the recommended minimum of 20 degrees of freedom (Appendix B), the values are based on a reasonably large number of independent sets and can be regarded as reasonable estimates of the population standard deviations. The pooled standard deviations for the 500 mL system were calculated for 19 - 22 degrees of freedom (Table 3.8) and may be regarded as good estimates of the population standard deviation.

From the Table 3.7 and Table 3.8 it can be seen indicate that pooled standard deviations of the final attenuation values for the 2 L and 500 mL systems were 0.12 and 0.07 respectively and those for the α-constants were 0.19 and 0.16 respectively (Table 3.7 and Table 3.8). This confirms that with regards to the attenuation, the 500 mL fermentations (Figure 3.5) were more reproducible than the 2 L fermentations (Figure 3.3).

There appears to be less scatter in the pH, diacetyl and acetaldehyde levels, but more scatter in the final SO_2 level of the final beer produced in the 2 L system (Table 3.7) compared to that produced in the 500 mL system (Table 3.8). Differences in the absolute values of these final beer quality indicators in the two systems produced the differences in the coefficients of variation. The large coefficient of variation ($> 8\%$) for the diacetyl, SO_2 and acetaldehyde levels in both systems, however, suggest that the beer quality indicators are not expected to be very sensitive to differences in the quality of the beer produced in the small scale fermentations.

The standard deviations of the beer quality indicators are larger after 12 days of fermentation than after 8 days of fermentation (Table 3.7). This is most marked for the acetaldehyde content. Changes that are independent of the quality of the pitching yeast thus appear to occur during this fermentation period. If differences in yeast quality are to be highlighted, it may be more appropriate to terminate the fermentations at an earlier stage. However, comparisons made at the end of fermentation (constant density over a 24 hour period) are be more representative of the brewery situation.

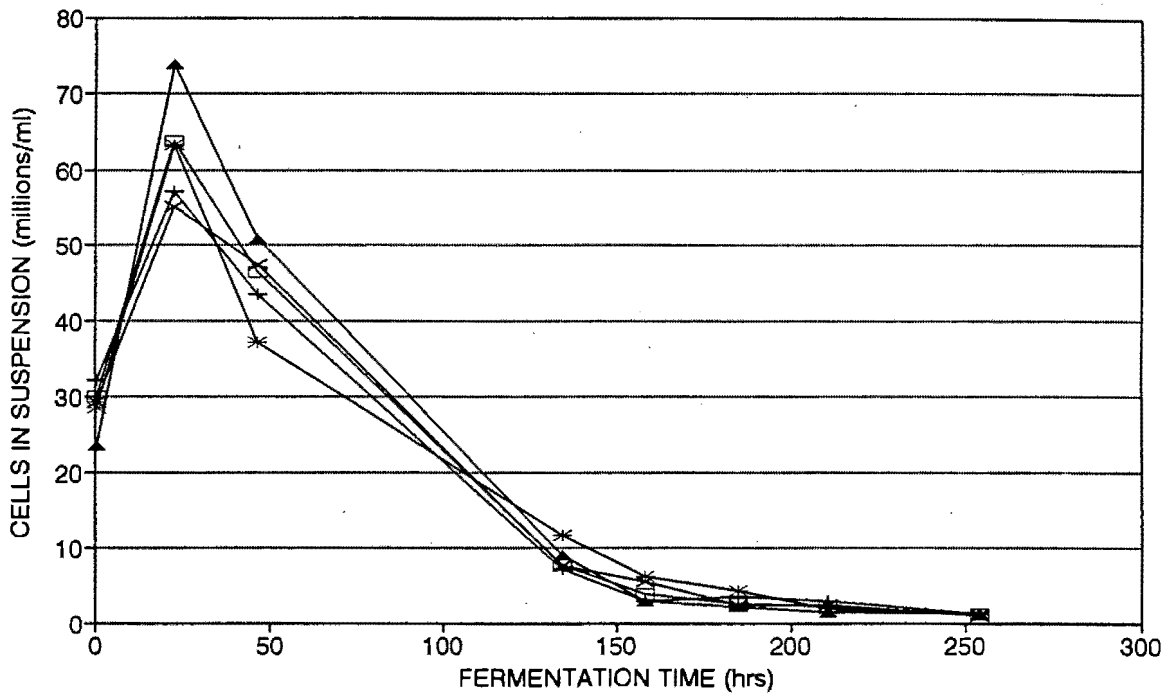

Figure 3.2 Cell count profiles for replicate fermentations done in 2 L EBC tube system

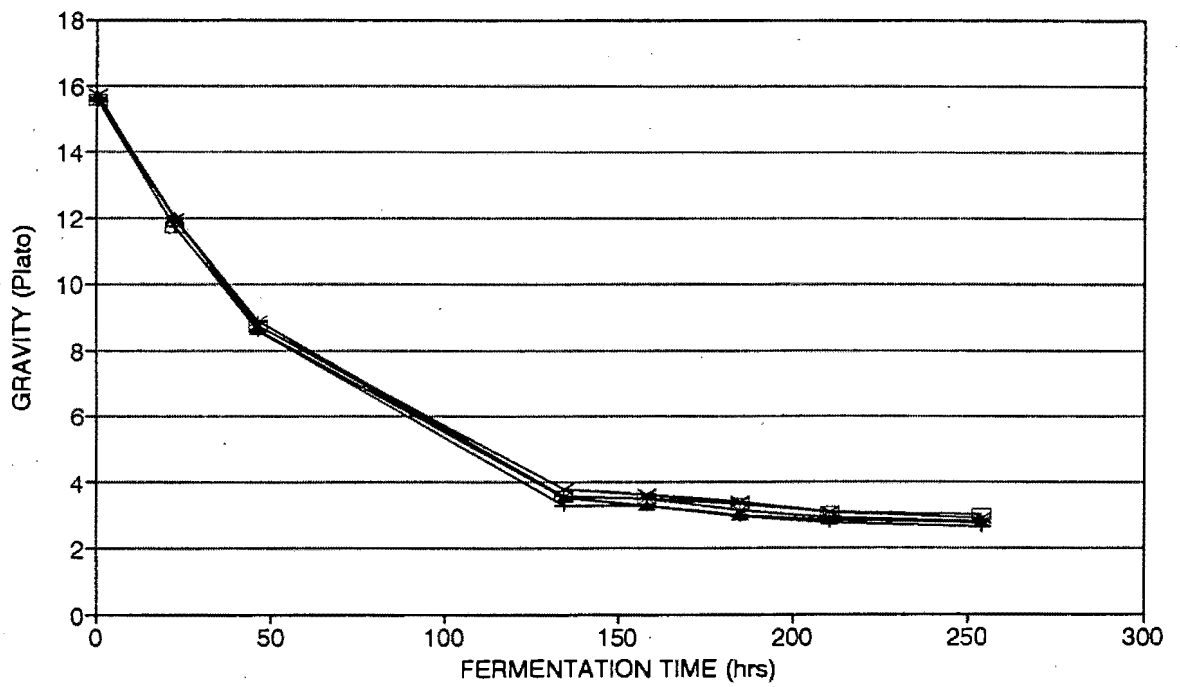

Figure 3.3 Attenuation profiles for replicate fermentations done in 2 L EBC tube system

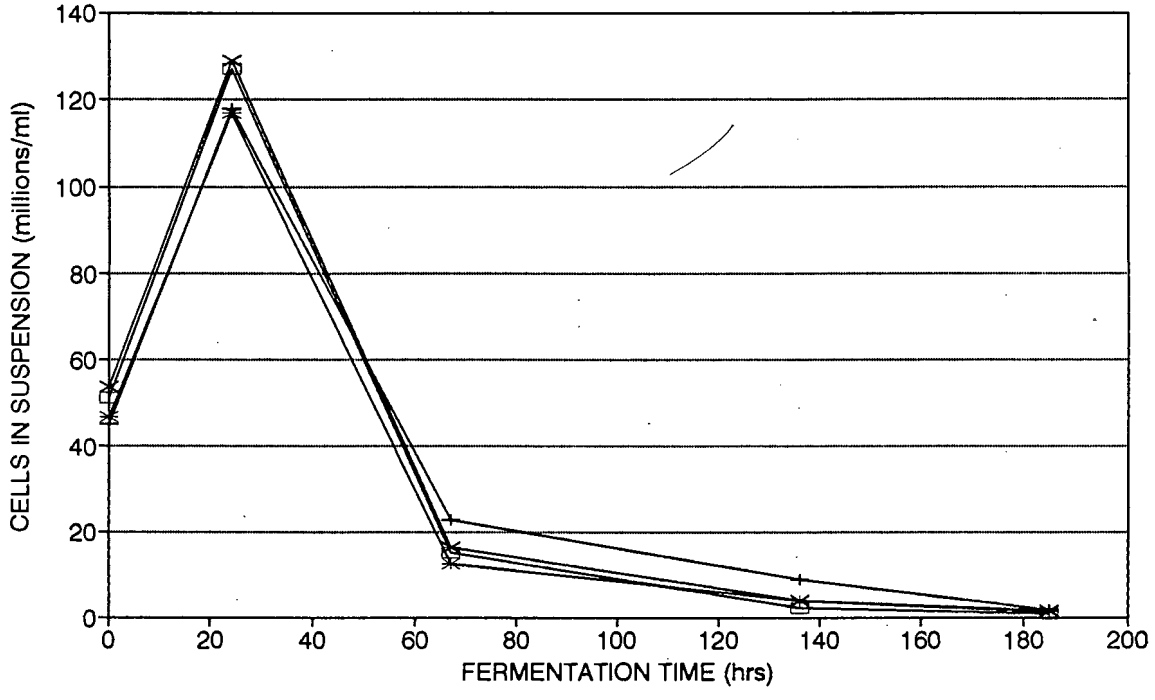

Figure 3.4 Cell count profiles for replicate fermentations done in 500 mL measuring cylinder system

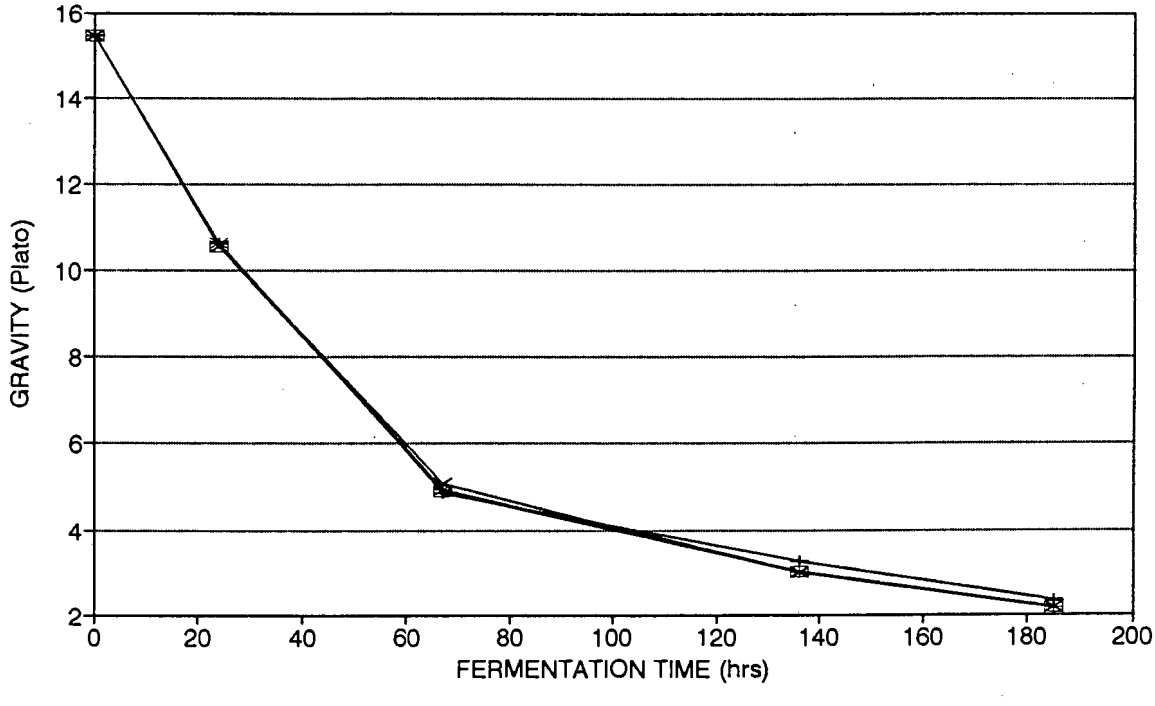

Figure 3.5 Attenuation profiles for replicate fermentations done in 500 mL measuring cylinder system

Table 3.7 Summary of statistical parameters indicating reproducibility of fermentations done in the 2 L EBC tube system

	Pooled Standard Deviation (s_{pooled})	Coefficient of Variation (%)	Degrees of Freedom (v)
Fermentation Indicators			
Increase in Biomass	0.2 (factor)	7	16
Final Attenuation	0.12 (°Plato)	5	16
α-Constant	0.19 (°Plato)	1	17
β-Constant	0.0003 (hr^{-1})	4	17
Beer Quality Indicators (Day 8)			
pH	0.03 (pH units)	1	16
Diacetyl	9 (ppb)	8	15
SO_2	2 (ppm)	19	16
Acetaldehyde	1 (ppm)	6	16
Beer Quality Indicators (Day 12)			
pH	0.04 (pH units)	1	16
Diacetyl	10 (ppb)	13	16
SO_2	2 (ppm)	20	16
Acetaldehyde	4 (ppm)	14	16

Table 3.8 Summary of statistical parameters indicating reproducibility of fermentations done in the 500 mL system

	Pooled Standard Deviation (s_{pooled})	Coefficient of Variation (%)	Degrees of Freedom (v)
Fermentation Indicators			
Increase in Biomass	0.2 (factor)	6	21
Final Attenuation	0.07 (°Plato)	4	21
α-Constant	0.16 (°Plato)	1	22
β-Constant	0.0003 (hr^{-1})	2	22
Beer Quality Indicators (Day 12)			
pH	0.05 (pH units)	1	21
Diacetyl	13 (ppb)	11	19
SO_2	1 (ppm)	8	20
Acetaldehyde	5 (ppm)	21	21

(d) Comparison of fermentations in the 2 L EBC tube and 500 mL measuring cylinder systems

Figure 3.6 and Figure 3.7 are examples of cell count and attenuation profiles (respectively) for fermentation of identical yeast samples using the 2 L EBC tube system and the 500 mL measuring cylinder system. The fermentation and beer quality indicators for the two systems are compared in Table 3.9.

Table 3.9 Comparison between fermentations done in 2 L EBC tube system and 500 mL measuring cylinder system

	2 L EBC Tube System	**Pooled Standard Deviation (s_{pooled})**	**500 mL Measuring Cylinder System**	**Pooled Standard Deviation (s_{pooled})**
Fermentation Indicators				
Increase in Biomass (factor)	3.00	0.2	3.1	0.2
Final Attenuation (°Plato)	2.64	0.12	2.08	0.07
α-Constant (°Plato)	15.95	0.19	15.86	0.16
β-Constant (hr^{-1})	-0.0096	0.0003	-0.0105	0.0003
Beer Quality Indicators (Day 12)				
pH (pH units)	3.97	0.04	4.13	0.05
diacetyl (ppb)	77	10	84	13
SO_2 (ppm)	9	2	13	1
Acetaldehyde (ppm)	19	4	23	5

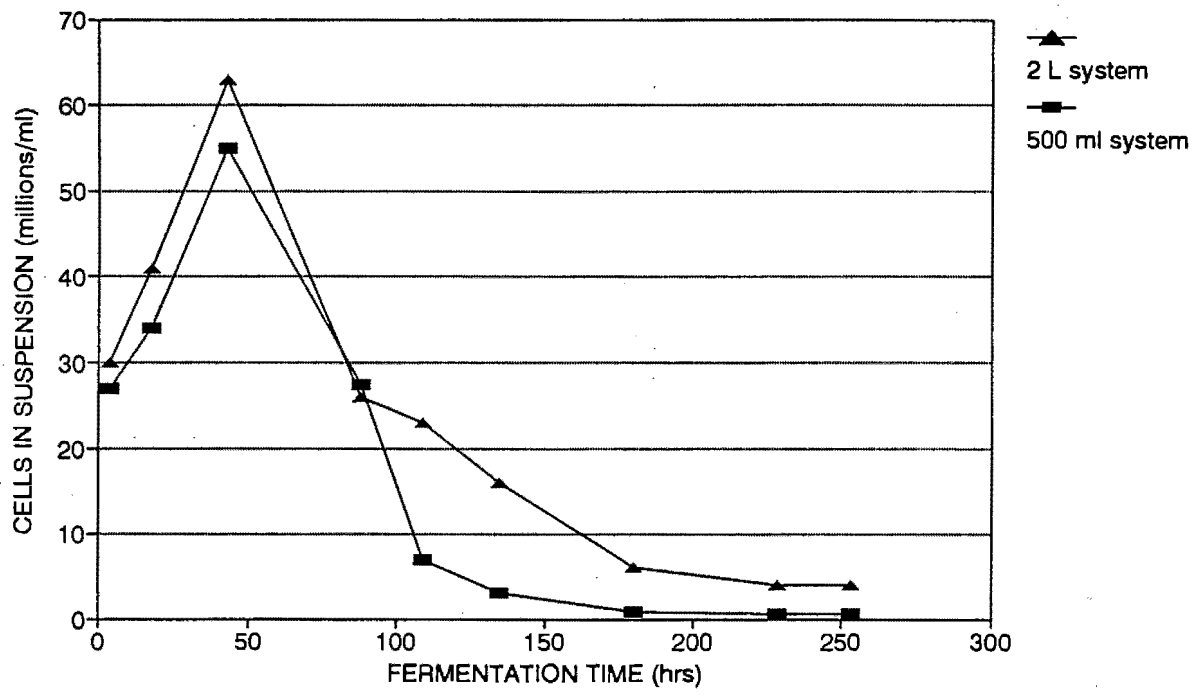

Figure 3.6 Cell count profiles for fermentations of identical yeast samples done in the 2 L and 500 mL systems

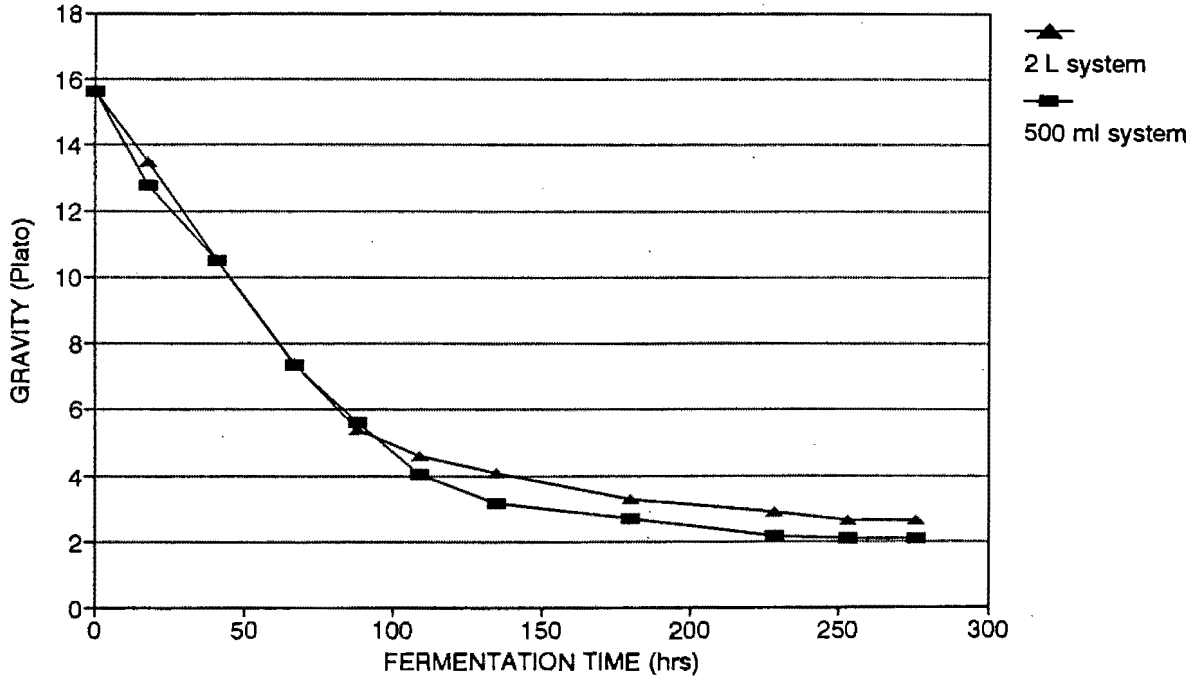

Figure 3.7 Attenuation profiles for fermentations of identical yeast samples done in the 2 L and 500 mL systems

Although the difference in peak cell counts was not large, more cells remained in suspension towards the end of fermentation in the 2 L system than in the 500 mL system (Figure 3.6). Despite this, a greater rate and extent of attenuation was achieved in the 500 mL system (Figure 3.7) as confirmed by the relative magnitudes of the β-constants and the lower specific gravity of the final product in the 500 mL system (Table 3.9). The differences in biomass growth and the end of fermentation diacetyl concentrations were insignificant (Table 3.9), suggesting that the differences in attenuation were not associated with cell growth or suspension characteristics of the yeast.

Sampling from the 500 mL system results in greater agitation of the fermentation vessel than sampling from the 2 L system. It is suggested that this results in greater degree of decarbonation of the medium, thus avoiding possible CO_2 toxicity which may have a deleterious effect on yeast performance. However, the final pH and SO_2 levels in the 500 mL system appear to have been higher than those in the 2 L system. This could indicate greater yeast stress in the 500 mL system. Alternatively, the production of volatile organics may be promoted in the 500 mL system which would reduce the specific gravity of the medium hence the greater rate and extent of attenuation observed.

The differences in fermentation performance resulting from the geometry of the laboratory fermentation vessels indicate that the systems may not be representative of large scale brewery operations. However, differences in fermentation performance resulting from yeast quality are expected to become apparent.

3.3 THE APPLICATION OF THE SELECTED YEAST QUALITY ASSAYS TO A LOSS OF YEAST QUALITY IN RESPONSE TO STRESS CONDITIONS

To illustrate the ability of the selected yeast quality assays to identify, characterise and quantify a loss of yeast quality resulting from stress conditions, the results of an experiment in which yeast was exposed to mechanical stress are presented. Correspondence between the results of the yeast quality assays and the subsequent fermentation performance of the yeast are also highlighted.

3.3.1 Experimental Procedure

A cropped yeast sample from a storage vessel in the brewery (stationary phase cells) was diluted (10x) in saline solution (0.9% w/v) containing EDTA (1.9 g/L) for deflocculation. 10 mL aliquots were exposed to ultrasonic cavitation (MSE Soniprep 150, frequency: 22.8 kHz, amplitude: 18 μm). To prevent excessive heat generation, the samples were placed on wetted ice and ultrasonication done for 1

minute intervals interspersed with cooling periods of 2.5 minutes. Samples were treated for a total of 1, 3 and 5 minutes.

Loss of yeast quality was assessed by total cell counts, the protease assay, methylene blue staining, slide counts and specific oxygen utilisation rate measurements (Method B). In addition, small scale (500 mL) fermentations at 1/4 the standard pitching rate (limited sample volume) were performed to assess the fermentation performance of the treated yeast. The fermentations were terminated after 160 hours in order to highlight differences in fermentation performance. Since only small volumes could be treated with ultrasound, the acidification power, glycogen content and trehalose content of the cells were not determined.

3.3.2 Results

The results of the yeast quality assays and small scale fermentations for the ultrasonically treated yeast are given in Figures 3.8 - 3.12. Figure 3.8 presents the results of the assays used to detect the extent of cell disruption (total cell counts and the protease assay). The results of the staining techniques (methylene blue and Mg-ANS) and slide counts used to detect the loss of "viability" through cell death and replicative deactivation are presented in Figure 3.9. The metabolic response to the ultrasonic treatment as indicated by the specific oxygen utilisation rate is presented in Figure 3.10. For comparison, results indicating the metabolic response of the yeast to physiological stress (storage at 30°C) are included in Figure 3.10. Figure 3.11 and Figure 3.12 are the cell count and attenuation profiles respectively for the 500 mL fermentations done with the ultrasonically treated yeast.

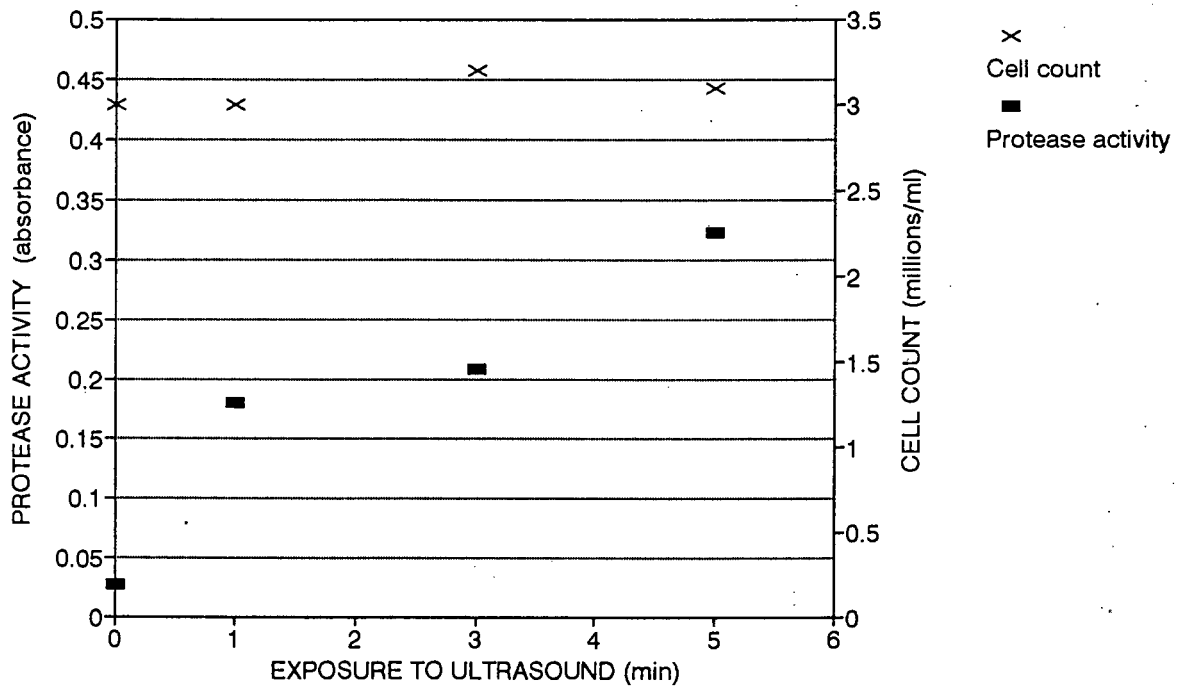

Figure 3.8 The extent of cell disruption on ultrasonic cavitation, determined by total cell count and extracellular protease activity

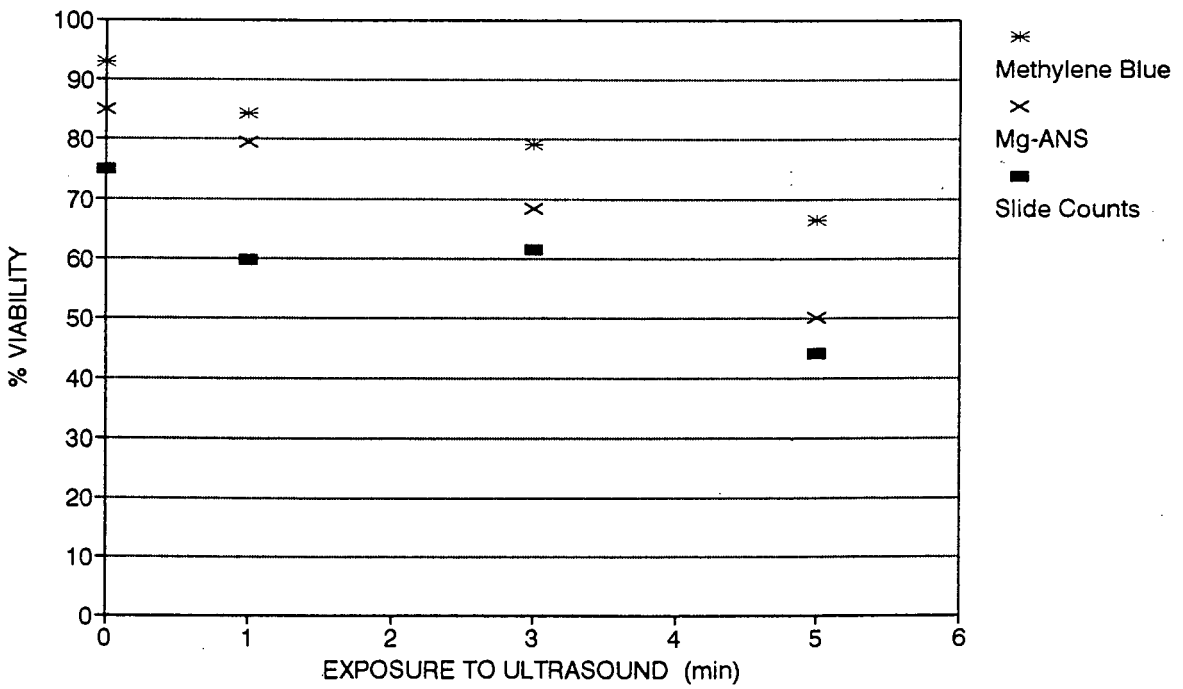

Figure 3.9 The extent of cell death and replicative deactivation on ultrasonic cavitation, determined by methylene blue staining, Mg-ANS staining and slide counts

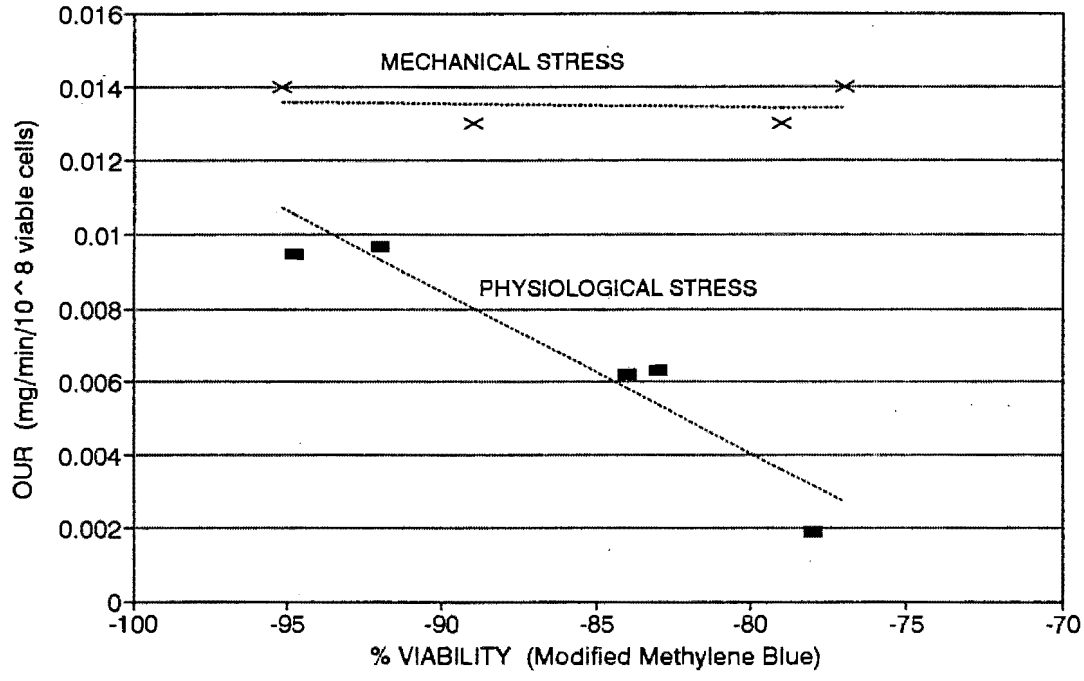

Figure 3.10 Changes in metabolic activity, measured as oxygen utilisation rate, in response to mechanical stress (induced by ultrasonic cavitation) and physiological stress (induced by storage at 30°C) (Note: The results were generated in separate experiments with the same strain of yeast).

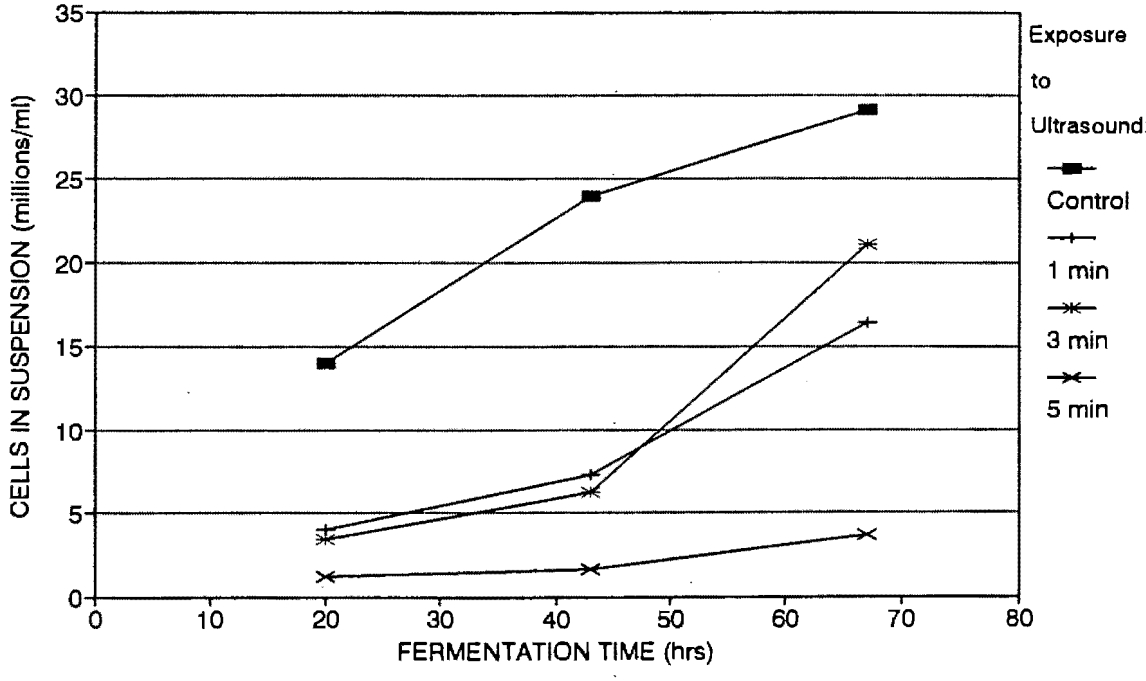

Figure 3.11 Cell count profiles for the first 62 hours of fermentation of yeast exposed to mechanical stress induced by ultrasonic cavitation

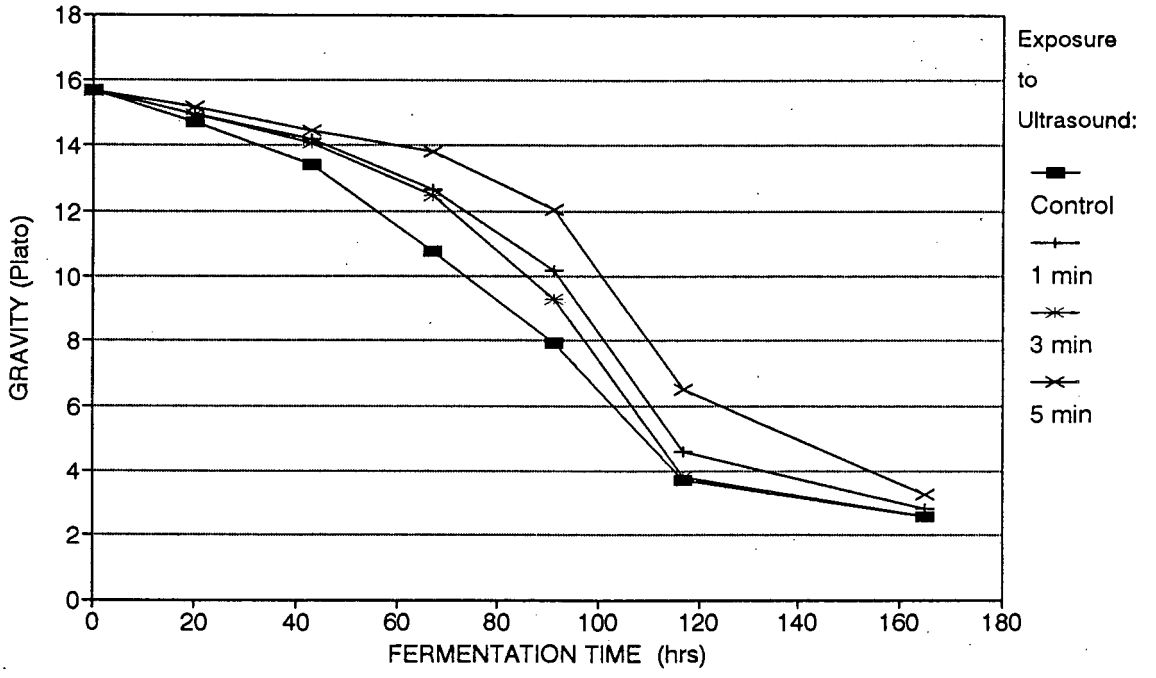

Figure 3.12 Attenuation profiles for the fermentations of yeast exposed to mechanical stress induced by ultrasonic cavitation

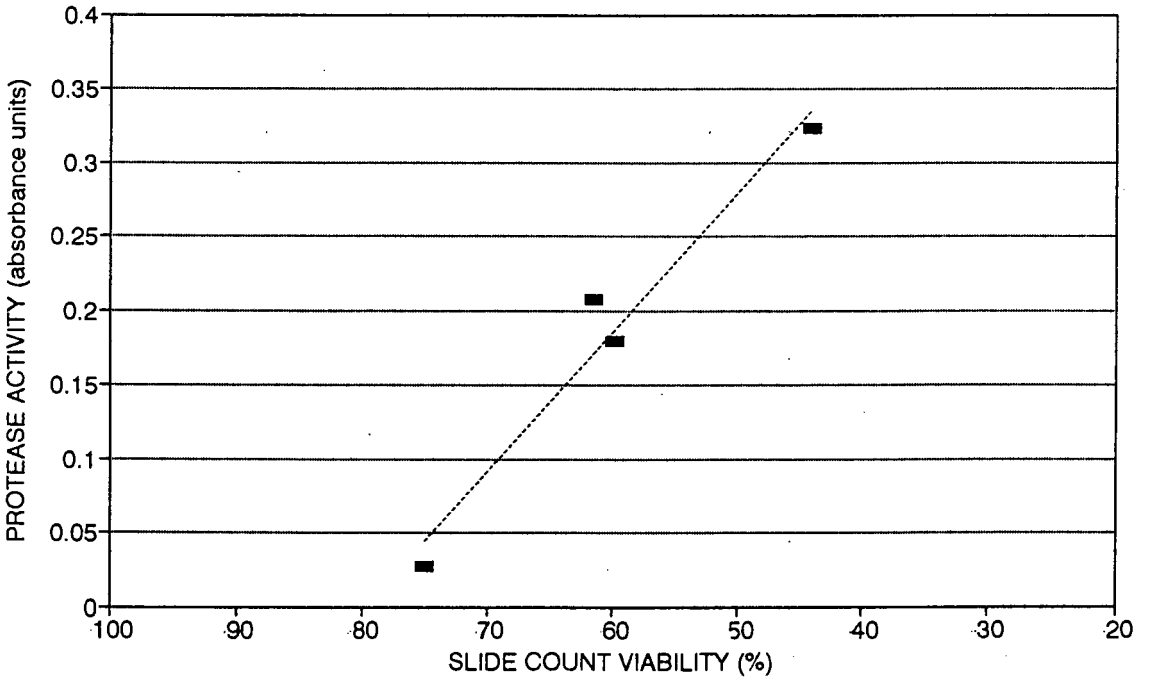

Figure 3.13 Correspondence between slide counts and protease activity for yeast exposed to ultrasonic cavitation

3.3.3 Discussion

Figure 3.8 shows that there was no significant difference in the total number of cells in suspension with increasing exposure to ultrasonic cavitation. This suggests that there was insufficient mechanical stress to cause total cell disruption. However, there was an increase in the external protease activity with increasing exposure to ultrasonic cavitation, suggesting the presence increasing numbers of cells with damaged envelopes. There was not a large difference between the external protease activity after 1 minute of exposure to ultrasound and after 3 minutes of exposure.

The results of the staining as well as the slide count technique (Figure 3.9) show that there was an increase in the number of dead cells (membrane damaged and metabolically inactive cells) and replicatively deactivated cells (respectively) with increasing exposure to ultrasound (indicated as a decrease in % "viability"). Close correspondence between the results of Mg-ANS staining and slide count results as suggested by the American Society of Brewing Chemists (1981), King *et al.* (1981) and McCaig (1990) was not observed in this instance. This trend was apparent in preliminary experiments (data not shown) and it is expected that the % "viability" indicated by Mg-ANS staining for the sample exposed to ultrasonic cavitation for 1 minute was artificially high due to experimental error.

There was not a large difference between the percentage of replicatively competent cells in the samples exposed to 1 and 3 minute of ultrasound (as indicated by the slide count results). Replicative competence is dependent on the presence of an intact and functioning cell membrane (Jones 1987b). The protease and slide count techniques indicate membrane intactness and functioning respectively. The increasing trend in the external protease activity upon increasing exposure to ultrasonic cavitation is reflected by an increase in the number of replicatively deactivated cells (Figure 3.13). This suggests that exposure to ultrasonic cavitation leads to membrane damage and that the extent of this damage is dependent on the duration of the exposure.

Figure 3.11 illustrates the relationship between the specific oxygen utilisation rate and the methylene blue "viability" for samples of yeast exposed to ultrasonic cavitation (mechanically stressed) and for a different batch of the same strain of yeast exposed to physiological stress (storage at 30°C). Unlike storage, exposure to ultrasonic cavitation does not affect the specific oxygen utilisation rate of the cells. This suggests that the metabolism of the remaining living cells was not compromised by the mechanical stress imposed by ultrasonic cavitation and highlights that yeast damage resulting from mechanical and physiological stresses occurs by different mechanisms.

The results of the small scale fermentations of the ultrasonically treated yeast suggest that mechanical stress decreased the attenuation rate (Figure 3.12). This effect was mediated through a reduced rate and extent of yeast growth during the fermentation (Figure 3.11). The shape of the attenuation profiles for the

fermentations of the ultrasonically treated yeast differ from typical attenuation profiles (Figure 3.1). There appeared to be a longer lag phase followed by more gradual attenuation of the wort. This may have been the result of the reduced pitching rate (1/4 the standard pitching rate). The equation $dS/dt = \alpha e^{\beta t}$ did not describe the data accurately ($0.84 < R^2$ 0.93). The accuracy of fit became worse with the increasing lag phase observed (Figure 3.12). Owing to the poor fit, the α and β values were not used to compare the attenuation profiles. The sample that had been exposed to ultrasonic cavitation for 5 minutes appeared to show the lowest rate and extent of attenuation, while there appeared to be little difference in the fermentations of the samples exposed to ultrasonic cavitation for 1 and 3 minutes.

Since the mechanism of the loss of yeast quality appeared to be through membrane damage, the methods which indicate membrane damage, the protease assay and slide counts, were able to give an indication of the subsequent fermentation performance of the yeast. As indicated by the different metabolic responses to mechanical and physiological stresses (Figure 3.10), the type of stress imposed on the yeast determined the mechanism of damage. It is postulated that the accuracy and predictive ability of a particular yeast quality assay depends on correspondence between the mechanism of damage and the physiological basis of the yeast quality assay, hence the selection of appropriate yeast quality assays to detect yeast damage should be based on an knowledge of the mechanism of the damage and the physiological basis of the yeast quality assays.

3.4 THE CHARACTERISATION OF A LOSS OF YEAST QUALITY

Within the brewing industry, the terms "viability" and "vitality" are used to describe the nature of a loss of yeast quality. It has been shown that these terms are too broad to allow accurate characterisation of a loss of yeast quality. The experimental work discussed above and the review of the different yeast quality assays highlighted that the response of yeast to stress conditions and the concomitant loss of yeast quality depend on the nature, duration and intensity of stress conditions. Furthermore, the need for a knowledge of the mechanism of yeast damage was identified. It is proposed that the physiological condition of yeast can be described in terms of certain physiological states and that a loss of yeast quality in response to stress conditions occurs via stepwise transition between these physiological states.

The following physiological states have been identified:

Healthy yeast cells are alive and able to withstand stress conditions. When inoculated into fresh wort, the cells have the ability to grow and reproduce which,

together with appropriate flocculation and sedimentation characteristics and a sufficiently active metabolism, allow them to attenuate the wort at a rate and to an extent which promote the efficient large scale production of beer. In addition, these cells do not produce off-flavours in the beer and are sufficiently intact so that intracellular or wall-associated materials are not released into the product.

Physiologically stressed yeast cells are intact, able to grow and reproduce, but show signs of altered metabolism which may manifest as a reduced metabolic rate, less rapid or extensive attenuation of wort or the production of off-flavours in the beer. Physiologically stressed cells may be characterised by low levels of intracellular storage reserves, loss of stress resistance or the presence of stress indicator compounds in the final product. As indicated by Smart *et al.* (1995), changes in the surface properties of the cells and associated ramifications in the flocculation and sedimentation characteristics of the yeast during fermentation may also be indicative of exposure to physiological stress.

Replicatively deactivated yeast cells may appear intact, but are no longer able to reproduce. The cell membranes are the loci of processes leading to replicative deactivation (Jones 1987b). While this is the first in a number of steps leading to final cell death, it may be reversible permitting the cells to return to a replicating state after a period of adaptation.

Yeast cells with minor damage to the cell envelope: Cell wall damage may occur to a varying extent ranging from the partial removal of cell wall glycoproteins to complete rupture of the cell envelope. In minor cell damage, intact cells are considered. Partial removal of some of the cell wall glycoproteins may result in haze formation (Lewis and Poerwantaro 1991). This represents the least extreme degree of damage to yeast and manifests rather as a loss in beer quality than a true loss of yeast quality.

Dead yeast cells may be intact, but show no growth or other metabolic activity. This state represents true, irreversible cell death.

Ruptured yeast cells: The yeast cell envelope may be ruptured either mechanically or by autolysis. Disruption of the cell envelope may be partial or complete. **Partially ruptured cells** have a point break in the cell envelope and are still visible as cellular entities under the microscope. **Completely ruptured cells** do not retain their shape sufficiently to be identified as cellular entities during microscopic analysis. This represents the most extreme degree of damage to yeast.

A scheme proposing the progression between these physiological states in response to stress conditions is presented as Figure 3.14.

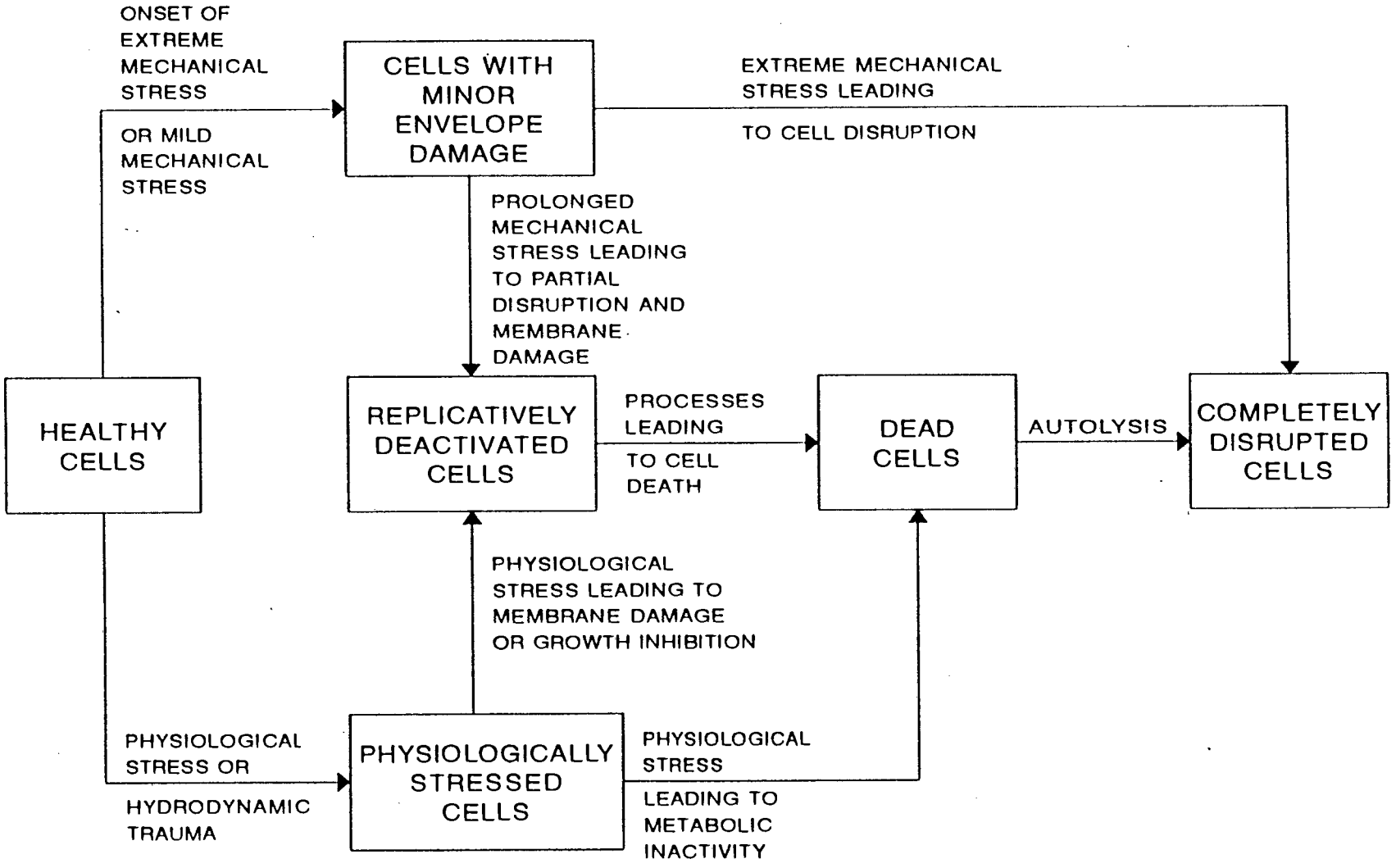

Figure 3.14 Proposed scheme for a loss of yeast quality

The scheme (Figure 3.14) provides a basis for the evaluation of loss of yeast quality. Based on an understanding of the physiological basis of yeast quality assays such as the techniques presented in this chapter and those reviewed by Jones (1987a and 1987b), Henschke and Eglinton (1991), Iserentant (1993) and Lentini (1993), these assays can be used to identify and quantify the different "cell states" or "changes in cell state". Table 3.10 summarises the yeast quality assays used in this investigation and indicates the "cell states" that the techniques are expected to identify. Although not used in this investigation, assays to identify minor cell damage have been included in Table 3.10 for completeness.

Table 3.10 Analytical methods to identify different "cell states"

Yeast Quality Assay	**Cell State**
Total cell counts	Completely ruptured cells
Assays for the release of intracellular compounds: (1) protease assay	Partially ruptured cells
Staining techniques: (1) methylene blue, (2) magnesium salt of 8-anilino-1-naphthalene sulfonic acid (Mg-ANS)	(1) Dead cells (2) Dead and replicatively deactivated cells
Assays for release of wall-associated material: (1) mannan, (2) glucan, (3) melibiase and (4) invertase	Cells with minor cell wall damage
Assays based on cell replication (1) plate counts (2) slide counts	Dead and replicatively deactivated cells
Metabolic activity indicators: (1) oxygen utilisation rate (2) acidification power	Physiologically stressed cells
Intracellular reserve indicators: (1) glycogen content	Physiologically stressed cells
Stress resistance indicators: (1) trehalose content	Physiologically stressed cells

Ideally, the critical conditions required to effect each "change of state" should be established. Based on a knowledge of the different physical conditions to which the yeast may be exposed in an operation, it would then be possible to evaluate the potential of the operation to effect "changes of state" and hence different degrees of yeast damage or loss of yeast quality.

Many interlinked parameters are expected to influence the critical conditions required to cause a "change of state". These include yeast strain, physiological status (lag phase, growing or stationary phase cells), yeast age, biomass concentration, the extracellular concentration of ethanol and dissolved gases, temperature and pressure. Determination of generalised critical conditions for "changes of state" is thus complex. However, the scheme can be used to inform more empirical studies of yeast damage or loss of yeast quality, aid in the selection of appropriate yeast quality assays and allow more accurate characterisation of a loss of yeast quality in brewing and other bioprocess applications.

3.5 CONCLUSIONS

Nine analytical parameters to identify and quantify a loss of yeast quality have been reviewed: an extracellular protease assay, methylene blue and Mg-ANS staining, plate and slide counts, oxygen utilisation rate, acidification power and intracellular glycogen and trehalose contents. The reproducibility of the methylene blue and Mg-ANS staining techniques, the slide count technique, the acidification power test and the assay for the glycogen content was good (coefficient of variation $< 5\%$).

The use of small scale fermentations to detect differences in yeast quality have also been reviewed. Two systems, one using 2 L EBC tubes and the other using 500 mL measuring cylinders were set up and the fermentations in these systems were compared. The small scale fermentations were reproducible: the coefficient of variation of the constant of the exponential curves fitted to the data was less than 5% in both systems. As the fermentations progressed, differences in the quality of the beer effected by differences in the quality of the pitching yeast became less apparent. If differences in pitching yeast quality are to be highlighted, beer quality should be assessed at an early stage during the fermentation. However, if the effects of such differences on the large scale brewery process are to be evaluated, beer quality should be determined at the end of the fermentation process.

An example of the application of the selected assays to a loss of yeast quality in response to mechanical stress induced by ultrasonic cavitation is presented. The loss of yeast quality was effected by cell membrane damage which affected fermentation performance by limiting the rate and extent of cell growth. The protease and slide count techniques, which indicate membrane intactness and functioning respectively, gave an indication of the subsequent fermentation of the pitching yeast. Unlike physiological stress (storage in a nutrient depleted environment), mechanical stress did not cause a change in specific oxygen utilisation rate, indicating that physiological stress and mechanical stress may effect a deterioration in yeast condition by different mechanisms. This highlights the need for an understanding of the mechanism of damage and suggests that the accuracy and predictive ability of a yeast quality assay depends on the correspondence between the nature of the damage and the physiological basis of

the assay. The response of yeast to stress conditions and the physiological basis of many of the yeast quality assay still require further elucidation. In research applications, small scale fermentations should be used to assess yeast quality.

Instead of describing yeast quality in terms of "viability" or "vitality" as is traditionally done in the brewing industry, it is proposed that the physiological condition of yeast be described in terms of certain physiological states and that a loss of yeast quality in response to physiological or mechanical stress occurs via the stepwise transition between these physiological states.

CHAPTER

4

EQUIPMENT AND EXPERIMENTAL PROCEDURE

4.1 INTRODUCTION

The aim of the third section of the study was to establish the extent and nature of the potential loss of yeast quality during yeast cropping and to determine the critical handling conditions for a loss of yeast quality.

The potential for a loss of yeast quality during cropping is a function of:

- the yeast strain
- the yeast generation number (*ie.* the number of times the yeast had been re-used)
- the type of wort fermented (*ie.* the brand of beer)
- the biomass concentration of the suspension
- the design and operating speed of the cropping pump
- the flow conditions (*ie.* the flow rate (linear velocity) and the nature of the

flow (laminar or turbulent) as indicated by the Reynolds number)
- the type of valves and the position of the valves (*ie.* fully open or throttled)
- the presence of constrictions and expansions (*eg.* in passing through the plate and frame heat exchanger)
- the duration of the exposure of the yeast to mechanical handling determined by the length of the pipes through which the yeast flows
- the product temperature and the magnitude and rate of temperature changes during handling (*eg.* potential temperature shocks in the heat exchanger)
- the line pressure, and the magnitude and rate of pressure releases

The parameters investigated in this study were the effect of cropping pump design and operation as well as that of the flow conditions in the yeast handling circuit. Yeast strain, yeast generation and wort type were kept constant. Where possible, fourth to sixth generation of the SAB lager yeast strain (AJL 2036) that had fermented Castle Lager in a 3000 hL (300 m^3) fermentation vessel was selected for the experiments.

Existing plant operation could be evaluated by taking samples on-line during routine operation in the brewery. However, the production environment did not lend itself to the investigation of individual variables and the determination of critical handling conditions. Laboratory scale equipment could be used to investigate many of the variables indicated above. The need for the experimental conditions to bear close resemblance to plant situation was highlighted by the review of the literature (Chapter 2). Since the simulation of the mechanical handling conditions of the large scale brewery at a laboratory scale was problematic and there was concern that laboratory results would not be representative of the large scale brewery process, a pilot scale test rig was designed. This rig was representative of the standard yeast handling circuit and could operate using brewery scale equipment. The rig was operated in one of the fermentation cellars of SA Breweries' Newlands Brewery (Cellar no. 3) and was used to crop production yeast directly from the 3000 hL fermentation vessels. This chapter describes the equipment and experimental procedures used to establish the effect of cropping pump design and operating speed as well as that of the flow conditions in the yeast handling circuit on the quality and subsequent fermentation performance of yeast.

4.2 EQUIPMENT

4.2.1 The Test Rig

The test rig (Figure 4.1) consisted of three separate sections : a holding tank (Figure 4.2), a pump platform (Figure 4.3) and a set of holding tubes (Figure 4.4). Each section of the rig was free-wheeling so that it could be moved and the rig assembled at the base of any fermentation vessel in Cellar no. 3. When assembled, the rig was 4 m long, 1.8 m wide and 2.5 m high.

The rig could be operated either directly from a fermentation vessel (FV) (Figure 4.2) or in recirculation mode with yeast in the 500 L holding tank. In this study, the former configuration was used. Cleaning and sterilising of the unit was done using the holding tank. The rig was designed to accommodate as many configurations of pump as possible. These could be installed using two flexible lengths of hose (2 or 3 m long). A bypass was included for cleaning-in-place (CIP) purposes and a pressure relief valve for safety reasons (not indicated on diagram). Three interchangeable sets of holding tubes with diameters (ϕ) of 25, 50 and 65 mm were provided to enable operation at different linear velocities and Reynolds numbers. The holding tube section had a total length of 13.5 m and contained eighteen 90° bends (long-radius elbows).

All piping on the rig (other than the holding tubes) had a diameter of 50 mm. Union fittings were provided for rig assembly and all valves were butterfly valves. The rig was fitted with three sampling points (butterfly valves): one before the pump, one after the pump and one after the holding tubes (Figure 4.4). Temperature and pressure gauges were provided next to the sampling points.

The rig was made of stainless steel (304L quality) . All welds were ground smooth to be crevice free and the piping polished to 180 grit finish.

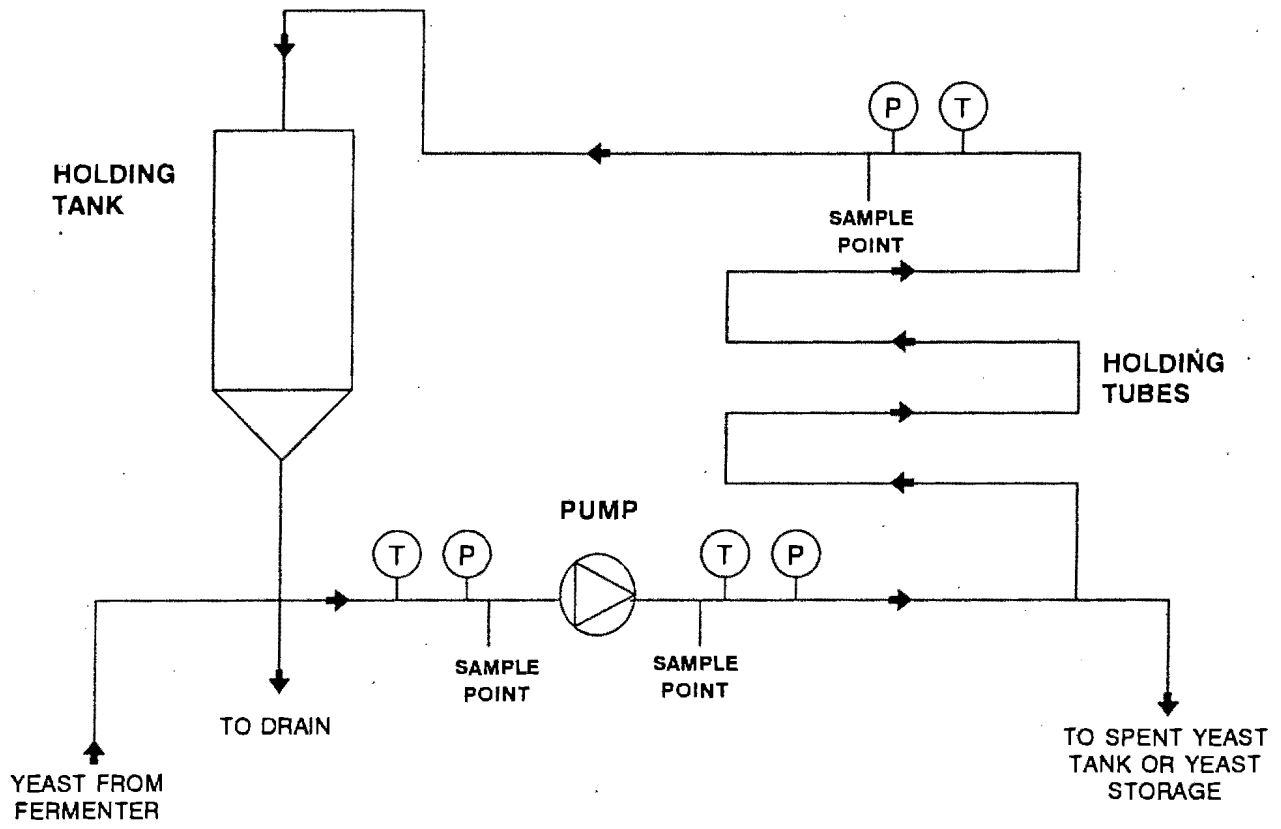

Figure 4.1 Schematic diagram of test rig

Figure 4.2 Holding tank of test rig showing operation directly from fermentation vessel

Figure 4.3 Pump platform of test rig showing pump (Bredel peristaltic SP/40), motor and electrical connections

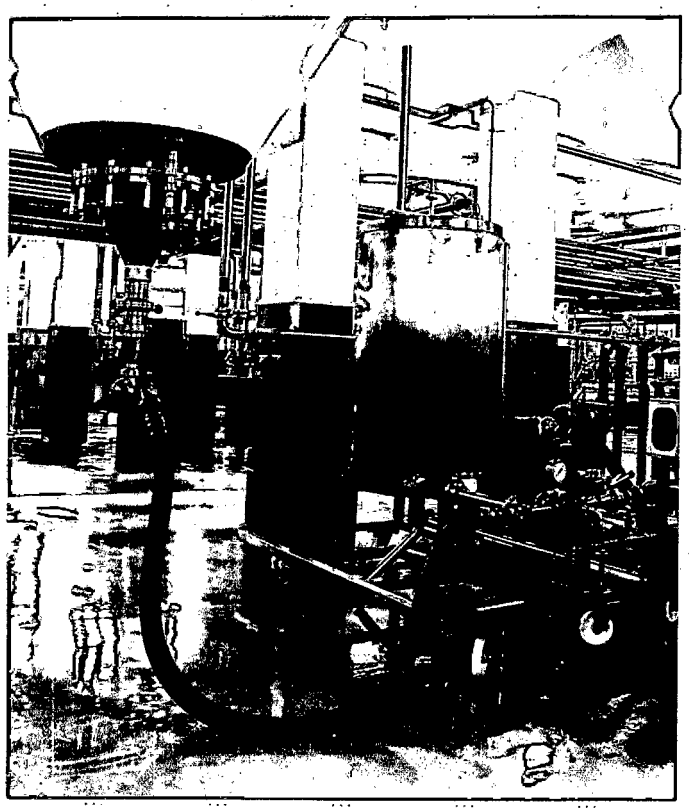

Figure 4.2 Holding tank of test rig showing operation directly from fermentation vessel

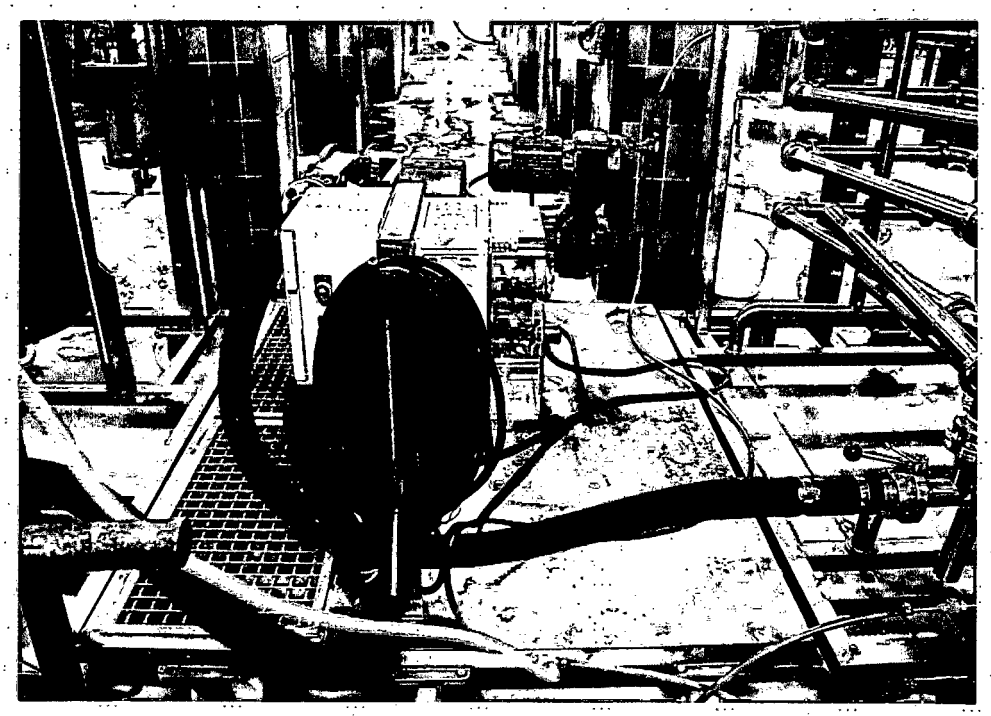

Figure 4.3 Pump platform of test rig showing pump (Bredel peristaltic SP/40), motor and electrical connections

Figure 4.4 Holding tubes of test rig with 50 mm tubes in place

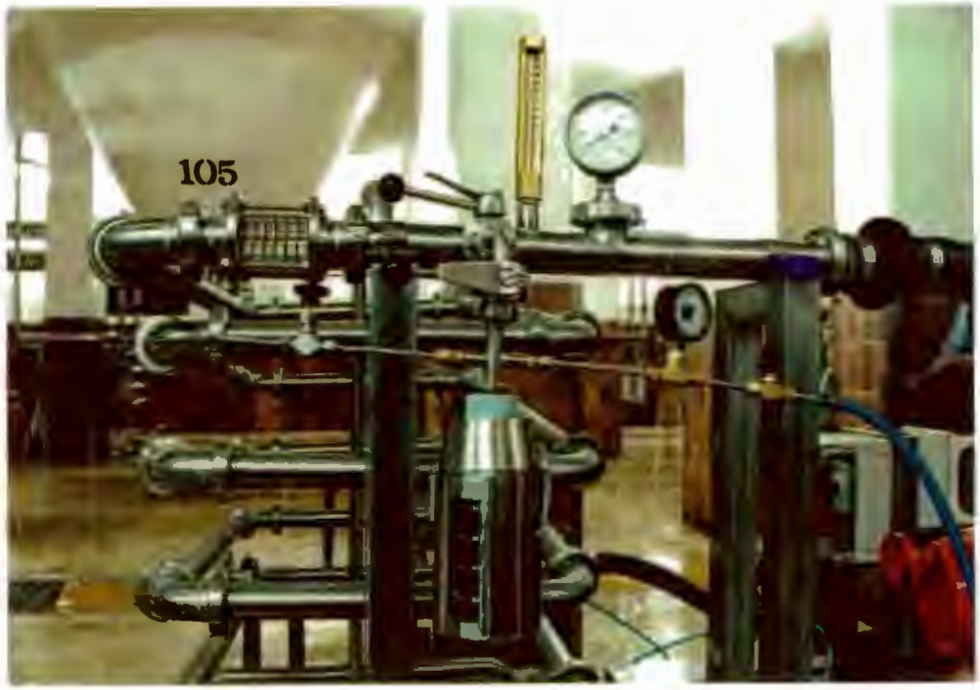

Figure 4.5 The sampling device used for pressurised sampling
(Note : pressure and temperature gauges of rig (top); sampling device pressure release valve (left), butterfly valve and pressure gauge (right).)

4.2.2 Cleaning and Sterilising the Rig

The cleaning and sterilising procedures used for the rig were based on standard procedures of SA Breweries using the available cleaning and sterilising reagents.

Before use, the rig was cleaned with a 4% cold, caustic solution. The holding tank was filled with 300 L of water, the caustic reagent added and the pump on the rig run at high speed to circulate the cleaning solution. The sampling valves were opened periodically for at least 1 minute at a time to clean them. After 20 - 30 minutes, the cleaning solution was drained from the rig. The rig was rinsed several times to remove the caustic reagent. Once the rinsing water was free of the caustic reagent, the holding tank was again filled with 300 L of water. Sterilant (concentrated Oxonia or Peroxol Plus) was added to this water to a final concentration of 0.5% and the solution was circulated for 20 minutes. The sterilant was displace by yeast when rig operation started. The initial yeast suspension (± 300 - 400 kg) was disposed of and no samples were taken during this period.

4.2.3 Sampling

Sampling could be done directly by opening the sampling valves. This constituted a rapid release of pressure, which caused the yeast to spray out of the sampling points. Sterile yeast sampling and prevention of exposure to oxygen was not possible. Pressurised sampling containers were designed to overcome these problems. The containers (Figure 4.5 and Figure 4.6) consisted of a stainless steel fitting which could be attached to the butterfly sampling valves on the rig. Each fitting was provided with its own valve and extended into a tube through which the yeast passed. A screw top lid was attached to the upper part of the tube to enable the attachment of an autoclavable glass bottle which functioned as the yeast receptacle. Two small tubes were welded to the interior of the large tube of the sampling device. The ends of these tubes were open to the interior of the receptacle above the intended sample level. The small tubes were extended to the upper part of the large tube beyond the lid of the bottle. A valve and pressure gauge were fitted to an extension of one of the tubes and a pressure release valve to the extension of the other. For safety reasons, the bottle was fitted with a stainless steel guard in case of failure under pressure.

Prior to sampling, the tube of the sampling device was rinsed with 70% ethanol solution and flamed. An autoclaved 1 L bottle was attached to the sampling device and the guard put in place. To maintain sterility, the neck of the bottle was flamed during this operation. Teflon tape was used to ensure an airtight seal. Sufficient time was allowed for the tube of the sampling device to cool before sampling commenced.

Sampling was always done with the pump off to avoid high shear which may occur during rapid flow through the narrower tubes of the butterfly sampling valves and the sampling devices (ϕ = 15 mm) and to allow representative sampling of the

yeast suspension. Once the pump was switched off, the sampling bottle was pressurised using nitrogen. Nitrogen was used instead of air to avoid exposing the yeast to oxygen. The nitrogen was passed through a membrane filter (0.22 μm) to ensure sterility. The pressure in the line was read from the pressure gauge next to the sampling point, while the pressure in the sample bottle was read on the gauge of the sampling device. The sample bottle was pressurised to $\pm$ 10 kPa below line pressure. The valve in the nitrogen line was closed to prevent further pressurisation of the sampling container. The butterfly valves on the rig and sampling device were opened. The pressure in the sampling bottle was then released slowly using the pressure release valve on the sampling device. This allowed the yeast to flow gently into the sampling bottle. Once sufficient yeast had been collected (500 mL), the valves on the rig and sampling device were closed. The sample bottle was removed, again flaming the neck to prevent entry of micro-organisms. The sampling device was rinsed with water to remove any remaining yeast and re-sterilised for the next sample.

To allow pressurised sampling directly from the yeast line during routine brewery operation, special sampling joints were constructed. These consisted of pipe sections with standard union couplings which could be inserted into the lines. As in the rig, these pipe sections were fitted with butterfly valves to which the sampling devices could be attached to allow aseptic, pressurised sampling as described above.

The quality of the yeast right at the base of the cone was expected to be inferior to that in the remainder of the cone. For this reason, no sampling was done for the first 10 - 15 minutes of cropping. allowing the first 700 - 1000 kg of yeast to be removed from the cone before sampling commenced.

To limit yeast metabolism, the samples were immediately placed on wetted ice after sampling. The samples were then stored on wetted ice in a refrigerator (4 °C) until analysed.

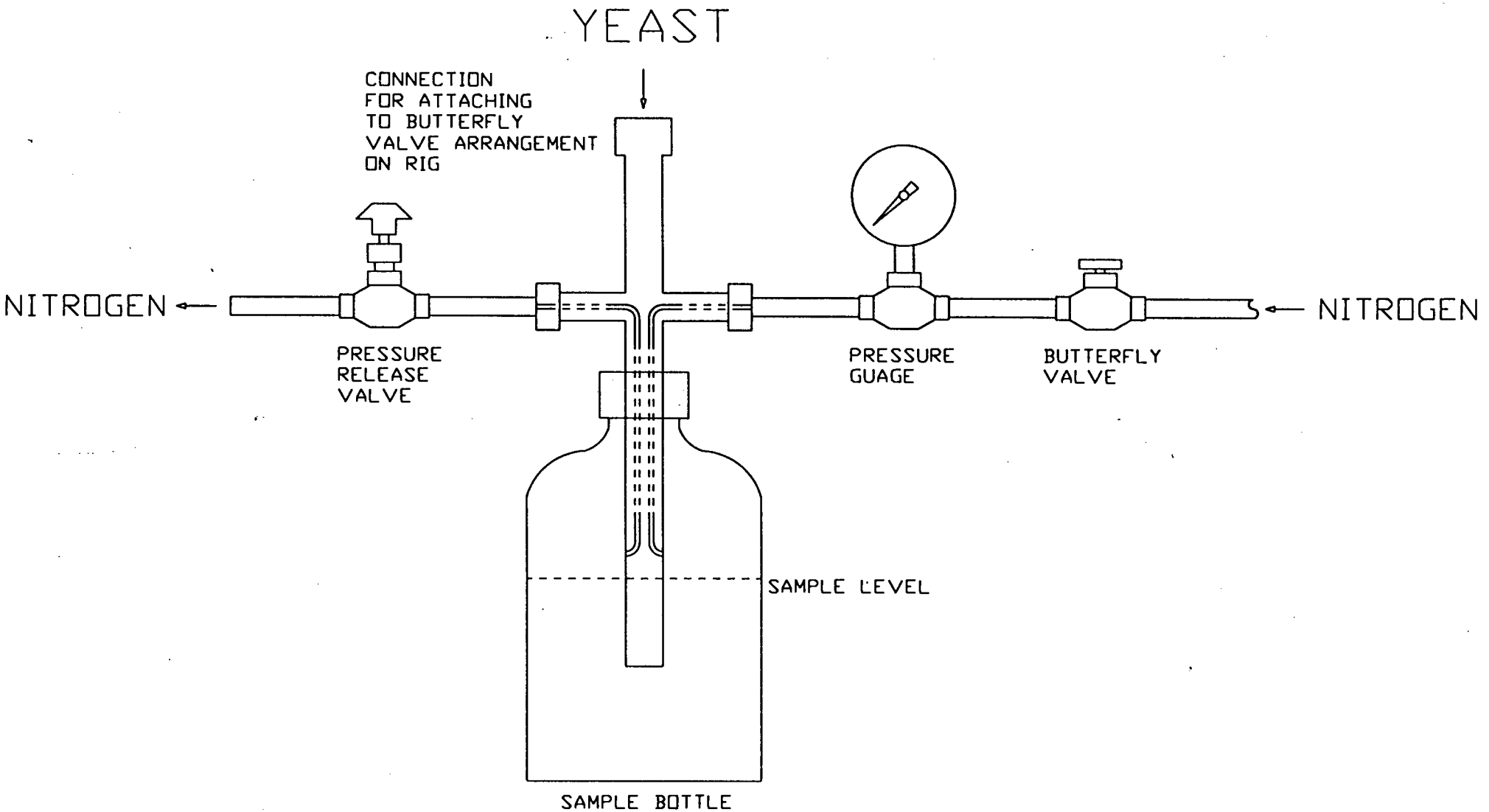

Figure 4.6 Schematic diagram of sampling device used for pressurised sampling

4.3 THE EVALUATION OF ROUTINE BREWERY OPERATION

4.3.1 Introduction

The evaluation of routine brewery operation was done by sampling directly from the yeast handling circuit of SA Breweries' Newlands Brewery. The evaluation was done on second generation yeast which had fermented Castle wort (BICPAP[1]) from the Huppmann brewhouse (Brewhouse 2) in a 3000 hL fermentation vessel (FV 79) in Cellar no. 3. The yeast handling procedures observed during the investigation, the sampling procedure and the yeast quality analyses used are described.

4.3.2 The Yeast Handling Procedures Observed

Six days after inoculation, settled yeast was transferred from the cone of the fermentation vessel (FV 79) to one of the two yeast collection vessels (YCV 1) in the yeast room. A tri-lobe positive displacement pump (Wilflo Model CL/2S/052/10) was used and the yeast was transferred at the standard cropping rate of 70 kg/min. Flexible hose and stainless steel couplings were used to connect the pump to the base of the cone and to the yeast mains. A 65 mm diameter hose and 65/50 mm reducer were used on the suction side of the pump to connect to the fittings at the base of the cone and a 50 mm hose and 50/65 mm expander were used on the discharge side of the pump to link up to the mains in the cellar and yeast room (ϕ = 65 mm). Before entering the yeast collection vessel, the yeast passed through a swing bend panel containing butterfly valves and a glycol-cooled plate and frame heat exchanger. The temperature of the yeast in the cone was 14 to 16°C. The setpoint for the yeast exit temperature from the chiller was 2.5°C. The yeast collection vessels are not insulated, but the ambient temperature of the room is 10°C (57% relative humidity). Depending on the position of the fermentation vessel relative to the yeast room, the total pipe length between the fermentation vessel and the yeast collection vessel may be between 85 and 145 m. For FV 79 this distance was 135 m of which 100 m was in the cellar (at an ambient temperature of ± 20°C) and 35 m in the yeast room (10°C).

After the first 400 kg of yeast was removed from the cone and discarded, a total of 12 800 kg of yeast was cropped over a period of approximately 4 hours (including sampling time)

1. BICPAP (Brewhouse Increased Capacity through Parallel Adjunct Preparation) is an alternative brewing method which increases brewhouse capacity by optimising malt input and blending the liquid adjunct into the FV at collection (Lodolo *et al.* 1996).

4.3.3 Sampling

Sampling from the yeast lines was done using the purpose-built sampling devices and sampling joints (described in Section 4.2.3). The four on-line sampling points were placed in the cropping line as follows : (1) before the pump, (2) before the swing bend panel (after 105 m of the yeast line), (3) at the inlet to the chiller and (4) at the outlet of the chiller. Two sets of four samples were taken in sequence starting at the cropping pump and ending at the outlet of the chiller. The first set of samples (Set A) were taken once 2000 kg of yeast had been cropped into the yeast collection vessel and the second set (Set B) 30 minutes later when 4000 kg of yeast had been cropped. Samples were placed on wetted ice immediately.

When the cropping pump was stopped during sampling, the chiller was also turned off to prevent the yeast in the chiller from freezing. The temperature of the yeast at the chiller outlet rose from 2.5 to 8.7°C during this period (< 10 minutes).

4.3.4 Analysis

Analysis of the samples was performed approximately 4 hours after sampling. The potential changes in yeast quality were assessed as indicated in Table 4.1. The samples were fermented using the 2 L EBC system. Yeast performance was assessed by determining cell count and attenuation profiles. Beer quality was evaluated after
12 days of fermentation by measuring the diacetyl, SO_2 and acetaldehyde contents as well as the pH of the final beer.

Table 4.1 Yeast quality assays used to evaluate routine brewery operation

NATURE YEAST QUALITY LOSS	ASSAY
Partial cell disruption	Protease assay
Cell death	Modified methylene blue staining technique
Physiological stress manifesting as a change in metabolic activity	Oxygen utilisation rate (Method B)
Physiological stress indicated by low levels of intracellular reserve compounds	Glycogen content by NIR spectrometry
Physiological stress leading to loss of stress resistance	Trehalose content by NIR spectrometry
Altered fermentation performance	Small scale fermentation (2 L)

4.4 ESTABLISHING THE EFFECT OF PUMP DESIGN AND OPERATING SPEED

4.4.1 Introduction

To assess the effect of different pump designs and operating speeds on the quality of yeast during yeast cropping, a number of pumps were obtained on loan from several industrial pump suppliers. Only pumps capable of sanitary operation were considered. The pumps presented were typically used for applications requiring positive displacement and gentle handling of the product. The pump suppliers were given the specifications of the rig and were asked to size the pumps accordingly. Table 4.2 presents the pumps that were submitted for evaluation. Five different pump designs are represented: peristaltic, lobe, sine, gear, diaphragm and centrifugal. In addition, it was decided to evaluate the yeast handling capability of a pump not normally used for yeast cropping purposes. A centrifugal pump which is used for yeast transfer during propagation at Newlands Brewery (OCB) was selected. The handling of this pump was expected to be less gentle than any of the displacement pumps on trial. A review of the pumps is given in Appendix A. In this review, the principle of operation of each type of pump is explained, the advantages and drawbacks of the individual pumps with regard to yeast handling applications highlighted and comments made on the experience with the pumps during the pump trials.

Table 4.2 Pumps submitted for evaluation

PUMP TYPE	SUPPLIER	MAKE	MODEL	MAXIMUM CAPACITY (for water) (L/hr)	MOTOR RATING (kW)	MAXIMUM ROTATIONAL SPEED (Gearbox) (rpm)	RESULTANT MAXIMUM CAPACITY (L/hr)
PERISTALTIC	Walter Becker (Becker Mining)	Bredel	SP/40	6000 (continuous duty)	1.5	46	3660 (61 L/min)
	OCB	Bredel	SP/50	10 500 (continuous duty)	4	46	8004 (133 L/min)
LOBE	APV	APV	CL/3S/156/7	-	5.5	174	16 286 (271 L/min)
	Alfa-Laval	Ibex	NMOG/622	± 35 000	1.1	273	± 9000 (± 150 L/min)
	NDE	Johnson	OL3/0108/07	64 800	4.0	131	8460 (141 L/min)
	OCB	Wilflo	CL/2S/052/10	-	3.0	333	10 380 (173 L/min)

Table 4.2 (cont.)

PUMP TYPE	SUPPLIER	MAKE	MODEL	MAXIMUM CAPACITY (for water) (L/hr)	MOTOR RATING (kW)	MAXIMUM ROTATIONAL SPEED (Gearbox) (rpm)	ESTIMATED MAXIMUM CAPACITY (of combination) (L/hr)
SINE	Aeromix	Maso	SP3"	17 000	4.0	1000	16 200 (270 L/min)
GEAR	Micro Matic	Scandi Brew	GP160	9600	3.0	114	9600 (160 L/min)
DIAPHRAGM	Alfa-Laval	DEPA	DL 40	11 500 with discharge head of 250 kPa using 700 kPa air	N/A	N/A	N/A
	Letaba Pumps	Wilden	M8/SP Foodmaster	26 100 with discharge head of 250 kPa using 700 kPa air	N/A	N/A	N/A
CENTRIFUGAL	OCB	Fristam	JP 15	_	5.5	2860 (motor)	-

Note : maximum capacity figures are theoretical values which have been calculated from the information provided by the pump suppliers

4.4.2 Experimental Procedure

When evaluating the effect of the different pumps on yeast quality, the rig was operated directly from the fermentation vessels in order to simulate the true cropping conditions. As discussed in Section 4.1, as far as possible the variables that could affect yeast quality during cropping were kept constant. For production reasons, however, the rig had to be operated using yeast that would no longer to be used for beer production, so in some cases during the first round of trials higher yeast generations (> 6) were used. After passing through the rig, the yeast was pumped to the spent yeast facilities for further processing.

The pumps were to be operated at two speeds: the standard cropping rate at Newlands Brewery (70 kg/min ≈ 66 L/min) and the highest cropping rate that could be achieved with the pump, motor and gearbox combination. Initially, no flowmeter was available for the trials and flow rate estimates were based on the rotational speed of the pumps and pump performance curves supplied by the manufacturers. The rotational speed of the pumps (where applicable) was measured using a tachometer. A viscosity of 88 cP and a density of 1050 kg/m^3 were assumed for these estimations. These are standard design values for the cropped yeast slurry used by SA Breweries. Once a flowmeter (Micromotion S150S141) was installed, the flow rate estimates were verified.

In the first series of trials, all the pumps except the Wilflo lobe and the centrifugal pump, were tested twice at the same operating conditions with different batches of yeast. This was done to avoid the possibility of false results due to discrepancies between the yeast quality of different batches of yeast. Since the Wilflo and centrifugal pumps were included in the investigation for reference only, these pumps were tested only once and at a single pump speed. Based on the results of this first round of trials (which will be discussed in Chapter 5), three of the pumps were selected for retrials. An additional Bredel peristaltic pump was included in the pump retrials. This pump, an SP/50 model, had a larger capacity than the SP/40 model used in the initial trials. The latter could only achieve the standard cropping rate when operated at the maximum rotational speed of its motor.

The operating conditions of the pumps are summarised in Table 4.3. A comparison between the estimated and measured flow rates is given in Table 4.4. The flow rate estimates were reasonable, except in the case of the APV, Ibex and Johnson lobe pumps where the measured flow rates were significantly lower than the estimated flow rates. During the initial pump trials, all three pumps were thus operated at rates under the standard cropping rate. Modifications could be made to the setup of the APV pump to allow operation in the range of the standard cropping rate during the retrials. Within the time frame of the investigation, this could not be done for the other two pumps. Since the maximum available air pressure was 600 kPa, the standard cropping rate could also not be achieved with the diaphragm pumps.

Table 4.3 Summary of pump operation during pump trials

PUMP TYPE	PUMP	INSTALLATION	PUMP SPEED (N) (rpm)	SUCTION HEAD* (kPa)	DISCHARGE HEAD* (kPa)
Peristaltic	Bredel SP/40	40 mm flange to 50 mm Union couplings (flow path 50↔40 mm directly)	25 47	130 - 180 100 - 200	300 - 370 290 - 385
	Bredel SP/50	50 mm flange to 50 mm Union couplings (uniform flow path)	23 42	130 - 180 90 - 230	280 - 980** 320 - 860
Lobe	APV	110 mm to 50 mm Union adaptors (flow path 110↔50 mm over 170 mm)	40 85	155 - 160 150 - 170	240 - 255 300 - 320
	Ibex	60 mm SMS to 50 mm Union adaptors (flow path 60↔50 mm over 150 mm), 90°bend at outlet	108 256	165 - 170 160 - 170	215 - 220 260 - 270
	Johnson	80 mm to 50 mm Union adaptors (flow path 80↔50 over 135 mm)	80 122	155 - 160 150 - 160	390 - 405 440 - 450
	Wilflo	65 mm to 50 mm Union adaptor (inlet) direct coupling (outlet)	127	150 - 160	360 - 370
Sine	Maso	80 mm to 50 mm Union adaptors (flow path 80↔50 over 135 mm)	244 400	150 - 155 150 - 155	465 - 475 485 - 490
Gear	Scandi Brew	direct coupling	4 8***	160 145	280 370
Diaphragm	DEPA	40 mm TriClover to 50 mm Union adaptors (flow path 50↔35 mm over 85 mm)	400 kPa air 600 kPa air	110 - 270 90 - 270	340 - 410 360 - 420
	Wilden	50 mm TriClover to 50 mm Union adaptors, 1.5 m S/S piping including 2 x 90° bends (long-radius elbows) on inlet side	400 kPa air 550 kPa air	85 - 225 80 - 315	300 - 470 430 - 550
Centrifugal	Fristam	direct coupling	-	135	420

* average value for all runs measured with pressure gauges on rig

** with 25 mm holding tube in place

*** unable to expose shafts to measure pump speed, numbers correspond to dial on pump

Table 4.4 Comparison between estimated and measured flow rates

		PUMP TRIALS		FLOW MEASUREMENT	
PUMP TYPE	PUMP	PUMP SPEED (N) (rpm)	ESTIMATED FLOW RATE (L/min)	PUMP SPEED (N) (rpm)	MEASURED FLOW RATE (L/min)
Peristaltic	Bredel SP/40	25 47	33 63	25 44	33 63
	Bredel SP/50	23 42	67 122	23 42	59 110
Lobe	APV	40 85 -	62 133 -	- - 157	- - 63
	Ibex	108 256	50 120	108 255	8 42
	Johnson	80 122	67 120	81 122	18 48
	Wilflo	243	66	243	54
Sine	Maso	244 400	58 133	244 400	89 162
Gear	Scandi Brew	4 8*	-	4 8*	99 193
Diaphragm	DEPA	400 kPa air 600 kPa air	-	400 kPa air 600 kPa air	44 56
	Wilden	400 kPa air 550 kPa air	-	400 kPa air 550 kPa air	19 42
Centrifugal	Fristam	3000	-	-**	-

* unable to expose shafts to measure pump speed, numbers correspond to dial on pump

** pump out of order (irreparable)

4.4.3 Sampling

In the first set of trials, duplicate samples were taken at the sampling points before and after the pump at both operating speeds. The pumps were stopped and sampling was done directly as described in Section 4.2.3. In the second set of trials, the pump was stopped at two separate instances at both operating speeds and single samples taken before and after the pump. Since small scale fermentations were done in the second set of trials, sample sterility was imperative, hence the sampling devices were used and sampling was done under pressure. In both the initial trials and the pump retrials, at least 15 minutes of cropping time was allowed before taking each set of samples. In a separate trial, samples were taken over 2.5 hour period of cropping to investigate the operation of a pump over

a longer period of cropping and to verify whether the quality of the yeast removed from the cone was consistent during the cropping period. The SP/40 Bredel peristaltic pump was selected for this since it was expected that, due to the friction between the pressing shoes and the hose, considerable heat generation would occur within the pump casing over an extended cropping period.

4.4.4 Analysis

Small scale fermentations take approximately two weeks to complete and for practical reasons the number of samples that could be fermented had to be limited. To enable testing of all the pumps within a realistic time scale, no small scale fermentations were done during the first round of pump trials. The yeast quality assays indicated in Table 4.5 were used for the evaluation of changes in yeast quality.

Table 4.5 Yeast quality assays selected for the first round of pump trials

NATURE OF YEAST QUALITY LOSS	ASSAY
Cell death	Methylene blue staining technique
Replicative Deactivation	Plate counts or slide counts
Physiological stress manifesting as a change in metabolic activity	(a) Oxygen utilisation rate (Method A) (b) Acidification power test
Physiological stress indicated by low levels of intracellular reserve compounds	Glycogen content by NIR spectrometry
Physiological stress leading to loss of stress resistance	Trehalose content by NIR spectrometry

When evaluating the performance of the four pumps during the retrials, focus was placed on detecting more subtle changes in yeast quality, hence small scale fermentations were used in addition to yeast quality assays (Table 4.6). The 2 L EBC tube system described in Section 3.3.7 was used for the small scale fermentations. Yeast performance was assessed by determining cell count profiles and total biomass growth as well as establishing the attenuation profiles and final level of attenuation. Beer quality was evaluated by measuring the diacetyl, SO_2 and acetaldehyde contents as well as the pH of the beer after 8 and 12 days of fermentation.

Table 4.6 Yeast quality assays selected for the pump retrials

NATURE YEAST QUALITY LOSS	ASSAY
Partial cell disruption	Protease assay
Cell death	Modified methylene blue staining technique
Physiological stress manifesting as a change in metabolic activity	Oxygen utilisation rate (Method B)
Altered fermentation performance	Small scale fermentation (2 l)

4.5 ESTABLISHING THE EFFECT OF DIFFERENT FLOW CONDITIONS

4.5.1 Introduction

Within SA Breweries, maximum linear velocities of 0.5 m/s are specified for yeast cropping. Based on the SA Breweries standard design values for viscosity (88 cP) and density (1050 kg/m^3), linear velocities of 0.3 and 0.5 m/s and Reynolds numbers of 260 and 340 were estimated to be typical of the yeast cropping conditions at the brewery where the experiments were done (Newlands Brewery). The yeast slurry is thus transferred under laminar flow conditions at relatively low flow rates.

To investigate the effect of linear velocity and Reynolds number on the quality of the cropped yeast suspension, a range of linear velocities and Reynolds numbers were set up in the rig.

4.5.2 Experimental Procedure

The rig was operated directly from the fermentation vessels. Using different holding tubes and operating over a range of pumping speeds, the flow conditions generated (detailed in Table 4.7) covered a range of flowrates from 17 to 110 L/min, linear velocities from 0.1 to 3.7 m/s and Reynolds numbers from 86 to 1114. Flow was restricted to the laminar flow region (owing to the equipment available).

Table 4.7 Flow conditions during the flow trials

PUMP	TUBE DIAMETER (mm)	PUMP SPEED (N) (rpm)	FLOW RATE (L/min)	REYNOLDS NUMBER	LINEAR VELOCITY (m/s)
APV Lobe	50	42	17	86	0.1
Peristaltic (SP/40)	50	25	30	152	0.3
Peristaltic (SP/40)	50	40	57	289	0.5
APV Lobe	50	168	67	339	0.6
Peristaltic (SP/50)	25	23	59	598	2.0
APV Lobe	25	187	75	760	2.5
Peristaltic (SP/50)	25	42	110	1114	3.7

4.5.3 Sampling

Pressurised sampling proceeded as in the pump retrials where the pump was stopped at two separate instances (15 minutes of cropping time apart) to obtain samples at the sampling points before and after the set of holding tubes (indicated on Figure 4.1). Between the sampling points, the yeast passed through 14 m of pipe, nineteen 90° bends (long-radius elbows) and a butterfly valve (fully open).

4.5.4 Analysis

Yeast quality was monitored as in the pump retrials (Table 4.6, Section 4.4.4), except that the fermentations were done using the 500 mL UCT system, described in Section 3.3.7.

CHAPTER

5

RESULTS AND DISCUSSION

5.1 INTRODUCTION

The results of the experimental work to establish the effect of cropping on yeast quality and subsequent fermentation performance are presented and discussed in this chapter. The data obtained during the evaluation of cropping during routine brewery operation, the effect of pump design and operation and the effect of flow conditions are given in Appendix D, Appendix E and Appendix F respectively. Included in these appendices are the results of the analyses done to establish the statistical significance of any differences in yeast quality. An explanation of the different statistical techniques used has been provided in Appendix B.

5.2 THE LOSS OF YEAST QUALITY DURING ROUTINE BREWERY CROPPING

5.2.1 Results

The effect of cropping on yeast quality during routine brewery operation was assessed by taking yeast samples on-line at the four points indicated in Section 4.3.3. The two sets of samples taken at these four sampling points, Set A and Set B, were taken 30 minutes apart after cropping 2000 and 4000 kg of yeast respectively. The results of the yeast quality assays for all 8 samples are presented in Table 5.1. For clarity, the cell count and attenuation profiles for the 2 L EBC tube fermentations of the two sets of samples have been plotted separately (Figures 5.1, 5.2, 5.3 and 5.4). The parameters for the exponential curves of the form $dS/dt = ae^{\beta t}$ fitted to the attenuation data in Figures 5.3 and 5.4 are given in Table 5.2. The beer quality indicators for the 2 L EBC fermentations are given in Table 5.3. The range of the data and the standard deviations normally associated with replicates have been included in all the tables. These standard deviations (s-values) may be regarded as good estimates of the population standard deviation (σ) since they represent the pooled standard deviations of replicates which have been determined for more than 20 degrees of freedom for the yeast quality assays and at least 16 degrees of freedom for the fermentation and beer quality indicators (see Appendix C).

Table 5.1 The effect of cropping on yeast quality during routine brewery operation: yeast quality assays

SAMPLING POINT	PROTEASE ACTIVITY (ΔA_{574nm})	VIABILITY (MODIFIED METHYLENE BLUE) (%)	OXYGEN UTILISATION RATE (mg/L/min/ 10^8 viable cells)	GLYCOGEN CONTENT (% w/w)	TREHALOSE CONTENT (% w/w)
Before Pump (A)	0.08	96	0.006	23.5	4.1
Before Pump (B)	0.05	96	0.006	24.3	4.7
After Line (A)	0.04	91	0.007	24.5	4.6
After Line (B)	0.06	91	0.006	24.7	4.0
Before Chiller (A)	0.05	89	0.007	24.3	5.0
Before Chiller (B)	0.06	91	0.005	25.6	3.9
After Chiller (A)	0.09	93	0.007	24.7	4.2
After Chiller (B)	0.05	94	0.005	25.0	4.8
Range	0.05	7	0.002	2.1	1.1
Standard Deviation for Replicates	0.02	2	0.002	1.0	1.0

5.2 THE LOSS OF YEAST QUALITY DURING ROUTINE BREWERY CROPPING

5.2.1 Results

The effect of cropping on yeast quality during routine brewery operation was assessed by taking yeast samples on-line at the four points indicated in Section 4.3.3. The two sets of samples taken at these four sampling points, Set A and Set B, were taken 30 minutes apart after cropping 2000 and 4000 kg of yeast respectively. The results of the yeast quality assays for all 8 samples are presented in Table 5.1. For clarity, the cell count and attenuation profiles for the 2 L EBC tube fermentations of the two sets of samples have been plotted separately (Figures 5.1, 5.2, 5.3 and 5.4). The parameters for the exponential curves of the form $dS/dt = ae^{\beta t}$ fitted to the attenuation data in Figures 5.3 and 5.4 are given in Table 5.2. The beer quality indicators for the 2 L EBC fermentations are given in Table 5.3. The range of the data and the standard deviations normally associated with replicates have been included in all the tables. These standard deviations (s-values) may be regarded as good estimates of the population standard deviation (σ) since they represent the pooled standard deviations of replicates which have been determined for more than 20 degrees of freedom for the yeast quality assays and at least 16 degrees of freedom for the fermentation and beer quality indicators (see Appendix C).

Table 5.1 The effect of cropping on yeast quality during routine brewery operation: yeast quality assays

SAMPLING POINT	PROTEASE ACTIVITY (ΔA_{574nm})	VIABILITY (MODIFIED METHYLENE BLUE) (%)	OXYGEN UTILISATION RATE (mg/L/min/ 10^8 viable cells)	GLYCOGEN CONTENT (% w/w)	TREHALOSE CONTENT (% w/w)
Before Pump (A)	0.08	96	0.006	23.5	4.1
Before Pump (B)	0.05	96	0.006	24.3	4.7
After Line (A)	0.04	91	0.007	24.5	4.6
After Line (B)	0.06	91	0.006	24.7	4.0
Before Chiller (A)	0.05	89	0.007	24.3	5.0
Before Chiller (B)	0.06	91	0.005	25.6	3.9
After Chiller (A)	0.09	93	0.007	24.7	4.2
After Chiller (B)	0.05	94	0.005	25.0	4.8
Range	0.05	7	0.002	2.1	1.1
Standard Deviation for Replicates	0.02	2	0.002	1.0	1.0

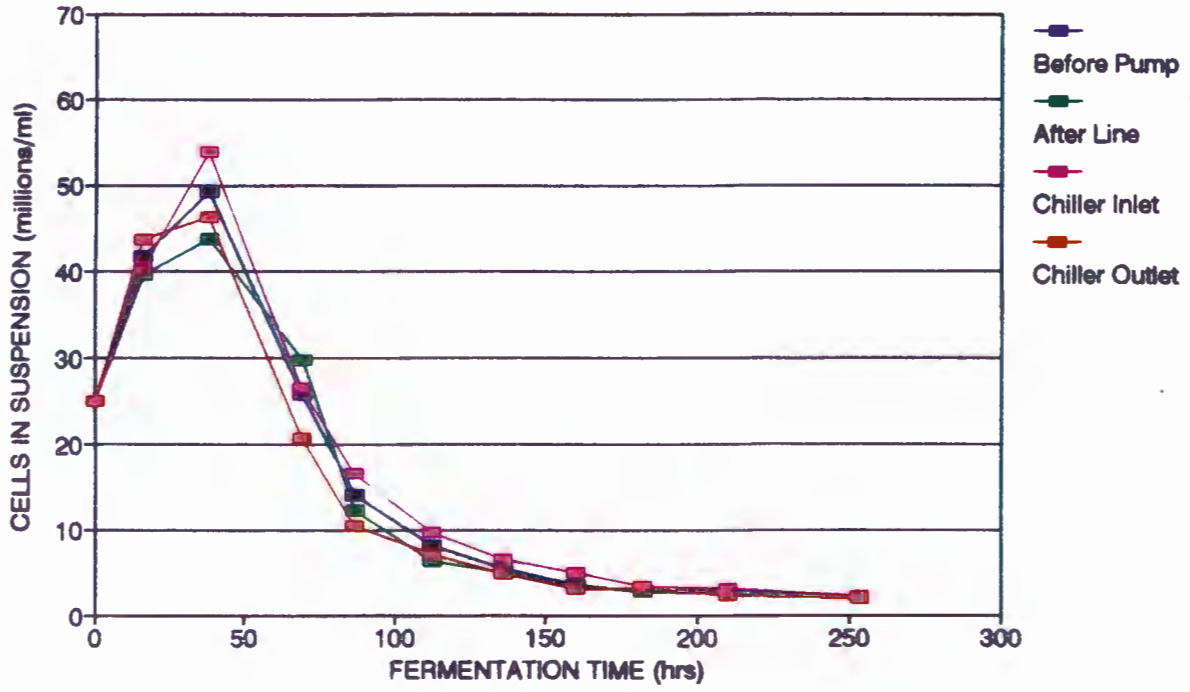

Figure 5.1 Cell count profiles for 2 L EBC tube fermentations of samples taken on-line to evaluate the effect of cropping on yeast quality during routine brewery operation (Set A)

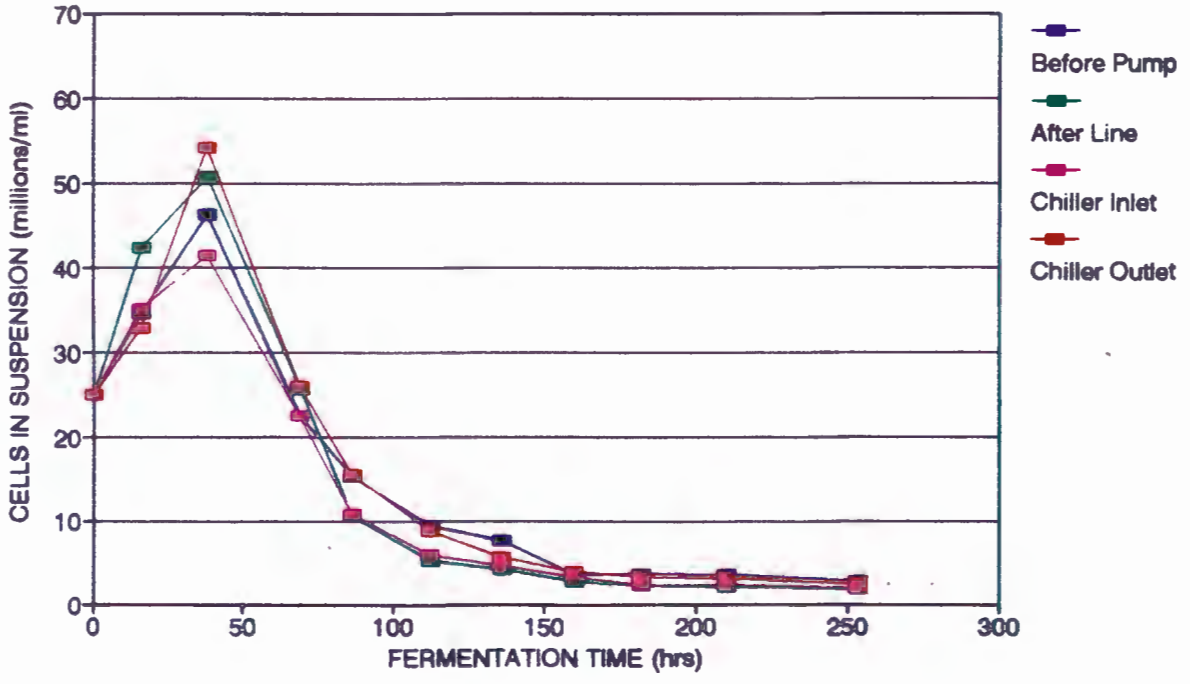

Figure 5.2 Cell count profiles for 2 L EBC tube fermentations of samples taken on-line to evaluate the effect of cropping on yeast quality during routine brewery operation (Set B)

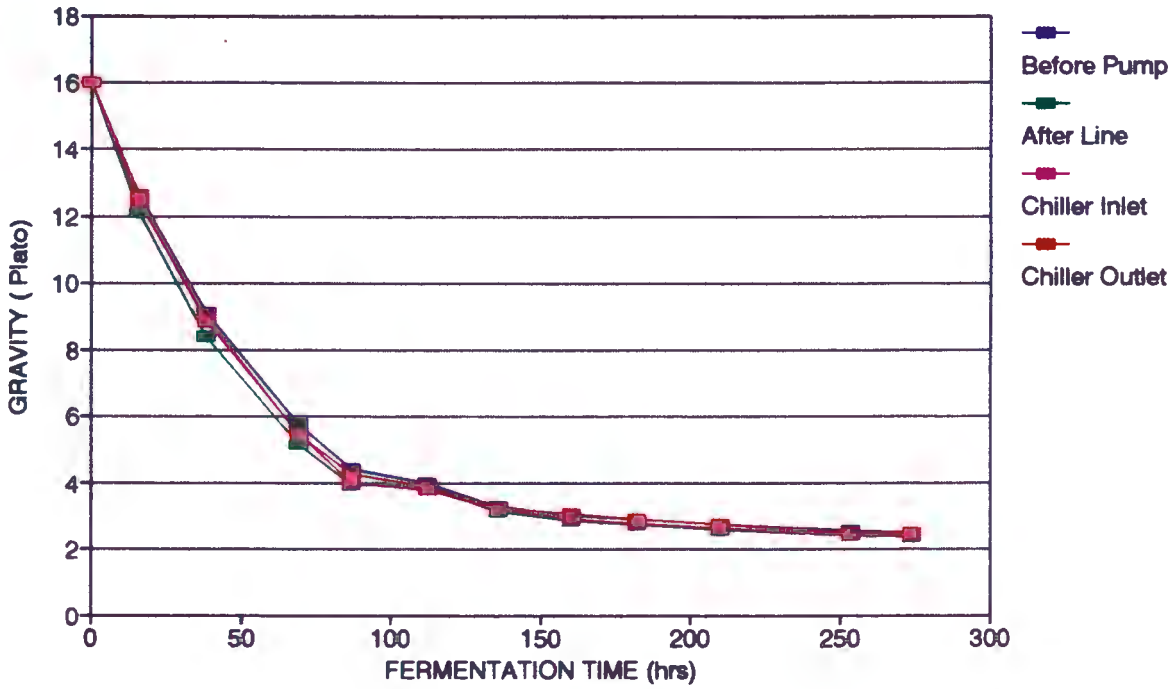

Figure 5.3 Attenuation profiles for 2 L EBC tube fermentations of samples taken on-line to evaluate the effect of cropping on yeast quality during routine brewery operation (Set A)

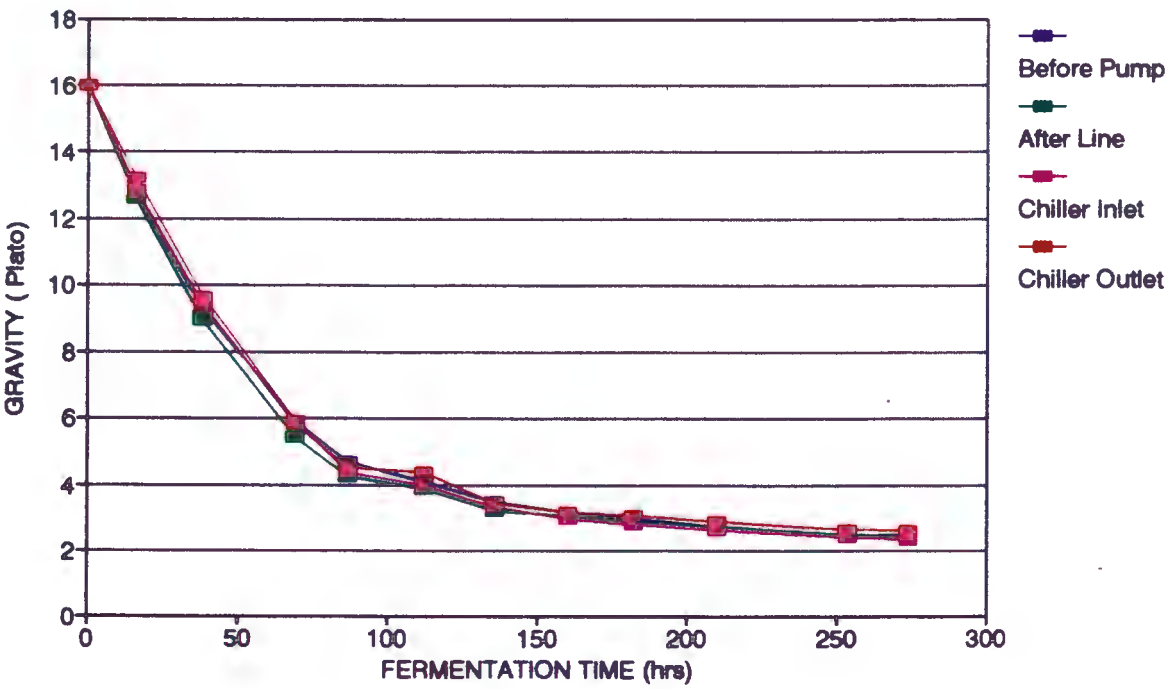

Figure 5.4 Attenuation profiles for 2 L EBC tube fermentations of samples taken on-line to evaluate the effect of cropping on yeast quality during routine brewery operation (Set B)

Table 5.2 The effect of cropping on yeast quality during routine brewery operation: parameters for exponential curves fitted to attenuation data for 2 L EBC tube fermentations

SAMPLING POINT	α-CONSTANTS (°Plato)	β-CONSTANTS (hr^{-1})	R^2 VALUES
Before Pump (A)	13.77	-0.0108	0.9537
Before Pump (B)	14.04	-0.0104	0.9955
After Line (A)	13.46	-0.0109	0.9310
After Line (B)	14.06	-0.0118	0.9513
Before Chiller (A)	13.61	-0.0110	0.9370
Before Chiller (B)	14.05	-0.0110	0.9538
After Chiller (A)	14.02	-0.0108	0.9392
After Chiller (B)	13.51	-0.0104	0.9471
Range	0.60	0.0014	-
Standard Deviation for Replicate Fermentations	0.19	0.0003	-

Table 5.3 The effect of cropping on yeast quality during routine brewery operation: results of beer quality indicators for 2 L EBC tube fermentations

SAMPLING POINT	pH (pH units)	DIACETYL (ppb)	SO_2 (ppm)	ACETALDEHYDE (ppm)
Before Pump (A)	4.19	84	8	21
Before Pump (B)	4.20	90	9	22
After Line (A)	4.20	88	8	23
After Line (B)	4.13	70	8	23
Before Chiller (A)	4.18	75	8	21
Before Chiller (B)	4.22	88	10	23
After Chiller (A)	4.24	93	9	23
After Chiller (B)	4.17	86	8	22
Range	0.11	23	2	2
Standard Deviation for Replicate Fermentations	0.04	10	2	4

5.2.2 Discussion

Statistical assessment was required to determine whether differences in yeast quality indicated by the results of the yeast quality assays (Table 5.1), fermentation indicators (Table 5.2) and beer quality indicators (Table 5.3) were significant. Since changes could occur in the quality of the yeast removed from the cone during the investigation and the two sets of samples (Set A and Set B) were taken after 2000 and 4000 kg of yeast had been cropped respectively, it could not be assumed that the samples taken at each sampling point were replicates. Paired-sample t tests could be done to determine whether the different steps in the yeast transfer process caused significant changes in the quality of the yeast. These tests focus on the (signed) differences (δ) between successive samples in each set and determine whether these differences can be regarded as random samples from a population for which the mean (μ) is zero. If the null hypothesis $\mu = 0$ were tested against the alternative hypothesis $\mu \neq 0$, acceptance of the null hypothesis would indicate that the apparent differences in yeast quality generated by the successive steps in the yeast transfer process were not significant. Each comparison, based on two points (one from Set A and one from Set B), would only have one degree of freedom (v). As discussed in Appendix B, comparison of small data sets are associated with large confidence intervals and would thus not be very sensitive to changes in yeast quality. To reduce the size of the confidence interval, the paired-sample t tests could be done by looking at the differences between successive sampling points simultaneously. In these tests, six differences would be compared, implying 5 degrees of freedom. This approach would not identify differences in yeast quality should the steps in the yeast transfer process have opposite effects.

An alternative approach would be to assume that the quality of the yeast removed from the cone did not change appreciably during the investigation and hence that the samples taken at the individual sampling points were replicates. The results could then be analysed using the standard techniques to compare means (Appendix B, Section 3) or by doing an analysis of variance (Appendix B, Section 4). The size of the confidence interval can be reduced significantly in both cases. In the former, it can be done by using z-values and the standard deviations normally associated with replicates (s_{pooled}) (provided these could be regarded as good estimates of the population standard deviation (σ)) and in the latter by looking at the body of data as a whole. The analysis of variance (ANOVA) would have the additional advantage that it would give an indication of the relative size of the variance associated with any change in the quality of the yeast during the investigation and changes resulting from mechanical handling of the yeast.

Both paired-sample t tests and an analysis of variance were done to establish whether the observed differences in yeast quality indicated by the results in Tables 5.1, 5.2 and 5.3 were significant.

5.2.2.1 Statistical Assessment by Paired-Sample *t* Tests

(a) The results of the yeast quality assays

Table 5.1 shows that for all the assays, other than the oxygen utilisation rate and trehalose content assays, the range of the eight samples is more than twice the standard deviations normally associated with replicates. This suggests possible differences in yeast quality between the samples. Two sets of paired-sample *t* tests were performed to determine whether the apparent changes in yeast quality were statistically significant. In the first, the differences in the yeast quality indicators obtained for samples taken at successive sampling points were treated as samples from a single population (simultaneous analysis). In the second, the differences obtained for each successive step were treated as samples from a population (analysis of individual sets). In the latter tests, the differences between the samples taken before the pump and after the chiller (the overall differences) were also considered. The results of the paired-sample *t* tests are given in Appendix D (page D1). Summaries of all the *t*-values obtained are presented in Table 5.4 (simultaneous analysis) and Table 5.5 (analysis of individual sets).

Table 5.4 Summary of *t*-values obtained in paired-sample *t* tests performed on results of yeast quality assays for samples taken on-line during routine brewery cropping (simultaneous analysis) (Critical *t*-value at 95% confidence level = 2.57)

YEAST QUALITY INDICATORS	*t*-VALUES
PROTEASE ASSAY	0.15
MODIFIED METHYLENE BLUE	-0.53
OXYGEN UTILISATION RATE	0.00
GLYCOGEN CONTENT	1.25
TREHALOSE CONTENT	0.12

When treating the differences taken from the successive sampling points simultaneously, the critical *t*-value for a 95% confidence level is 2.57 ($v = 5$). None of the *t*-values in Table 5.4 exceed this, hence the null hypothesis can not be rejected at a 95% confidence level. This suggests that none of the handling steps caused a change in the quality of the yeast.

The paired-sample *t*-tests on the individual sets of differences (summarised in Table 5.5) were done to identify if the different handling steps had opposing effects. In these tests, when the successive samples have identical values in all cases (denoted as VI), the average difference and standard deviation become zero; hence the expression for *t* is mathematically undefined. Intuitively, the null hypothesis can not be rejected. This is the case for the oxygen utilisation rate of the samples taken before and after the chiller. Hence, it is reasonable to conclude that the chiller does not effect a difference in oxygen utilisation rate of the yeast.

Where the differences between samples taken in each set are identical, the standard deviation of the differences becomes zero (denoted as SDZ) and the expression for t again becomes mathematically undefined. This is seen for difference between the fraction of living cells in the samples taken before the pump and before the swing bend panel ('after line') as indicated by the modified methylene blue staining technique and for the overall difference in trehalose content between the samples taken before the pump and after the chiller. A standard t test to compare two means (Appendix B, Section 3) was used for these results. Each value was treated as a mean and the pooled standard deviation for the assay applied. The differences in methylene blue results are not significant at 95% confidence level (difference = 5%, RHS value = 5.5%), but are significant at a 90% confidence level (difference = 5%, RHS value = 4.5%). Since the differences in the trehalose contents within each set of samples (0.1% w/w) are less than the standard deviations normally associated with trehalose assays (1% w/w), the overall differences in trehalose contents may be regarded as insignificant.

Table 5.5 Summary of t-values obtained in paired-sample t tests performed on results of yeast quality assays for samples taken on-line during routine brewery cropping (analysis of individual sets) (Critical t-value at 95% confidence level = 12.70)

SAMPLING POINTS	PROTEASE ASSAY	MODIFIED METHYLENE BLUE	OXYGEN UTILISATION RATE	GLYCOGEN CONTENT	TREHALOSE CONTENT
Pump/Line	-0.60	SDZ*	1.00	2.33	-0.17
Line/Chiller Inlet	1.00	-1.00	-1.00	0.64	0.60
Chiller Inlet/Outlet	0.60	7.00	VI**	-0.20	0.06
Overall (Pump/Chiller Outlet)	1.00	-5.00	0.00	3.80	SDZ*

* SDZ = standard deviation of differences = zero (ie. differences identical)
** VI = values identical (ie. all differences = zero)

For the analysis of individual sets of differences, the critical t-value for a 95% confidence level (v = 1) is 12.70. In all cases where the t-values are defined, they are less than 12.70 (Table 5.5), hence the null hypothesis can not be rejected. As before, it could be concluded at a 95% confidence level that none of the steps in the transfer of yeast from the cone to the outlet of the chiller caused a detectable difference in the quality of the yeast. As indicated, these tests are not very sensitive due to the large confidence interval associated with comparisons made on the basis of two points only (*ie.* with only one degree of freedom). However, except for three differences indicated by the modified methylene blue method (two by the paired-sample t test and one by the standard t test) and one difference indicated by the glycogen content of the cells, the null hypothesis can not be rejected at an 80% confidence level where the critical t-value is 3.08. In addition,

these *t*-values are all reasonably close to zero, suggesting that it is reasonable not to reject the null hypothesis.

If the differences in yeast quality indicated by the modified methylene blue method are regarded as significant (at an 80% confidence level), then it is suggested that there is a decrease in the number living cells in the population (% "viability") as the yeast passes through the pump and flows through 105 m of pipe to the swing bend panel (as indicated by the standard *t* test); there is no difference in % "viability" between the sample taken at the swing bend panel and at the chiller inlet (t = -1.00) and that the "viability" is greater after passing through the chiller (t = 7.00). This apparent increase in "viability" over the chiller is counter-intuitive. During sampling the pump was switched off and the on-line samples were taken in sequence starting at the sampling point before the pump and ending at the chiller outlet. During this period, the chiller was switched off to prevent the yeast from freezing and the temperature of the yeast at the chiller outlet increased from 3.3°C to 8.7°C. The yeast leaving the cone of the fermenter is usually at 14 to 16°C. During the five minutes that elapsed between taking the samples before the pump and taking those at the swing bend panel some metabolism may have occurred ultimately leading to the death of some of the cells. Cooling in the chiller may have limited the metabolism of the yeast at the chiller outlet. The apparent changes in the number of living cells during transfer of the yeast from the cone to the chiller outlet would thus be a function of the sampling procedure rather than the handling of the yeast. However, five minutes is not a large time scale for such an occurrence hence the apparent changes in yeast "viability" may rather be the result of analytical error.

The *t*-value for the "viability" results for the overall process of transferring yeast from the cone to the chiller outlet (t = -5.00) is significant at an 80% confidence level which suggests a decrease in yeast "viability". This decrease is not significant at a 90% confidence level (critical *t*-value = 6.31). The glycogen content of the samples taken before the pump appears to be less than that taken after the chiller as the *t*-value for the overall process (t = 3.80) is significant at an 80% confidence level (critical *t*-value = 3.08), but not at a 90% confidence level (critical *t*-value = 6.31). This apparent increase in glycogen is not consistent with a loss of yeast quality indicated by the modified methylene blue assay. Since neither of these differences are significant at a 90% confidence level, it can not be said conclusively that the transfer of yeast from the cone to chiller outlet causes a change in yeast quality.

(b) The results of the small scale fermentations

The apparent differences in the cell count profiles (Figures 5.1 and 5.2) and attenuation profiles (Figure 5.3 and 5.4) for the 2 L EBC tube fermentations of the samples taken on-line during cropping appear to be comparable to those observed for replicate fermentations (Figure 3.2 and Figure 3.3). However, the ranges of the α- and β-constants for the exponential curves fitted to the attenuation profiles over the first 160 hours of fermentation (0.60 and 0.0014 respectively) are greater than twice the standard deviations associated with replicate fermentations (s = 0.19

and 0.0003). To establish whether there were significant differences in the attenuation profiles of the fermentations, paired-sample *t* tests were done as described above. The paired-sample *t* tests are given in detail in Appendix D (page D2) and summaries of all the *t*-values obtained are given in Table 5.6 (simultaneous analysis) and Table 5.7 (analysis of individual sets).

Table 5.6 Summary of *t*-values obtained in paired-sample *t* tests performed on α- and β-constant for exponential fits of the attenuation profiles following routine brewery cropping (simultaneous analysis) (Critical *t*-value at 95% confidence level = 2.57)

FERMENTATION PERFORMANCE INDICATORS	*t*-VALUES
α-CONSTANTS	-0.34
β-CONSTANTS	0.00

Table 5.7 Summary of *t*-values obtained in paired-sample *t* tests performed on α- and β-constant for exponential fits of the attenuation profiles following routine brewery cropping (analysis of individual sets) (Critical *t*-value at 95% confidence level = 12.70)

SAMPLING POINTS	α-CONSTANTS	β-CONSTANTS
Pump/Line	-0.83	-1.15
Line/Chiller Inlet	0.84	0.78
Chiller Inlet/Outlet	-0.14	2.00
Overall (Pump/Chiller Outlet)	-0.36	VI*

* VI = values identical (ie. all differences = zero)

Both the *t*-values in Table 5.6 for the simultaneous analysis of all the differences are less than the appropriate critical *t*-value 2.57 (v = 5) for a 95% confidence level. This suggests that there are no significant differences in the attenuation profiles of the 2 L EBC fermentations done with the samples taken during the investigation.

As can be seen from Table 5.2, the values of the β-constant for the fermentations of the samples taken before the pump and after the chiller are identical in Set A and in Set B, hence the overall difference in the β-constants and the standard deviation (s) of their average are zero. When paired-sample *t* tests are done individually on the corresponding sets of differences, the expression for *t* thus becomes mathematically undefined for the overall difference in the β-constants. As discussed above, the null hypothesis should not be rejected. In all other cases, the *t*-values are less than 12.70, the critical *t*-value (v = 1) (Table 5.7), hence the null

hypothesis can not be rejected in any of the cases. This confirms the conclusion that (at a 95% confidence level) none of the steps in the transfer of yeast from the cone to the outlet of the chiller cause a detectable difference in the attenuation profiles of the yeast. All the *t* values in Table 5.7 are reasonably close to zero (especially those for the α-constants), suggesting that this conclusion is valid despite the limitations of *t* tests with only one degree of freedom.

For the beer quality indicators after 12 days of fermentation (Table 5.3), the range of all eight samples that were fermented is greater than twice the standard deviation associated with replicate fermentations for the final pH and diacetyl levels, equal to the normal standard deviation for the SO_2 level and less than that for the acetaldehyde level. There may thus be differences in the final pH and diacetyl levels. As before, paired sample *t*-tests were done to test the significance of the apparent differences in the beer quality indicators. The paired-sample *t* tests are presented in detail in Appendix D (page D2) and summaries of all the *t*-values obtained are presented in Table 5.8 (simultaneous analysis) and Table 5.9 (analysis of individual sets).

Table 5.8 Summary of *t*-values obtained in paired-sample *t* tests performed on the results of the beer quality indicators (simultaneous analysis) (Critical *t*-value at 95% confidence level = 2.57)

FERMENTATION PERFORMANCE INDICATORS	*t*-VALUES
pH	0.13
DIACETYL	0.13
SO_2	0.00
ACETALDEHYDE	0.50

Table 5.9 Summary of *t*-values obtained in paired-sample *t* tests performed on the results of the beer quality indicators (analysis of individual sets) (Critical *t*-value at 95% confidence level = 12.70)

SAMPLING POINTS	pH	DIACETYL	SO_2	ACETALDEHYDE
Pump/Line	-0.75	-0.67	-1.00	3.00
Line/Chiller Inlet	0.64	0.16	1.00	-1.00
Chiller Inlet/Outlet	0.09	0.80	-0.33	0.33
Overall (Pump/Chiller Outlet)	0.25	0.38	-0.00	1.00

All the *t*-values in Table 5.8 for the simultaneous analysis of all the differences are less than the appropriate critical *t*-value 2.57 (v = 5) for a 95% confidence level. This suggests that, after 12 days of fermentation, there were no significant

differences in the quality of the beer produced in the 2 L EBC fermentations done with the samples taken during the investigation.

As indicated by Table 5.9, when the sets of differences are analysed individually, none of the differences are significant at a 95% confidence level (critical *t*-value = 12.70, v = 1) which confirms that no significant differences could be detected in the beer quality indicators following fermentation of samples taken to evaluate the effect of cropping on yeast quality during routine brewery operation.

(c) Changes in the quality of the yeast being removed from the cone during the investigation

To determine whether the quality of the yeast being removed from the cone changed significantly over the period of investigation, another set of paired-sample *t* tests were done. These tests compared the differences in the quality and fermentation performance of the yeast sampled after 2000 kg and 4000 kg of yeast had been cropped. Differences at four points were compared, hence the critical *t*-statistic for 3 degrees of freedom (v) at a 95% confidence level applies (t = 3.18). The paired-sample *t* tests are presented in detail in Appendix D (page D3) and the *t*-values obtained summarised in Table 5.10. All these *t*-values are less than 3.18. At a 95% confidence level, no difference could thus be detected in the quality or fermentation performance of the yeast removed from the cone during the investigation.

Table 5.10 Summary of *t*-values obtained in paired-sample *t* tests performed on yeast quality and fermentation performance indicators to determine the quality of the yeast as a function of cropping time (Critical *t*-value at 95% confidence level = 3.18)

YEAST QUALITY INDICATORS	*t*-VALUES
PROTEASE ASSAY	-0.68
MODIFIED METHYLENE BLUE	1.57
OXYGEN UTILISATION RATE	-2.61
GLYCOGEN CONTENT	2.57
TREHALOSE CONTENT	-0.29
FERMENTATION PERFORMANCE INDICATORS	***t*-VALUES**
α-CONSTANTS	0.81
β-CONSTANTS	-0.08
pH	-0.08
DIACETYL	-0.22
SO_2	0.77
ACETALDEHYDE	0.77

5.2.2.2 Statistical Assessment by an Analysis of Variance (ANOVA)

An analysis of variance was done to verify the conclusions drawn from the paired-sample *t* tests. The null hypothesis assumed that the variance of the samples taken at each sampling point (*the within-sample variance*) was equal to the variance between the samples taken at all the sampling points (*the between-sample variance*). This was tested against the alternative hypothesis that the latter variance exceeded the former. The ANOVA tables are presented in Appendix D (pages D4 and D5). A summary of the F-values obtained is given in Table 5.11. All the F-values are based on 3 degrees of freedom for the *between-sample variance* and 4 degrees of freedom for the *within-sample variance* (*ie.* total number of degrees of freedom = 7). The appropriate critical value of F for a 95% level of confidence is 6.59. Only the F-value calculated for the "viability" (as indicated by modified methylene blue staining) was significant at a 95% confidence level. The apparent differences in the number of living cells in the different samples have been explored (Section 5.2.2.1) and could be attributed either to analytical error or to the sampling procedure followed.

Table 5.11 Summary of F-values obtained during the analysis of variance for the yeast quality and fermentation performance indicators for the samples taken on-line during routine brewery cropping (Critical F-value at a 95% confidence level = 6.59)

YEAST QUALITY INDICATORS	F-VALUES
PROTEASE ASSAY	0.44
MODIFIED METHYLENE BLUE	23.13
OXYGEN UTILISATION RATE	0.11
GLYCOGEN CONTENT	1.46
TREHALOSE CONTENT	0.05
FERMENTATION PERFORMANCE INDICATORS	**F-VALUES**
α-CONSTANTS	0.08
β-CONSTANTS	1.85
pH	0.45
DIACETYL	0.65
SO_2	0.44
ACETALDEHYDE	1.11

The results presented by Table 5.11 thus indicate that the variance associated with the samples taken at the same sampling points after 2000 and 4000 kg of yeast had been cropped (the *within-sample variance*) can be regarded as equal to the variance associated with the differences between the samples taken at the different sampling points (the *between-sample variance*). Hence from the ANOVA, it can be

concluded that the mechanical handling of yeast during its transfer from the base of the cone to the chiller outlet does not affect yeast quality to a greater extent than the changes in the quality of the yeast removed from the cone during the investigation. However, looking at the results in Tables 5.1 to 5.3 and Figures 5.1 to 5.4, it is reasonable to conclude that mechanical handling of yeast during its transfer from the base of the cone to the chiller outlet did not affect yeast quality, neither was there a change in the quality of the next 2000 kg of the yeast removed from the cone once the first 2400 kg of yeast had been removed.

5.3 THE EFFECT OF PUMP DESIGN AND OPERATION ON YEAST QUALITY DURING CROPPING

5.3.1 Results of the Initial Pump Trials

As indicated in Section 4.4, during the initial pump trials, ten pumps were used to investigate the effect of cropping pump design and operation on yeast quality. The pumps were operated at two speeds and samples were taken in duplicate at the sampling points before and after each pump. The complete set of results of the yeast quality assays for all ten pumps is given in Appendix E (pages E1 - E10). As an example, the results obtained for the Maso sine pump are presented in this section (Table 5.12). The values in Table 5.12 are averages of the duplicate samples taken at each sampling point.

Table 5.12 Results of yeast quality assays for Maso sine pump evaluated during the initial pump trials

RUN NO.	PUMP SPEED	SAMPLING POINT	METHYLENE BLUE (% viability)	PLATE COUNTS (% viability)	OXYGEN UTILISATION RATE (mg/min/g cells)	SPONTANEOUS ACIDIFICATION POWER (pH units)	SPONTANEOUS CUMULATIVE ACIDIFICATION POWER (x 10^5)	ACIDIFICATION POWER (pH units)	CUMULATIVE ACIDIFICATION POWER (x 10^5)	TREHALOSE CONTENT (% w/w)	GLYCOGEN CONTENT (% w/w)
4	244 rpm (89 L/min)	Before Pump	89	55	0.119	1.98	2.8	2.38	14	-	-
		After Pump	92	52	0.117	2.03	2.8	2.46	15	-	-
	713 rpm (289 L/min)	Before Pump	90	60	0.122	1.92	1.7	2.42	13	-	-
		After Pump	88	46	0.117	1.99	2.8	2.49	16	-	-
5	244 rpm (89 L/min)	Before Pump	90	-	0.142	2.15	4.1	2.53	18	6.9	26.5
		After Pump	90	-	0.154	1.91	2.5	2.43	15	5.4	25.4
	400 rpm (162 L/min)	Before Pump	94	-	0.156	2.12	5.0	2.47	16	5.7	26.0
		After Pump	91	-	0.153	2.11	3.0	2.61	21	5.6	27.0

5.3.2 Discussion of the Results the Initial Pump Trials

Since the samples taken at each sampling point were true replicates, an analysis of the variance (ANOVA) was done to assess the significance of the differences in yeast quality. Since all the pumps (except the Wilflo lobe pump and the Fristam centrifugal pump) were tested at two operating speeds and duplicate samples were taken before and after the pumps, eight samples were taken for each pump during each run in which the pump was tested. The analysis of variance was done simultaneously on each set of eight data points for each of the yeast quality assays performed. The null hypothesis that the *between-sample variance* was equal to the *within-sample variance,* was tested against the alternative hypothesis that the *between-sample variance* was greater than the *within-sample variance*. Rejection of the null hypothesis and acceptance of the alternative hypothesis would indicate that the observed difference in the quality of the yeast sampled before and after a particular pump was significant for at least one of the operating speeds or that the quality of the yeast removed from the cone changed during the testing period. For the Wilflo lobe and Fristam centrifugal pumps, which were tested at one pump speed only, rejection of the null hypothesis would indicate that the pump effected a change in yeast quality at that operating speed.

The analysis of variance for all the results obtained for all ten pumps tested during the initial pump trials is given in detail in Appendix E (pages E11 - E39). The samples are coded according the speed at which the pumps were tested and whether the samples were taken before or after the pump: S1B, for example, would correspond to the samples taken before the pump (B) at the first speed (S1) at which the pump was operated during the trial. The exact pump speeds can be obtained from the tables which contain the complete sets of results (Appendix E, pages E1 to E10). As an example, the results of the analysis of variance for the second run with the Maso sine pump (Run 5) are given in this section (Table 5.13 and Table 5.14). The appropriate critical F-value for each analysis at a 95% confidence level is 6.59 (v = 3 for the *between-sample variance* and v = 4 for the *within-sample variance*). As indicated by the shaded regions in Table 5.13, only the F-values calculated for the cumulative spontaneous acidification power (F = 7.03), acidification power (F = 16.45) and cumulative acidification power (7.03) exceed the critical F-value of 6.59. The null hypothesis can be rejected at a 95% confidence level for these three cases which suggests that there may have been differences in yeast quality between the sets of samples taken before and after the pumps at the two operating speeds.

To identify whether these apparently significant changes in yeast quality indicated in Table 5.13 could be attributed to the operation of the pumps or to changes in the quality of the yeast being removed from the cone, the variances for the data obtained at the two pump speeds were analysed separately. The results of these analyses of variance are given in Table 5.14. Here the appropriate critical F-value at a 95% confidence level was 18.5 (v = 1 for the *between-sample variance* and v = 2 for the *within-sample variance*).

Table 5.13 Results of ANOVA for second trial of Maso sine pump (simultaneous analysis)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	93	87	180	90	2	Between Samples	27.38	3	9.13	1.28
Blue	S1A	88	92	180	90	2	Within Samples	28.50	4	7.13	
	S2B	95	94	189	95	2	Total	55.88	7		
	S2A	90	92	182	91	2					
Oxygen	S1B	-	0.142	0.142	0.142	1	Between Samples	0.00014	3	0.00005	2.23
Utilisation	S1A	0.159	0.149	0.308	0.154	2	Within Samples	0.00006	3	0.00002	
Rate	S2B	0.156	0.156	0.312	0.156	2	Total	0.00020	6		
	S2A	0.150	0.155	0.305	0.153	2					
Spontaneous	S1B	2.20	2.10	4.30	2.15	2	Between Samples	0.075	3	0.025	3.72
Acidification	S1A	1.85	1.96	3.81	1.91	2	Within Samples	0.027	4	0.007	
Power	S2B	2.20	2.04	4.24	2.12	2	Total	0.102	7		
	S2A	2.15	2.07	4.22	2.11	2					
Cumulative	S1B	3E-05	5E-05	8E-05	4E-05	2	Between Samples	9E-10	3	3E-10	7.03
Spontaneous	S1A	3E-05	2E-05	5E-05	3E-05	2	Within Samples	2E-10	4	4E-11	
Acidification	S2B	5E-05	5E-05	1E-04	5E-05	2	Total	1E-09	7		
Power	S2A	3E-05	3E-05	6E-05	3E-05	2					
Acidification	S1B	2.54	2.52	5.06	2.53	2	Between Samples	0.038	3	0.013	16.45
Power	S1A	2.41	2.45	4.86	2.43	2	Within Samples	0.003	4	0.001	
	S2B	2.49	2.44	4.93	2.47	2	Total	0.041	7		
	S2A	2.59	2.63	5.22	2.61	2					
Cumulative	S1B	2E-04	2E-04	4E-04	2E-04	2	Between Samples	4E-09	3	1E-09	7.03
Acidification	S1A	1E-04	1E-04	3E-04	1E-04	2	Within Samples	8E-10	4	2E-10	
Power	S2B	2E-04	2E-04	3E-04	2E-04	2	Total	5E-09	7		
	S2A	2E-04	2E-04	4E-04	2E-04	2					
Glycogen	S1B	25.4	27.6	53.0	26.5	2	Between Samples	2.7	3	0.9	1.22
Content	S1A	25.2	25.6	50.8	25.4	2	Within Samples	3.0	4	0.7	
	S2B	25.5	26.4	51.9	26.0	2	Total	5.7	7		
	S2A	26.8	27.1	53.9	27.0	2					
Trehalose	S1B	7.7	6.2	13.9	7.0	2	Between Samples	3.01	3	1.00	2.08
Content	S1A	5.5	5.3	10.8	5.4	2	Within Samples	1.93	4	0.48	
	S2B	6.2	5.1	11.3	5.7	2	Total	4.94	7		
	S2A	5.9	5.3	11.2	5.6	2					

Table 5.14 Results of ANOVA for second trial of Maso sine pump (analysis for individual pump speeds)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Cumulative Spontaneous Acidification Power	S1B	3E-05	5E-05	8E-05	4E-05	2	Between Samples	3E-10	1	3E-10	3.18
	S1A	3E-05	2E-05	5E-05	3E-05	2	Within Samples	2E-10	2	8E-11	
							Total	4E-10	3		
	S2B	5E-05	5E-05	1E-04	5E-05	2	Between Samples	5E-10	1	5E-10	86.84
	S2A	3E-05	3E-05	6E-05	3E-05	2	Within Samples	1E-11	2	6E-12	
							Total	5E-10	3		
Acidification Power	S1B	2.54	2.52	5.06	2.53	2	Between Samples	0.010	1	0.010	20.00
	S1A	2.41	2.45	4.86	2.43	2	Within Samples	0.001	2	0.000	
							Total	0.011	3		
	S2B	2.49	2.44	4.93	2.47	2	Between Samples	0.021	1	0.021	20.51
	S2A	2.59	2.63	5.22	2.61	2	Within Samples	0.002	2	0.001	
							Total	0.023	3		
Cumulative Acidification Power	S1B	2E-04	2E-04	4E-04	2E-04	2	Between Samples	8E-10	1	8E-10	3.23
	S1A	1E-04	1E-04	3E-04	1E-04	2	Within Samples	5E-10	2	2E-10	
							Total	1E-09	3		
	S2B	2E-04	2E-04	3E-04	2E-04	2	Between Samples	2E-09	1	2E-09	14.31
	S2A	2E-04	2E-04	4E-04	2E-04	2	Within Samples	3E-10	2	2E-10	
							Total	3E-09	3		

As can be seen from Table 5.14, the null hypothesis can be rejected and the alternative hypothesis that the *between-sample variance* exceeds the *within-sample variance* can be accepted at a 95% confidence level in three cases. This suggests that there was a significant decrease in the acidification power of the yeast samples taken before and after the pump at the first pump speed (244 rpm) (F = 20.00) and a significant decrease in cumulative spontaneous acidification power (F = 86.84), but an increase in the acidification power at the second pump speed (400 rpm) (F = 20.51).

A summary of the F-values obtained during the analysis of variance for all ten pumps evaluated during the initial pump trials is given in Table 5.15. For all the pumps except the Wilflo lobe pump and the Fristam centrifugal pump, the appropriate critical F-value for each analysis at a 95% confidence level was 6.59 (v = 3 for the *between-sample variance* and v = 4 for the *within-sample variance*). For the Wilflo lobe pump and the Fristam centrifugal pump, the appropriate critical F-value at a 95% confidence level was 18.5 (*v* = 1 for the *between-sample variance* and v = 2 for the *within-sample variance*). In some cases, fewer data points were collected. In Table 5.15, such cases are indicated and the appropriate critical F-values given at the base of the table.

Where the *between-sample variance* was significant relative to the *within-sample variances*, the blocks in Table 5.15 have been shaded. As illustrated for the Maso sine pump, the variances for the data collected at the two pump speeds were then analysed separately to identify whether the apparently significant changes in yeast quality could be attributed to the operation of the pumps or to changes in the quality of the yeast being removed from the cone. The results of these analyses of variance, for which the appropriate critical F-value at a 95% confidence level was 18.5 (v = 1 for the *between-sample variance* and *v* = 2 for the *within-sample variance*), are given in detail in Appendix E (pages E11 - E39). A summary of the apparent changes in yeast quality which could be attributed to mechanical handling of the yeast by the pumps is given in Table 5.16. The presence of such changes in yeast quality have also been indicated with dotted margins in Table 5.15.

Table 5.15 Summary of F-values obtained for analysis of variance of results obtained during initial pump trials (simultaneous analysis)

PUMP TYPE	MAKE	TRIAL	METHYLENE BLUE	PLATE/SLIDE COUNTS	OXYGEN UTILISATION RATE	SPONTANEOUS ACIDIFICATION POWER	CUMULATIVE SPONTANEOUS ACIDIFICATION POWER	ACIDIFICATION POWER	CUMULATIVE ACIDIFICATION POWER	GLYCOGEN CONTENT	TREHALOSE CONTENT
PERISTALTIC	Bredel	1	1.04	6.38 (plate)	15.59**	7.66	1.03	6.95	3.40	-	-
		2	0.50	1.60 (plate)	3.56	1.69	0.10	3.18	1.27	1.40	1.61
LOBE	APV	1	14.44	-	5.05	0.22	0.75	1.72	5.63	0.23	1.67
		2	0.24	-	0.07	2.04	3.70	5.66	3.01	9.30	0.75
	Ibex	1	0.44	0.25 (plate)	0.35	2.01**	10.34**	1.15**	1.73**	0.42	2.84
		2	1.04	0.76 (plate)**	0.77	0.37	9.02	0.84	3.14	-	-
	Johnson	1	1.67	-	2.55	2.26	0.38	1.69	1.86	1.41	0.90
		2	2.38	1.27 (slide)	3.53	-	-	-	-	0.52	0.46
	Wilflo*	1	3.24	0.53 (slide)	0.01	-	-	-	-	33.11	0.86
SINE	Maso	1	2.13	0.21 (plate)	0.18**	1.69	2.58	6.71	3.63	-	-
		2	1.28	-	2.23**	3.72	7.03	16.45	7.03	1.22	2.08
GEAR	Scandi Brew	1	3.36	2.10 (slide)	0.07	-	-	-	-	-	-
		2	1.70	0.12 (slide)	1.90***	-	-	-	-	0.07	0.65
DIAPHRAGM	DEPA	1	3.38	10.15 (plate)	0.51****	1.64	6.37	0.13	0.60	7.11	1.95
		2	3.21	0.03 (plate)	1.74****	0.89	1.02	2.58	1.70	96.33*****	2.07***
	Wilden	1	6.44	0.17 (slide)	1.46	-	-	-	-	0.67	4.52
		2	0.19	0.14 (slide)	0.76	-	-	-	-	1.72	0.15
CENTRIFUGAL	Fristam*	1	0.50	9.00 (slide)	50.00	-	-	-	-	0.05	0.00

* pumps tested at one speed only hence $v = 1,2$ $F_{95\%} = 18.5$

** $v = 3,3$ $F_{95\%} = 9.28$

*** $v = 1,2$ $F_{95\%} = 18.5$

**** $v = 3,1$ $F_{95\%} = 216$

***** $v = 1,1$ $F_{95\%} = 161$

Table 5.16 Changes in yeast quality effected by pumping (95% confidence level)

PUMP	TRIAL	PUMP SPEED	YEAST QUALITY INDICATOR	CHANGE
Bredel peristaltic	1	25 rpm (33 L/min)	oxygen utilisation rate	16% increase
		50 rpm (72 L/min)	acidification power	5% increase
APV lobe	1	40 rpm (16 L/min)	% viability (indicated by methylene blue staining technique)	3% decrease
Wilflo lobe	1	243 rpm (66 L/min)	glycogen content	9% increase
Maso sine	2	244 rpm (89 L/min)	acidification power	4% decrease
		400 rpm (162 L/min)	cumulative spontaneous acidification power	50% decrease
			acidification power	6% increase
DEPA diaphragm	1	400 kPa air pressure	glycogen content	18% decrease
Fristam centrifugal	1	3000 rpm	oxygen utilisation rate	22% increase

The Wilflo lobe pump was evaluated at the same operating speed (243 rpm) during the investigation of cropping during routine brewery operation in which no significant changes in the glycogen content of the yeast could be attributed to mechanical handling of the yeast by the pump (Table 5.5). The apparent increase in glycogen content observed for the Wilflo lobe pump (Table 5.16), which is counter-intuitive, could more likely be attributed to experimental error than a true change in yeast quality. The decrease in "viability" indicated by methylene blue staining for yeast pumped with the APV lobe pump and the decrease in the glycogen content of the yeast pumped with the DEPA diaphragm pump would be consistent with a loss of yeast quality. Mechanical handling of the yeast by the pumps appeared to effect increases in oxygen utilisation rate in two cases (the Bredel peristaltic pump and the Fristam centrifugal pump). Both increases and decreases in acidification power were observed upon mechanical handling of the yeast (Bredel peristaltic pump and Maso sine pumps).

None of the pumps that were tested twice, however, produced consistent and apparently significant changes in yeast quality in both trials. In addition, the apparently significant change in the quality of the yeast was not evident across several yeast quality assays in any case. The results in Table 5.16 were therefore not regarded as conclusive proof that the pumps effected changes in yeast quality and the following pumps were selected for retrial: the Bredel peristaltic pump, the APV lobe pump and the Maso sine pump. No further trials were done with the DEPA diaphragm and the Fristam centrifugal pumps, since these pumps were regarded as unsuitable for yeast cropping applications. Additional data for the Wilflo pump was provided by the assessment of routine brewery operation (Tables 5.1 - 5.3, Figures 5.1 - 5.4).

5.3.3 Results of the Pump Retrials

In addition to the three pumps selected for retrial (Bredel peristaltic (SP/40), APV lobe and Maso sine), the performance of an SP/50 Bredel peristaltic pump which had greater capacity than the SP/40 pump tested during the initial trials, was evaluated during the second set of pump trials (as indicated in Section 4.4.2). The results of the yeast quality assays and small scale fermentations performed on the samples taken during these trials are presented in detail in Appendix E (pages E40 - E51). As an example, the results obtained for the Maso sine pump are presented in this section. The results of the yeast quality assays are given in Table 5.17. Figure 5.5 and Figure 5.6 present the cell count and attenuation profiles for the samples taken when the pump was operated at 244 rpm, while the profiles for the samples taken when the pump was operated at 400 rpm are given in Figure 5.7 and Figure 5.8. Table 5.18 presents the results of the fermentation indicators: increase in biomass, final attenuation and the constants for the exponential curve fits to the attenuation profiles. The results of the beer quality indicators after 8 and 12 days of fermentation are given in Table 5.19 and Table 5.20 respectively. As indicated in Section 4.4.3, the pump was stopped and samples taken at two separate instances approximately 15 minutes of cropping time apart. The results in the tables and Appendix E (pages E40 - E51) have been coded accordingly. For example, 'before 1' and 'after 1' refer to the samples taken before the pump and after the pump at the first instance when the pump was stopped for sampling after operation at the particular pumping speed.

Table 5.17 Results of yeast quality assays for the retrial of the Maso sine pump

PUMP SPEED	SAMPLE	PROTEASE ACTIVITY (ΔA_{574nm})	VIABILITY (MODIFIED METHYLENE BLUE) (%)	OXYGEN UTILISATION RATE (mg/L/min/ 10^8 viable cells)
240 rpm (89 L/min)	before 1	0.05	92	0.015
	before 2	0.06	89	0.008
	after 1	0.08	93	0.009
	after 2	0.07	90	0.008
404 rpm (164 L/min)	before 1	0.04	92	0.015
	before 2	0.10	94	0.011
	after 1	0.10	93	0.007
	after 2	0.10	93	0.008

Table 5.18 Results of fermentation indicators for the 2 L EBC tube fermentations of the samples taken to evaluate the effect of the Maso sine pumps on yeast quality

				ATTENUATION PROFILE $dS/dt = \alpha e^{\beta t}$		
PUMP SPEED	SAMPLE	INCREASE IN BIOMASS (factor)	FINAL ATTENUATION (°Plato)	α-CONSTANT (° Plato)	β-CONSTANT (hr^{-1})	R^2 VALUE
240 rpm (89 L/min)	before 1	3.0	2.55	16.26	-0.0101	0.9777
	before 2	3.0	2.51	15.96	-0.0103	0.9777
	after 1	3.3	2.52	16.20	-0.0103	0.9788
	after 2	3.0	2.43	15.97	-0.0105	0.9788
404 rpm (164 L/min)	before 1	3.4	2.48	16.40	-0.0104	0.9749
	before 2	3.3	2.41	15.98	-0.0106	0.9767
	after 1	3.3	2.36	16.11	-0.0105	0.9794
	after 2	3.5	2.51	15.81	-0.0103	0.9803

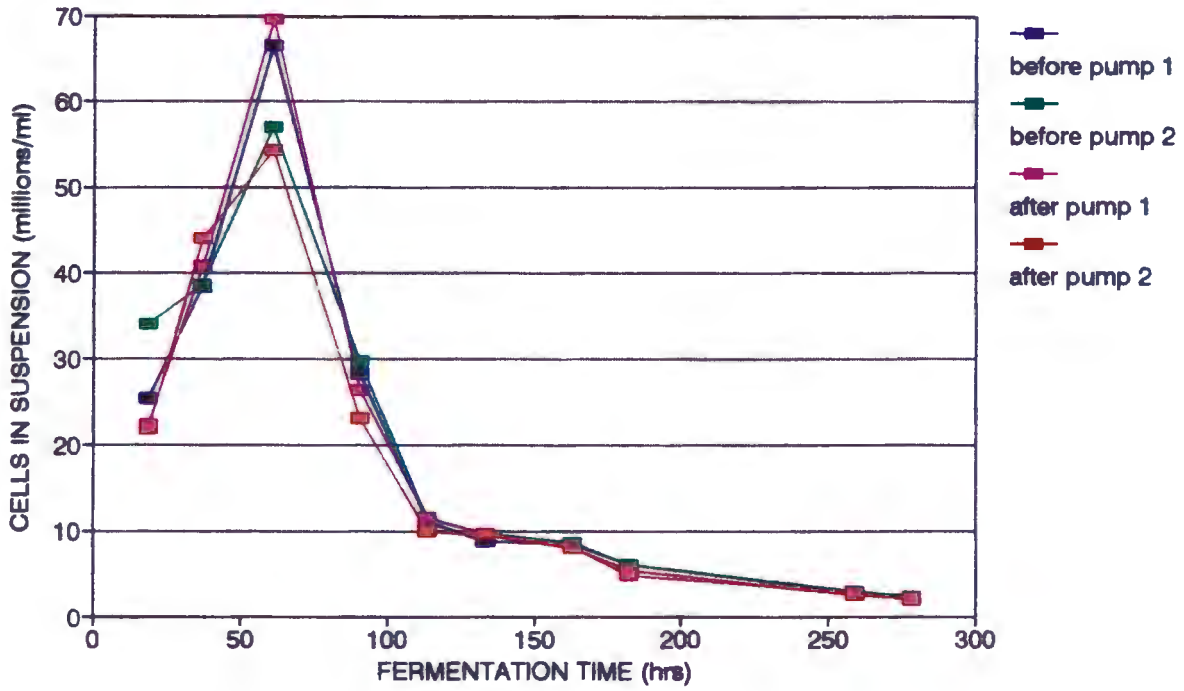

Figure 5.5 Cell count profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Maso sine pump on yeast quality (Pump speed = 244 rpm ≈ 89 L/min)

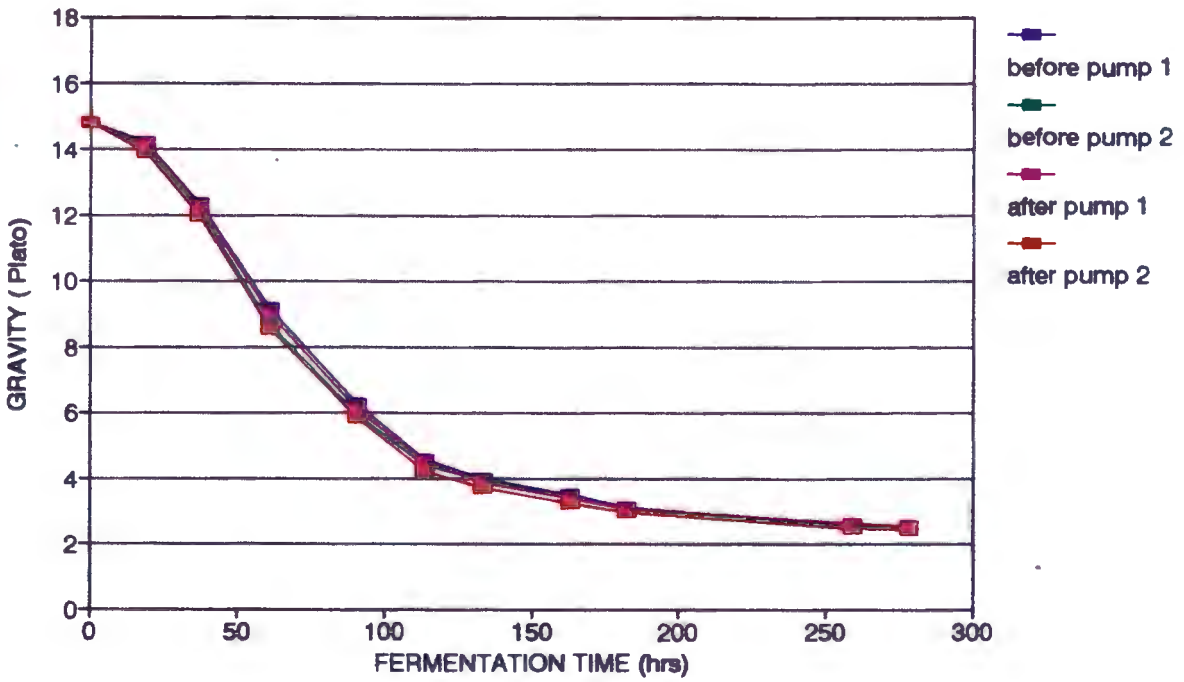

Figure 5.6 Attenuation profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Maso sine pump on yeast quality (Pump speed = 244 rpm ≈ 89 L/min)

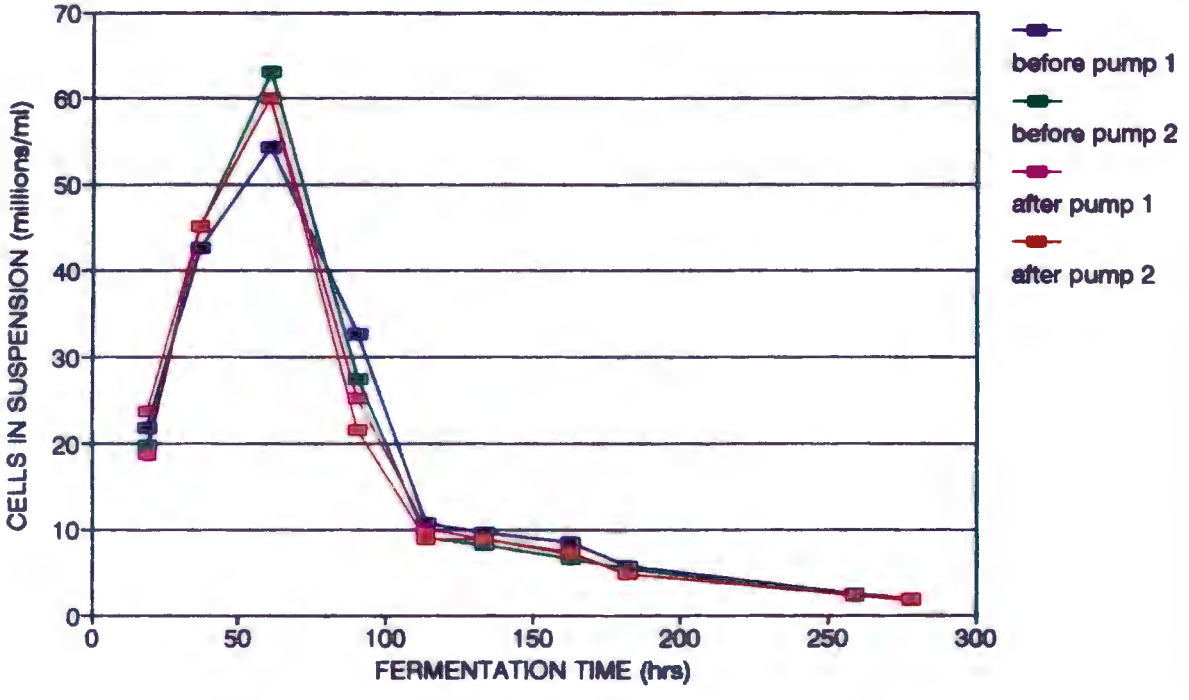

Figure 5.7 Cell count profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Maso sine pump on yeast quality (Pump speed = 404 rpm ≈ 164 L/min)

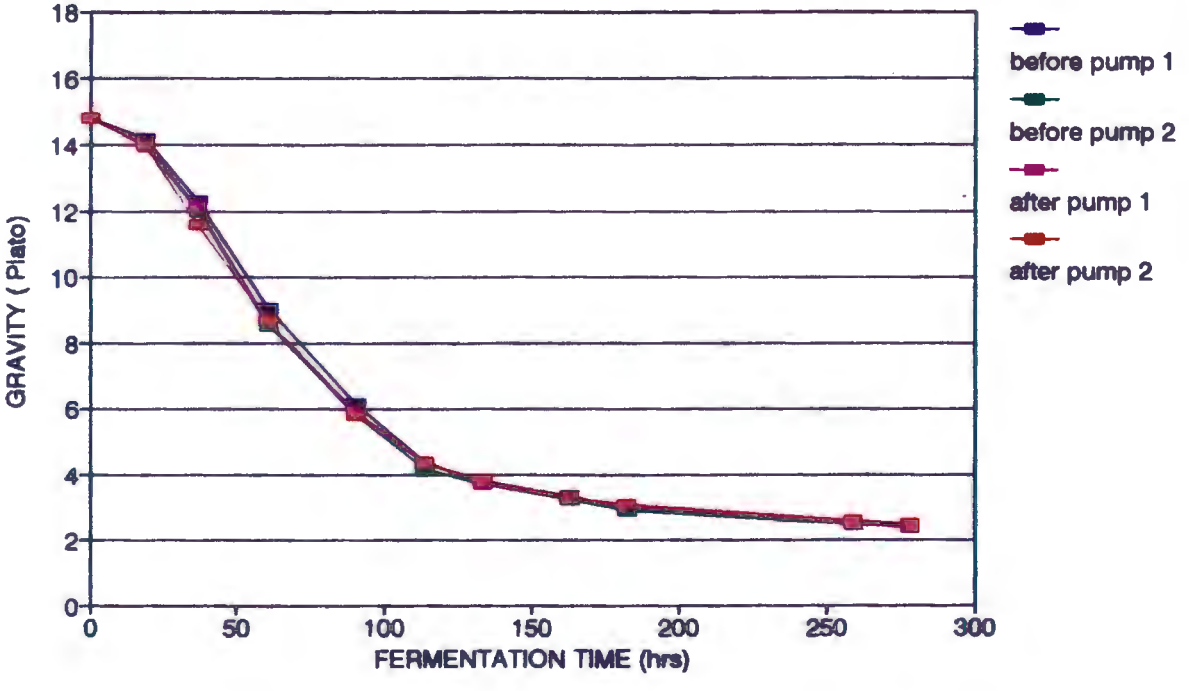

Figure 5.8 Attenuation profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Maso sine pump on yeast quality (Pump speed = 404 rpm ≈ 164 L/min)

Table 5.19 Results of beer quality indicators for the 2 L EBC tube fermentations of the samples taken to evaluate the effect of the Maso sine pumps on yeast quality (after 8 days of fermentation)

PUMP SPEED	SAMPLE	pH (pH units)	DIACETYL (ppb)	SO_2 (ppm)	ACETALDEHYDE (ppm)
240 rpm (89 L/min)	before 1	3.94	121	15	23
	before 2	3.92	141	12	23
	after 1	9.93	139	14	24
	after 2	3.94	156	15	24
404 rpm (164 L/min)	before 1	3.95	-	16	21
	before 2	3.92	152	15	24
	after 1	3.94	148	20	24
	after 2	3.95	169	13	22

Table 5.20 Results of beer quality indicators for the 2 L EBC tube fermentations of the samples taken to evaluate the effect of the Maso sine pumps on yeast quality (after 12 days of fermentation)

PUMP SPEED	SAMPLE	pH (pH units)	DIACETYL (ppb)	SO_2 (ppm)	ACETALDEHYDE (ppm)
240 rpm (89 L/min)	before 1	3.97	81	14	42
	before 2	3.94	76	13	38
	after 1	3.95	76	13	45
	after 2	3.95	85	14	34
404 rpm (164 L/min)	before 1	3.98	68	10	36
	before 2	3.96	76	13	41
	after 1	3.97	62	14	48
	after 2	3.98	80	16	34

5.3.4 Discussion of the Results of the Pump Retrials

The samples before and after the pumps at each pump speed could not be regarded as true replicates since 15 minutes of cropping time had passed between sampling. To establish whether the pumps effected a difference in the quality of the yeast, paired-sample *t* tests could be done. In these tests, the differences between the results of the yeast quality assays, the fermentation indicators and the beer quality indicators for each set of 'before' and 'after' samples taken at a particular instance would be calculated. To determine the statistical significance of the apparent changes in yeast quality caused by the pump, these differences (δ) could be regarded as random samples from a population for which the mean (μ) is zero. The null hypothesis $\mu = 0$ would be tested against the alternative hypothesis $\mu \neq 0$. Acceptance of the null hypothesis would indicate that the pump did not effect a change in yeast quality.

In the above paired-sample *t* tests, the differences obtained at both pumps speeds could be analysed together. This would allow a comparison based on 4 differences which would improve the sensitivity of the tests. The critical *t*-value at a 95% confidence level is 3.18 ($v = 3$). This treatment relies on the reasonable assumption that the pumps would not effect opposite changes in yeast quality at the different pump speeds. To avoid inappropriate conclusions if this were not the case, the paired-sample *t* test could also done for the individual pump speeds. However, these tests would based on two differences and the critical *t*-value at a 95% confidence level is 12.70 ($v = 1$), hence these tests would be less sensitive to changes in yeast quality.

As an alternative to the above approach, based on the results of the on-line investigation and the initial pump trials, it would be reasonable to assume that the quality of the yeast being removed from the cone did not change appreciably over the period that the each pump was operated at a particular pumping speed ($\pm$ 30 minutes of cropping time). It could then be assumed that the samples taken at each point at each pump speed were replicates. As for the samples taken during the evaluation of routine brewery operation (Section 5.2), the results could then be analysed using the standard techniques to compare means (Appendix B, Section 3) or by doing an analysis of variance (Appendix B, Section 4). For the analysis of variance technique the sensitivity of the analysis can be improved as for the initial pump trials, by analysing the data collected at both pump speeds simultaneously. Where apparently significant changes are observed, the data for each pump speed can be analysed separately. In each case, the null hypothesis that the *between-sample variance* was equal to the *within-sample variance* (no significant change in yeast quality) would be tested against the alternative hypothesis that the *between-sample variance* exceeded the *within-sample variance* (a significant change in yeast quality). In the former case, the critical F-value for 95% confidence is 6.59 ($v = 3$ for the *between-sample variance* and $v = 4$ for the *within-sample variance*) and, in the latter case, 18.5 ($v = 1$ for the between-sample variance and $v = 2$ for the within-sample variance).

Both paired-sample *t*-tests and the analysis of variance technique were used to determine whether the observed differences in yeast quality during the pump retrials were statistically significant.

5.3.4.1 Statistical Assessment by Paired-Sample *t* Tests

The results of the paired-sample *t* tests are given in detail in Appendix E (pages E52 - 75). As an example, the results obtained for the Maso sine pump are given in this section. The paired-sample *t* tests done for the differences obtained at both pump speeds are presented in Table 5.21, while those for the differences obtained at the individual pumps speeds are presented in Table 5.22. The results obtained for the yeast quality assays and the growth and final attenuation indicators for the small scale fermentations are presented in Table 5.21(a) and Table 5.22(a). In Table 5.21(b) and Table 5.22(b) the results obtained for the constants of the exponential curves fitted to the attenuation profiles and the beer quality indicators after 8 days of fermentation are given, while the beer quality indicators after 12 days of fermentation are given in Table 5.21(c) and Table 5.22(c).

As indicated above, for the results of the simultaneous analysis of samples taken at both pump speeds, the critical *t*-value at a 95% confidence level was 3.18 (v = 3). Critical *t*-values for exceptions (diacetyl content of the beer after 8 days) are indicated as a footnote to the table. It can be seen in Table 5.21(a) to (c), that the only *t*-value that exceeds its appropriate critical *t*-value was that for the diacetyl content of the beer after 8 days of fermentation for which t was equal to 18.90 (indicated by the shaded region). Due to the absence of a data point, the *t*-value for the paired-sample *t*-test for the individual pumps speeds could not be calculated for the higher pump speed (404 rpm). The *t*-value for the lower pump speed was high (t = 11.00), but did not exceed the critical *t*-value of 12.70 (v = 1) for these comparisons (Table 5.22(b)). The apparent differences in diacetyl content were not present after 12 days of fermentation (Table 21(c)). This suggests any difference in fermentation performance at an early stage in the fermentation was not significant enough to persist in the fermentation and to have an impact on the final quality of the beer produced. Since no other *t*-values for the simultaneous analysis of samples taken at both pump speeds exceed 3.18 (Table 5.21) and no *t*-values calculated for the individual pump speeds exceed 12.70 (Table 5.22), it can be concluded at a 95% confidence level that cropping with the Maso sine pump did not effect a detectable change in the quality or fermentation performance of the yeast.

Table 5.21(a) Results of paired-sample *t* tests on yeast quality, growth and final attenuation indicators obtained for samples during the retrial of the Maso sine pump (simultaneous analysis of samples taken at both pump speeds) (Critical *t*-value at 95% confidence level = 3.18)

RETRIAL		1																		
DATE		31/8/95																		
YEAST		CASTLE Y5 EX FV 112																		
YEAST QUALITY																				
PUMP SPEED	SAMPLE	INTEGRITY						VIABILITY						VITALITY						
		Protease	Statistical Parameters					Mod MeBl	Statistical Parameters					OUR	Statistical Parameters					
		(absorbance)	Diff	Avg	Stds	t	SS?	(% viability)	Diff	Avg	Stds	t	SS?	(mg/l/min/10^6 viable cells)	Diff	Avg	Stds	t	SS?	
240 rpm	before 1	0.05						92						0.015						
(89 l/min)	before 2	0.06						89						0.008						
	after 1	0.08	0.03					93	1					0.009	-0.006					
	after 2	0.07	0.01					90	1					0.008	0.000					
404 rpm	before 1	0.04						92						0.015						
(164 l/min)	before 2	0.10						94						0.011						
	after 1	0.10	0.06					93	1					0.007	-0.008					
	after 2	0.10	0.00	0.03	0.03	1.89	NSS	93	-1	1	1	1.00	NSS	0.008	-0.003	-0.004	0.004	-2.43	NSS	
FERMENTATION																				
PUMP SPEED	SAMPLE	GROWTH						ATTENUATION												
		Inc in Biomass	Statistical Parameters					Final Gravity	Statistical Parameters											
		(factor)	Diff	Avg	Stds	t	SS?	(Plato)	Diff	Avg	Stds	t	SS?							
240 rpm	before 1	3.0						2.55												
(89 l/min)	before 2	3.0						2.51												
	after 1	3.3	0.3					2.52	-0.03											
	after 2	3.0	0.0					2.43	-0.08											
404 rpm	before 1	3.4						2.48												
(164 l/min)	before 2	3.3						2.41												
	after 1	3.3	-0.1					2.36	-0.12											
	after 2	3.5	0.2	0.1	0.2	1.08	NSS	2.51	0.10	-0.0	0.1	-0.68	NSS							

Table 5.21(b) Results of paired-sample *t* tests on attenuation profile curve fit constants and beer quality indicators (day 8) obtained for samples during the retrial of the Maso sine pump (simultaneous analysis of samples taken at both pump speeds) (Critical *t*-value at 95% confidence level = 3.18)

RETRIAL		1																			
DATE		31/8/95																			
YEAST		CASTLE Y5 EX FV 112																			
BEER QUALITY (DAY 8)																FERMENTATION					
PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters					Alpha Constants	Statistical Parameters						
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t *	SS?		Diff	Avg	Stds	t	SS?		
240 rpm (89 l/min)	before 1	3.94						121						16.27							
	before 2	3.92						141						15.96							
	after 1	3.93	-0.01					139	18					16.20	-0.06						
	after 2	3.94	0.02					156	15					15.97	0.01						
404 rpm (164 l/min)	before 1	3.95						-						16.40							
	before 2	3.92						152						15.98							
	after 1	3.94	-0.01					148	-					16.11	-0.29						
	after 2	3.95	0.03	0.01	0.02	0.73	NSS	169	17	16.67	1.53	18.90	SS	15.81	-0.18	-0.13	0.13	-1.95	NSS		
PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters					Beta Constants	Statistical Parameters						
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?		
240 rpm (89 l/min)	before 1	15						23						-0.0101							
	before 2	12						23						-0.0103							
	after 1	14	-1					24	1					-0.0103	-2E-04						
	after 2	15	3					24	1					-0.0105	-2E-04						
404 rpm (164 l/min)	before 1	16						21						-0.0104							
	before 2	15						24						-0.0106							
	after 1	20	4					24	3					-0.0105	-1E-04						
	after 2	13	-2	1	3	0.68	NSS	22	-2	1	2	0.73	NSS	-0.0103	3E-04	-5E-05	2E-04	-0.42	NSS		

* $v = 2$, $t_{95\%} = 4.30$

Table 5.21(c) Results of paired-sample *t* tests on beer quality indicators (day 12) obtained for samples during the retrial of the Maso sine pump (simultaneous analysis of samples taken at both pump speeds) (Critical *t*-value at 95% confidence level = 3.18)

RETRIAL		1											
DATE		31/8/95											
YEAST		CASTLE Y5 EX FV 112											
BEER QUALITY (DAY 12)													
PUMP SPEED	SAMPLE	pH	Statistical Parameters					Diacetyl	Statistical Parameters				
		(pH units)	Diff	Avg	Stds	t	SS?	(ppb)	Diff	Avg	Stds	t	SS?
240 rpm	before 1	3.97						81					
(89 l/min)	before 2	3.94						76					
	after 1	3.95	-0.02					76	-5				
	after 2	3.95	0.01					85	9				
404 rpm	before 1	3.98						68					
(164 l/min)	before 2	3.96						76					
	after 1	3.97	-0.01					62	-6				
	after 2	3.98	0.02	0.00	0.02	0.00	NSS	80	4	1	7	0.14	NSS
PUMP SPEED	SAMPLE	SO2	Statistical Parameters					Acetaldehyde	Statistical Parameters				
		(ppm)	Diff	Avg	Stds	t	SS?	(ppm)	Diff	Avg	Stds	t	SS?
240 rpm	before 1	14						42					
(89 l/min)	before 2	13						38					
	after 1	13	-1					45	3				
	after 2	14	1					34	-4				
404 rpm	before 1	10						36					
(164 l/min)	before 2	13						41					
	after 1	14	4					48	12				
	after 2	16	3	2	2	1.58	NSS	34	-7	1	8	0.24	NSS

Table 5.22(a) Results of paired-sample *t* tests on yeast quality, growth and final attenuation indicators obtained for samples during the retrial of the Maso sine pump (individual analysis of samples at the two pump speeds) (Critical *t*-value at 95% confidence level = 12.70)

RETRIAL		1																
DATE		31/8/95																
YEAST		CASTLE Y5 EX FV 112																
YEAST QUALITY																		
PUMP SPEED	SAMPLE	INTEGRITY						VIABILITY						VITALITY					
		Protease	Statistical Parameters					Mod MeBl	Statistical Parameters					OUR	Statistical Parameters				
		(absorbance)	Diff	Avg	Stds	t	SS?	(% viability)	Diff	Avg	Stds	t	SS?	(mg/l/min/10^8 viable cells)	Diff	Avg	Stds	t	SS?
240 rpm	before 1	0.05						92						0.015					
(89 l/min)	before 2	0.06						89						0.008					
	after 1	0.08	0.03					93	1					0.009	-0.006				
	after 2	0.07	0.01	0.02	0.01	2.00	NSS	90	1	1	0	-	SDZ	0.008	0.000	-0.003	0.004	-1.00	NSS
404 rpm	before 1	0.04						92						0.015					
(164 l/min)	before 2	0.10						94						0.011					
	after 1	0.10	0.06					93	1					0.007	-0.008				
	after 2	0.10	0.00	0.03	0.04	1.00	NSS	93	-1	0	1	0.00	NSS	0.008	-0.003	-0.006	0.004	-2.20	NSS
FERMENTATION																			
PUMP SPEED	SAMPLE	GROWTH						ATTENUATION											
		Inc in Biomass	Statistical Parameters					Final Gravity	Statistical Parameters										
		(factor)	Diff	Avg	Stds	t	SS?	(Plato)	Diff	Avg	Stds	t	SS?						
240 rpm	before 1	3.0						2.55											
(89 l/min)	before 2	3.0						2.51											
	after 1	3.3	0.3					2.52	-0.03										
	after 2	3.0	0.0	0.2	0.2	1.06	NSS	2.43	-0.08	-0.05	0.04	-2.20	NSS						
404 rpm	before 1	3.4						2.48											
(164 l/min)	before 2	3.3						2.41											
	after 1	3.3	-0.1					2.36	-0.12										
	after 2	3.5	0.2	0.0	0.2	0.27	NSS	2.51	0.10	-0.01	0.16	-0.09	NSS						

Table 5.22(b) Results of paired-sample *t* tests on attenuation profile curve fit constants and beer quality indicators (day 8) obtained for samples during the retrial of the Maso sine pump (individual analysis of samples at the two pump speeds) (Critical *t*-value at 95% confidence level = 12.70)

RETRIAL		1																	
DATE		31/8/95																	
YEAST		CASTLE Y5 EX FV 112																	
BEER QUALITY (DAY 8)														FERMENTATION					
PUMP SPEED	SAMPLE																		
		pH	Statistical Parameters					Diacetyl	Statistical Parameters					Alpha	Statistical Parameters				
		(pH units)	Diff	Avg	Stds	t	SS?	(ppb)	Diff	Avg	Stds	t	SS?	Constants	Diff	Avg	Stds	t	SS?
240 rpm	before 1	3.94						121						16.27					
(89 l/min)	before 2	3.92						141						15.96					
	after 1	3.93	-0.01					139	18					16.20	-0.06				
	after 2	3.94	0.02	0.01	0.02	0.33	NSS	156	15	17	2	11.00	NSS	15.97	0.01	-0.02	0.06	-0.64	NSS
404 rpm	before 1	3.95						-						16.40					
(164 l/min)	before 2	3.92						152						15.98					
	after 1	3.94	-0.01					148	-					16.11	-0.29				
	after 2	3.95	0.03	0.01	0.03	0.50	NSS	169	17	-	-	-	-	15.81	-0.18	-0.23	0.08	-4.19	NSS
PUMP SPEED	SAMPLE																		
		SO2	Statistical Parameters					Acetaldehyde	Statistical Parameters					Beta	Statistical Parameters				
		(ppm)	Diff	Avg	Stds	t	SS?	(ppm)	Diff	Avg	Stds	t	SS?	Constants	Diff	Avg	Stds	t	SS?
240 rpm	before 1	15						23						-0.0101					
(89 l/min)	before 2	12						23						-0.0103					
	after 1	14	-1					24	1					-0.0103	-2E-04				
	after 2	15	3	1	3	0.50	NSS	24	1	1	0	-	SDZ	-0.0105	-2E-04	-2E-04	0E+00	-	SDZ
404 rpm	before 1	16						21						-0.0104					
(164 l/min)	before 2	15						24						-0.0106					
	after 1	20	4					24	3					-0.0105	-1E-04				
	after 2	13	-2	1	4	0.33	NSS	22	-2	1	4	0.20	NSS	-0.0103	3E-04	1E-04	3E-04	0.50	NSS

Table 5.22(c) Results of paired-sample *t* tests on beer quality indicators (day 12) obtained for samples during the retrial of the Masò sine pump (individual analysis of samples at the two pump speeds) (Critical *t*-value at 95% confidence level = 12.70)

RETRIAL	1
DATE	31/8/95
YEAST	CASTLE Y5 EX FV 112

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
240 rpm	before 1	3.97						81					
(89 l/min)	before 2	3.94						76					
	after 1	3.95	-0.02					76	-5				
	after 2	3.95	0.01	-0.00	0.02	-0.33	NSS	85	9	2	10	0.29	NSS
404 rpm	before 1	3.98						68					
(164 l/min)	before 2	3.96						76					
	after 1	3.97	-0.01					62	-6				
	after 2	3.98	0.02	0.01	0.02	0.33	NSS	80	4	-1	7	-0.20	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
240 rpm	before 1	14						42					
(89 l/min)	before 2	13						36					
	after 1	13	-1					45	3				
	after 2	14	1	0	1	0.00	NSS	34	-4	-1	5	-0.14	NSS
404 rpm	before 1	10						36					
(164 l/min)	before 2	13						41					
	after 1	14	4					48	12				
	after 2	16	3	4	1	7.00	NSS	34	-7	3	13	0.26	NSS

Summaries of the *t*-values calculated for the four pumps using both types of paired-sample *t* tests (presented in detail in Appendix E, pages E52 - E75) are provided in Table 5.23 and Table 5.24. The presence of statistically significant differences are indicated by the shaded regions.

Table 5.23 Summary of *t*-values obtained during paired-sample *t*-tests on results of pump retrials (simultaneous analysis of samples taken at both pump speeds) (Critical *t*-value at 95% confidence level = 3.18)

PUMP	MASO SINE	APV LOBE	BREDEL PERISTALTIC (SP/50)	BREDEL PERISTALTIC (SP/40)
YEAST QUALITY				
Protease Activity	1.89	3.00	1.73	5.00
Viability (Modified Methylene Blue)	1.00	-0.41	-0.40	0.87
Oxygen Utilisation Rate	-2.43	-0.56	-0.77	3.58
FERMENTATION				
Increase in Biomass	1.08	1.10	0.78	-0.63
Final Attenuation	-0.68	-1.12	0.21	-1.57
α-Constants	-1.95	-0.13	-1.04	-1.54
β-Constants	-0.42	-2.32	0.18	-0.23
BEER QUALITY (DAY 8)				
pH	0.73	1.67	1.08	-1.81
Diacetyl	18.90	-2.41	0.33	-0.96
SO_2	0.68	0.78	0.29	-0.88
Acetaldehyde	0.73	1.00	2.22	-0.93
BEER QUALITY (DAY 12)				
pH	0.00	3.46	0.81	-2.31
Diacetyl	0.14	0.54	0.31	-1.57
SO_2	1.58	0.33	0.77	-1.99
Acetaldehyde	0.24	0.68	1.00	-0.48

Table 5.24 Summary of *t*-values obtained during paired-sample *t*-test on results of pump retrials (individual analysis of samples at the two pump speeds) (Critical *t*-value at 95% confidence level = 12.70)

PUMP	MASO SINE		APV LOBE		BREDEL PERISTALTIC (SP/50)		BREDEL PERISTALTIC (SP/40)	
PUMP SPEED	244 rpm	404 rpm	40 rpm	86 rpm	23 rpm	42 rpm	25 rpm	40 rpm
YEAST QUALITY								
Protease Activity	2.00	1.00	1.00	SDZ*	1.00	1.00	3.00	SDZ*
Viability (Modified Methylene Blue)	SDZ*	0.00	-0.45	1.00	-1.00	1.00	SDZ*	SDZ*
Oxygen Utilisation Rate	-1.00	-2.20	-1.67	0.14	1.00	-3.00	3.00	3.00
FERMENTATION								
Increase in Biomass	1.06	0.27	-1.00	5.00	2.50	-0.09	0.00	-0.67
Final Attenuation	-2.20	0.16	-0.33	-1.00	-6.20	0.70	-0.28	-2.65
α-Constants	-0.64	-4.19	-1.38	0.28	2.22	-4.81	-6.73	0.38
β-Constants	SDZ*	0.50	-5.00	-2.00	SDZ*	0.53	0.67	-0.54
BEER QUALITY (DAY 8)								
pH	0.33	0.50	1.00	1.00	SDZ*	0.88	-1.00	-1.57
Diacetyl	11.00	-	-3.57	-0.82	-0.43	0.41	-1.00	-0.43
SO_2	0.50	0.33	0.33	-1.00	1.00	0.00	0.00	-1.00
Acetaldehyde	SDZ*	0.20	VI**	1.00	9.00	0.60	1.00	-1.67
BEER QUALITY (DAY 12)								
pH	-0.33	0.33	2.00	2.00	1.00	0.57	-0.67	-8.00
Diacetyl	0.29	-0.20	-1.67	1.20	0.33	0.23	-3.00	-0.58
SO_2	0.00	7.00	1.00	0.09	1.00	0.56	-1.00	-2.00
Acetaldehyde	-0.14	0.26	0.20	1.00	3.00	0.33	1.00	-2.00

* SDZ = standard deviation of differences = zero (ie. differences identical)
** VI = values identical (ie. all differences = zero)

From Table 5.23, it can be seen that apart from *t*-value for the differences in the diacetyl concentration after 8 days of fermentation for the sample taken during the evaluation of the Maso sine pump (discussed above), only three of the 60 *t*-values calculated during the simultaneous analysis of samples taken at both pump speeds

5.3.4.2 Statistical Assessment by Analysis of Variance

The results of the analysis of variance of the results of the yeast quality assays and small scale fermentations for the samples taken during the pump retrials are presented in detail in Appendix E (E76 - E86). A summary of the F-values obtained for the simultaneous analysis of the data obtained at both pump speeds is given in Table 5.25.

Table 5.25 Summary of F-values obtained during analysis of variance of results of the pump retrials (simultaneous analysis of samples taken at both pump speeds) (Critical F-value at 95% confidence level = 6.59 for v = 3,4)

PUMP	MASO SINE	APV LOBE	BREDEL PERISTALTIC (SP/50)	BREDEL PERISTALTIC (SP/40)
YEAST QUALITY				
Protease Activity	1.47	1.22	9.04	9.33
Viability (Modified Methylene Blue)	1.09	0.14	2.33	19.33
Oxygen Utilisation Rate	1.57	0.76	14.00	10.11
FERMENTATION				
Increase in Biomass	3.90	1.33	1.07	0.57
Final Attenuation	0.79	1.44	0.76	1.83
α-Constants	0.36	0.34	1.62	1.71
β-Constants	1.58	4.92	0.67	0.29
BEER QUALITY (DAY 8)				
pH	0.42	1.13	0.99	0.48
Diacetyl	1.42*	4.09	0.16	0.25
SO_2	0.44	0.70	0.41	0.49
Acetaldehyde	0.49	9.00	4.51	0.85
BEER QUALITY (DAY 12)				
pH	1.62	1.03	0.48	1.69
Diacetyl	0.72	1.87	0.09	0.82
SO_2	2.20	0.29	0.51	2.33
Acetaldehyde	0.05	0.78	0.96	0.62

* v = 3,3 $F_{95\%}$ = 9.28

exceeded the critical *t*-value for a 95% confidence level ($t = 3.18$). These are the protease activity and oxygen utilisation rate during the evaluation of the SP/40 Bredel peristaltic pump and the pH of the beer after 12 days of fermentation of the samples taken during the evaluation of the APV lobe pump.

For the protease and oxygen utilisation rate assays done on samples taken during the evaluation of the SP/40 Bredel peristaltic pump the *t*-values were 5.00 and 3.58 indicating apparent increases in protease level and oxygen utilisation rate upon pumping. When the results of the protease assay were treated separately for the different pump speeds, a *t*-value of 3.00 was obtained for the lower pump speed (25 rpm) (Table 5.24). This is not significant at a 95% confidence level where the critical *t*-value is 12.70 or even at an 80% confidence level where the critical *t*-value is 3.08. The differences for the higher pump speed were both 0.01, hence the standard deviation for their average was zero which made the expression for *t* mathematically undefined. These differences were less than the standard deviation normally associated with replicate samples (0.02) which suggest that the differences were not significant. For the APV lobe pump, a *t*-value of 3.46 was calculated for the pH of the beer after 12 days of fermentation (Table 5.23). As indicated in Table 3.24, the *t*-values calculated for the individual pump speeds (2.00 in both cases) did not exceed the critical *t*-values for both the 80% and 95% confidence levels (critical *t*-value of 3.08 and 12.70 respectively).

During the analysis of the data for the individual pump speeds for all the pumps, in several cases, the differences between the two sets of 'before' and 'after' data points were identical as for the pH of the beer after 12 days of fermentation during the evaluation of the APV lobe pump, hence no *t*-values could be calculated (indicated by SDZ in Table 5.24). In all these cases, the differences were less than or identical to the standard deviation normally associated with replicate samples, except for the modified methylene blue staining done on samples taken during the evaluation of the SP/40 Bredel peristaltic pump at the higher pump speed (40 rpm) where the difference was 1.5 times the standard deviation normally associated with replicate samples. This suggests that none of the differences indicated by SDZ in Table 5.24 were significant. For the samples taken during the evaluation of the APV lobe pump, the acetaldehyde content of the beer produced in the small scale fermentations after 8 days of fermentation were identical (indicated by VI in Table 5.24), hence the expression for the *t*-value again became mathematically undefined. It is reasonable to accept that there were no significant differences in the acetaldehyde contents of the beer produced by fermentations of the yeast samples taken before and after the pump after 8 days of fermentation.

The results of the paired-sample *t* tests thus indicate that no difference could be demonstrated in the quality and fermentation performance of the yeast in response to pumping by any of the four pumps evaluated during the pumps retrials.

The analysis of variance summarised in Table 5.25, indicated that the *between-sample variance* was larger than the *within-sample variance* in six cases ($F > 6.59$).

For samples taken during the evaluation of the APV pump, an F-value of 9.00 was obtained for the acetaldehyde content of the beer produced after 8 days of fermentation. The identical values obtained for the all four samples at the lower pump speed (40 rpm) reduced the *within-sample variance* making the *between-sample variance* seem significant relative to it. The analysis of the variance of the samples taken at the individual pump speeds indicated that the pump did not effect any change in acetaldehyde content of the beer (day 8) at the higher pump speed (86 rpm) (Appendix E, page E80).

For the SP/50 Bredel peristaltic pump apparently significant changes in yeast quality were observed for the protease ($F = 9.04$) and oxygen utilisation rate ($F = 14.00$) assays. These changes were not significant in the analysis for the samples for the individual pump speeds (Appendix E, page E82). The effect in the simultaneous analysis of variance was produced by an apparent increase in protease activity and decrease in oxygen utilisation rate during the investigation which indicates a change in yeast quality of yeast removed from the cone during the investigation.

For the SP/40 Bredel peristaltic pump, the simultaneous analysis of variance for both pump speeds indicated apparently significant changes in protease activity ($F = 9.33$), "viability" indicated by methylene blue staining ($F = 19.33$) and oxygen utilisation rate ($F = 10.11$). The apparent increase in protease activity and oxygen utilisation rate for the SP/40 Bredel peristaltic pump were the only differences highlighted as significant by both the paired-sample *t* tests and the analysis of variance done simultaneously for both pump speeds. However, none of these changes were significant for the individual pump speeds and there were no clear trends to suggest any change in yeast quality during the investigation (Appendix E, page E85). The performance of SP/40 Bredel peristaltic pump was evaluated further over an extended cropping period.

5.3.5 The Evaluation of Cropping over an Extended Period

Over a long cropping period, heat generation and a concomitant increase in the temperature within the pump casing could occur. As indicated in Section 4.4.3, this was expected to be most likely for the SP/40 Bredel peristaltic pump, hence it was selected to evaluate cropping over an extended period. The results of the yeast quality assays and small scale fermentations of samples collect during this evaluation are presented in this section. The results of the yeast quality assays are given in Table 5.26. The cell count and attenuation profiles for the 500 mL fermentations are given in Figure 5.9 and Figure 5.10 respectively. The fermentation indicators are given in Table 5.27 and the end of fermentation (Day 12) beer quality indicators are presented in Table 5.28. The samples are coded according to the sampling point at which they were taken (before or after the

pump) and the time after the commencement of cropping (15 min or 140 min). Each table indicates the range of the data obtained and the standard deviation normally associated with replicates (s_{pooled}) which may be regarded as good estimates of the population standard deviation (σ). An indication of the size of the difference required between two samples for statistical significance at a 95% confidence level is also given. These differences were calculated from the pooled standard deviations using a standard method to compare means (Appendix B, Equation 12).

Table 5.26 Results of yeast quality assays for samples taken to evaluate the effect of cropping over an extended period (140 min) (SP/40 Bredel peristaltic pump)

SAMPLING POINT	PROTEASE ACTIVITY (ΔA_{574nm})	VIABILITY (MODIFIED METHYLENE BLUE) (%)	OXYGEN UTILISATION RATE (mg/L/min/ 10^8 viable cells)
Before Pump (15 min)	0.24	93	0.006
After Pump (15 min)	0.26	94	0.006
Before Pump (140 min)	0.25	92	0.010
After Pump (140 min)	0.22	94	0.009
Range	0.04	2	0.004
Standard Deviation for Replicates	0.02	2	0.002
Statistically significant difference at a 95% confidence level	0.06	6	0.006

Table 5.27 Fermentation indicators for 500 mL fermentations of samples taken during the evaluation of cropping over an extended period (140 min) (SP/40 Bredel peristaltic pump)

SAMPLING POINT	INCREASE IN BIOMASS (factor)	FINAL ATTENUATION (°Plato)	α-CONSTANTS (°Plato)	β-CONSTANTS (hr^{-1})	R^2 VALUES
Before Pump (15 min)	2.9	2.24	13.21	-0.0128	0.9586
After Pump (15 min)	3.1	2.25	12.88	-0.0128	0.9554
Before Pump (140 min)	3.0	2.23	12.89	-0.0125	0.9552
After Pump (140 min)	3.3	2.24	12.67	-0.0128	0.9551
Range	0.4	0.02	0.54	0.0003	-
Standard Deviation for Replicate Fermentations	0.2	0.07	0.16	0.0003	-
Statistically significant difference at a 95% confidence level	0.6	0.19	0.44	0.0008	-

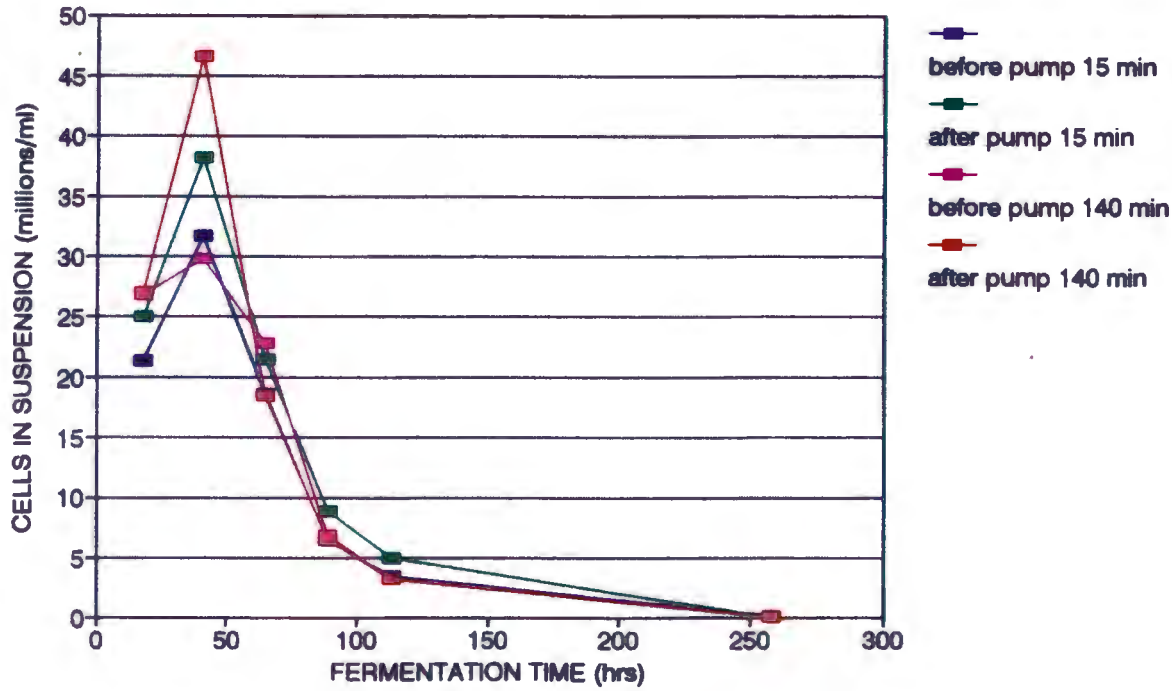

Figure 5.9 Cell count profiles for 500 mL fermentations of samples taken during the evaluation of cropping over an extended period (140 min) (SP/40 Bredel peristaltic pump)

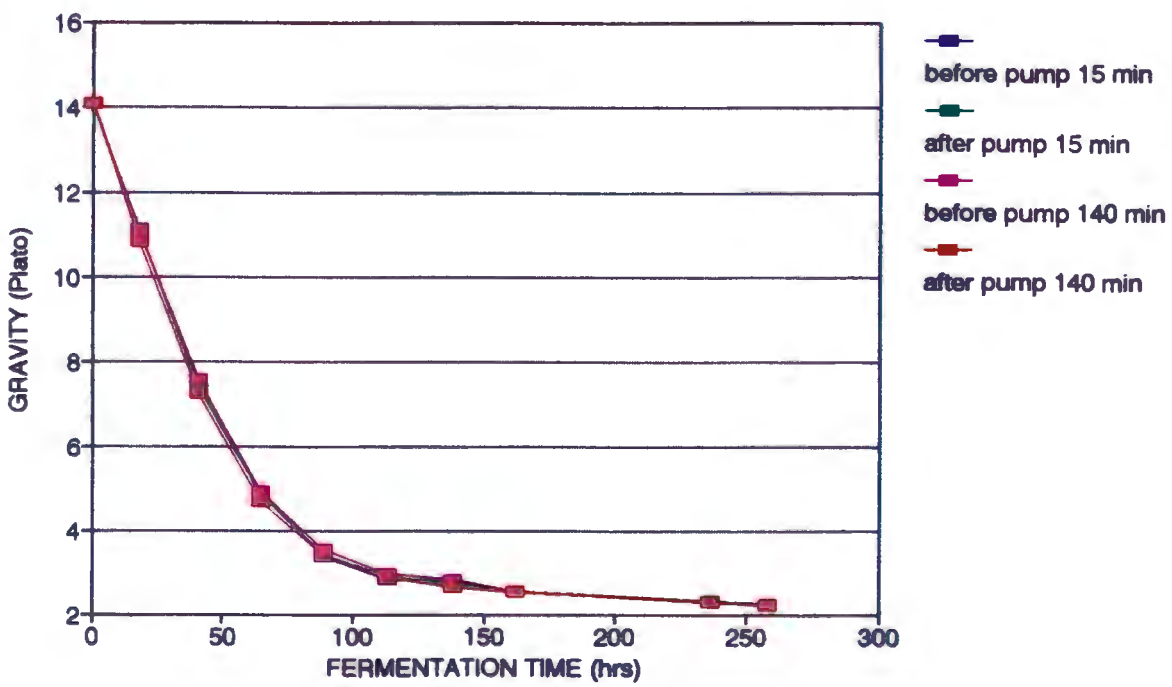

Figure 5.10 Attenuation profiles for 500 mL fermentations of samples taken during the evaluation of cropping over an extended period (140 min) (SP/40 Bredel peristaltic pump)

Table 5.28 Fermentation indicators for 500 mL fermentations of samples taken during the evaluation of cropping over an extended period (140 min) (SP/40 Bredel peristaltic pump)

SAMPLING POINT	pH (pH units)	DIACETYL (ppb)	SO_2 (ppm)	ACETALDEHYDE (ppm)
Before Pump (15 min)	4.59	328	17	27
After Pump (15 min)	4.58	339	16	27
Before Pump (140 min)	4.60	320	20	21
After Pump (140 min)	4.56	320	21	31
Range	0.04	19	5	10
Standard Deviation for Replicate Fermentations	0.05	13	1	5
Statistically significant difference at a 95% confidence level	0.14	36	3	14

5.3.6 Discussion of the Results Obtained During the Evaluation of Cropping over an Extended Period

Since the differences required for statistical significance were calculated for a comparison of two data points, the confidence intervals were large. Only in the case of the α-constants and the final SO_2 level, did the range of the data exceed the difference required for statistical significance. No clear trend with cropping time is apparent in the cell count profiles for the 500 mL fermentations (Figure 5.9) and no differences can be seen in the attenuation profiles (Figure 5.10). Since the range of the α-constants exceeds the difference required for statistical significance, some difference in the fermentation performance of the samples is suggested. The fermentation of the sample taken after the pump after 140 min of cropping appears to show the greatest increase in biomass (3.3 fold) and the fastest attenuation rate (indicated by the lower α-constant (12.67), suggesting that the quality of this yeast sample was the best of the four samples taken. This is not confirmed by the beer quality indicators. The final SO_2 and acetaldehyde level for the sample taken after 140 min of cropping indicates a degree of yeast stress.

Since the ranges of the yeast quality indicators are small relative to the standard deviations and there appears to be no trend consistent with a loss of yeast quality in the fermentation performance indicators, it seems reasonable to conclude that the pump effected no difference in the quality of the yeast nor did the quality of the next 9500 kg of yeast change substantially after the first 400 kg of yeast had been removed from the cone.

5.4 THE EFFECT OF FLOW CONDITIONS ON YEAST QUALITY DURING CROPPING

5.4.1 Results of the Flow Trials

As indicated in Section 4.5.2, linear velocities from 0.1 to 3.7 m/s and Reynolds numbers from 86 to 1114 were generated to investigate the effect of flow conditions during cropping on yeast quality. The results of the yeast quality assays and small scale fermentations performed on the samples taken during the investigation are presented in Appendix F (pages F1 - F7). As an example, the results obtained for the lowest and highest linear velocity and Reynolds number combinations (v = 0.1 m/s and Re = 86 and v = 3.7 m/s and Re = 1114) are given in this section. The results of the yeast quality assays are presented in Table 5.29. Figures 5.11 and 5.12 are the attenuation profiles for the 500 mL fermentations of these samples. The fermentation indicators are summarised in Table 5.30 and the beer quality indicators in Table 5.31. The samples are coded according to the sampling point (before or after the holding tubes) and whether the samples were taken at the first instance or the second instance when the pump was stopped for sampling at the particular flow conditions (eg. 'before tubes 1' would correspond to a sample taken before the holding tubes at the first instance when the pump was stopped for sampling).

Table 5.29 Results of yeast quality assays for mildest and most extreme conditions during the investigation of the effect of different flow conditions on yeast quality

YEAST QUALITY INDICATOR	PROTEASE ACTIVITY (ΔA_{574nm})	VIABILITY (MODIFIED METHYLENE BLUE) (%)	OXYGEN UTILISATION RATE (mg/L/min/10^8 viable cells)
SAMPLING POINT	FLOW CONDITIONS	v = 0.1 m/s	Re = 86
Before Tubes (1)	0.06	94	0.007
Before Tubes (2)	0.09	93	0.006
After Tubes (1)	0.11	93	0.006
After Tubes (2)	0.06	92	0.006
Range	0.05	2	0.001
SAMPLING POINT	FLOW CONDITIONS	v = 3.7 m/s	Re = 1114
Before Tubes (1)	0.24	96	0.008
Before Tubes (2)	0.20	96	0.008
After Tubes (1)	0.29	94	0.008
After Tubes (2)	0.25	94	0.009
Range	0.09	2	0.001
Standard Deviation for Replicates	0.02	2	0.002

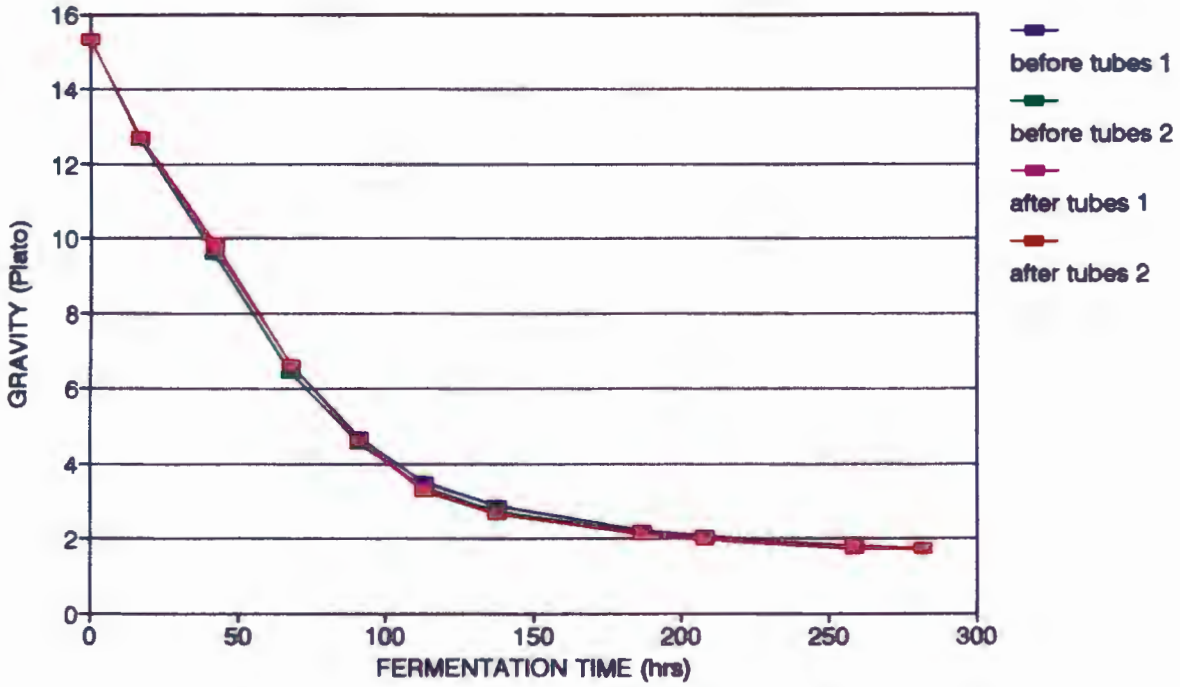

Figure 5.11 Attenuation profiles for the 500 mL fermentations of samples taken during operation at v = 0.1 m/s and Re = 86

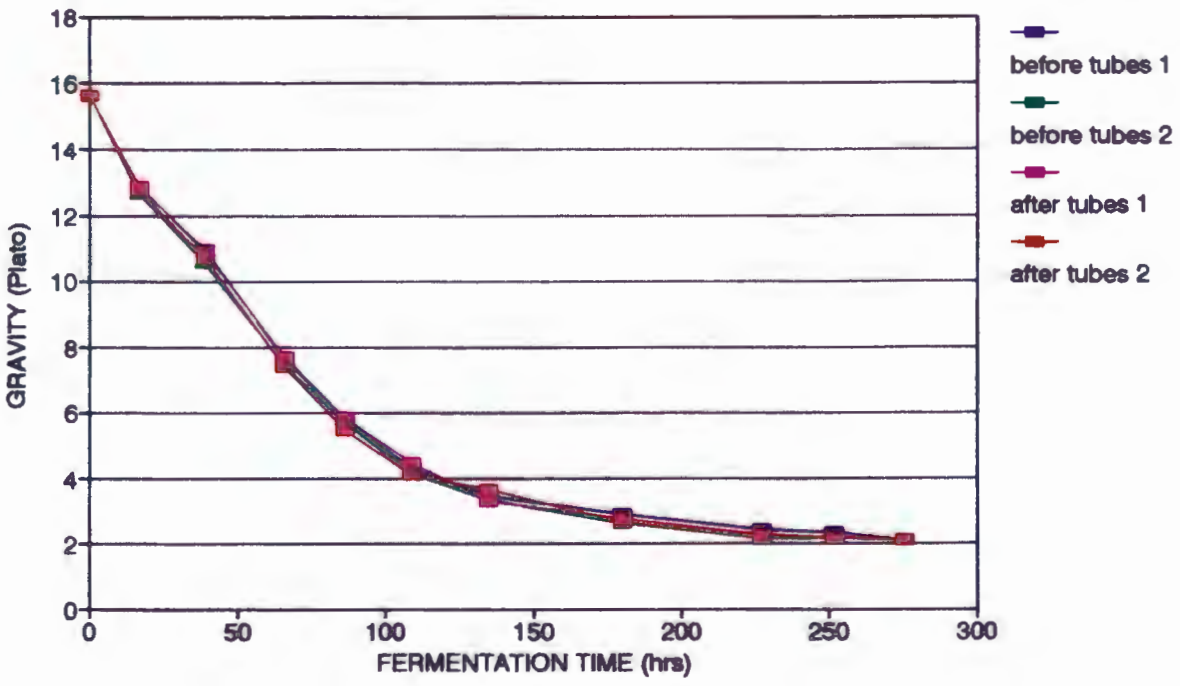

Figure 5.12 Attenuation profiles for the 500 mL fermentations of samples taken during operation at v = 3.7 m/s and Re = 1114

Table 5.30 Results of fermentation indicators for 500 mL fermentations of samples taken for the mildest and most extreme conditions during the investigation of the effect of different flow conditions on yeast quality

FERMENTATION INDICATOR	INCREASE IN BIOMASS (factor)	FINAL ATTENUATION (°Plato)	α-CONSTANTS (°Plato)	β-CONSTANTS (hr^{-1})
SAMPLING POINT	**FLOW CONDITIONS**	v = 0.1 m/s	Re = 86	
Before Tubes (1)	2.8	1.73	14.46	-0.0111
Before Tubes (2)	2.7	1.73	14.40	-0.0114
After Tubes (1)	2.7	1.72	14.62	-0.0115
After Tubes (2)	2.7	1.76	14.62	-0.0115
Range	0.1	0.04	0.22	0.0004
SAMPLING POINT	**FLOW CONDITIONS**	v = 3.7 m/s	Re = 1114	
Before Tubes (1)	3.0	2.08	15.00	-0.0101
Before Tubes (2)	3.0	2.14	14.99	-0.0105
After Tubes (1)	3.0	2.09	15.16	-0.0103
After Tubes (2)	3.1	2.09	14.88	-0.0104
Range	0.1	0.06	0.28	0.0004
Standard Deviation for Replicates	0.2	0.07	0.158	0.0003

Table 5.31 Results of beer quality indicators for 500 mL fermentations of samples taken for the mildest and most extreme conditions during the investigation of the effect of different flow conditions on yeast quality

BEER QUALITY INDICATOR	pH (pH units)	DIACETYL (ppb)	SO_2 (ppm)	ACETALDEHYDE (ppm)
SAMPLING POINT	FLOW CONDITIONS	v = 0.1 m/s	Re = 86	
Before Tubes (1)	4.33	156	13	21
Before Tubes (2)	4.27	140	14	23
After Tubes (1)	4.34	156	15	22
After Tubes (2)	4.34	168	13	19
Range	0.07	28	2	4
SAMPLING POINT	FLOW CONDITIONS	v = 3.7 m/s	Re = 1114	
Before Tubes (1)	4.19	88	12	25
Before Tubes (2)	4.16	88	13	18
After Tubes (1)	4.15	89	13	27
After Tubes (2)	4.15	89	13	20
Range	0.04	1	1	9
Standard Deviation for Replicates	0.05	13	1	5

5.4.2 Discussion of the Flow Trial Results

To establish whether the observed differences in yeast quality were significant, a statistical analysis of the results was done. The results of the evaluation of routine brewery operation and the pump trials, indicated that the quality of the yeast being removed from the cone was not expected to change significantly over the period in which the samples were taken. For this reason yeast quality indicators for the samples taken at each sampling point at a particular pump speed were treated as replicates and an analysis of variance was done. Since the flow conditions were set up with three different batches of yeast and three different pumps, the data for each batch of yeast and pump were analysed together. The null hypothesis that the *between-sample variance* was equal to the *within-sample variance* was tested against the alternative hypothesis that the *between-sample variance* exceeded the *within-sample variance*. The analysis would thus indicate whether operation at the different flow conditions effected a change in yeast quality and would also indicate if the quality the yeast changed significantly during the investigation.

The detailed results of the analysis of variance is presented in Appendix F (pages F8 - F13). The samples are coded according to the speed at which they were tested and whether the samples were taken before or after the holding tubes (*eg.* S1B would be a sample taken before the tubes at the first pump speed used in the

trial). The exact flow conditions can be found in the table with the complete set of results (Appendix F, pages F1 - F7). A summary of the F-values obtained is given in Table 5.32 which includes the appropriate critical F-values for a 95% confidence level.

Table 5.32 Summary of F-values obtained during analysis of variance of results of results obtained during the investigation of the effect of different flow conditions on yeast quality during cropping

TRIAL	FLOW CONDITIONS		YEAST QUALITY				
	Linear Velocity (m/s)	Reynolds Number	Protease Activity	% Viability (Modified Methylene Blue)	Oxygen Utilisation Rate		Critical F-Value
1	2.0 3.7	598 1114	10.27	5.53	6.07		6.59
2	0.3 0.5	152 289	4.00	6.44	0.27		6.59
3	0.1 0.6 2.5	86 339 760	0.74	3.73	0.82		4.39
TRIAL	FLOW CONDITIONS		FERMENTATION				
	Linear Velocity (m/s)	Reynolds Number	Increase in Biomass	Final Attenuation	α-Constants	β-Constants	Critical F-Value
1	2.0 3.7	598 1114	2.67	1.13	2.10	1.04	6.59
2	0.3 0.5	152 289	0.96	2.67	3.08	3.77	6.59
3	0.1 0.6 2.5	86 339 760	3.22	0.78	2.70	1.66	5.05
TRIAL	FLOW CONDITIONS		BEER QUALITY (DAY 12)				
	Linear Velocity (m/s)	Reynolds Number	pH	Diacetyl	SO_2	Acetaldehyde	Critical F-Value
1	2.0 3.7	598 1114	2.35	1.24	0.41	0.75	6.59
2	0.3 0.5	152 289	1.09	2.03	1.33	1.59	6.59
3	0.1 0.6 2.5	86 339 760	6.13	4.17	0.55	1.39	5.05

As indicated by the shaded regions in Table 5.32, only two of the 33 F-values calculated exceeded the critical F-values. These are the protease activity during the first trial and the final pH of the beer produced in the 500 mL fermentations during the third trial. When the analysis of variance was calculated for the individual flow conditions, none of the differences were significant at a 95% confidence level (Appendix F, pages F9 and F13).

The results of the flow trials thus suggest that at a 95% confidence level no difference in yeast quality could be detected over the range of linear velocities and Reynolds numbers investigated.

5.5 CONCLUSIONS

During the evaluation of routine brewery operation, mechanical handling of yeast during its transfer from the base of the cone to the chiller outlet did not affect yeast quality or fermentation performance. The selected yeast quality assays (methylene blue staining, slide counts, oxygen utilisation rate, acidification power, glycogen content and trehalose content) indicated that in the flowrate range of 16 to 193 L/min (± 1 to 11.6 m^3/hr), achievable with the available pump and motor combinations, cropping with the ten pumps under investigation had no significant effect on the quality of the cropped yeast. These pumps include examples of peristaltic, lobe, sine, gear, diaphragm and centrifugal pumps. Over a range of flowrates from 16 to 162 L/min (± 1 to 9.7 m^3/hr), no significant effect on the fermentation performance (rate and extent of growth, rate and extent of attenuation and beer quality) in small scale fermentations (2 L) was confirmed for four pumps: the Bredel peristaltic pump (model SP/40 and SP/50), the APV lobe pump (model CL/3/156/7) and the Maso Sine pump (model SP3"). In addition, intracellular protease release (recognised as an indicator of yeast quality) was not observed. Over a range of flowrates from 17 to 110 L/min, linear velocities from 0.1 to 3.7 m/s and Reynolds numbers from 86 to 1114, no difference in yeast quality (indicated by an assay for extracellular protease activity, methylene blue staining and oxygen utilisation rate) or subsequent fermentation performance in small scale fermentations (500 mL) could be detected.

CHAPTER

6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

In the brewing industry, a loss of yeast quality is characterised in terms of a "loss of viability" or a "loss of vitality", where the term "loss of viability" indicates loss of replicative ability, while the term "loss of vitality" generally refers to metabolic activity. A loss of yeast quality may occur via several mechanisms:

- cell death
- cell disruption
- minor wall damage
- cell membrane damage leading to a loss of replicative ability
- reduced growth potential (associated with low levels of the intracellular reserve compound glycogen)
- reduced metabolic activity
- changes in the metabolic pathways expressed
- changes in flocculation and sedimentation characteristics
- loss of stress resistance

Hence, the phrases "loss of viability" and "loss of vitality" do not provide an accurate description of the nature of a loss of yeast quality. In this thesis, it is suggested that the physiological condition of yeast can be described more accurately in terms of certain physiological states and that a loss of yeast quality in response to stress conditions occurs as stepwise changes in the physiological state of the yeast. Based on literature and experimental evidence, a scheme is proposed for a loss of yeast quality in response to mechanical and physiological stress. The scheme can be used as a basis for the characterisation of a loss of yeast quality.

Since yeast quality is associated with the fermentative capacity of the yeast and quality of the beer produced, small scale fermentations can be done to assess yeast quality. Since vessel geometry and scale affect the progress of a fermentation, small scale fermentations do not provide a direct reflection of the production situation; however, differences in the yeast quality become apparent. In this investigation, two small scale fermentation systems, one using 2 L EBC tubes and the other using 500 mL measuring cylinders, were set up. The small scale fermentations were reproducible: the coefficients of variation of the constants of the exponential curves fitted to the attenuation data were less than 5% in both systems.

In the small scale fermentations, differences in the quality of the beer effected by differences in the quality of the pitching yeast became less apparent as the fermentations progressed. To highlight differences in pitching yeast quality, beer quality should be assessed at an early stage during the fermentation. To determine the effect of differences in pitching yeast quality on the large scale brewery process, beer quality should be evaluated at the end of fermentation.

More rapid analytical techniques to assess yeast quality have been developed. Nine yeast quality assays were selected for this investigation:

- an extracellular protease assay
- methylene blue and Mg-ANS staining techniques
- plate and slide counts
- oxygen utilisation rate
- acidification power
- intracellular glycogen and trehalose contents

The reproducibility of the methylene blue and Mg-ANS staining techniques, the slide count technique, the acidification power test and the assay for the glycogen content was good (coefficient of variation $< 5\%$). The oxygen utilisation rate, trehalose content and intracellular protease assays require improvement.

The ability of the yeast quality assays to predict the fermentation performance of the yeast depends on the correspondence between the nature of the loss of yeast quality and the physiological basis of the assays. Hence the selection of appropriate yeast quality assays should be based in an understanding of the response of yeast

to stress conditions causing the loss of yeast quality and the physiological basis of the yeast quality assays.

Mechanical stress affects the cell envelope leading to the release of wall associated material, cell disruption and concomitant release of intracellular compounds, replicative deactivation and cell death. The release of intracellular and wall-associated material reduces beer quality. Replicative deactivation and cell death lead to reduced rate and extent of biomass growth and hence to a reduced rate and extent of attenuation. Since replicative competence relies on the functional and physical integrity of the cell membrane, the intracellular protease assay, which gives an indication of the extent of membrane damage and the slide count technique, which reflects replicative competence, are appropriate assays for the assessment of the loss of yeast quality in response to mechanical stress. This was validated experimentally.

Physiological stress, such as prolonged exposure to a nutrient depleted environment, is expected to result in reduced growth potential via depletion of the intracellular reserve compound glycogen, reduced metabolic activity, changes in the metabolic pathways expressed and loss of stress resistance. The rate and extent of attenuation is reduced and undesirable fermentation products may be present in the beer. Specific oxygen utilisation rate, intracellular glycogen and intracellular trehalose contents are proposed as appropriate indicators of the response of yeast to physiological stress. The inapplicability of the specific oxygen utilisation rate as an indication of loss os yeast quality resulting from mechanical stress and its ability to identify loss of yeast quality resulting from storage was validated experimentally.

The evaluation of the effect of mechanical handling on yeast during re-circulation, centred on yeast cropping (the transfer of yeast from the fermentation vessel to the storage vessel). The following was demonstrated at a 95% confidence level:

- At the production scale, transfer of yeast from the base of the cone to the chiller outlet during routine brewery operation did not affect yeast quality as indicated by small scale fermentations and selected yeast quality assays (extracellular protease activity, methylene blue staining, oxygen utilisation rate, intracellular glycogen content and intracellular trehalose content)

- Over the range of flow rates that could be achieved with the available pump and motor combinations (16 to 193 L/min $\approx$ 1 to 11.6 m^3/hr), cropping with the ten pumps under investigation had no significant effect on the quality of the cropped yeast as indicated in pilot scale experiments using the selected yeast quality assays (methylene blue staining, slide counts, oxygen utilisation rate, acidification power, intracellular glycogen content and intracellular trehalose content). The pumps evaluated include examples from the following categories: peristaltic, lobe, sine, gear, diaphragm and centrifugal pumps.

- No significant effect on the fermentation performance (rate and extent of growth, rate and extent of attenuation and beer quality) in small scale fermentations was confirmed for four pumps, namely the Bredel peristaltic pumps (model SP/40 and SP/50), the APV lobe pump (model CL/3/156/7) and the Maso Sine pump (model SP3"). These were operated at a range of flow rates from 16 to 162 L/min (± 1 to 9.7 m^3/hr).

- Over a range of flowrates from 17 to 110 L/min (± 1 to 6.6 m^3/hr), linear velocities from 0.1 to 3.7 m/s and Reynolds numbers from 86 to 1114, no difference in yeast quality (indicated by an assay for extracellular protease activity, methylene blue staining and oxygen utilisation rate) or subsequent fermentation performance in small scale fermentations could be detected.

Since pump design and operation under brewery conditions do not affect yeast quality, the selection of cropping pumps can be based on an assessment of the operating characteristics, wear, maintenance requirements, cleanability, sanitary operation, efficiency and an economic evaluation of the pumps. Such an assessment was not within the scope of this investigation, however, based on the experience with the pumps tested the following observations can be made:

- Air-driven diaphragm pumps and peristaltic pumps should not be used for yeast cropping since these pumps produce unacceptable flow pulsations. The cleanability and suitability of pulsation dampeners for use with these pumps in yeast handling is questioned.

- The Maso Sine pump and the Scandi Brew gear pump both cause negligible flow pulsations and appear to be good pumps for yeast cropping. Cleanability and seal life require investigation.

- The lobe pumps tested (APV, Ibex, Johnson and Wilflo) all produce slight flow pulsations (10 - 15 kPa). However, based on an evaluation of yeast quality during routine brewery operation using a Wilflo lobe pump, flow pulsations of this magnitude are not expected to jeopardise the effective cooling of the yeast.

6.2 RECOMMENDATIONS

In this investigation, only laminar flow conditions, which are representative of conditions in the brewery yeast handling circuit, were investigated. Investigation of turbulent flow conditions may identify critical flow conditions for a loss of yeast quality. It should be recognised, however, that flowrates during yeast cropping will always be limited by the risk of vortex formation in the cone of the fermentation vessel and the associated risk of beer losses.

The conditions which occur in the yeast handling process require study. Characterisation of the rheology of the various yeast suspensions (cropped, stored, re-pitched and propagated yeast) would not only aid in the specification of yeast handling equipment, but would improve the understanding of the conditions to which the yeast is exposed and allow the study of loss of yeast quality at a more fundamental level.

The investigation was limited to aspects of yeast quality that have a direct impact on fermentation performance. Through this, the difference in the effect of mechanical and physiological stress on yeast quality became evident. The need to understand the mechanism of loss of yeast quality and the principle of the yeast quality assay for selection of appropriate analytical techniques has been discussed. It is recognised that under the conditions in the yeast handling circuit, wall damage leading to the release of wall-associated material into the medium may occur. This requires investigation, since the carry over of wall-associated material into a subsequent fermentation may lead to a reduced beer quality. Assays for glucan, mannan, invertase and melibiase (Lewis and Poerwantaro 1991) would be appropriate.

The study was limited to cropping. Other areas important for mechanical stress are yeast propagation, pitching, storage with agitation and acid washing. Different responses of micro-organisms as a function of growth phase, level of intracellular storage products, floc size and biomass concentration are reported in literature. The brewery system requires study. Yeast appears to be more susceptible to mechanical damage at lower biomass concentrations (Harrison and Pandit 1992). In the yeast handling circuits of SA Breweries, the biomass concentrations of the suspensions transferred during pitching and propagation and the suspension agitated during acid washing are lower than the biomass concentration of the cropped yeast suspensions examined in this investigation. Furthermore, growing cells, such as those transferred during propagation, are more sensitive to mechanical damage than stationary phase cells (Gray *et al.* 1972, Vraná *et al.* 1982). Since propagation, pitching and acid washing are central aspects of yeast handling, the study should be extended to the potential loss of yeast quality during these processes.

REFERENCES

Ahlquist, E. (1986). 'Process and Quality Management of Yeast. *Brewers' Guardian*, **115** (8), 22-23, 26-28.

Ahvenainen, J. and Mäkinen, V. (1981). 'The Effect of Pitching Yeast Aeration on Fermentation and Beer Flavour'. In *Proceedings of the EBC Congress*, Lisbon, 285-291.

American Society of Brewing Chemists (1976). 'Microbiology. Yeast 3A: Dead Yeast Cell Stain'. *Methods of Analysis*. 7th ed. St Paul: American Society of Brewing Chemists.

American Society of Brewing Chemists (1980). 'Microbiology'. *ASBC Journal*, **38** (3), 109-110.

American Society of Brewing Chemists (1981). 'Microbiology'. *ASBC Journal*, **39** (3), 86-89.

American Society of Brewing Chemists (1984). 'Microbiology'. *ASBC Journal*, **42** (3), 132-135 .

Avis, J.W. (1990). 'Yeast Quality Hazard Analysis'. *Ferment*, **3** (5), 284-287.

Axcell, B., Kruger, L. and Allan, G. (1988). 'Some Investigative Studies with Yeast Foods'. In *Proceedings of the 20th Convention of the Institute of Brewing (Australia and New Zealand Section)*, Brisbane, 201-208.

Ball, A. (1994). 'Brewery Yeast Routing Principles'. *The Brewer*, **80**, 53-56.

Becker, J.U. (1978). 'A Method for Glycogen Determination in Whole Cells'. *Analytical Biochemistry*, **86**, 56-64.

Boughton, R.A. (1983). 'Are You Getting the "Best" Out of Your Yeast?' *The Brewer*, **69**, 260-264.

Boughton, R. (1987). 'Practically Managing Yeast'. *MBAA Technical Quarterly*, **24**, 133-136.

Boulton, C. (1991). 'Yeast Management and the Control of Brewery Fermentations'. *Brewers' Guardian*, **120** (4), 25-29.

Boulton, C.A. and Quain, D.E. (1987). 'Yeast, Oxygen and the Control of Brewery Fermentations'. In *Proceedings of the EBC Congress*, Madrid, 401-407.

Casey, G.P., Magnus, C.A. and Ingledew, W.M. (1983). 'High Gravity Brewing: Nutrient Enhanced Production of High Concentrations of Ethanol by Brewing Yeast'. *Biotechnol. Lett.*, **5** (6), 429-434.

Casey, G.P., Magnus, C.A. and Ingledew, W.M. (1984). 'High Gravity Brewing: Effects of Nutrition on Yeast Composition, Fermentative Ability and Alcohol Production'. *Appl. Environ. Microbiol.*, **48** (3), 639-646.

Chilver, M.J., Harrison, J. and Webb, T.J.B. (1978). 'Use of Immuno Fluorescence and Viability Stains in Quality Control'. *ASBC Journal*, **36** (1), 13-18.

Cumming, R.H., Tuffnell, J. and Street, G. (1985). 'The Dependence of Protein Release from *Bacillus amyloliquefaciens* on the Growth Phase in Batch Culture'. *Biotechnol. Bioeng.*, **27**, 887-889.

D'Amore, T., Crumplen, R. and Stewart, G. (1991). 'The Involvement of Trehalose in Yeast Stress Tolerance'. *Journal of Industrial Microbiology*, **7**, 191-196.

Daoud, I. (1990). 'Assessment of Yeast Quality'. *Brewers' Guardian*, **119** (4), 10-11.

Daoud, I.S. and Searle, B.A. (1986). 'Yeast Vitality and Fermentation Performance'. In *European Brewing Convention Monograph XII, Symposium on Brewing Yeast*, 108-121.

Dunlop, E.H. and Namdev, P.K. (1993). 'Effect of Fluid Forces on Plant Cell Suspensions'. In *Proceedings of the 3rd International Conference on Bioreactor and Bioprocess Fluid Dynamics*, Cambridge, 447-455

EBC Analytica Microbiologica (1977). *J. Inst. Brew.*, **83**, 109-118.

Edwards, D.G. and Wiseman, A. (1971). 'Novel Shock Tube Cell Disrupter'. *Process Biochemistry*, **6** (11), 32-34.

Engler, C.R. (1985). 'Disruption of Microbial Cells'. In *Comprehensive Biotechnology, Volume 2*. (ed. M. Moo-Young). London: Pergamon Press. 305-324.

European Brewing Convention (1962). 'Estimation of Yeast Viability'. *J. Inst. Brew.*, **68**, 14-20.

Fernández, S., González, G. and Sierra, A. (1991). 'The Acidification Power Test and the Behaviour of yeast in Brewery Fermentations'. *MBAA, Technical Quarterly*, **28** (1), 89-95.

Fernández, S., González, G. and Sierra, J.A. (1993). 'Evaluation of the Effect of Acid Washing on the Fermentation and Respiratory Behaviour of Yeast by the Acidification Power Test'. *MBAA Technical Quarterly*, **30**, 1-8.

Fish, N.M., and Lilly, M.D. (1984). 'The Interactions Between Fermentation and Protein Recovery'. *Biotechnology*, **2** (1), 623-627.

Fisher, D.J. (1975). 'Flocculation - Some Observations on the Surface Charges of Yeast Cells'. *J. Inst. Brew.*, **81**, 107-110.

Fowler, J.D. and Robertson, C.R. (1991). 'Metabolic Behaviour of Immobilised Aggregates of *Escherichia coli* on Varying Mechanical Stress'. *Appl. Environ. Microbiol.*, **57** (1), 93-101.

Fraser, D. (1951). 'Bursting Bacteria by Release of Gas Pressure'. *Nature*, **167** (4236), 33-34.

Gervais, P., Marechal, P.A. and Molin, P. (1992). 'Effects of the Kinetics of Osmotic Pressure Variation on Yeast Viability'. *Biotechnol. Bioeng.*, **40**, 1435-1439.

Gilliland, R.B. (1959). 'Determination of Yeast Viability'. *J. Inst. Brew.*, **65**, 424-429.

Gray, P.P., Dunnill, P. and Lilly, M.D. (1972). 'The Continuous Flow Isolation of Enzymes'. In *Fermentation Technology Today*. (ed. G. Terui). Japan: Society for Fermentation Technology, 347-351.

Hammond, J.R.M. (1986). 'Brewer's Yeast'. In *The Yeasts, Volume V.* (ed. A.H. Rose and J.S. Harrison). London: Academic Press, 8-56.

Harrison, S.T.L. (1990). 'The Extraction and Purification of PHB from *Alcaligenes eutrophus*'. PhD dissertation, University of Cambridge, UK.

Harrison, S.T.L. (1991). 'Bacterial Cell Disruption: A Key Unit Operation in the Recovery of Intracellular Products'. *Biotech. Adv.*, **9**, 217-240.

Harrison, S.T.L., Chase, H.A. and Dennis, J.S. (1991). 'The Disruption of *Alcaligenes eutrophus* by High Pressure Homogenisation: Key Factors Involved in the Process'. *Bioseparation*, **2**, 155-166.

Harrison, S.T.L. and Pandit, A.B. (1992). 'The Disruption of Microbial Cells by Hydrodynamic Cavitation'. *9th International Biotechnology Symposium of the ACS*, Washington DC, USA.

Harrison, S.T.L. and Pearce, S.J.A. (1994). 'The Effect of the Presence of Particulates on Micro-organisms in Stirred Tank Bioreactors'. In *Proceedings of the 7th National Meeting of SAIChE*, Johannesburg, 442-450.

Henschke, P.A. and Eglington, J.M. (1991). 'Yeast Starter Cultures: II Measurement of Metabolic Status'. *Australian and New Zealand Wine Industry Journal,* May, 123-127.

Hough, J.S. (1985). *The Biotechnology of Malting and Brewing.* Cambridge: Cambridge University Press.

Institute of Brewing (1970). 'Measurement of Yeast Viability'. *J. Inst. Brew.*, **76**, 442-443.

Iserentant, D. (1993). 'Yeast Handling: The Key to Optimal Performance in Fermentation'. In *Proceedings of the International Symposium on Malting and Brewing Technology,* Brussels, 29-30.

Jones, R.P. (1987a). 'Measures of Yeast Death and Deactivation and their Meaning: Part I'. *Process Biochemistry,* **22** (4), 118 - 128.

Jones, R.P. (1987b). 'Measures of Yeast Death and Deactivation and their Meaning: Part II'. *Process Biochemistry,* **22** (5), 130 - 134.

Kara, B.V., Daoud, I. and Searle, B. (1987). 'Assessment of Yeast Quality'. In *Proceedings of the EBC Congress,* Madrid, 409-416.

Kara, B.V., Simpson, W.J. and Hammond, J.R.M. (1988). 'Prediction of the Fermentation Performance of Brewing Yeast with the Acidification Power Test'. *J. Inst. Brew.,* **94**, 153-158.

Kienle, I., Burgert, M. and Holzer, H. (1993). 'Assay of Trehalose with Acid Trehalase Purification from *Saccharomyces cerevisiae*'. *Yeast,* **9**, 607-611.

King, L.M., Schisler, D.O. and Ruocco, J.J. (1981). 'Epifluorescent Method for Detection of Nonviable Yeast'. *ASBC Journal,* **39** (2), 52-54.

Knudsen, F.B. (1985). 'Fermentation Variables and their Control'. *ASBC Journal,* **43** (2), 91-95.

Kreutzfeldt, C. and Witt, W. (1991). 'Structural Biochemistry'. In *Biotechnology Handbooks: Volume 4: Saccharomyces.* (ed. M.F. Tuite and S.G. Oliver). London: Plenum Press. 5-58.

Kronlof, J. (1986). 'Optimisation of Yeast Handling in a Brewery'. *Brewers' Digest,* **61** (12), 28-30.

Kruger, L., Ryder, D.S., Alcock, B.A. and Murray, J.P. (1982). 'Malt Quality: Prediction of Malt Fermentability. Part I'. *MBAA Technical Quarterly,* **19** (1), 45-51.

Kula, M-R. and Schütte, H. (1987). 'Purification of Protein and the Disruption of Microbial Cells'. *Biotechnology Progress*, **3** (1), 31-42.

Lee, S.S., Robinson, F.M. and Wang, H.Y. (1981). 'Rapid determination of yeasts viability'. *Biotech. Bioeng. Symp.*, **11**, 641-649.

Lenoël, M., Meunier, J-P., Moll, M. and Midoux, N. (1987). 'Improved System for Stabilizing Yeast Fermenting Power During Storage'. In *Proceedings of the EBC Congress*, Madrid, 425-432.

Lentini, A., Hawthorne, D.B. and Kavanagh, T.E. (1992). 'A Rheological Study of Yeast during Handling and Storage'. In *Proceedings of 22nd Convention of the Institute of Brewing, Australia and New Zealand Section*, Melbourne, 198-199.

Lentini, A. (1993). 'A Review of the Various Methods Available for Monitoring Physiological Status of Yeast: Yeast Viability and Vitality'. *Ferment*, **6** (5), 321-327.

Lewis, M.J. and Poerwantaro, W.M. (1991). 'Release of Haze Material from Cell Walls of Agitated Yeast'. *ASBC Journal*, **49** (2), 43-46.

Lillie, S.H. and Pringle, J.R. (1980). 'Reserve Carbohydrate Metabolism in *Saccharomyces cerevisiae*: Responses to Nutrient Limitation'. *Journal of Bacteriology*, **143**, 1384-1394.

Lodolo, E.J., Basson, L., O'Connor-Cox, E.S.C. and Harrison, S.T.L. (1996). *An Investigation into the Newlands Yeast Handling Plant*. Johannesburg: South African Breweries (Beer Division).

Logan, B.E. and Dettmer, J.W. (1990). 'Increased Mass Transfer to Micro-organisms with Fluid Motion'. *Biotechnol. Bioeng.*, **35**, 1135-1144.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). 'Protein Measurement with the Folin Reagent'. *Journal of Biological Chemistry*, **193**, 265-275.

Macleod, A.M. (1977). 'Beer'. In *Economic Microbiology, Volume I: Alcoholic Beverages.* (ed. A.H. Rose). London: Academic Press. 43-137.

Manson, D.H. and Slaughter, J.C. (1986). 'Methods for Predicting Yeast Fermentation Activity'. In *Proceedings of the 2nd Aviemore Conference on Malting, Brewing and Distillation,* Aviemore, 295-297.

Martens, F.B., Egberts, G.T.C., Kempers, J., Robles de Medina, M.H.L. and Welten, H.G.J. (1986). 'Yeast Storage Methods and their Effect on Fermentation'. In *European Brewery Convention Monograph VII, Brewers' Yeast*, 95-105.

Mathieu, C., Van den Berg, L. and Iserentant, D. (1991). 'Prediction of Yeast Fermentation Performance using the Acidification Power Test'. In *Proceedings of the EBC Congress,* Lisbon, 273-280.

McCaig, R. and Bendiak, D.S. (1985a). 'Yeast Handling Studies I: Agitation of Stored Pitching Yeast'. *ASBC Journal,* **43** (2), 114-118.

McCaig, R. and Bendiak, D.S. (1985b). 'Yeast Handling Studies II: Temperature of Storage of Pitching Yeast'. *ASBC Journal,* **43** (2), 119-122.

McCaig, R. (1990). 'Evaluation of the Fluorescent Dye 1-anilino-8-Naphthalene Sulfonic Acid for Yeast Viability Determination'. *ASBC Journal,* **48** (1), 22-25.

Mochaba, F., Torline, P.A. and Axcell, B. (1993). 'Resorufin-labelled Casein as a Substrate for Protease Activity in Beer'. In *Proceedings of the EBC Congress,* Oslo, 533-541.

Moonsamy, N., Mochaba, F., Majara, M., O'Connor-Cox, E.S.C. and Axcell, B.C. (1995). 'Rapid yeast trehalose measurement using near infrared reflectance spectrometry'. *J. Inst. Brew.*, **101**, 203-206.

Mukataka, S., Kobayashi, N., Sato, S. and Takahashi, J. (1988). 'Variation in Cellulase-Constituting Components from *Trichoderma reesei* with Agitation Intensity'. *Biotechnol. Bioeng.*, **32**, 760-763.

Murray, C.R., Barich, T. and Taylor, D. (1984). 'The Effect of Yeast Storage Conditions on Subsequent Fermentations'. *MBAA Technical Quarterly,* **21** (4), 189-194.

O'Connor-Cox, E.S.C. (1994). *Handling AJL 2036: Current Impressions.* Johannesburg: South African Breweries (Beer Division).

O'Connor-Cox, E.S.C. (1995). *Yeast Vitality Measurement: Progress Report, Recommendations and Future Directions.* Johannesburg: South African Breweries (Beer Division).

Oliver, S.G. (1991). '"Classical" Yeast Biotechnology'. In *Biotechnology Handbooks: Volume 4: Saccharomyces.* (ed. M.F. Tuite and S.G. Oliver). London: Plenum Press. 213-248.

Opekarová, M. and Sigler, K. (1982). 'Acidification Power: Indicator of Metabolic Activity and Autolytic Changes in *Saccharomyces cerevisiae'. Folia Microbiologica,* **27**, 395-403.

Ormrod, I.H.L., Lalor, E.F. and Sharpe, F.R. (1991). 'The Release of Proteolytic Enzymes into Beer'. *J. Inst. Brew.,* **97**, 441-443.

Parkkinen, E., Oura, E. and Suomalainen, H. (1976). 'Comparison of Methods for the Determination of Cell Viability in Stored Baker's Yeast. *J. Inst. Brew.*, **82**, 283-285.

Pascual, C., Romay, C. and Sigler, K. (1983). 'Proton Extrusions in *Saccharomyces cerevisiae* Mutants in Very Dilute Suspensions'. *Folia Microbiologica,* **28**, 353-362.

Patel, G.B. and Ingledew, W.M. (1973). 'Internal Carbohydrates of *Saccharomyces carlsbergensis* during Commercial Lager Brewing'. *J. Inst. Brew.*, **79**, 392-396.

Patino, H., Edelen, C. and Miller, J. (1993). 'Practical New Measures of Yeast Vitality Using pH and Conductance Probes'. *MBAA Technical Quarterly,* **30**, 98-103.

Pauls' Malt (1989). *Brewing Room Book.* 82nd ed. Ipswich: Pauls' Malt.

Peddie, F. (1988). 'Observations on Laboratory Scale Methods for Assessing Vitality and their Relevance to Yeast Physiology'. *BRF Quarterly,* April.

Peddie, F.L., Simpson, W.J., Kara, B.V., Robertson, S.C. and Hammond, J.R.M. (1991). 'Measurement of Endogenous Oxygen Uptake Rates of Brewers' Yeast'. *J. Inst. Brew.,* **97**, 21-25.

Pickerell, A.T.W., Hwang, A. and Axcell, B.C. (1991). 'Impact of Yeast Handling Procedures on Beer Flavour Development during Fermentation'. *ASBC Journal*, **49** (2), 87-92.

Quain, D.E. (1981). 'The Determination of Glycogen in Yeasts'. *J. Inst. Brew.*, **87**, 289-291.

Quain, D.E. (1991). 'Stress and the Yeast Cell'. *Ferment*, **4** (3), 155-156.

Quain, D.E., Thurston, P.A. and Tubb, R.S. (1981). 'The Structural and Storage Carbohydrates of *Saccharomyces cerevisiae*: Changes during Fermentation of Wort and a Role for Glycogen Catabolism in Lipid Biosynthesis'. *J. Inst. Brew.*, **87**, 108-111.

Quain, D.E. and Tubb, R.S. (1982). 'The Importance of Glycogen in Brewing Yeasts'. *MBAA Technical Quarterly*, **19** (1), 29-33.

Quain, D.E. and Tubb, R.S. (1983). 'A Rapid and Simple Method for the Determination of Glycogen in Yeast'. *J. Inst. Brew.*, **89**, 38-40.

Quain, D.E. (1988). 'Studies on Yeast Physiology - Impact on Fermentation Performance and Product Quality'. *J.Inst. Brew.*, **95**, 315-323.

Quain, D.E. (1991). 'Stress and the Yeast Cell'. *Ferment*, **4** (3), 155-156.

Renger, R. (1991). *Carbon Dioxide and its Relevance to Large-Scale Brewery Fermentation.* PhD dissertation, TU Delft, The Netherlands.

Reuss, M., Josic, D., Popivic, M. and Bronn, W.K. (1979). 'Viscosity of Yeast Suspensions'. *Eur. J. Appl. Microbiol. Biotechnol.*, **8**, 167-175.

Rodrigues, U.M. and Kroll, R.G. (1986). 'Use of the Direct Epifluorescent Filter Technique for the Enumeration of Yeasts'. *J. Appl. Bact.*, **61**, 139-144.

Rose, A.H. (1977). 'History and Scientific Basis of Alcoholic Beverage Production'. In *Economic Microbiology, Volume I: Alcoholic Beverages.* (ed. A.H. Rose). London: Academic Press. 1-41.

Ryder, D.S., Woods, D.R., Murray, J.P. and Masschelein, C.A. (1983). 'Some Practical Implications of Yeast Growth and Yeast Performance'. *MBAA Technical Quarterly*, **20** (10), 9-21.

SAB (1992). *Functional Specification : Yeast Handling*. Johannesburg: South African Breweries (Beer Division).

SAB (1993). *Microbiological Methods Manual.* 1st ed. Johannesburg: South African Breweries (Beer Division).

Sadie, F. (1992). *Systems Requirements: Yeast Collection and Pitching*. Project A508/4. Johannesburg: South African Breweries (Beer Division).

Sall, C.J., Seipp, J.F. and Pringle, A.T. (1988). 'Changes in Brewer's Yeast during Storage and the Effect of these Parameters on Subsequent Fermentation Performances'. *ASBC Journal,* **46** (2), 23-25.

Salle, A.J. (1971). *Fundamental Principles of Bacteriology*, 7th ed., 311-315. New York: McGraw-Hill.

Schöffel, F. (1978a). 'Kreiselpumpen in der Brauerei (Getränkeindustrie)'. *Brauwelt*, no. 45, 1709-1714.

Schöffel, F. (1978b). 'Kreiselpumpen in der Brauerei (Getränkeindustrie)'. *Brauwelt*, no. 49, 1871-1878.

Schöffel, F. (1979a). 'Verdrängerpumpen in der Brauerei (Getränkeindustrie)'. *Brauwelt*, no. 15, 499-506.

Schöffel, F. (1979a). 'Verdrängerpumpen in der Brauerei (Getränkeindustrie)'. *Brauwelt*, no. 16, 545-551.

Schur, F. (1990). 'Yeast Treatment and Beer Quality'. *Brau Rundsch*, **101** (9), 147-151.

Siebel Institute of Technology (1978). *Beer Flavour Descriptions.* Chicago: Siebel Institute of Technology.

Simpson, W.J. and Hammond, J.R.M. (1989). 'The Response of Brewing Yeast to Acid Washing'. *J. Inst. Brew.*, **95**, 347-354

Slaughter, J.C. and Nomura, T. (1992a). 'Intracellular Glycogen and Trehalose Contents as Predictors of Yeast Viability'. *Enzyme Microb. Technol.*, **14**, 64-67.

Slaughter, J.C. and Nomura, T. (1992b). 'Activity of the Vacuolar Proteases of Yeast and the Significance of the Cytosolic Protease Inhibitors during the Post-Fermentation Decline Phase'. *J. Inst. Brew.*, **98**, 335-338.

Smart, A.C., Boulton, C.A., Hinchliffe, E. and Molzahn, S. (1995).'Effect of Physiological Stress on the Surface Properties of Brewing Yeasts'. *ASBC Journal*, **53**, 33-38.

Smith, J.J., Lilly, M.D. and Fox, R.I. (1990). 'The Effect of Agitation on the Morphology and Penicillin Production of *Penicillium chrysogenum*'. *Biotechnol. Bioeng.*, **35**, 1011-1023.

Stewart, G.G. (1977). 'Fermentation - Yesterday, Today and Tomorrow'. *MBAA Technical Quarterly*, **14** (1), 1-14.

Toma, M.K., Ruklisha, M.P., Vanags, J.J., Zeltina, M.O., Leite, M.P., Galinina, N I., Viesteurs, U.E. and Tengerdy, R.P. (1991). 'Inhibition of Microbial Growth and Metabolism by Excess Turbulence'. *Biotechnol. Bioeng.*, **38**, 552-556.

Trevelyan, W.E. and Harrison, J.S. (1956). 'A Method for Glycogen Determination'. *Nature*, **166**, 444-445.

Trevors, J.T., Merrick, R.L., Russell, I. and Stewart, G.G. (1983). 'A Comparison of Methods for Assessing Yeast Viability'. *Biotechnol. Lett.*, **5** (2), 131-134.

Van Dijck, P., Colavizza, D., Smet, P. and Thevelein, J.M. (1995). 'Differential Importance of Trehalose in Stress Resistance in Fermenting and Non-fermenting *Saccharomyces cerevisiae* Cells'. *Applied and Environmental Microbiology,* **61** (1), 109-115.

Vogel, A.I. (1989). *Vogel's Textbook of Quantitative Chemical Analysis*. 5th ed. (ed. G.H. Jeffery, J. Basset, J. Mendham and R.C. Denney). Harlow: Longman Scientific and Technical.

Vraná, D., Vortuba, J. and Placek, J. (1982). 'Age-Related Changes in the Physiological State of Budding Yeast Cells'. *Experimental Cell Research*. **138**, 57-62.

Wecker, A. and Onken, U. (1991). 'Influence of Dissolved Oxygen Concentration and Shear Rate on the Production of Pullulan by *Aureobasidium pullulans*'. *Biotechnol. Lett.*, **13** (3), 155-160.

Wheatcroft, R., Lim, Y.H., Hawthorne, D.B., Clarke, B.J. and Kavanagh, T.E. (1988). 'An Assessment of the Use of Specific Oxygen Uptake Measurements to Predict the Fermentation Performance of Brewing Yeast'. In *Proceedings of the 20th Convention of the Institute of Brewing (Australia and New Zealand Section)*, Brisbane, 193-199.

Wheatcroft, R., Lentini, A., Tai, L., Shaw, R., Hawthorne, D.B. and Kavanagh, T.E. (1993). 'Critical Control Points Analysis for Optimising Yeast Handling'. In *Proceedings of 4th Scientific and Technical Convention of the Institute of Brewing*, South Africa, 153-165.

Wiemken, A. (1990). 'Trehalose in Yeast: Stress Protectant rather than Reserve Carbohydrate'. *Antonie van Leeuwenhoek,* **58**, 209-217.

Yan, Y. (1989). 'Cavitation Phenomena and the Effect of the Admittance of Air in the Flow Through an Orifice'. PhD dissertation, University of Cambridge, UK.

APPENDIX A

A REVIEW OF THE PUMPS SUBMITTED FOR EVALUATION

1. INTRODUCTION

The evaluation of the performance of pumps in this thesis is centred on yeast handling. Provided the yeast handling criteria are met, the selection of specific pumps for yeast cropping requires a complete assessment of all the characteristics of the pumps. Such an assessment includes an evaluation of mechanical components, wear, maintenance requirements, cleanability, sanitary operation, efficiency and an economic evaluation of the pumps. This review highlights typical applications of the pumps that were submitted for evaluation and explains the principle of operation of each pump type. Some favourable features and drawbacks of the various pumps tested and the experience with them during the investigation are briefly discussed with a view to providing a basis for the complete evaluation of the pumps. The review is preceded by a brief outline of the classification of pumps and the requirements for pumps in the food and beverage industries.

2. THE CLASSIFICATION OF PUMPS

Pumps can be classified in several different ways. A classification based on the principle by which energy is added to the fluid divides pumps into two major categories, namely, dynamic and displacement pumps (Karrassik *et al.* 1976). In dynamic pumps, energy is added continuously to increase the fluid velocity within

the pump to velocities higher than those at the pump discharge. The subsequent velocity reduction within and beyond the pump produces a pressure increase. Centrifugal and certain special effect pumps are classed as dynamic pumps. In displacement pumps, energy is periodically added by applying a force to one or more moveable boundaries of a number of enclosed, fluid containing volumes. This results in a direct increase in pressure up to the pressure required to move the fluid through valves or ports into the discharge line. Displacement pumps are divided into reciprocating and rotary type pumps depending on the nature of the movement of the pressure-producing members. In reciprocating pumps, the main motion of the moving elements is reciprocating, while the main pumping action of rotary displacement pumps is caused by the relative movement between the rotating and stationary elements of the pump. Figure A1 illustrates the further classification of displacement pumps.

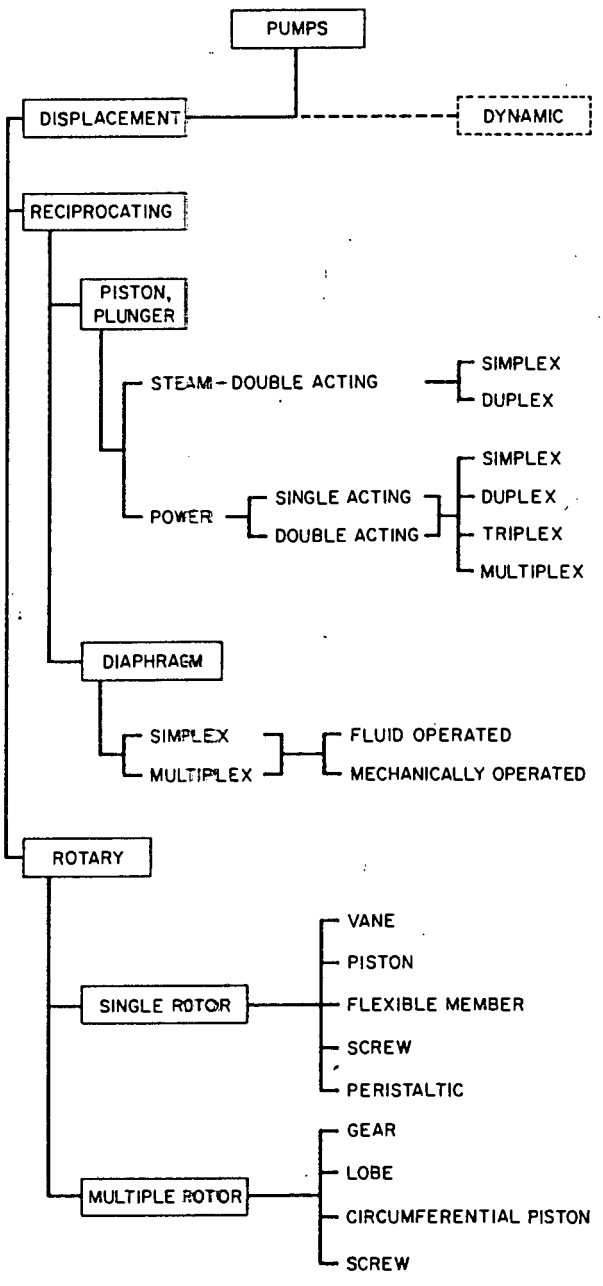

Figure A1 The classification of displacement pumps (from Karassik *et al.* 1976)

3. THE CHARACTERISTICS OF CENTRIFUGAL AND DISPLACEMENT PUMPS

In centrifugal pumps, the discharge pressure of the pump is dependent on the speed of the pump and on operating conditions such as the resistance to flow in the lines. The discharge pressure is also proportional to the density of the liquid being pumped. The presence of air or gas reduces the discharge pressure since this lowers the density of the mixture. Since more viscous fluids offer greater resistance to flow than less viscous fluids, flow rate and head are reduced as viscosity increases.

In contrast, a displacement pump develops a discharge pressure equal to the resistance to be overcome, irrespective of the speed of the pump. The pump speed, however, determines the output of the pump. Displacement pumps are thus better suited for pumping more viscous fluids and are able to produce constant flow against varying discharge conditions. These characteristics of displacement pumps make them suitable for yeast cropping where downstream equipment, such as heat exchangers used to cool the yeast, provide considerable resistance to flow.

In contrast with centrifugal pumps, where liquid has to be present at the first impeller before the pump can impart a force on the liquid to make it flow, displacement pumps are self-priming, since they automatically evacuate the suction line resulting in flow to the suction chamber.

4. PUMPS IN THE FOOD AND BEVERAGE INDUSTRIES

Pumps for the food and beverage industry should meet rigid sanitation codes. One of these codes, which was established by several American and international food, beverage and health associations, is known as the 3A Standards. The purpose of the specifications is to prevent the accumulation of food or beverage in isolated points and the subsequent putrefication of such food or beverage and to allow the cleaning of the internal components of the pump as quickly and effectively as possible (Karassik *et al.* 1976).

The specifications of the 3A Standards require that the wetted parts of any pump must be of type 300 stainless steel or equivalent material with the same corrosion-resistant properties. No cracks and crevices are permitted in any of the wetted parts of the pump. No internal threads are permitted, except where no alternative fastener is possible for the proper functioning of the pump. The Standards specify the finish and minimum radius of curvature of any wetted surface. In addition, the pump must be designed in such a way that the wetted parts can be readily disassembled for inspection and cleaning.

Centrifugal, rotary and reciprocating pumps are used throughout the food and beverage industry. Single stage end suction centrifugal pumps are widely used, but as discussed above, centrifugal pumps are less suited to pumping highly viscous products. Positive displacement pumps are used for this purpose with the rotary lobe pumps being most commonly used. Sanitary lobe pumps are limited to 1000 kPa discharge pressure (Karassik *et al.* 1976).

Mechanical seals are almost exclusively used in the food and beverage industry. Since "cleaning in place" (CIP) is most widely practised, the seals need to be cleanable under CIP conditions. Where "cleaning out of place" (COP) occurs, the seals need to be readily accessible (Karassik *et al.* 1976).

5. RECIPROCATING DISPLACEMENT PUMPS

In reciprocating displacement pumps, the pumping action occurs as a result of a reciprocating motion within the pump chamber. There are two main types of reciprocating displacement pumps, namely, piston/plunger pumps and diaphragm pumps. The two air-driven double diaphragm pumps submitted for evaluation are discussed in more detail below.

5.1 Air-Driven Double-Diaphragm Pumps

Double diaphragm pumps are used to pump liquids and fine dry powders in several industries including the chemical, pharmaceutical, paint, ceramic, construction, mining and aerospace industries. In the food and beverage industries, the pumps are used to pump several products including yeast, chocolate, fruit pulp, fruit yogurt, wine and other gas-liquid mixtures. Within SA Breweries, bulk acids and detergents as well as waste Kieselguhr (a filter-aid) are pumped using air-driven diaphragm pumps. These pumps are never used to pump any product (beer, wort or yeast).

For the trials, a DEPA stainless steel DL 40 pump was supplied by Alfa-Laval and a Wilden M8 Foodmaster by Letaba Pumps and Flo-Mart.

5.1.1 Principle of Operation

A double diaphragm pump consists of two chambers and two flexible diaphragms. The diaphragms are clamped in sandwich fashion at their outer edges and are connected to a shaft. The operation of the pump is depicted in Figure A2(a) - (c).

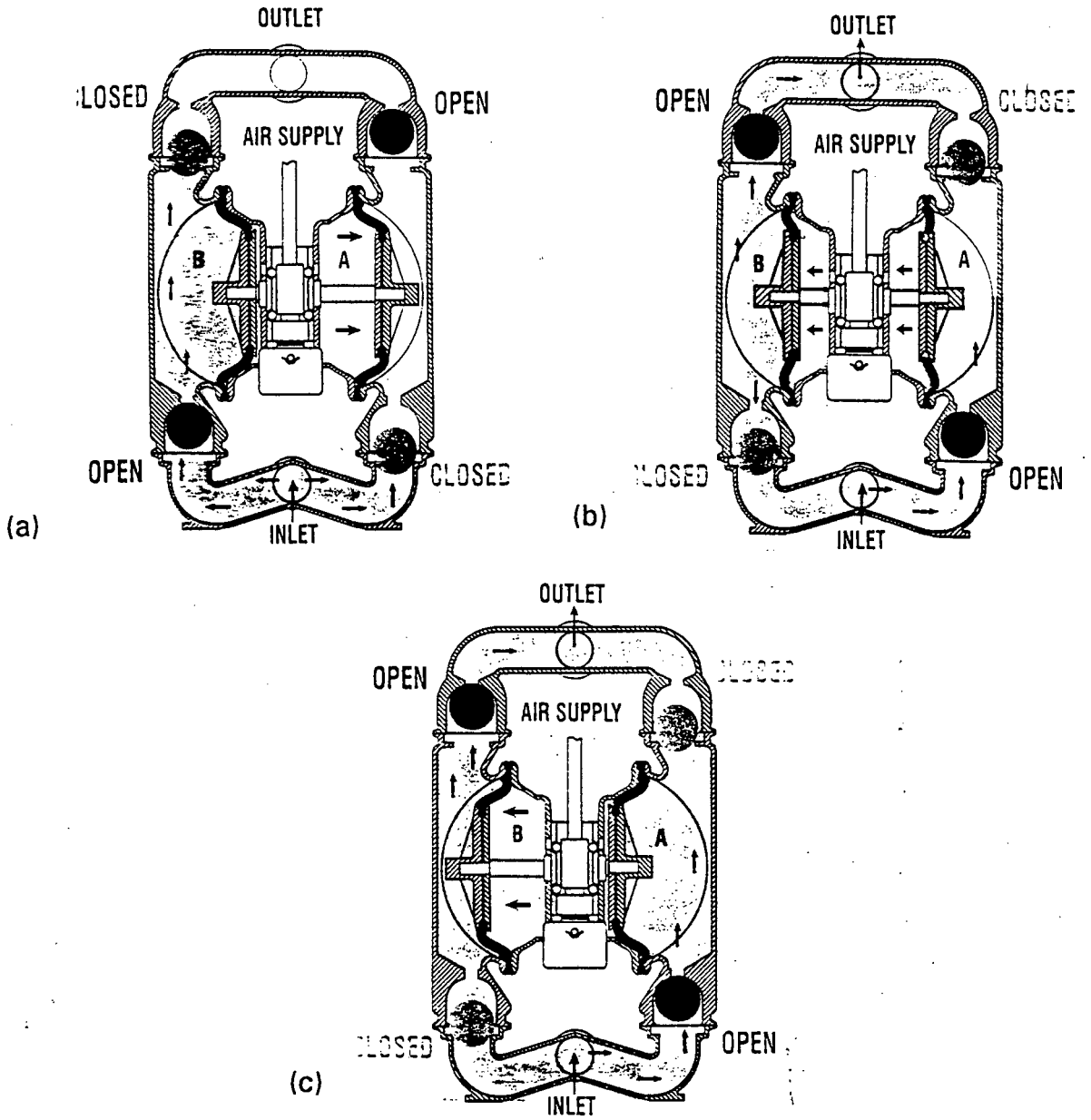

Figure A2 Operation of an air-driven double diaphragm pump

Compressed air which is directed to the back side of Diaphragm A moves the diaphragm away from the centre block, while the opposite diaphragm is pulled toward the centre block by the shaft. Air is exhausted from behind Diaphragm B via an exhaust port. The movement of Diaphragm B creates a vacuum within Chamber B allowing fluid to force the inlet valve off its seat and fill the chamber (Figure 2(a)). Upon completion of the stroke, an air distribution valve automatically transfers compressed air to the back side of Diaphragm B and exhausts air from the back of Diaphragm A. The shaft thus moves in the opposite direction, compressing the fluid in chamber B. This closes the inlet valve and opens the discharge valve,

forcing fluid to flow through the pump discharge. Fluid enters Chamber A as it did Chamber B in the previous stroke (Figure A2(b) and Figure A2(c)). This continuous reciprocating motion creates alternate intake and discharge of pumped fluid into and out of each chamber and results in near continuous pumping action from the combined chambers. The flow pulsations in the discharge line occur when fluid returns to the pump to displace the vacuum created by outlet valves returning to their seats. On the suction side, flow pulsation occur when the inlet valves are forced back to their seats displacing fluid away from the chamber inlet.

The pumps can be made to be bottom discharging or top discharging. Bottom discharge is suited to pumping solids in suspension which may tend to settle out while top discharge has the advantage of allowing air or vapour to be easily expelled from the chamber.

Figure A3 is a composite performance chart of a typical air-operated double-diaphragm pump. While air-operated diaphragm pumps are displacement pumps, they are not positive displacement pumps. At a constant supply pressure of compressed air, the pump discharge pressure decreases with increasing capacity. In this respect the pumps are similar to centrifugal pumps. However, unlike centrifugal pumps, the pump discharge pressure remains the same for a given capacity and air inlet pressure regardless of the specific gravity of the liquid being pumped. The maximum pumping pressure, however, cannot exceed the pressure of the compressed air powering the pump. Air consumption is approximately proportional to the pumping rate. This allows control of the flow rate of the fluid over a wide range of flow.

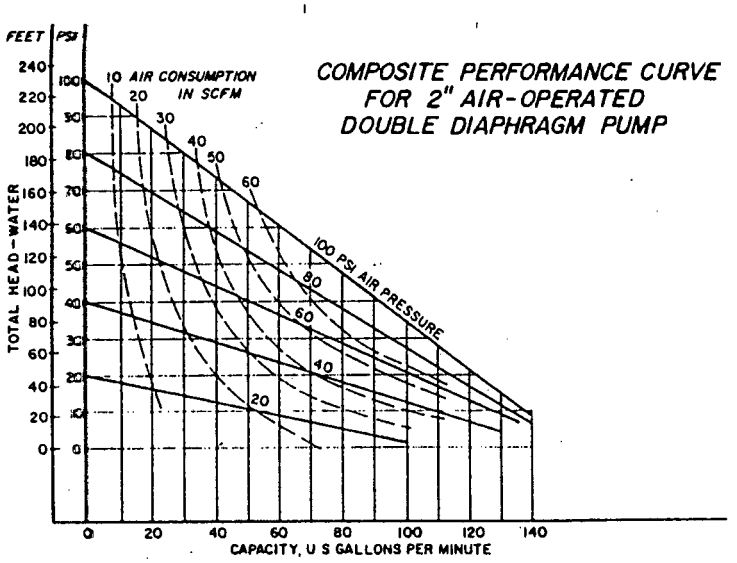

Figure A3 Composite performance chart for a typical air-driven double diaphragm pump

The size of air-driven diaphragm pumps are limited by a practical consideration. Above a capacity of 60 m^3/hr, the cost of compressed air and components become excessive. There is no real limit to the outlet pressure that these pumps can achieve, although compressed air above 860 kPa is not usually available.

The diaphragms in air-operated double-diaphragm pumps are essentially balanced and act simply as membranes separating the compressed air from the product being pumped. The diaphragms are customarily made of elastomers and a wide variety of rubber, thermoplastic and Teflon diaphragms are available for various applications.

5.1.2 Favourable Features of Air-Driven Double Diaphragm Pumps

Air-operated double-diaphragm pumps have several favourable features for both general brewery use and for yeast handling.

- Diaphragm pumps can pump viscous liquids up to 11 000 cSt (1.1×10^{-2} m^2/s).
- The pumps can pump abrasive slurries and solids in suspension.
- The pumps can pump fine, dry powders in air suspension.
- The pumps have no close-fitting, sliding or rotating parts in contact with the fluid to be pumped.
- The pumps have no seals or rotary bearings.
- The pumps have an infinitely variable pumping rate and discharge pressure within the pressure and capacity range of pump.
- No electric motors are required, making the pumps safe for use in wet, explosive and hazardous areas.
- The pumps can be used in confined areas since there is no heat build up.
- No leakage occurs.
- The pumps are self-priming from dry start.
- The pumps can run dry indefinitely.
- Discharge can be throttled to zero indefinitely.
- No bypass is required.
- The pumps are simple to maintain and repair.

In addition, KWW GmbH, the manufacturer of DEPA pumps claim the following with regard to DEPA diaphragm pumps:

- Stainless steel pumps are available with capacity up to 45 m^3/hr (750 l/min).
- The pumps are suitable for shear and compression sensitive media, since transport is exclusively by displacement.
- The pumps are made of highly polished type 304 stainless steel.
- The pumps have DIN 11 851 threaded connections and can be quickly and easily disassembled for visual inspection.
- The pumps are CIP compatible and can be steam sterilised.
- The pumps are provided with a lubricant-free control valve.

- The pumps are easily portable.
- The pumps can operate against closed discharge lines without increased heat generation, wear or leakage.
- Units are exceptionally rugged. Damage due to incorrect operation is virtually impossible

Wilden Pump and Engineering Company claim the following with regard to Wilden food-processing air-driven double-diaphragm pumps:

- Food pumps are available up to a capacity of 55 m^3/hr (908 l/min).
- The pumps provide very gentle pumping action, keeping shear to a minimum.
- The pumps are made of type 316 stainless-steel.
- Product contact parts have a bead-blasted finish.
- M8 Foodmaster is approved by the United States Food and Drug Administration (FDA).
- M8 Foodmaster has a maximum capacity of 530 l/min.
- All elastomeric materials are of FDA-approved Saniflex or Teflon TFE.
- The pumps have tri-clamp type ports and the clamp band has wing-nut fasteners for easy disassembly.
- The pumps are completely portable.
- No efficiency loss occurs due to wear.
- No efficiency loss occurs due to changing pressure or flow.

Wilden replaced electrically operated pumps (type unspecified) with M8 Foodmaster pumps in Schoenling Brewing Company in Cincinnati, Ohio. The pumps are used to transfer yeast-slurry from fermentation tanks to the yeast culture room for re-use. An increase in operating efficiency and a 75% reduction in maintenance cost are claimed. The plant has a capacity of 260 m^3/day and appears to do COP as opposed to CIP, hence the increase in efficiency.

5.1.3 Drawbacks of Double Diaphragm Pumps

During yeast cropping, the pumps operate under positive suction head (approximately 150-180 kPa). Although air-driven double-diaphragm pumps can work under conditions of positive section head, there is a reduction in efficiency. Wilden suggest that the inlet pressure be limited to 48-68 kPa. The large positive suction head produces large pressure fluctuation on the suction side. Suction side pressure fluctuations of up to 180 kPa in the DEPA pump and up to 250 kPa in the Wilden pump were observed under the operating conditions employed.

In addition, the large positive suction head appeared to affect the ability of the elastomeric balls to return to their seats. The apparent disruption of characteristic pump operation was audible. Although balls with heavy, solid cores and elastomeric exteriors can be installed, this would constitute an increase in capital cost and would not necessarily solve the problem.

Noise pollution was another problem. Although the pumps can be fitted with mufflers, the pumps made considerably more noise than electrically operated pumps.

5.1.4 General Comments

The design of the DEPA pumps is superior to that of the Wilden. The DEPA design used thread connections and is extremely easy to disassemble. The standard issue of union couplings (as opposed to Tri-clamp fittings) is also advantageous. Depending on pump size, reducers and expanders may have to be attached, but the connections on the pump are standard to the Breweries. In the case of the Wilden tri-clamp connections, adaptors need to be provided. The design of the DEPA pumps makes the pumps more portable and easier to work with than the Wilden pumps. The DEPA pumps use a non-lubricated air valve assembly, while the Wilden requires a separate lubricator with the air regulator. The Saniflex diaphragms, which are US FDA approved, are a trademark of Wilden. The Wilden M8 Foodmaster is capable of creating a large suction vacuum. Reinforced suction hose or stainless steel tubing would be required to install the pump.

6. ROTARY DISPLACEMENT PUMPS

In rotary positive displacement pumps, relative motion between the rotating and stationary elements of the pump constitutes the main pumping action of the pumps. The pumping chamber is the space inside the pump that contains the fluid to be pumped. The body of the pump, which is sometimes referred to as the casing or housing, is that part of the pump which surrounds the boundaries of the pumping chamber. In most types of rotary displacement pumps the body is the stationary element of the pump, but in some pumps it may also be part of the rotating assembly - a term which encompasses all parts of the pump that rotate when the pump is operating. The part of the rotating assembly that rotates within the pumping chamber is called the rotor. The rotor may be given specific names (eg. vane, screw, gear, lobe) in the different types of rotary pumps.

Being positive displacement pumps, the amount of liquid displaced by the pumps during each revolution is independent of the speed of the pump. The action and position of the pumping elements and the close running clearances of the pump maintain a liquid seal between the inlet and outlet ports obviating the need for inlet and outlet valve arrangement of reciprocating displacement pumps. The pumping sequence in all rotary positive displacement pumps includes three elementary actions. The rotating and stationary parts of the pumps act to define a volume, sealed from the pump outlet and open to the pump inlet. As the element rotates this volume grows. The pumps then establishes a seal between the pump inlet and some of this volume, cutting the volume off from both the inlet and outlet of the

pump chamber. The seal to the outlet chamber is then opened and the volume which is now open to the outlet is constricted by the cooperative action of the moving and stationary elements of the pump. In all rotary displacement pumps three conditions thus exist: closed-to-outlet open to inlet (CTO), closed-to-outlet closed-to-inlet (CTIO), open-to-outlet closed-to-inlet (OTO). Near continuous flow can be achieved if the change in open-to-inlet and open-to-outlet conditions occurs smoothly and if the volume of the closed-to-inlet, closed-to-outlet condition remains constant.

To be true positive displacement pumps, there should be no fluid in the pumping chamber which is open to the inlet and outlet simultaneously. However, the clearances between the rotating and stationary members of the path allow a certain quantity of fluid to leak from the OTO volume to the OTI volume. The quantity of fluid that leaks per unit time is known as slip and is dependent on the size of the clearances, the differential pressure between the OTO and OTI volume and the properties of the fluid being handled (particularly its viscosity). Where liquid velocity is high, slip may be secondarily dependent on pump speed. Slip is an important factor in rotary pump performance and applications and one of the common causes of flow pulsations in rotary pumps.

The majority of pumps in the trials were rotary displacement pumps : two single rotor pumps (a sine pump and a peristaltic pump) and five multiple rotor pumps (four lobe pumps and an internal gear pump). These are discussed in turn below.

6.1 Single Rotor Screw Pumps - The Sine Pump

Sine pumps are novel rotary displacement pumps used in the food, soft and fruit drink, dairy, confectionary, candy, cosmetic and pharmaceutical industries. Fluids pumped include shear sensitive or particulate materials such as salads, frozen fruit juice concentrates, butter, chocolate, shampoos and face creams.

A Maso sine pump (model SP3") was provided for evaluation by Aeromix (Pty) Ltd.

6.1.1 Principle of Operation

Sine pumps are single rotor screw-type displacement pumps (Figure A4 and A5). The principle of operation is typical of rotary displacement pumps. The pump rotor which has a sinusoidal shape rotates within the stationary housing creating chambers for product movement. At any given time there are four chambers : one chamber open-to-inlet (OTI), two chambers closed-to-inlet-and-outlet (CTIO) and one chamber open-to-outlet. As the pump rotates the volume of the open-to-inlet chamber grows, creating a suction and allowing the entry of product into the pump chamber. After a quarter of a revolution, the rotor (by virtue of its shape) seals this volume from the inlet and creates a chamber which is closed to both inlet and outlet. This chamber moves through the pump and becomes open-to the outlet

after another half a revolution. As the pump continues to rotate, the volume of the now open-to-outlet chamber decreases forcing product into the discharge line. The suction (inlet) and pressure (outlet) sides of the pump are divided by a scraper which also forms the seal (Figure A5(D)).

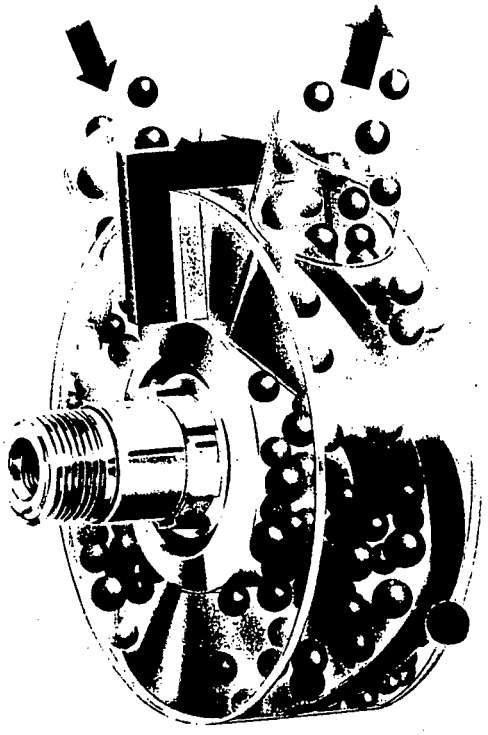

Figure A4 Operation of the sine pump

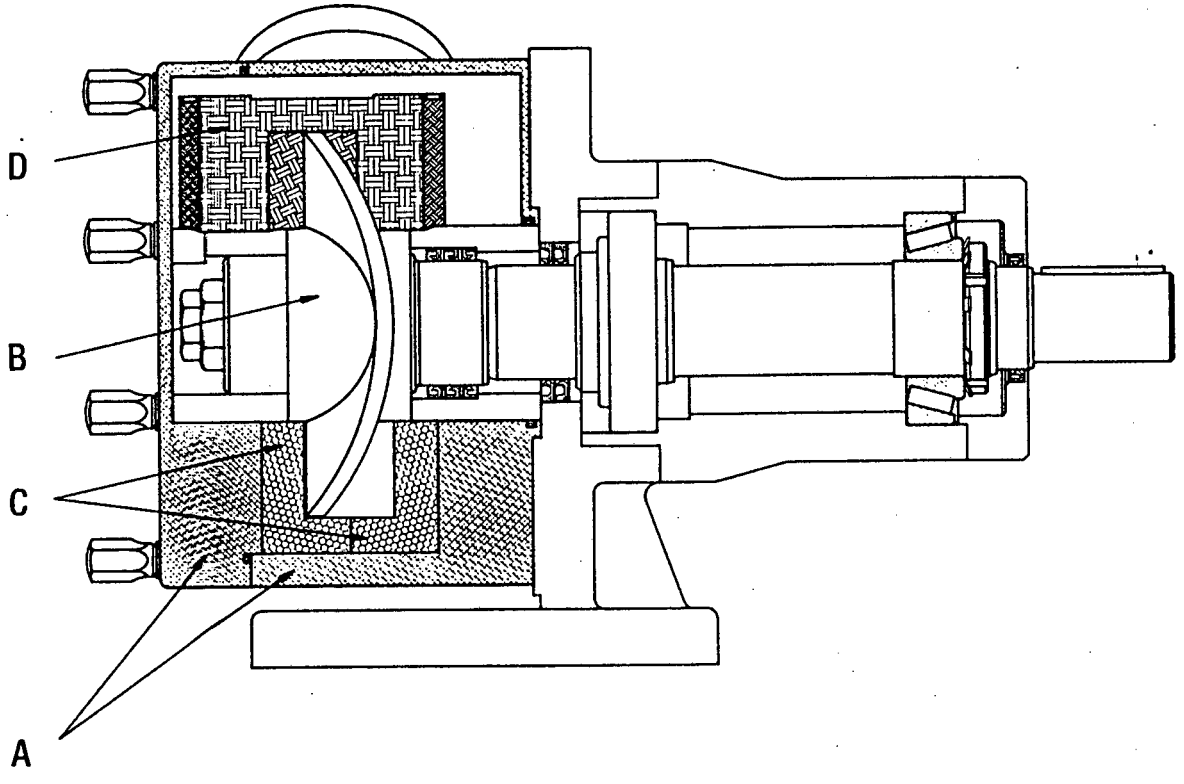

Figure A5 Section through the sine pump (A = pump housing, B = sinusoidal Rotor, C = separate liners, D = scraper)

6.1.2 Favourable Features of Sine Pumps

The main feature of the sine pump that makes suitable for yeast handling applications is that the design of the rotor allows ideal rotary displacement pump operation (*i.e.* smooth growth and shrinking of chambers open to inlet and outlet and a constant volume in the chambers closed to both outlet and inlet). This results in near continuous flow (*i.e.* almost no flow pulsations).

In addition, the following are claimed by Maso Dickstoffpumpen GmbH for the Maso sine pump

- A wide range of pump sizes are available with maximum capacities from 1 m^3/hr to 90 m^3/hr and maximum operating pressure of 1500 kPa.
- The pump housing is made of 316 stainless steel.
- All parts, including the rotor are machined from solid blocks.
- Liners are available in alternative materials (Polyamid, Peek or stainless steel) to suit the application.
- The scrapers are available in alternative materials to suit the application.
- The pump can be provided with any of three different port locations.
- The pump is CIP compatible and can be cleaned automatically. It can also be dismantled and cleaned by hand.
- During CIP, the scraper gate forces CIP fluids into all areas of the housing to ensure complete cleaning.
- Aseptic pumps are available on request.
- The pump design allows dismantling in-line and all main wearing parts can be replaced *in situ*.
- The pumps have minimal rotational parts which ensures ease of maintenance.
- There are no gear boxes or timing gears.
- The main pump body and cover are not subject to wear, eliminating machining, shimming and oversize components.
- Constantly open suction and discharge ports and screw feeding allow powerful suction for the pumping of highly viscous materials.
- Shear sensitive and particulate bearing products can be pumped without damage or deterioration.
- Low slip of the rotor and liners allows constant reduction on the suction side to 85 kPa resulting in efficient product transfer.
- The pumps can run dry for several minutes without damage.

In addition, Maso claim applications in biotechnology. Mechanically sensitive cell cultures used for Insulin and Interferon production are harvested and pumped to ultra-filtration systems using Maso sine pumps.

6.1.3 Drawbacks of the Maso Sine pump

The precision manufacturing of the pump components for solid stainless steel is a

costly process which adds to the initial capital cost of the pumps. The evaluation of wear characteristics and maintenance fall beyond the scope of this investigation, but the claimed minimal wear and reduced maintenance costs may offset the initial capital cost.

Although pumps can run dry, the sealing system is at risk during dry operation. The pumps therefore need to be primed before operation. Unlike diaphragm pumps, the sine pumps run the risk of severe damage should they be operated against a throttled or closed valve. With a set configuration, the pump operates in one direction only. However, it is possible to change the direction of rotation by changing the installation of the scrapper gate and scrapper guide.

6.1.4 General comments

The Maso sine pump provided for testing was an SP3" model with a maximum capacity of 17 m^3/hr at 600 rpm. The pump is issued with 80 mm union fittings. In order to install the pump for testing, asymmetric 80 mm to 50 mm union fittings were attached. As suggested in the operation manual, the fittings were orientated to allow fluid to return to the pump when the pump was stopped. Despite the contraction and expansion created by these fittings, no cavitation was audible. The pump provided near continuous flow with negligible flow pulsations (5 - 10 kPa on the discharge side).

Concern has been expressed about the cleanability of the pump, with particular reference to the scraper. As indicated above, the manufacturers claim that they are able to provide aseptic pumps and claim full CIP cleanability. Cleanability and sterility of pump surfaces was never directly assessed. However, during a trial with the Maso sine pump, fermentations that had been inoculated with yeast samples taken were checked for contamination. No contamination was found implying that the pump was fully cleanable and sterilisable under the test conditions.

The scraper forms the seal and should prevent the contamination of the product by grease or oils used for pump and motor lubrication. The durability and effectiveness of this seal has not been validated over long term operation.

6.2 Peristaltic pumps

Peristaltic pumps are used in several industries such as the paper, ceramic, building and mining industries as well as in the food and beverage industries. In breweries, peristaltic pumps are used to pump yeast, diatomaceous earth, flocculants, stabilizers and filter press feed. Within SA Breweries, peristaltic pumps are commonly used for additive dosing where positive displacement into varying line pressures is essential. These pumps are not used to pump any product (beer, wort or yeast).

Two Bredel peristaltic pumps (supplied by Walter Becker SA) were used in the trials, the SP/40 and the SP/50 pumps.

6.2.1 Principle of Operation

Peristaltic or hose pumps are single rotor rotary positive displacement pumps. Figure A6 is a diagram of a typical peristaltic pump. The pump consists of a composite, reinforced hose enclosed in a casing. The casing is flanged at both ends to enable connection to the suction and discharge lines of the system. The pump has a rotor with two pressing shoes at opposite points about its centre line. As the rotor rotates, the hose is totally compressed by the shoes and the product within the hose is pushed forward. Once the shoe has passed and compression ceases, the hose returns to its original shape instantaneously as a result of the rigidity imparted by the reinforcing element within the hose. This action provides the self-priming capability of the pump and allows product to enter the hose. The pushing action of the pressing shoe which forces product into the discharge line at each half rotation of the rotor, sets up the pressure differential across the pump. The output of the pump is determined by the contents of the hose and the rotational speed of the rotor.

The operation of the peristaltic pump is characteristic of rotary lobe pumps. The OTI volume is the tube volume between the inlet and the first compression or "nip" point, the CTIO volume is that contained between the two nip points and the OTO volume is that contained in the tube volume between the pump outlet and the adjacent nip point.

Peristaltic pumps produce pulsating flow. During compression, the product is pushed into the discharge line by the pressing shoe. When compression ceases and the shoe moves away from the hose, an increase in volume occurs in the hose and discharge line. This increase is equivalent to the volume of the pressing shoe. Flow into the discharge line ceases momentarily, until the fluid in the discharge line and the hose meet as a result of the pressure in the discharge line. Hereafter the velocity in the discharge line returns to normal. The successive deceleration and acceleration of mass, results in pressure peaks during the pulsations.

Pulsations may also occur on the suction side of the pump. The decrease in volume when compression of the hose starts, stops the flow of product for a moment whereafter the flow returns to normal. Pressure peaks also occur as on the discharge side.

Pulsation has a negative effect on pump performance. As shock pulse levels in the suction and discharge lines increase, pump performance may become unstable and a fall off in capacity may occur due to high acceleration losses.

During pumping, compression must be total in order to avoid slip and loss of self-priming capability. Slip results in the retention of a film of fluid on the wall of the

hose causing a fall off in the output of pump. The degree of compression is dependent on the operating pressure and the pump speed. To achieve total compression, the pressing shoes can be adjusted with shims. For high pressure and low pump speeds more shims will be necessary. Shimming guides are generally provided by the manufacturers.

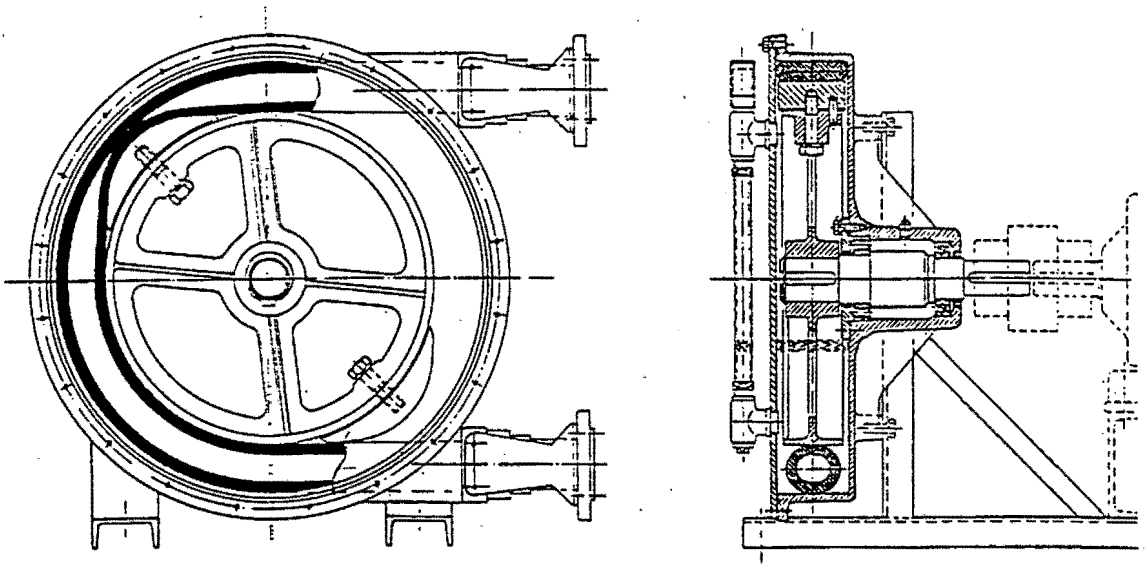

Figure A6 The peristaltic pump

6.2.2 Favourable Features of Peristaltic Pumps

From a general brewery point of view and for yeast handling purposes, peristaltic pumps provide the following favourable features:

- Simple construction allows operation in either direction and in four mounting positions (Figure A7).
- There is only one wearing part - the hose.
- Replacement of the hose can be done without dismantling the pump, hence maintenance is simple and downtime short.
- The pumps are easy to clean since there are no valves, cavities, glands or seals.
- The pumps can be designed and built for high discharge pressures.
- The pumps can run dry without damage.
- The product does not contact any mechanical parts or seals.
- The noise level is low.

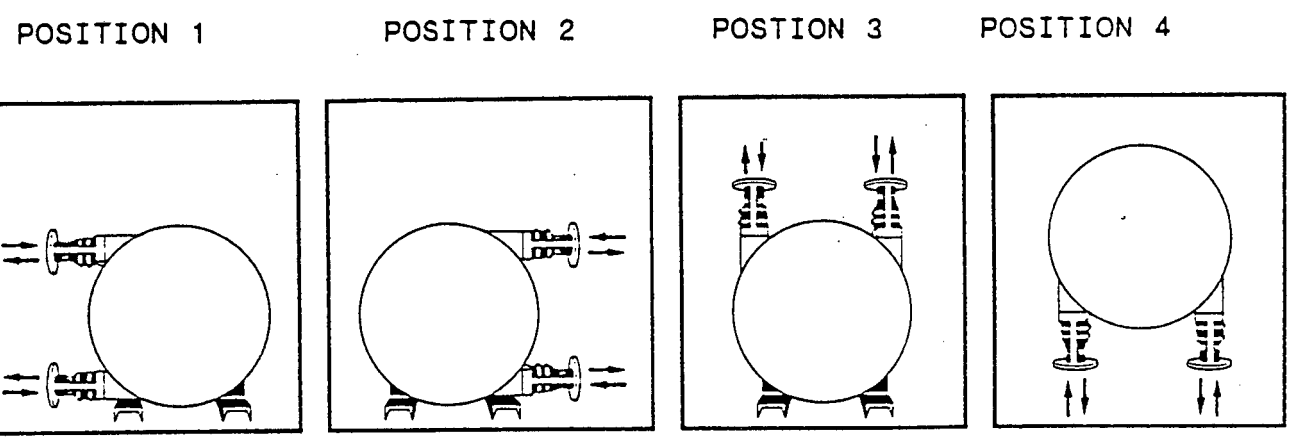

Figure A7 Different mounting positions of the Bredel peristaltic pump

In addition the manufacturer, Waukesha Bredel Fluid Handling B.V. claim that Bredel peristaltic pumps have the following favourable features

- A wide range of pump sizes are available.
- The pumps are suitable for shear sensitive products and for viscous products.
- The pumps are suitable for abrasive, corrosive and high density products
- The pumps have 100% positive flow (ie. no slip).
- Depending on pump speed, operating pressure and the temperature of the pumped media, hose life is between 2000 and 3000 hours or longer under more favourable operating conditions.
- There is a choice of hose material depending on the product and cleaning agents used.
- EPDM hose is resistant to 50% caustic and 10, 30 and 88% hydrogen peroxide (agents commonly used in SA Breweries).
- Rigidity of the hose guarantees vacuums up to 95 kPa on the suction side. (This is not of consequence if used under suction head conditions such as yeast cropping).
- The hose life is prolonged at lower pump speeds.
- Any drive system can be used.
- A special rotor with retractable pressing shoes can be installed upon request allowing CIP at full velocity and obviating the need for a bypass.
- Food grade hose and food grade lubricant can be provided.
- Stainless steel inlet and outlet fittings to DIN/ISO are supplied on request.

6.2.3 Drawbacks of Peristaltic Pumps

Pulsating flow is the main drawback of peristaltic pumps. Pulsation dampeners may alleviate this problem. This is discussed in more detail in Section 8.

Pulsation makes accurate flow measurement and control difficult. For applications such as yeast cropping and pitching (where the required amount of yeast is weighed off before pumping), accurate flow measurement is not required.

The physical dimensions and mass of the pumps may be problematic. For yeast cropping, the Bredel SP/40 and SP/50 would typically be used. The Bredel SP/40 pump which has a maximum output of 6000 l/hr under continuous duty at 75 rpm has a gross mass of 150 kg and a crate volume of 0.36 m^3 while the SP/50 with a maximum capacity of 10 500 l/hr under continuous duty at 60 rpm has a gross mass of 240 kg and a crate volume of 0.56 m^3. The largest pump in the range, the SP/100, with a maximum output of 36 000 l/hr under continuous duty at 30 rpm weighs 1055 kg and has crate dimensions of 2.64 m^3.

6.2.4 General Comments

The pumps provided for testing had flange couplings, hence stainless steel flange to Union adaptors were made to install the pumps. In the case of the SP/40 where the pump ports are 40 mm, the adaptors resulted in rapid reduction and expansion of the 50 mm flow path.

The pulsating flow created by the peristaltic pumps is the greatest concern with regard to their suitability for yeast cropping. There was concern that an increase in pump temperature will occur due to friction between the pressing shoes and the hose and that this could have a negative effect on yeast quality. However, despite the fact that the pump casing of the SP/40 pump used to investigate this became extremely hot after 2 hours of pumping at 46 rpm and caused a 1 °C increase in the temperature of the yeast passing through the pump, no significant effect on yeast quality could be detected.

6.3 Internal Gear Pumps

Gear pumps are well known displacement pumps which are used for a variety of applications amongst other in the brewing, dairy and food industries. An internal gear pump, the Scandi Brew gear pump (Model GP160), was provided for the testwork by Micro Matic Liquid Transfer.

6.3.1 Principle of Operation

Gear pumps are rotary displacement pumps in which two or more gears mesh to provide the pumping action. In gear pumps, unlike lobe pumps which will be discussed below, one of the gears is able to drive the others. Figure A8 and A9 presents the two different types of gear pumps namely external and internal gear pumps. In external gear pumps, the centre of rotation of each element is external to the major diameter of an adjoining gear and all gears have external teeth. In internal gear pumps, such as the Scandi Brew yeast pump, the centre of rotation of at least one gear is inside the major diameter of an adjoining gear. This implies that at least one gear has to be of the internal-tooth type or crown-tooth type. The Scandi Brew yeast pump consists of two gears. The outer gear with internal toothing is known as the star wheel and it is driven by the smaller central gear, the rotor.

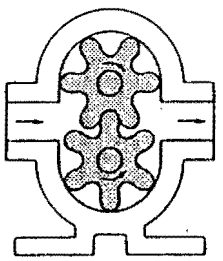

Figure A8 An external gear pump

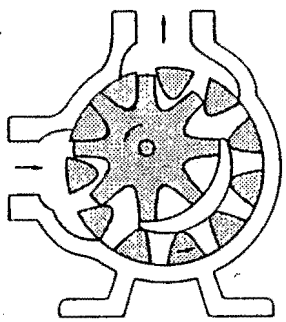

Figure A9 An internal gear pump

In gear pumps, the OTI volume of the pump chamber is defined by the body walls and by the gear tooth surfaces between the fluid seal point where the teeth mesh and the fluid seal points where each tooth tip meets and seals with the body walls as it leaves the OTI volume. The CTIO volume is the fluid trapped between the gear teeth and the body walls where it is sealed from both the inlet and outlet chambers. Similarly to the OTI volume, the OTO volume is defined by the body walls and those gear tooth surfaces between the fluid seal where each tooth leaves the body wall and enters the OTO volume and the fluid seal points where the gears

mesh. Small clearance contact occurs between the gear teeth where they mesh, between the gears and the radial surfaces of the pump chamber and between the axial surfaces of the gears and the end faces of the pump. During operation under differential pressure, slip occurs as a result of these clearances. These pumps are thus not full positive displacement pumps and a slight drop off in theoretical volume output occurs at higher discharge pressures.

6.3.2 Favourable Features of Internal Gear Pumps

Gear pumps do not require external timing gears, making them simpler to maintain than pumps such as lobe pumps. When pumping non-lubricating fluids, external timing gears may be required to transfer torque from one rotating assembly to the other to reduce wear between rotor teeth.

Scandi Brew claim the following with regard to the Scandi Brew internal gear yeast pump:

- Pumps are available with standard pumping rates of 40, 60, 100, 160 or 200 l/min (12 m^3/hr), but can be provided with variable speed control to regulate capacity (eg. for dosing purposes).
- The pump housing and rotor are made of type 304 stainless steel.
- Star wheels are supplied in acid-proof bronze (or acid proof stainless steel at extra cost).
- The pump are self-priming and have high suction power when filled with liquid (up to 50 kPa vacuum).
- Pumps have been developed to pump fluids with high viscosity, emulsions and fluids with suspended particles.
- The flow direction of the product is changed only slightly when passing through the pump which secures gentle transport without whips and pulsations.
- The pumps fulfil the stringent hygienic demands of the brewing, dairy and food industries.
- CIP can be done with any detergent up to a maximum temperature of 90°C.

In addition, the pumps have a built-in combined regulation and CIP bypass valve, which also acts as pressure release valve, opening when the pressure reaches 850 kPa. The valve can be opened manually or pneumatically during CIP. The channels created in this way allow effective cleaning of the back of the rotor and effective CIP of the stuffing box.

6.3.3 Drawbacks of Internal Gear Pumps

The cost of internal gear pumps such as the Scandi Brew yeast pump generally exceeds that of other displacement pumps used for the same application.

6.3.4 General Comments

The Scandi Brew gear pump provided steady, non-pulsating flow. The pump, which was mounted on a stainless steel trolley, was easy to relocate. The pump can also be fixed onto a stainless steel console, if required. The end cover can be removed easily should one want to inspect parts of the pump.

6.4 Rotary Lobe Pumps

Lobe pumps are used for a variety of applications in the food, diary, beverage, paper, chemical and pharmaceutical industries. In SA Breweries, yeast cropping is generally done with lobe pumps.

Three lobe pumps were provided for the testwork : an APV lobe pump (APV), a Johnson On-Line lobe pump (NDE) and an Ibex lobe pump (Alfa-Laval). In addition, a Wilflo lobe pump, used for yeast cropping at Newlands Brewery, was also tested.

6.4.1 Principle of Operation

Operation of a typical rotary lobe pump is illustrated in Figure A10. The OTI volume is defined by the body surfaces, the rotor surfaces, the contact between the rotors and the contact between the rotor lobe ends and the body. The CTIO volume is defined by the contacts between lobe ends and the body wall and the adjoining body wall and lobe surfaces. The OTO volume is defined by the body walls, the rotor surfaces, the lobe-to-body-wall contacts and the lobe-to-lobe contacts.

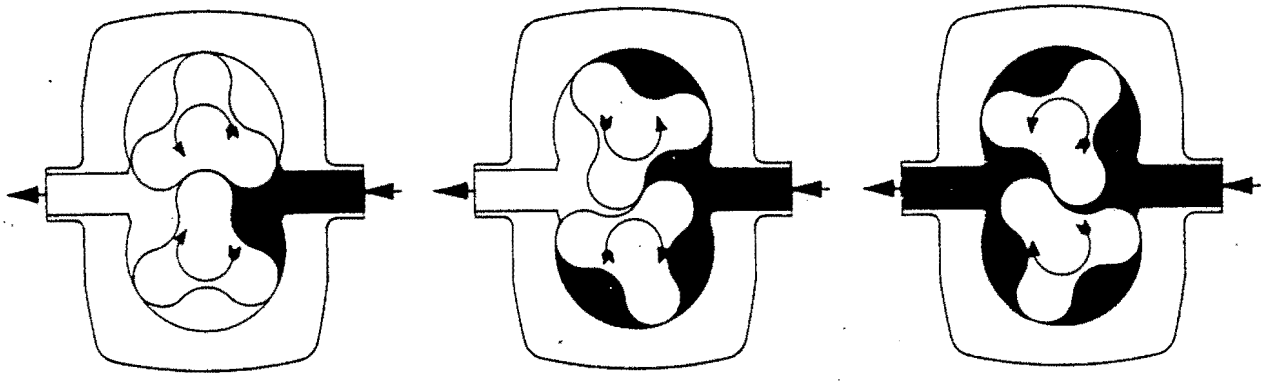

Figure A10 Operation of a typical rotary lobe pump

As with gear pumps, clearances between the rotors, the rotor ends and the pump body as well as the clearance between the axial surfaces of the lobes and the end plate allow a certain amount of slip which is dependent on the viscosity of the fluid being pumped and the pressure differential across the pump. The pumps are thus not 100% positive displacement pumps.

The rotors of lobe pumps have a rounded shape. This allows the rotors to be in continuous contact with each other as they rotate. A variety of lobe shapes are used including bi-lobe, tri-lobe and scimitar (bi-wing) rotors. Since the rounded shape of the rotors prevents them from driving each other, lobe pumps require external timing gears.

6.4.2 Favourable Features of Rotary Lobe Pump

Lobe pumps have the following features which make them good pumps for brewery applications.

- The pumps provide smooth flow with very little pulsation.
- They pump with low agitation and low shear.
- The pumps have good solids handling characteristics.
- They are able to pump fluids with high, medium and low viscosity.
- The pumps cope well with liquids with entrained or dissolved gases
- The pumps have good cleanablity and sanitary operation.
- The pumps are self-priming.
- They can run dry.
- The pumps can be fitted with by-pass valves to ensure cleanability during CIP.
- Pressure relief valves can be fitted for protection against overload.

The APV lobe pumps have the following features

- The pumps are designed to meet the 3A Standards and materials of construction are FDA approved.
- All metal parts are made of 316 stainless steel and all standard gasket and seal materials are food grade EPDM rubber or carbon.
- Four of the twelve seal types are 3A approved for hygienic food processing.
- A wide variety of lobe pumps are available with capacities ranging from 6 to 620 l/100 rev.
- The pumps have flexible configuration with a selection of gear boxes, end-covers and seals.
- End-covers may be plain, have by-pass valves or pressure relief valves.
- Rotors have tri-lobe design.
- For each pump, there are four rotor sizes to achieve appropriate clearances depending on the maximum temperature within the pump chamber (70°C, 110°C, 150°C or 200°C).

- For sanitary operation, the pump configuration is such that rotation is possible in one direction only.
- Corrosion resistant surfaces and crevice free construction allow easy in place cleaning.
- Relief and by-pass valve arrangements allow effective CIP.
- The pumps can be dismantled easily for manual cleaning or inspection.

For the Ibex lobe pumps:

- A range of pump sizes with bi-lobe and tri-lobe rotor designs are available.
- Flow is in the vertical direction.
- Mechanical seals and packed glands are used.
- High viscosity products can be pumped.
- The pumps can be cleaned in place (CIP).
- The pumps can be fitted with relief valves on request.

Johnson Pump (UK) Ltd claim the following with regards to Johnson On Line lobe pumps:

- A wide range of pumps with maximum capacities from 56 to 2592 l/min (at 4 to 325 l/100 rev) and differential pressures up to 1500 kPa are available.
- All product wetted parts are type 316 stainless steel and the rotor case and front cover are fully machined.
- The pumps have bi-wing rotor design which reduces slip and improves pump efficiency.
- They have unique front-loading product seal positioning which allows rapid maintenance, easy cleaning and reduces downtime.
- Minimum product entrapment and effective cleaning is ensured by the fully swept pump chamber, flush front cover and a rotor retention device.
- The pumps can handle fluids of varying viscosity, delicate liquids (with minimum agitation and shear), as well as aggressive chemicals, slurries and pastes.
- Precise alignment and concentricity between the shaft, rotorcase and rotor is guaranteed by the shaft cartridge assembly which is mounted directly onto the back of the rotorcase.
- Shaft cartridges can be withdrawn for inspection or replacement while the pump is on-line.
- Single point shimming provides quick and easy on-line adjustment of rotor clearances.
- Suction and discharge ports can be selected to correspond with standard fittings in plant.

6.4.3 Drawbacks of Lobe pumps

Slip, which is dependent on the clearances within the pump, the viscosity of the fluid being pumped and the differential pressure across the pump, reduces the accuracy and efficiency of lobe pumps. Yeast suspensions are thixotropic (*i.e.* viscosity is dependent on shear rate and time) and the viscosity may vary significantly between different batches of yeast. It is also difficult to obtain accurate viscosity data for yeast suspensions. Sizing lobe pumps correctly and ensuring efficient operation is thus difficult.

The design of the pumps, with seals and timing gears, means that the pumps require a certain amount of maintenance to prolong pump life and ensure efficient operation. Seal design is critical to ensure that product contamination does not occur.

6.4.4 General Comments

The APV lobe pump supplied was a model CL/3S/156/7 which has a theoretical capacity of 1.56 liter/revolution when pumping water. However, measurement of the flow rate and rotational speed showed that the actual capacity was significantly less ($\approx$ 0.4 liter/revolution). This suggests that considerable slip occurs in the pumps. The pump was fitted with 110 mm Union fittings. Asymmetric 110 mm to 50 mm Union fittings which were 170 mm long were made to install the pump. These fittings caused considerable contraction and expansion of the flow path at the inlet and outlet ports of the pump. Some cavitation was audible when operating at the higher pumps speeds (80 - 85 rpm). This may have contributed to the lower pump efficiency. Very slight pulsation in flow (10 - 15 kPa) was observed.

The NMOG/622 model Ibex lobe pump which was supplied for the testwork was fitted with 60 mm SMS fittings. The pump was thus installed with 150 mm long 60 mm to 50 mm adaptors. The suction and discharge ports of the Ibex lobe pump are in the vertical plane. A 90° bend was placed directly after the discharge port to enable the attachment of the flexible hose. This bend results in an increase in the flow resistance down stream from the pump. The vertical orientation of the suction and discharge ports makes for less convenient pump installation. Steady flow with slight pulsation (10 - 15 kPa) was achieved.

The OL3/0108/07 On-Line lobe pump supplied by NDE has a capacity of 1.08 liters/revolution when pumping water. It is issued with 80 mm Union fittings thus asymmetric 80 to 50 mm Union fittings with a length of 135 mm were used to install the pump. No cavitation was audible. Steady flow with only slight flow pulsation (10 - 15 kPa) was produced.

The Newlands Brewery production cropping pump, a Wilflo (model CL/2S/052/10) produced near steady flow with pulsations of 10 - 15 kPa at the standard cropping rate.

7. DYNAMIC PUMPS

7.1 Centrifugal Pumps

Centrifugal pumps are not generally used for yeast cropping due to the thixotropic nature and the high viscosity of cropped yeast suspension as well as the relatively large and variable pressures on the discharge side of the pump. Centrifugal pumps are however used during yeast propagation to transfer the more dilute yeast suspensions from one tank to another.

A Fristam centrifugal pump from the propagation plant at Newlands Brewery was used in the testwork with the aim of subjecting the yeast to more extreme handling conditions than could be achieved in the displacement pumps.

7.1.1 Principle of Operation

Figure A11 is a diagram of a typical centrifugal pump. Centrifugal pumps consist of an impeller which carries a number of backward curved vanes. The impeller rotates within a casing. When rotating, the impeller draws a partial vacuum at the centre into which the fluid from the suction side flows. The liquid passes centrifugally outwards so that it leaves the impeller at a higher velocity. The kinetic energy of the fluid is partly converted into pressure energy by the pump casing before the fluid leaves the pump via the delivery pipe.

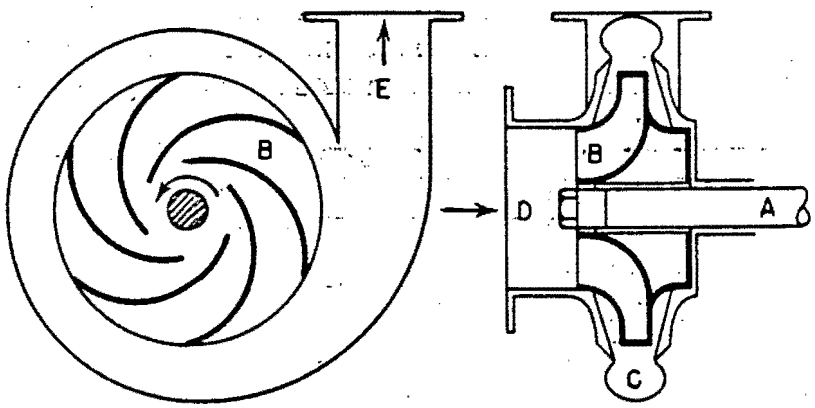

Figure A11 A centrifugal pump

7.1.2 Favourable Features of Centrifugal Pumps

Centrifugal pumps are cheaper to purchase, operate and maintain than displacement pumps. They require no gearboxes, operate without valves and produce steady flow.

7.1.3 Drawbacks of Centrifugal Pumps

In contrast to displacement pumps, the discharge pressure of the pump is dependent on the speed of the pump and on operating conditions. The density and viscosity of the liquid being pumped affect the discharge head: head decreases with a decrease in fluid density and an increase in fluid viscosity. Centrifugal pumps are better suited to pumping low viscosity products which do not require gentle treatment. The pumps are not self-priming and are not as efficient as displacement pumps.

7.1.4 General Comments

The Fristam centrifugal pump (model JP 15) was able to pump the yeast suspension. A differential pressure of 285 kPa was achieved. Although centrifugal pumps do not share the flexibility of displacement pumps in coping with varying discharge pressures, it appears that correctly sized centrifugal pumps may be used for yeast cropping.

8. THE USE OF PULSATION DAMPENERS

As discussed, peristaltic and diaphragm pumps produce considerable pulsation in the flow on both the suction and discharge sides of the pump. Significant flow pulsation is undesirable when cropping yeast, particularly on the discharge side of the pump where the yeast has to pass through a plate and frame heat exchanger. Pulsating flow may jeopardise the effective cooling of the yeast prior to storage. Pulsation dampeners can be used to reduce the pulsations. Suction line pulsation dampeners generally consist of T-pieces in the pipeline to which flexible hose is fitted. There is concern that this "dead-leg" and accumulation of yeast during transfer may have a detrimental effect on the quality of yeast. The cleanability and subsequent sterility of such systems is also questioned. Discharge side pulsation dampeners consist of diaphragm systems or thick wall hose in a steel housing which can be pressurised. No long term accumulation of material should occur in these systems and in-place cleaning should not be a problem.

9. FURTHER COMMENTS

The pumps submitted for evaluation represent the most commonly used displacement pumps. A clear omission, however, is the single-screw pump which is also known as the mono pump. Concern was expressed that mono pumps do not comply with the sanitary standards required for yeast cropping, however, an Inoxpa mono pump which meets the 3A sanitary standards can be supplied by Vintec (Pty) Ltd.

10. BIBLIOGRAPHY

Information leaflets supplied by

- Alfa Laval Pumps Limited - Ibex Division (Ibex lobe pump)
- APV Crepaco Pumps Limited (APV lobe pump)
- Johnson Pump (UK) Limited (Johnson On Line lobe pump)
- KWW GmbH (DEPA air-driven diaphragm pump)
- Maso Dickstoffpumpen GmbH (Maso Sine pump)
- Scandi Brew (Scandi Brew gear pump)
- Wilden Pump and Engineering Company (Wilden air-driven diaphragm pump)
- Waukesha Bredel Fluid Handling B.V. (Peristaltic/Hosepump)

Karassik, I.J., Krutzsch, W.C., Fraser, W.H., and J.P. Messina. *Pump Handbook*. New York: Mc Graw-Hill, 1976.

Karassik, I.J., Krutzsch, W.C., Fraser, W.H., and J.P. Messina. *Pump Handbook*. 2nd ed. New York: Mc Graw-Hill, 1986.

Molloy, E. *Modern Pumps*. London: George Newnes Limited, 1953.

APPENDIX B

STATISTICAL ANALYSIS OF EXPERIMENTAL DATA

1. INTRODUCTION

A set of N measurements or observations may be expressed in terms of their ***arithmetic mean*** or ***average*** ***($\bar{x}$)*** which is defined by the following formula:

$$\bar{x} = \frac{\sum_{i=1}^{N} x_i}{N} \qquad (1)$$

where x_i represents the individual values of $\bar{x}$ making up the set of N measurements.

Precision is a measure of the spread of two or more measurements made in exactly the same way (replicates) and can be expressed in terms of the ***standard deviation (s)*** from the mean. This is as defined as follows:

$$s = \sqrt{\frac{\sum_{i=1}^{N} (x_i - \bar{x})^2}{N - 1}} \qquad (2)$$

where $x_i - \bar{x}$ is the deviation from the mean of the ith measurement.

The ***variance*** (s^2) of a set of data is the square of the standard deviation.

The precision of a set of data can also be expressed in terms of the ***range*** of the data or the ***coefficient of variation (CV)***, where the ***range*** is the difference between the largest and smallest value in the set and the ***coefficient of variation (CV)*** is defined as follows:

$$CV = (s / \bar{x}) \times 100\% \qquad (3)$$

Accuracy is a measure of the agreement between a result and its true value. Inaccuracy in data implies bias (***systematic error*** or ***determinate error***) due to instrument error, non-representative sampling or personal errors (mistakes).

Accuracy can not be determined exactly since it requires knowledge of the true value which is what the experimenter attempts to determine. On the other hand, precision describes the agreement among several results measured in the same way and can be determined by replicating the measurement. Uncontrollable variables which are inevitably part of measurement cause the data from a set of replicate measurements to be scattered more or less symmetrically around the mean of the set. This is known as ***random*** or ***indeterminate error***. It has been found empirically that for most quantitative analytical experiments the distribution of the replicate data points approaches a bell-shaped curve called the Gaussian or normal error curve. Since chance is involved in indeterminate errors, statistical laws which deal with chance variations are used to extract information from experimental data and allow objective judgements to be made concerning the validity of the results.

2. STATISTICAL TREATMENTS OF INDETERMINATE ERRORS

2.1 Populations and Samples

It is assumed that the set of experimental measurements represent a ***sample*** which is a subset of the infinite ***population*** of data that in principle exist if an infinite number of results could be collected. Statistical laws apply to populations only, however, it is assumed that the sample or set of measurements done is representative of the population. Since there is no guarantee that this assumption is valid, statistical inferences about the magnitude of indeterminate errors are subject to uncertainty and can only be made in terms of probabilities.

The sample mean ($\bar{x}$), which is defined by equation (1) when N is small, is the mean of the limited sample drawn from the population. In contrast, the ***population***

mean (μ) is the true mean of the population and is also defined by equation (1) when N approaches infinity. If the data is free of systematic or determinate error, the sample mean is also the true mean. Most often, when N is small, $\bar{x}$ differs from μ because a small sample does not exactly represent its population.

The true standard deviation of the population is given by the ***population standard deviation (σ)*** which is defined as follows:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{N} (x_i - \mu)^2}{N}} \tag{4}$$

Figure B1(a) shows two normal error curves in which the relative frequency of deviations from the mean is plotted as a function of the deviation from the mean $(x - \mu)$. The difference in the curves results from the differences in standard deviation (σ) where σ of the population represented by curve B (the broader curve) is twice that of the population represented by curve A. Figure B1(b) is an alternative normal error curve in which the abscissa is a new variable ***z***, the deviation from the mean in units of the population standard deviation, defined as follows:

$$z = \frac{(x - \mu)}{\sigma} \tag{5}$$

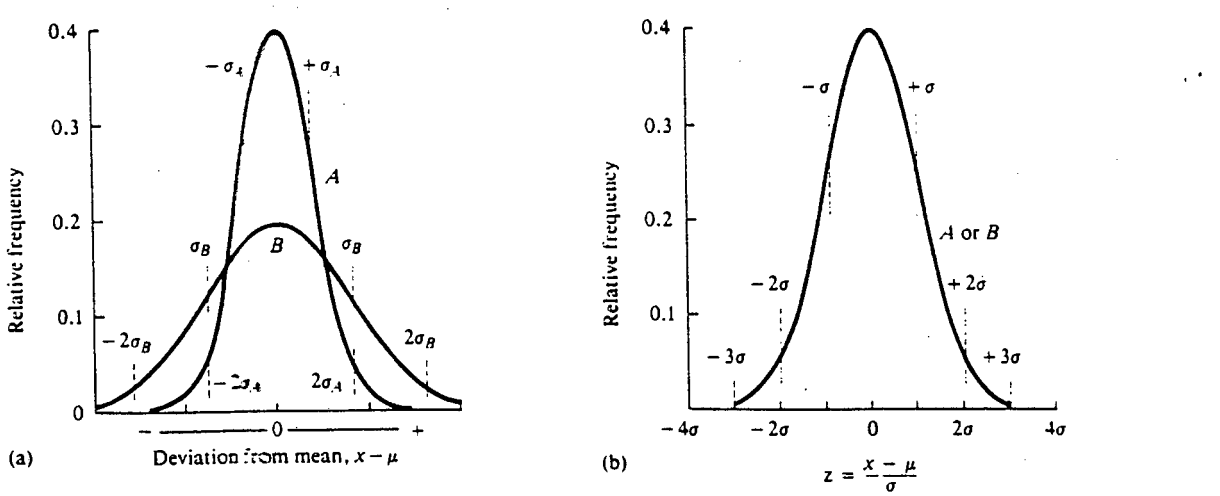

Figure B1 Normal error curves.

The standard deviation for curve B is twice that for curve A ($\sigma_B = 2\sigma_A$). (a) The abscissa is the deviation from the mean in the units of measurement. (b) The abscissa is the deviation from the mean in units of σ, thus the two curves A and B are identical here (Skoog *et al.* 1992).

If a series of samples, each containing N data points, are taken randomly from a population of data, the mean of each set will show less and less scatter as N increases. The standard deviation of each mean is known as the ***standard error*** (σ_m) of the mean. The standard error is inversely proportional to the number of data points used to calculate the mean:

$$\sigma_m = \frac{\sigma}{\sqrt{N}} \tag{6}$$

An analogous equation can be written for a sample standard deviation (s):

$$s_m = \frac{s}{\sqrt{N}} \tag{7}$$

The uncertainty in the calculated value of the sample standard deviation (s) deceases as N increases. When N is greater than about 20, the sample standard deviation (s) and the population standard deviation (σ) can be assumed to be identical for all practical purposes.

A good approximation of s can be obtained by doing numerous replicate measurements. If the method is too time-consuming, data from a series of samples can be accumulated over time and pooled to provide an estimate of s, the pooled standard deviation (s_{pooled}) that is superior to the value for any individual subset. An assumption is made that the samples have the same sources of indeterminate error. If the samples have similar compositions and have been analysed in exactly the same way, this assumption is usually valid. The pooled standard deviation (s_{pooled}) is defined as follows:

$$s_{pooled} = \sqrt{\frac{\sum_{i=1}^{N_1}(x_i - \overline{x_1})^2 + \sum_{j=1}^{N_2}(x_j - \overline{x_2})^2 + \sum_{k=1}^{N_3}(x_k - \overline{x_3})^2 + \ldots}{N_1 + N_2 + N_3 + \ldots - N_s}} \tag{8}$$

where N_1 is the number of data in set 1, N_2 is the number of data in set 2 and so on. The term N_s is the number of data sets being pooled. If the denominator (number of degrees of freedom) is greater than 20, s_{pooled} is regarded as a good estimate of σ.

2.2 Confidence Limits

The exact value of the mean of a population (μ) can never be determined since an

infinite number of measurements would be required. Using statistical theory a limit can be set around the determined sample mean ($\bar{x}$) such that the true mean μ will lie within these limits with a given degree of probability. These limits are called ***confidence limits (CL)*** and the interval they define is known as the ***confidence interval.***

The size of the confidence interval is derived from the standard deviation and depends on the certainty with which s is known. If there is reason to believe that s is a good approximation of σ, then the confidence interval can be significantly narrower than if the estimate of s is based upon only a few measurements.

Figure B2 shows a series of normal error curves in which the relative frequency is plotted as a function of the quantity z (the deviation from the mean in units of the population standard deviations). The shaded area in each plot lies between the values of $-z$ and $+z$ that are indicated to the right and left of the curve. The number within this shaded area is the percentage of the total area which is included within the z-values. 50% of the area under any normal curve lies between -0.67σ and $+0.67\sigma$, 95% between -1.96σ and $+1.96\sigma$, etc. It may thus be assumed, for example, that 95 times out of a 100, the true mean (μ) will be within $\pm 1.96\sigma$ of any of the measurements made. In this case, the confidence level is 95% and the confidence interval is $\pm z\sigma = \pm 1.96\sigma$.

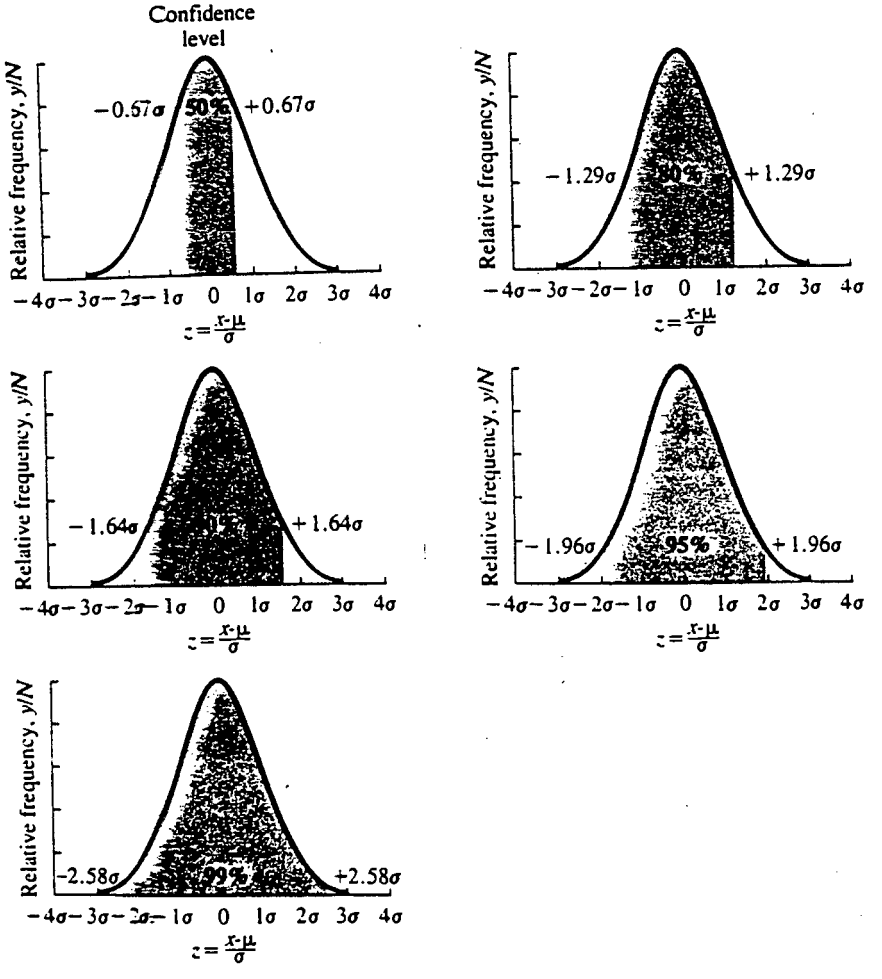

Figure B2 Areas under the normal error curve for various values of $\pm z$ (Skoog *et al.* 1992).

When σ is known, the ***confidence limits (CL)*** for an average of N replicate measurements are defined as follows:

$$CL \text{ for } \mu = \bar{x} \pm \frac{z\sigma}{\sqrt{N}} \quad (9)$$

This applies in the absence of determinate errors. The equation is usually applied to large samples where $N > 30$ or when it can be assumed that s is a good estimate of σ. Values for z at various confidence levels can be found in standard statistical texts.

Limitations of time and the availability of sample may prevent the use of large samples and determination of accurate estimations of σ. The calculation of s based on a small set of data is uncertain hence the confidence limits are necessarily broader. Analogous to z, a value t is defined as follows to account for the variability of s:

$$t = \frac{(x - \mu)}{s} \quad (10)$$

As for z, the value t depends on the desired confidence level but is also dependent on the number of degrees of freedom (v) in the calculation of s, where $v = N - 1$. Values for t can be found in statistical texts. As the number of degrees of freedom become infinite, $t \rightarrow z$.

In the absence of a good estimate of σ, the confidence limits for the mean x of N replicate measurements can be derived from t by the following equation:

$$CL \text{ for } \mu = \bar{x} \pm \frac{ts}{\sqrt{N}} \quad (11)$$

3. THE COMPARISON OF TWO EXPERIMENTAL MEANS

In scientific studies, it is often necessary to determine whether the difference between the means of two sets of measurements is real and can be taken as evidence that the samples are different or whether the apparent difference is a

consequence of indeterminate errors in the two sets. This can be done by hypothesis testing. It is assumed that the samples are identical (*ie.* null hypothesis: $\overline{x}_1 - \overline{x}_2 = 0$) and hence that the observed difference is the result of indeterminate errors. To test this hypothesis against the alternative hypothesis ($\overline{x}_1 - \overline{x}_2 \neq 0$), the following equation can be used:

$$\overline{x}_1 - \overline{x}_2 = \pm t s_{pooled} \frac{\sqrt{N_1 + N_2}}{N_1 N_2} \qquad (12)$$

where $\overline{x}_1$ and $\overline{x}_2$ are the means of N_1 replicate measurements done on one sample (1) and N_2 replicate measurements done on the other sample (2) respectively. Equation 12 assumes that the standard deviations of the samples are not significantly different. If the data is collected in the same way, this is a reasonable assumption. Equation 12 is fairly robust, but statistical tests based on the F-distribution can be used to check the validity this assumption.

The value of t in Equation 12 is computed for the desired confidence level. The number of degrees of freedom (v) for t is $N_1 + N_2 - 2$. If the experimental difference ($\overline{x}_1 - \overline{x}_2$) is smaller than the value computed in the right hand side (RHS) of the equation, the null hypothesis can not be rejected and no significant difference between the two means can be demonstrated at the selected confidence level. An experimental difference greater than the value computed from t indicates that there is a significant difference between the means. If a good estimate of σ is available, then Equation 12 can be modified by replacing t with z and s with σ.

When dealing with "before and after" kind of data, ***paired-sample t testing*** can be done. Here the signed differences (δ's) in the paired before and after data may be regarded as a random sample from a population for which $\mu = \delta$. The null hypothesis $\mu = 0$ is tested against the alternative hypothesis $\mu \neq 0$. The critical value for the rejection of the null hypothesis is calculated as follows:

$$\overline{x} - \mu = \pm \frac{ts}{\sqrt{N}} \qquad (13)$$

where $\overline{x}$ is the mean and s the standard deviation of the differences and N the number of differences. If the mean of the differences exceeds the critical value calculated from the t value at $N - 1$ degrees of freedom for the desired level of confidence, the null hypothesis can be rejected and the difference between the before and after situation can be regarded as a real difference at that level of confidence.

Alternatively, Equation 13 can be rearranged as follows:

$$t = \frac{(\bar{x} - \mu)}{s/\sqrt{N}} \qquad (14)$$

and the *t*-value obtained can be compared with *t* for $N - 1$ degrees freedom at the desired confidence level. If the calculated *t*-value is less than the selected *t*-value, the null hypothesis can not be rejected and the difference observed can be attributed to random error.

4. THE ANALYSIS OF VARIANCE

To determine whether several means are significantly different from one another, as a group, a statistical technique called the analysis of variance (ANOVA) can be used. This test uses the F-distribution to assess the significance of the variances due to the means. It essentially partitions the total data variance into its components and makes comparisons between them. The one-way ANOVA allows the comparison between the variance associated with replicate measurements or observations (the *within-sample variance*) and the variance associated with different samples (*between-sample variance*) each of which has a sum of squared errors associated with it. For a set of k samples and N observations, the following table can be set up:

SAMPLE NUMBER	OBSERVATIONS	TOTAL	MEAN	NUMBER OF OBSERVATIONS*
1	x11 x12 x13 ..... $x1N_1$	S_1	x_1	N_1
2	x21 x22 x23 ..... $x2N_2$	S_2	x_2	N_2
3	x31 x32 x33 ..... $x3N_2$	S_3	x_3	N_3
.				
.				
.				
k	xk1 xk2 xk3 ..... xkN_2	S_k	x_k	N_k
Σ	-	S	-	N_{total}

* not necessarily equal

The following procedure is used:

1. Correction for mean (C) = S^2/N_{total}
2. Total sum of squares (SS_{total}) = $((x11)^2 + (x12)^2 + ... + (xkn_k)^2) - C$
3. Sum of squares between samples ($SS_{between}$) = $(S_1^2/n_1 + S_2^2/n_2 + .. + S_k^2/n_k) - C$
4. Sum of squares within samples (SS_{within}) = $SS_{total} - SS_{between}$
5. Mean square for each component (MS) = SS/degrees of freedom

A calculation table is set up:

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F
BETWEEN SAMPLES	$SS_{between}$	k - 1	$M_{between}$	$M_{between}/M_{within}$
WITHIN SAMPLES	SS_{within}	N_{total} - k	M_{within}	-
TOTAL	SS_{total}	N_{total} - 1	-	-

The *between-sample* mean square ($M_{between}$) results from real differences between the samples and the *within-sample* mean square (M_{within}) is due to the testing or analytical procedure (*ie.* indeterminate error). The null hypothesis $M_{between} = M_{within}$ is tested against the alternative hypothesis $M_{between} > M_{within}$. If F, which equals $M_{between}/M_{within}$ exceeds the value of F with (k-1) and N_{total} - k degrees of freedom for the desired confidence level, the null hypothesis is rejected and the alternative hypothesis accepted *ie.* the differences between the means of the different samples can be regarded as significant at that level of confidence.

5. BIBLIOGRAPHY

Miller, I., and J.E. Freud. *Probability and Statistics for Engineers*. 3rd ed. Englewood Cliffs, New Jersey: Prentice-Hall, 1985.

Skoog, D.A., West, M.W., and F.J. Holler. *Fundamentals of Analytical Chemistry*. 6th ed. New York: Saunders College Publishing, 1992.

Napier-Munn, T.J. *An Introduction to Comparative Statistics and Experimental Design for Minerals Engineers*. 2nd ed. Brisbane: University of Queensland, 1995.

APPENDIX C

STANDARD DEVIATIONS OF YEAST QUALITY ASSAYS AND FERMENTATION PERFORMANCE INDICATORS

CONTENTS

PROTEASE

SOURCE	PUMP RETRIALS + FLOW TRIAL														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	0.05		0.000	0.08		0.000	0.16		0.000	0.01		0.000	0.08		0.001
B	0.06	0.06	0.000	0.07	0.07	0.000	0.17	0.17	0.000	0.00	0.01	0.000	0.14	0.11	0.001
C	0.06		0.000	0.06		0.000	0.16		0.000	0.02		0.000	0.07		0.000
D	0.07	0.06	0.000	0.06	0.06	0.000	0.16	0.17	0.000	0.02	0.02	0.000	0.06	0.07	0.000
E	0.04		0.001	0.06		0.000	0.23		0.000	0.02		0.000	0.06		0.000
F	0.10	0.07	0.001	0.07	0.06	0.000	0.21	0.22	0.000	0.02	0.02	0.000	0.09	0.07	0.000
G	0.10		0.000	0.09		0.000	0.24		0.000	0.03		0.000	0.11		0.001
H	0.10	0.10	0.000	0.06	0.06	0.000	0.21	0.22	0.000	0.03	0.03	0.000	0.06	0.09	0.001
I													0.07		0.001
J													0.12	0.09	0.001
K													0.11		0.000
L													0.11	0.11	0.000
TOTALS	0.60		0.002	0.63		0.000	1.55		0.001	0.15		0.000	1.10		0.005
TOTAL SQUARED ERRORS															0.009
NUMBER OF POINTS															44
NUMBER OF SUBSETS															22
POOLED STANDARD DEVIATION															0.02
COEFFICIENT OF VARIATION (%)															21

METHYLENE BLUE

SOURCE	INITIAL PUMP TRIALS																											
RUN	RUN 1			RUN 2			RUN 3			RUN 4			RUN 5			RUN 6			RUN 7			RUN 8			RUN 9		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	92		0	94		0	90		9	93		0	93		9	92		3	94		0	95		1	93		1
B	91	91	0	94	94	0	96	93	9	93	93	0	87	90	9	89	90	3	95	94	0	93	94	1	96	94	1
C	90		1	94		0	95		4	90		5	88		3	92		0	93		1	95		0	92		1
D	92	91	1	94	94	0	91	93	4	94	92	5	92	90	3	92	92	0	95	94	1	95	95	0	90	91	1
E	92		2	94		0	93		1	92		0	95		0	92		2	94		1	90		2	94		1
F	95	93	2	95	95	0	95	94	1	93	93	0	94	94	0	89	90	2	93	93	1	93	91	2	92	93	1
G	91		1	92		6	94		4	91		1	90		1	89		3	91		0	95		0	91		1
H	93	92	1	87	90	6	98	96	4	90	91	1	92	91	1	93	91	3	91	91	0	95	95	0	93	92	1
I				94		1	96		4	90		0	95		0	94		1	93		0	94		0	92		1
J				96	95	1	100	98	4	88	89	0	95	95	0	92	93	1	92	92	0	93	93	0	94	93	1
K				90		4	95		4	91		0	91		1	89		0	93		0	94		0	93		1
L				94	92	4	91	93	4	92	92	0	92	91	1	90	89	0	93	93	0	93	93	0	91	92	1
M				91		0	93		0	92		1	89		0	88		0	92		1	92		0	95		2
N				97	94	8	92	93	0	89	90	1	90	90	0	89	89	0	90	91	1	93	93	0	92	93	2
O				94		4	93		2	89		3	89		1	91		12	96		3	95		4	87		4
P				90	92	4	90	92	2	86	88	3	91	90	1	84	87	12	93	94	3	91	93	4	91	89	4
TOTALS	735		9	1490		49	1502		57	1453		21	1462		29	1445		44	1487		12	1495		14	1474		24
TOTAL SQUARED ERRORS																											258
NUMBER OF POINTS																											136
NUMBER OF SUBSETS																											68
POOLED STANDARD DEVIATION																											2
COEFFICIENT OF VARIATION (%)																											2

MODIFIED METHYLENE BLUE

SOURCE	PUMP RETRIALS + FLOW TRIALS														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	92		3	94		5	96		1	93		0	92		0
B	89	91	3	89	92	5	94	95	1	93	93	0	91	92	0
C	93		1	88		7	94		0	92		0	94		1
D	90	91	1	92	89	7	94	94	0	92	92	0	91	93	1
E	92		0	87		11	95		0	93		1	94		0
F	94	93	0	93	90	11	96	96	0	92	92	1	93	94	0
G	93		0	88		6	96		0	96		0	93		0
H	93	93	0	93	90	6	96	96	0	95	95	0	92	92	0
I													88		1
J													90	89	1
K													91		0
L													90	90	0
TOTALS	736		8	721		59	761		2	746		2	1099		6
TOTAL SQUARED ERRORS															77
NUMBER OF POINTS															44
NUMBER OF SUBSETS															22
POOLED STANDARD DEVIATION															2
COEFFICIENT OF VARIATION (%)															2

PLATE COUNTS

SOURCE	INITIAL PUMP TRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	59		[illegible]	30		8	80		384	100		807
B	57	58	[illegible]	35	32	8	40	60	384	43	72	807
C	59		[illegible]	38		0	72		234	-		-
D	64	62	[illegible]	38	37	0	42	57	234	50	-	-
E	37		[illegible]	30		31	60		101	64		274
F	32	34	[illegible]	41	35	31	40	50	101	31	48	274
G	44		[illegible]	54		17	48		557	33		6
H	21	32	[illegible]	62	58	17	95	72	557	29	31	6
I				65		12	70		159	53		3
J				58	62	12	45	58	159	57	55	3
K				37		355	52		59	38		213
L				75	56	355	68	60	59	67	52	213
M				54		4	59		4	47		161
N				50	52	4	55	57	4	72	60	161
O				28		19	71		231	61		222
P				37	32	19	40	55	231	31	46	222
TOTALS	373		[illegible]	730		893	937		3458	725		3372
TOTAL SQUARED ERRORS												6006
NUMBER OF POINTS												54
NUMBER OF SUBSETS												27
POOLED STANDARD DEVIATION												17
COEFFICIENT OF VARIATION (%)												53

SLIDE COUNTS

SOURCE	INITIAL PUMP TRIALS								
RUN	RUN 7			RUN 8			RUN 9		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	90		5	94		1	93		3
B	95	92	5	96	95	1	90	92	3
C	92		0	94		0	90		0
D	91	91	0	94	94	0	92	91	0
E	93		4	90		3	94		21
F	89	91	4	93	92	3	84	89	21
G	90		7	94		1	94		13
H	96	93	7	92	93	1	87	90	13
I	94		2	93		0	85		3
J	91	92	2	94	94	0	89	87	3
K	91		4	93		1	93		0
L	87	89	4	95	94	1	93	93	0
M	93		1	93		0	88		4
N	94	93	1	94	94	0	92	90	4
O	88		7	94		0	89		1
P	93	90	7	93	94	0	88	89	1
TOTALS	1464		50	1495		11	1440		87
TOTAL SQUARED ERRORS									158
NUMBER OF POINTS									48
NUMBER OF SUBSETS									24
POOLED STANDARD DEVIATION									3
COEFFICIENT OF VARIATION (%)									3

OUR (METHOD A)

SOURCE	INITIAL PUMP TRIALS																										
RUN	RUN 1			RUN 2			RUN 3			RUN 4			RUN 5			RUN 6			RUN 7			RUN 8			RUN 9		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	0.118		4E-06	-		-	0.146		2E-04	0.109		2E-05	-		-	0.110		2E-05	0.087		9E-06	0.097		6E-05	-		-
B	0.122	0.120	4E-06	0.147	-	-	0.117	0.132	2E-04	0.118	0.114	2E-05	0.142	-	-	0.116	0.114	2E-05	0.093	0.090	9E-06	0.082	0.090	6E-05	0.116	-	-
C	0.133		-	0.175		3E-04	0.119		4E-05	0.121		2E-06	0.159		3E-05	0.111		9E-06	0.094		4E-05	0.094		2E-05	-		-
D	-	-	-	0.143	0.159	3E-04	0.131	0.125	4E-05	0.124	0.123	2E-06	0.149	0.154	3E-05	0.117	0.114	9E-06	0.108	0.100	4E-05	0.085	0.090	2E-05	-	-	-
E	0.114		1E-05	-		-	0.132		2E-05	0.107		3E-05	0.158		0.0	0.114		1E-06	0.094		2E-06	0.086		6E-06	0.109		2E-05
F	0.121	0.118	1E-05	0.159	-	-	0.141	0.137	2E-05	0.117	0.112	3E-05	0.156	0.156	0.0	0.112	0.113	1E-06	0.097	0.096	2E-06	0.091	0.089	6E-06	0.117	0.113	2E-05
G	0.137		2E-06	0.185		-	0.137		9E-06	0.112		2E-04	0.150		6E-06	0.110		8E-06	0.096		1E-06	0.088		1E-06	0.104		6E-06
H	0.140	0.139	2E-06	-	-	-	0.131	0.134	9E-06	0.139	0.126	2E-04	0.155	0.153	6E-06	0.115	0.113	8E-06	0.098	0.097	1E-06	0.086	0.087	1E-06	0.109	0.107	6E-06
I				0.114		1E-04	-		-	0.114		2E-05	0.141		7E-05	0.110		9E-06	0.098		4E-06	0.087		3E-05	0.096		1E-05
J				0.138	0.126	1E-04	0.136	-	-	0.124	0.119	3E-05	0.156	0.150	7E-05	0.116	0.113	9E-06	0.102	0.100	4E-06	0.097	0.092	3E-05	0.103	0.100	1E-05
K				0.150		4E-04	-		-	0.110		5E-05	0.163		1E-06	0.117		8E-06	0.109		9E-06	0.093		4E-05	0.125		3E-07
L				0.190	0.170	4E-04	0.121	-	-	0.124	0.117	5E-05	0.161	0.162	1E-06	0.112	0.115	8E-06	0.103	0.106	9E-06	0.106	0.100	4E-05	0.124	0.125	3E-07
M				-		-	0.130		2E-05	0.125		9E-06	0.147		4E-05	0.118		2E-05	0.110		1E-04	0.085		1E-05	0.139		2E-04
N				-	-	-	0.122	0.126	9E-06	0.119	0.122	9E-06	0.160	0.154	4E-06	0.120	0.122	9E-06	0.130	0.120	1E-04	0.092	0.089	1E-05	0.109	0.124	2E-04
O				-	-	-	-		-	0.117		-	0.160		6E-06	0.111		1E-06	0.123		8E-06	0.090		9E-05	0.127		2E-06
P				0.155	-	-	0.135	-	-	-	-	-	0.175	0.178	8E-06	0.113	0.112	1E-06	0.118	0.121	8E-06	0.104	0.097	5E-05	0.124	0.126	2E-06
TOTALS	0.752		4E-05	0.910		2E-03	1.306		6E-04	1.663		6E-04	2.210		3E-04	1.630		1E-04	1.658		3E-04	1.463		4E-04	1.386		5E-04
TOTAL SQUARED ERRORS																											0.005
NUMBER OF POINTS																											110
NUMBER OF SUBSETS																											55
POOLED STANDARD DEVIATION																											0.009
COEFFICEINT OF VARIATION (%)																											6

OUR (METHOD B)

SOURCE	PUMP RETRIALS + FLOW TRIALS														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	0.015		0.0000	0.010		0.0000	0.011		0.0000	0.008		0.0000	0.005		0.0000
B	0.008	0.012	0.0000	0.012	0.011	0.0000	0.010	0.011	0.0000	0.007	0.008	0.0000	0.006	0.005	0.0000
C	0.009		0.0000	0.009		0.0000	0.011		0.0000	0.010		0.0000	0.006		0.0000
D	0.008	0.009	0.0000	0.008	0.009	0.0000	0.011	0.011	0.0000	0.011	0.011	0.0000	0.007	0.007	0.0000
E	0.015		0.0000	0.009		0.0000	0.010		0.0000	0.008		0.0000	0.007		0.0000
F	0.011	0.013	0.0000	0.012	0.011	0.0000	0.009	0.010	0.0000	0.008	0.008	0.0000	0.006	0.006	0.0000
G	0.007		0.0000	0.013		0.0000	0.008		0.0000	0.009		0.0000	0.006		0.0000
H	0.008	0.008	0.0000	0.009	0.011	0.0000	0.008	0.008	0.0000	0.010	0.010	0.0000	0.006	0.006	0.0000
I													0.007		0.0000
J													0.008	0.007	0.0000
K													0.005		0.0000
L													0.008	0.006	0.0000
TOTALS	0.081		0.0000	0.082		0.0000	0.078		0.0000	0.071		0.0000	0.074		0.0000
TOTAL SQUARED ERRORS															0.0001
NUMBER OF POINTS															44
NUMBER OF SUBSETS															22
POOLED STANDARD DEVIATION															0.002
COEFFICIENT OF VARIATION (%)															18

ACIDIFICATION POWER - AP

SOURCE	INITIAL PUMPS TRIALS																	
RUN	RUN 1			RUN 2			RUN 3			RUN 4			RUN 5			RUN 6		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	2.15		2E-05	2.47		3E-05	2.34		2E-05	2.43		2E-04	2.54		1E-04	2.46		9E-04
B	2.14	2.15	2E-05	2.46	2.47	2E-05	2.35	2.35	3E-05	2.40	2.42	2E-04	2.52	2.53	1E-04	2.52	2.49	9E-04
C	2.26		0.00	2.48		9E-04	-		-	2.40		3E-05	2.41		4E-04	2.50		2E-03
D	2.26	2.26	0.00	2.42	2.45	9E-04	2.40	-	-	2.41	2.41	2E-05	2.45	2.43	4E-04	2.60	2.55	3E-03
E	2.38		2E-04	2.46		2E-04	2.43		3E-02	2.30		4E-03	2.49		6E-04	2.47		2E-04
F	2.33	2.35	2E-04	2.49	2.48	2E-04	2.80	2.62	3E-02	2.42	2.36	4E-03	2.44	2.47	6E-04	2.50	2.49	2E-04
G	2.38		4E-03	2.42		2E-03	2.37		2E-03	2.40		2E-03	2.59		4E-04	2.65		5E-03
H	2.25	2.32	4E-03	2.51	2.47	2E-03	2.45	2.41	2E-03	2.49	2.45	2E-03	2.63	2.61	4E-04	2.79	2.72	5E-03
I				2.29		2E-03	2.37		9E-04	2.35		6E-04	2.46		2E-04	2.60		9E-04
J				2.39	2.34	3E-03	2.43	2.40	9E-04	2.40	2.38	6E-04	2.43	2.45	2E-04	2.68	2.63	9E-04
K				2.50		1E-04	2.29		2E-03	2.47		2E-04	2.46		4E-04	2.64		4E-04
L				2.48	2.49	1E-04	2.39	2.34	3E-03	2.44	2.46	2E-04	2.50	2.48	4E-04	2.68	2.66	4E-04
M				2.38		2E-03	2.40		2E-05	2.40		2E-04	2.40		4E-03	2.68		4E-04
N				2.48	2.43	3E-03	2.39	2.40	2E-05	2.43	2.42	2E-04	2.53	2.47	4E-03	2.64	2.66	4E-04
O				2.45		6E-04	2.29		1E-04	2.51		4E-04	2.55		0.00	2.62		9E-04
P				2.50	2.48	6E-04	2.31	2.30	1E-04	2.47	2.49	4E-04	2.55	2.55	0.00	2.58	2.59	9E-04
TOTALS	18.13		2.01	39.18		0.02	33.61		0.08	38.72		0.01	39.95		0.01	41.57		0.02
TOTAL SQUARED ERRORS																		0.18
NUMBER OF POINTS																		86
NUMBER OF SUBSETS																		43
POOLED STANDARD DEVIATION																		0.06
COEFFICEINT OF VARIATION (%)																		2

SPONTANEOUS ACIDIFICATION POWER - AP(10)

SOURCE	INITIAL PUMP TRIALS																	
RUN	RUN 1			RUN 2			RUN 3			RUN 4			RUN 5			RUN 6		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	1.64		4E-03	1.74		3E-03	1.80		5E-03	1.90		3E-03	2.20		2E-03	2.05		2E-03
B	1.51	1.58	4E-03	1.84	1.79	3E-03	1.94	1.87	5E-03	2.00	1.95	3E-03	2.10	2.15	3E-03	2.15	2.10	3E-03
C	1.81		2E-04	1.85		4E-03	-		-	1.98		6E-04	1.85		3E-03	2.15		2E-04
D	1.78	1.80	2E-04	1.73	1.79	4E-03	1.84	-	-	2.01	1.99	6E-04	1.96	1.91	3E-03	2.18	2.17	2E-04
E	1.86		3E-05	1.85		9E-04	2.04		2E-02	1.85		8E-03	2.20		6E-03	2.10		3E-03
F	1.85	1.86	2E-05	1.91	1.88	9E-04	2.30	2.17	2E-02	2.03	1.94	8E-03	2.04	2.12	5E-03	2.21	2.16	3E-03
G	1.90		4E-03	1.87		4E-04	1.84		8E-03	2.02		1E-04	2.15		2E-03	2.22		2E-02
H	1.77	1.84	4E-03	1.91	1.89	4E-04	2.02	1.93	8E-03	2.00	2.01	1E-04	2.07	2.11	2E-03	2.50	2.36	2E-02
I				1.54		3E-02	1.85		2E-03	1.96		2E-04	2.11		4E-03	2.25		2E-05
J				1.88	1.71	3E-02	1.93	1.89	2E-03	1.99	1.98	2E-04	1.99	2.05	4E-03	2.24	2.25	2E-05
K				1.89		2E-04	1.76		3E-03	2.04		2E-04	2.03		1E-04	2.18		3E-03
L				1.92	1.91	2E-04	1.88	1.81	3E-03	2.01	2.03	2E-04	2.05	2.04	1E-04	2.29	2.24	3E-03
M				1.84		2E-03	1.87		2E-04	1.90		2E-04	1.99		6E-03	2.23		2E-04
N				1.92	1.88	2E-03	1.84	1.86	2E-04	1.93	1.92	2E-04	2.17	2.08	8E-03	2.20	2.22	2E-04
O				1.98		0.00	1.77		2E-03	2.05		4E-03	2.11		2E-04	2.17		3E-03
P				1.98	1.98	0.00	1.86	1.82	2E-03	1.92	1.99	4E-03	2.08	2.10	2E-04	2.06	2.12	3E-03
TOTALS	14.12		0.02	29.65		0.08	26.68		0.07	31.57		0.03	33.10		0.05	35.16		0.08
TOTAL SQUARED ERRORS																		0.31
NUMBER OF POINTS																		86
NUMBER OF SUBSETS																		43
POOLED STANDARD DEVIATION																		0.09
COEFFICEINT OF VARIATION (%)																		4

CUMULATIVE ACIDIFICATION POWER (SPONTANEOUS) - CAP(10)

SOURCE	INITIAL PUMP TRIALS																	
RUN	RUN 1			RUN 2			RUN 3			RUN 4			RUN 5			RUN 6		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	1E-05		9E-11	2E-05		[illegible]	2E-05		9E-12	5E-05		4E-12	3E-05		6E-11	5E-05		1E-10
B	3E-05	2E-05	9E-11	3E-05	2E-05	[illegible]	2E-05	2E-05	9E-12	6E-05	6E-05	4E-12	5E-05	4E-05	6E-11	7E-05	6E-05	1E-10
C	1E-05		3E-10	2E-05		[illegible]	-		-	2E-05		5E-11	3E-05		2E-11	2E-05		7E-11
D	4E-05	3E-05	3E-10	1E-05	2E-05	[illegible]	1E-05	-	-	4E-05	3E-05	5E-11	2E-05	3E-05	2E-11	3E-05	3E-05	7E-11
E	3E-05		4E-11	2E-05		[illegible]	5E-05		4E-11	1E-05		3E-11	5E-05		4E-12	5E-05		4E-11
F	5E-05	4E-05	4E-11	2E-05	2E-05	[illegible]	4E-05	5E-05	4E-11	3E-05	2E-05	3E-11	5E-05	5E-05	4E-12	4E-05	4E-05	4E-11
G	2E-05		6E-11	2E-05		[illegible]	3E-05		4E-13	3E-05		3E-11	3E-05		2E-12	4E-05		5E-12
H	8E-06	2E-05	6E-11	2E-05	2E-05	[illegible]	3E-05	3E-05	4E-13	2E-05	2E-05	3E-11	3E-05	3E-05	2E-12	4E-05	4E-05	5E-12
I				2E-05		[illegible]	1E-05		3E-11	3E-05		6E-14	6E-05		2E-10	3E-05		3E-12
J				3E-05	3E-05	[illegible]	2E-05	2E-05	3E-11	3E-05	3E-05	6E-14	3E-05	4E-05	2E-10	2E-05	2E-05	3E-12
K				2E-05		[illegible]	2E-05		6E-12	3E-05		6E-12	4E-05		0.00	4E-05		2E-10
L				2E-05	2E-05	[illegible]	1E-05	2E-05	6E-12	3E-05	3E-05	6E-12	4E-05	4E-05	0.00	2E-05	3E-05	2E-10
M				2E-05		[illegible]	2E-05		4E-12	2E-05		1E-14	5E-05		5E-11	3E-05		2E-11
N				3E-05	2E-05	[illegible]	1E-05	2E-05	4E-12	2E-05	2E-05	1E-14	3E-05	4E-05	5E-11	2E-05	3E-05	2E-11
O				1E-05		[illegible]	2E-05		3E-10	3E-05		4E-11	3E-05		9E-12	4E-05		7E-11
P				4E-05	3E-05	[illegible]	5E-05	4E-05	3E-10	2E-05	3E-05	4E-11	3E-05	3E-05	9E-12	3E-05	4E-05	7E-11
TOTALS	2E-04		9E-10	4E-04		[illegible]	4E-04		8E-10	5E-04		3E-10	6E-04		7E-10	6E-04		9E-10
TOTAL SQUARED ERRORS																		4E-09
NUMBER OF POINTS																		86
NUMBER OF SUBSETS																		43
POOLED STANDARD DEVIATION																		1E-05
COEFFICEINT OF VARIATION (%)																		32

CUMULATIVE ACIDIFICATION POWER - CAP

SOURCE	INITIAL PUMP TRIALS																	
RUN	RUN 1			RUN 2			RUN 3			RUN 4			RUN 5			RUN 6		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	8E-05		3E-11	2E-04		[illegible]	1E-04		3E-11	2E-04		4E-12	2E-04		2E-10	2E-04		3E-10
B	9E-05	8E-05	3E-11	2E-04	2E-04	[illegible]	1E-04	1E-04	3E-11	2E-04	2E-04	4E-12	2E-04	2E-04	2E-10	2E-04	2E-04	3E-10
C	9E-05		2E-10	2E-04		[illegible]	-		-	1E-04		5E-11	1E-04		2E-12	2E-04		9E-10
D	1E-04	1E-04	2E-10	1E-04	1E-04	[illegible]	1E-04	-	-	2E-04	2E-04	5E-11	1E-04	1E-04	2E-12	2E-04	2E-04	9E-10
E	1E-04		0.00	2E-04		[illegible]	2E-04		8E-09	1E-04		4E-10	2E-04		2E-12	2E-04		1E-11
F	1E-04	1E-04	0.00	2E-04	2E-04	[illegible]	3E-04	3E-04	8E-09	1E-04	1E-04	4E-10	2E-04	2E-04	2E-12	2E-04	2E-04	1E-11
G	1E-04		4E-10	2E-04		[illegible]	1E-04		3E-11	1E-04		1E-10	2E-04		2E-10	2E-04		5E-10
H	9E-05	1E-04	4E-10	2E-04	2E-04	[illegible]	1E-04	1E-04	3E-11	2E-04	2E-04	1E-10	2E-04	2E-04	2E-10	3E-04	3E-04	5E-10
I				1E-04		[illegible]	1E-04		8E-11	1E-04		2E-11	2E-04		2E-11	2E-04		2E-10
J				2E-04	1E-04	[illegible]	1E-04	1E-04	8E-11	1E-04	1E-04	3E-11	2E-04	2E-04	2E-11	2E-04	2E-04	2E-10
K				2E-04		[illegible]	1E-04		1E-11	2E-04		1E-12	2E-04		2E-11	2E-04		8E-11
L				2E-04	2E-04	[illegible]	1E-04	1E-04	1E-11	2E-04	2E-04	1E-12	2E-04	2E-04	2E-11	2E-04	2E-04	8E-11
M				1E-04		[illegible]	1E-04		2E-11	1E-04		2E-12	2E-04		4E-11	2E-04		1E-10
N				2E-04	2E-04	[illegible]	1E-04	1E-04	2E-11	1E-04	1E-04	2E-12	2E-04	2E-04	4E-11	2E-04	2E-04	1E-10
O				2E-04		[illegible]	1E-04		9E-11	2E-04		1E-10	2E-04		9E-12	2E-04		1E-10
P				2E-04	2E-04	[illegible]	1E-04	1E-04	9E-11	1E-04	2E-04	1E-10	2E-04	2E-04	9E-12	2E-04	2E-04	1E-10
TOTALS	9E-04		1E-09	3E-03		[illegible]	2E-03		1E-08	2E-03		1E-09	3E-03		1E-09	3E-03		4E-09
TOTAL SQUARED ERRORS																		2E-08
NUMBER OF POINTS																		86
NUMBER OF SUBSETS																		43
POOLED STANDARD DEVIATION																		2E-05
COEFFICEINT OF VARIATION (%)																		15

GLYCOGEN

SOURCE	INITIAL PUMP TRIALS																				
RUN	RUN 2			RUN 3			RUN 5			RUN 6			RUN 7			RUN 8			RUN 9		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	27.7		0.1	24.1		0.9	25.4		1.3	28.4		0.1	24.3		0.8	-		-	24.5		0.4
B	27.2	27.4	0.1	25.9	25.0	0.9	27.6	26.5	1.3	27.9	28.2	0.1	26.1	25.2	0.8	-	-	-	23.2	23.8	0.4
C	22.0		0.8	24.2		0.0	25.2		0.0	25.3		0.8	22.2		1.8	-		-	25.8		2.2
D	23.8	22.9	0.8	24.5	24.4	0.0	25.8	25.4	0.0	23.4	24.4	0.8	24.7	23.5	1.8	-	-	-	22.6	24.1	2.2
E	25.8		0.3	24.1		0.0	25.0		0.2	24.8		0.2	25.8		0.5	-		-	22.7		1.8
F	24.8	25.3	0.3	25.9	25.0	0.8	25.4	25.0	0.2	25.5	25.0	0.2	24.3	24.9	0.5	-	-	-	25.3	24.0	1.8
G	23.5		1.0	25.5		0.0	26.8		0.0	26.4		0.0	25.8		0.7	-		-	24.0		0.3
H	25.4	24.4	1.0	25.2	25.3	0.0	27.1	27.0	0.0	26.1	26.3	0.0	23.8	24.7	0.7	-	-	-	25.0	24.5	0.3
I	23.2		0.7	24.3		0.0	25.9		0.0	22.8		3.5	25.1		0.1	24.0		0.0	24.1		0.2
J	24.8	24.0	0.7	24.4	24.4	0.0	26.1	26.0	0.0	26.3	24.5	3.5	25.8	25.3	0.1	23.9	24.0	0.0	23.2	23.8	0.2
K	23.1		0.6	-		-	25.5		0.2	27.3		0.1	25.3		0.1	22.7		0.0	23.1		0.8
L	21.5	22.3	0.6	25.2	-	-	26.3	25.9	0.2	26.7	27.0	0.1	24.8	24.9	0.1	23.1	22.9	0.0	24.6	23.9	0.8
M	24.7		0.3	23.3		-	27.2		0.2	27.0		0.1	25.7		0.0	22.7		0.5	21.8		0.1
N	23.7	24.2	0.3	-	-	-	26.3	26.8	0.2	26.5	26.8	0.1	25.6	25.7	0.0	24.1	23.4	0.5	22.2	21.9	0.1
O	24.4		0.8	-		-	24.7		2.1	24.2		0.9	24.2		0.9	23.7		0.3	23.7		0.0
P	22.9	23.8	0.6	-	-	-	27.6	26.2	2.1	26.0	25.1	0.9	26.1	25.1	0.9	24.7	24.2	0.3	23.6	23.7	0.0
TOTALS	388.5		8.4	248.1		3.5	419.3		8.0	414.4		11.4	398.6		9.5	188.9		1.7	379.2		10.7
TOTAL SQUARED ERRORS																					53.1
NUMBER OF POINTS																					98
NUMBER OF SUSETS																					49
POOLED STANDARD DEVIATION																					1.0
COEFFICEINT OF VARIATION																					4

TREHALOSE

SOURCE	INITIAL PUMP TRIALS																				
RUN	RUN 2			RUN 3			RUN 5			RUN 6			RUN 7			RUN 8			RUN 9		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	6.2		0.5	3.8		0.9	7.7		0.6	4.6		0.1	3.3		0.3	-		-	1.1		2.1
B	4.7	5.5	0.5	5.7	4.7	0.9	6.2	6.9	0.6	5.1	4.8	0.1	4.4	3.8	0.3	-	-	-	4.0	2.5	2.1
C	3.1		0.1	3.9		0.1	5.5		0.0	6.4		0.3	4.0		1.1	-		-	2.8		0.2
D	3.7	3.4	0.1	3.1	3.5	0.1	5.3	5.4	0.0	5.3	5.8	0.3	6.1	5.1	1.1	-	-	-	1.7	2.1	0.2
E	7.6		2.1	1.2		0.4	6.2		0.3	4.5		0.5	3.1		0.2	-		-	2.0		0.0
F	4.9	6.3	2.1	2.4	1.8	0.4	5.1	5.7	0.3	5.9	5.2	0.5	2.3	2.7	0.2	-	-	-	2.1	2.1	0.0
G	5.2		0.6	5.8		1.3	5.9		0.1	5.1		0.1	1.7		0.1	-		-	3.5		0.0
H	3.7	4.5	0.6	3.6	4.6	1.3	5.3	5.6	0.1	5.6	5.4	0.1	2.2	2.0	0.1	-	-	-	3.3	3.4	0.0
I	3.3		0.1	5.5		1.5	6.0		0.9	6.4		0.2	1.7		0.4	4.2		0.6	2.7		0.4
J	3.8	3.5	0.1	3.0	4.2	1.5	4.1	5.0	0.9	5.5	5.9	0.2	0.5	1.1	0.4	5.8	5.0	0.6	1.4	2.0	0.4
K	4.2		0.6	2.4		0.0	3.3		0.7	5.5		0.2	1.5		0.1	3.7		0.7	2.3		0.1
L	5.7	5.0	0.6	2.5	2.5	0.0	5.0	4.2	0.7	4.7	5.1	0.2	2.2	1.8	0.1	5.4	4.6	0.7	1.8	2.0	0.1
M	5.1		0.1	2.7		-	4.1		0.2	3.5		1.5	0.5		0.6	4.8		0.1	1.3		1.1
N	5.8	5.5	0.1	-	-	-	3.2	3.7	0.2	5.9	4.7	1.5	2.0	1.2	0.8	4.3	4.5	0.1	3.4	2.4	1.1
O	5.0		1.2	-		-	5.5		0.0	5.2		3.4	0.4		0.3	4.5		0.0	4.0		0.3
P	2.8	3.9	1.2	2.8	-	-	5.8	5.7	0.0	1.5	3.4	3.4	1.5	1.0	0.3	4.5	4.5	0.0	2.9	3.4	0.3
TOTALS	75.0		10.8	43.1		8.8	84.2		5.6	80.8		12.5	37.4		6.1	37.3		2.9	39.9		8.4
TOTAL SQUARED ERRORS																					54.7
NUMBER OF POINTS																					100
NUMBER OF SUSETS																					50
POOLED STANDARD DEVIATION																					1.0
COEFFICEINT OF VARIATION (%)																					26

2L EBC FERMENTATIONS - GROWTH

SOURCE	PUMP RETRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	3.0		0.0	2.9		0.0	2.3		0.0	2.7		0.0
B	3.0	3.0	0.0	2.8	2.9	0.0	2.6	2.5	0.0	2.9	2.8	0.0
C	3.3		0.0	2.8		0.0	3.0		0.0	2.9		0.0
D	3.0	3.2	0.0	2.8	2.8	0.0	2.9	3.0	0.0	2.7	2.8	0.0
E	3.4		0.0	2.5		0.0	2.3		0.1	2.7		0.0
F	3.3	3.4	0.0	2.8	2.7	0.0	2.9	2.6	0.1	2.9	2.8	0.0
G	3.3		0.0	2.8		0.0	2.8		0.1	2.8		0.0
H	3.5	3.4	0.0	3.0	2.9	0.0	2.3	2.6	0.1	2.4	2.6	0.0
I												
J												
K												
L												
TOTALS	25.9		0.1	22.4		0.1	21.1		0.4	22.0		0.1
TOTAL SQUARED ERRORS												0.6
NUMBER OF POINTS												32
NUMBER OF SUBSETS												18
POOLED STANDARD DEVIATION												0.2
COEFFICIENT OF VARIATION (%)												7

2L EBC FERMENTATIONS - FINAL ATTENUATION

SOURCE	PUMP RETRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	2.55		0.00	2.75		0.00	2.82		0.00	2.43		0.00
B	2.51	2.53	0.00	2.72	2.74	0.00	2.75	2.79	0.00	2.55	2.49	0.00
C	2.52		0.00	2.71		0.00	2.84		0.00	2.52		0.00
D	2.43	2.48	0.00	2.74	2.73	0.00	2.62	2.63	0.00	2.39	2.46	0.00
E	2.46		0.00	2.71		0.00	2.30		0.06	2.52		0.00
F	2.41	2.45	0.00	2.74	2.73	0.00	2.80	2.55	0.06	2.42	2.47	0.00
G	2.38		0.01	2.71		0.00	2.86		0.01	2.38		0.02
H	2.51	2.44	0.01	2.58	2.65	0.00	2.70	2.78	0.01	2.11	2.25	0.02
TOTALS	19.77		0.02	21.66		0.01	21.49		0.14	19.32		0.06
TOTAL SQUARED ERRORS												0.23
NUMBER OF POINTS												32
NUMBER OF SUBSETS												16
POOLED STANDARD DEVIATION												0.12
COEFFICIENT OF VARIATION (%)												5

2L EBC FERMENTATIONS - ALPHA CONSTANTS

SOURCE	PUMP RETRIALS + REPRODUCIBILITY TRIALS														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			REPRO		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	16.27		0.02	14.72		0.00	14.69		0.00	15.24		0.00	14.69		0.00
B	15.96	16.11	0.02	14.68	14.70	0.00	14.75	14.82	0.00	15.16	15.21	0.00	14.73		0.00
C	16.20		0.01	14.47		0.01	14.95		0.00	15.04		0.00	14.86		0.01
D	15.97	16.09	0.01	14.64	14.56	0.01	14.92	14.93	0.00	15.04	15.04	0.00	14.67		0.01
E	16.40		0.04	14.12		0.11	15.47		0.07	15.24		0.00	14.88		0.02
F	15.98	16.19	0.04	14.78	14.45	0.11	14.93	15.20	0.07	15.10	15.17	0.00	14.77		0.00
G	16.11		0.02	14.57		0.00	14.88		0.03	15.27		0.01	14.49		0.07
H	15.81	15.96	0.02	14.53	14.55	0.00	14.54	14.71	0.03	15.09	15.18	0.01	14.90	14.75	0.02
TOTALS	128.70		0.23	116.52		0.23	119.32		0.22	121.20		0.03	118.00		0.13
TOTAL SQUARED ERRORS															0.81
NUMBER OF POINTS															40
NUMBER OF SUBSETS															17
POOLED STANDARD DEVIATION															0.19
COEFFICIENT OF VARIATION (%)															1

2L EBC FERMENTATIONS - BETA CONSTANTS

SOURCE	PUMP RETRIALS + REPRODUCIBILITY TRIALS														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			REPRO		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	-0.0101		0.0000	-0.0096		0.000	-0.0093		0.0000	-0.0110		0.0000	-0.0098		0.0000
B	-0.0103	-0.0102	0.0000	-0.0101	-0.010	0.000	-0.0093	-0.0093	0.0000	-0.0110	-0.0110	0.0000	-0.0103		0.0000
C	-0.0103		0.0000	-0.0101		0.000	-0.0096		0.0000	-0.0105		0.0000	-0.0098		0.0000
D	-0.0105	-0.0104	0.0000	-0.0103	-0.010	0.000	-0.0096	-0.0096	0.0000	-0.0111	-0.0108	0.0000	-0.0097		0.0000
E	-0.0104		0.0000	-0.0103		0.000	-0.0108		0.0000	-0.0109		0.0000	-0.0103		0.0000
F	-0.0106	-0.0105	0.0000	-0.0103	-0.010	0.000	-0.0093	-0.0101	0.0000	-0.0107	-0.0108	0.0000	-0.0095		0.0000
G	-0.0105		0.0000	-0.0104		0.000	-0.0095		0.0000	-0.0108		0.0000	-0.0102		0.0000
H	-0.0103	-0.0104	0.0000	-0.0106	-0.011	0.000	-0.0097	-0.0096	0.0000	-0.0117	-0.0112	0.0000	-0.0101	-0.0100	0.0000
TOTALS	-0.0830		0.0000	0.0000		0.000	-0.0771		0.0000	-0.0875		0.0000	-0.0797		0.0000
TOTAL SQUARED ERRORS															0.0000
NUMBER OF POINTS															40
NUMBER OF SUBSETS															17
POOLED STANDARD DEVIATION															0.0003
COEFFICIENT OF VARIATION (%)															4

2L EBC FERMENTATIONS - pH (DAY 8)

SOURCE	PUMP RETRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	3.94		0.00	4.03		0.00	3.93		0.00	4.04		0.00
B	3.92	3.93	0.00	4.06	4.05	0.00	3.93	3.93	0.00	4.07	4.06	0.00
C	3.93		0.00	4.05		0.00	3.94		0.00	4.01		0.00
D	3.94	3.94	0.00	4.06	4.06	0.00	3.94	3.94	0.00	4.07	4.04	0.00
E	3.95		0.00	4.04		0.00	3.86		0.00	4.09		0.00
F	3.92	3.94	0.00	4.02	4.03	0.00	3.92	3.89	0.00	4.05	4.07	0.00
G	3.94		0.00	4.04		0.00	4.02		0.00	4.07		0.00
H	3.95	3.95	0.00	4.05	4.05	0.00	3.91	3.97	0.00	3.96	4.02	0.00
TOTALS	31.49		0.00	32.35		0.00	31.45		0.01	32.36		0.01
TOTAL SQUARED ERRORS												0.02
NUMBER OF POINTS												32
NUMBER OF SUBSETS												16
POOLED STANDARD DEVIATION												0.03
COEFFICEINT OF VARIATION (%)												1

2L EBC FERMENTATIONS - DIACETYL (DAY 8)

SOURCE	PUMP RETRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	121		100	125		1	88		42	120		0
B	141	131	100	123	124	1	75	82	42	119	120	0
C	139		72	116		20	83		9	111		16
D	156	148	72	107	112	20	77	80	9	119	115	16
E	-		-	118		20	72		49	118		6
F	152	-	-	109	114	20	86	79	49	113	116	6
G	148		110	108		1	91		42	124		169
H	169	159	110	110	109	1	78	85	42	98	111	169
TOTALS	874		669	910		85	650		355	922		383
TOTAL SQUARED ERRORS												1318
NUMBER OF POINTS												30
NUMBER OF SUBSETS												15
POOLED STANDARD DEVIATION												9
COEFFICEINT OF VARIATION (%)												8

2L EBC FERMENTATIONS - SO2 (DAY 8)

SOURCE	PUMP RETRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	15		2	15		0	9		0	10		0
B	12	14	2	14	15	0	9	9	0	10	10	0
C	14		0	12		18	10		0	9		1
D	15	15	0	20	16	16	9	10	0	11	10	1
E	16		0	11		2	9		1	11		0
F	15	16	0	14	13	2	11	10	1	11	11	0
G	20		13	11		1	11		1	11		2
H	13	16	13	13	12	1	9	10	1	8	10	2
TOTALS	120		32	110		39	77		5	81		7
TOTAL SQUARED ERRORS												82
NUMBER OF POINTS												32
NUMBER OF SUBSETS												16
POOLED STANDARD DEVIATION												2
COEFFICEINT OF VARIATION (%)												19

2L EBC FERMENTATIONS - ACETALDEHYDE (DAY 8)

SOURCE	PUMP RETRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	23		0	21		0	15		0	19		0
B	23	23	0	21	21	0	18	16	0	20	20	0
C	24		0	21		0	19		1	19		1
D	24	24	0	21	21	0	21	20	1	21	20	1
E	21		2	19		0	16		2	21		0
F	24	23	2	20	20	0	19	18	2	20	21	0
G	24		1	20		0	20		1	20		4
H	22	23	1	20	20	0	18	19	1	18	18	4
TOTALS	185		7	163		1	144		9	158		11
TOTAL SQUARED ERRORS												27
NUMBER OF POINTS												32
NUMBER OF SUBSETS												16
POOLED STANDARD DEVIATION												1
COEFFICEINT OF VARIATION (%)												6

2L EBC FERMENTATIONS - pH (DAY 12)

SOURCE	PUMP RETRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	3.97		0.00	4.05		0.00	3.97		0.00	4.07		0.00
B	3.94	3.96	0.00	4.09	4.07	0.00	3.95	3.96	0.00	4.09	4.08	0.00
C	3.95		0.00	4.08		0.00	3.97		0.00	4.02		0.00
D	3.95	3.95	0.00	4.10	4.09	0.00	3.98	3.98	0.00	4.10	4.06	0.00
E	3.96		0.00	4.07		0.00	3.98		0.00	4.11		0.00
F	3.98	3.97	0.00	4.06	4.07	0.00	4.00	3.95	0.00	4.07	4.09	0.00
G	3.97		0.00	4.08		0.00	4.08		0.00	4.04		0.00
H	3.98	3.98	0.00	4.09	4.09	0.00	3.95	4.02	0.00	3.98	4.01	0.00
TOTALS	31.70		0.00	32.62		0.00	31.80		0.01	32.46		0.01
TOTAL SQUARED ERRORS												0.02
NUMBER OF POINTS												32
NUMBER OF SUBSETS												16
POOLED STANDARD DEVIATION												0.04
COEFFICEINT OF VARIATION (%)												1

2L EBC FERMENTATIONS - DIACETYL (DAY 12)

SOURCE	PUMP RETRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	81		8	67		4	79		1	78		16
B	76	78	8	63	65	4	77	78	1	86	82	16
C	76		20	63		0	77		4	72		1
D	85	81	20	62	63	0	81	79	4	74	73	1
E	86		13	61		4	83		210	76		4
F	76	72	13	67	59	4	92	78	210	80	78	4
G	62		79	62		9	101		272	81		100
H	80	71	79	68	65	9	68	85	272	61	71	100
TOTALS	606		240	503		35	638		975	608		242
TOTAL SQUARED ERRORS												1491
NUMBER OF POINTS												32
NUMBER OF SUBSETS												16
POOLED STANDARD DEVIATION												10
COEFFICEINT OF VARIATION (%)												13

2L EBC FERMENTATIONS - SO2 (DAY 12)

SOURCE	PUMP RETRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	14		0	9		0	9		0	10		0
B	13	13	0	9	9	0	8	9	0	10	10	0
C	13		0	9		1	10		1	9		0
D	14	13	0	11	10	1	8	9	1	10	10	0
E	10		2	9		4	8		1	11		0
F	13	12	2	13	11	4	10	9	1	11	11	0
G	14		1	15		12	15		12	10		1
H	16	15	1	8	12	12	8	12	12	8	9	1
TOTALS	106		7	83		35	76		29	79		3
TOTAL SQUARED ERRORS												73
NUMBER OF POINTS												32
NUMBER OF SUBSETS												16
POOLED STANDARD DEVIATION												2
COEFFICEINT OF VARIATION (%)												20

2L EBC FERMENTATIONS - ACETALDEHYDE (DAY 12)

SOURCE	PUMP RETRIALS											
RUN	RUN 1			RUN 2			RUN 3			RUN 4		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
A	42		3	26		1	18		0	21		0
B	38	40	3	24	25	1	17	18	0	22	22	0
C	45		28	24		2	19		0	21		2
D	34	40	28	27	26	2	19	19	0	24	23	2
E	36		6	23		0	20		0	23		1
F	41	38	6	24	24	0	20	20	0	21	22	1
G	48		54	25		0	24		9	22		4
H	34	41	54	24	25	0	18	21	9	18	20	4
TOTALS	317		182	197		8	155		19	172		15
TOTAL SQUARED ERRORS												223
NUMBER OF POINTS												32
NUMBER OF SUBSETS												16
POOLED STANDARD DEVIATION												4
COEFFICEINT OF VARIATION (%)												14

500 ml FERMENTATIONS - GROWTH

SOURCE	PUMP RETRIALS + FLOW TRIALS														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
1	3.4		0.0	3.1		0.1	3.1		0.0	2.7		0.0	2.9		-
2	3.0	3.2	0.0	2.4	2.8	0.1	3.1	3.1	0.0	2.8	2.8	0.0	-	-	-
3	3.5		0.0	2.7		0.0	3.0		0.0	2.9		0.0	2.9		0.0
4	3.3	3.4	0.0	3.0	2.8	0.0	2.8	2.9	0.0	2.7	2.8	0.0	2.9	2.9	0.0
5	2.8		0.0	3.0		0.0	3.0		0.0	2.7		0.0	2.8		0.0
6	3.2	3.0	0.0	2.9	3.0	0.0	3.0	3.0	0.0	2.8	2.8	0.0	2.7	2.8	0.0
7	3.2		0.0	2.9		0.0	3.0		0.0	2.8		0.0	2.7		0.0
8	3.1	3.2	0.0	3.0	3.0	0.0	3.1	3.1	0.0	3.1	3.0	0.0	2.7	2.7	0.0
9													2.7		0.0
10													2.7	2.7	0.0
11													2.8		0.0
12													2.8	2.7	0.0
TOTALS	25.5		0.2	23.0		0.3	24.1		0.0	22.5		0.1	27.5		0.0
TOTAL SQUARED ERRORS															0.6
NUMBER OF POINTS															42
NUMBER OF SUBSETS															21
POOLED STANDARD DEVIATION															0.2
COEFFICIENT OF VARIATION (%)															8

500 ml FERMENTATIONS - FINAL ATTENUATION

SOURCE	PUMP RETRIALS + FLOW TRIALS														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
1	1.87		0.00	1.80		0.02	2.08		0.00	1.94		0.00	1.78		-
2	1.88	1.88	0.00	2.09	1.95	0.02	2.08	2.08	0.00	1.83	1.89	0.00	-	-	-
3	1.78		0.00	1.85		0.00	2.10		0.00	1.87		0.00	1.73		0.00
4	1.80	1.79	0.00	1.95	1.90	0.00	2.19	2.15	0.00	1.82	1.85	0.00	1.71	1.72	0.00
5	1.83		0.00	1.73		0.02	2.08		0.00	1.92		0.00	1.73		0.00
6	1.94	1.89	0.00	1.99	1.96	0.02	2.14	2.11	0.00	1.90	1.91	0.00	1.73	1.73	0.00
7	2.05		0.00	1.90		0.00	2.09		0.00	1.97		0.00	1.72		0.00
8	1.94	2.00	0.00	1.81	1.86	0.00	2.09	2.09	0.00	1.96	1.97	0.00	1.76	1.74	0.00
9													1.73		0.00
10													1.78	1.75	0.00
11													1.74		0.00
12													1.69	1.72	0.00
TOTALS	15.09		0.01	15.12		0.06	18.85		0.01	15.21		0.01	17.30		0.00
TOTAL SQUARED ERRORS															0.11
NUMBER OF POINTS															42
NUMBER OF SUBSETS															21
POOLED STANDARD DEVIATION															0.07
COEFFICIENT OF VARIATION (%)															4

500 ml FERMENTATIONS - ALPHA CONSTANTS

SOURCE	PUMP RETRIALS + FLOW TRIALS + REPRODUCIBILITY TRIALS																	
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW			REPRO		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
1	16.52		0.00	15.67		0.06	14.86		0.00	16.24		0.00	14.25		-	14.00		0.01
2	16.48	16.50	0.00	16.17	15.92	0.06	14.93	14.89	0.00	16.29	16.26	0.00	-	-	-	13.74		0.03
3	16.53		0.00	15.39		0.00	14.71		0.00	16.52		0.02	14.42		0.00	13.95		0.00
4	16.70	16.70	0.00	15.43	15.41	0.00	14.92	14.77	0.00	16.25	16.38	0.02	14.29	14.35	0.00	13.90	13.93	0.00
5	16.89		0.00	15.01		0.13	15.00		0.00	16.69		0.01	14.40		0.00			
6	16.65	16.67	0.00	15.73	15.37	0.13	14.99	15.00	0.00	16.52	16.61	0.01	14.40	14.43	0.00			
7	16.76		0.00	16.02		0.01	15.16		0.02	16.45		0.00	14.62		0.00			
8	16.69	16.72	0.00	15.68	15.85	0.01	14.88	15.02	0.02	16.40	16.42	0.00	14.62	14.62	0.00			
9													14.38		0.00			
10													14.40	14.39	0.00			
11													14.28		0.02			
12													14.54	14.41	0.02			
TOTALS	133.18		0.01	125.30		0.40	119.36		0.05	131.35		0.05	144.41		0.05	55.59		0.04
TOTAL SQUARED ERRORS																		0.60
NUMBER OF POINTS																		46
NUMBER OF SUBSETS																		22
POOLED STANDARD DEVIATION																		0.16
COEFFICIENT OF VARIATION (%)																		1

500 ml FERMENTATIONS - BETA CONSTANTS

SOURCE	PUMP RETRIALS + FLOW TRIALS + REPRODUCIBILITY TRIALS																	
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW			REPRO		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
1	-0.0120		0.0000	-0.0123		0.0000	-0.0105		0.0000	-0.0119		0.0000	-0.0115		-	-0.0120		0.0000
2	-0.0119	-0.0120	0.0000	-0.0119	-0.0121	0.0000	-0.0110	-0.0108	0.0000	-0.0122	-0.0121	0.0000	-	-	-	-0.0115		0.0000
3	-0.0123		0.0000	-0.0116		0.0000	-0.0106		0.0000	-0.0121		0.0000	-0.0115		0.0000	-0.0121		0.0000
4	-0.0122	-0.0123	0.0000	-0.0116	-0.0116	0.0000	-0.0103	-0.0105	0.0000	-0.0126	-0.0124	0.0000	-0.0114	-0.0115	0.0000	-0.0121	-0.0119	0.0000
5	-0.0118		0.0000	-0.0128		0.0000	-0.0101		0.0000	-0.0119		0.0000	-0.0111		0.0000			
6	-0.0113	-0.0116	0.0000	-0.0118	-0.0123	0.0000	-0.0105	-0.0103	0.0000	-0.0116	-0.0118	0.0000	-0.0114	-0.0113	0.0000			
7	-0.0119		0.0000	-0.0118		0.0000	-0.0103		0.0000	-0.0115		0.0000	-0.0115		0.0000			
8	-0.0115	-0.0117	0.0000	-0.0121	-0.0120	0.0000	-0.0104	-0.0104	0.0000	-0.0117	-0.0116	0.0000	-0.0115	-0.0115	0.0000			
9													-0.0113		0.0000			
10													-0.0110	-0.0112	0.0000			
11													-0.0113		0.0000			
12													-0.0115	-0.0114	0.0000			
TOTALS	-0.0949		0.0000	-0.0959		0.0000	-0.0837		0.0000	-0.0955		0.0000	-0.1135		0.0000	-0.0477		0.0000
TOTAL SQUARED ERRORS																		0.0000
NUMBER OF POINTS																		46
NUMBER OF SUBSETS																		22
POOLED STANDARD DEVIATION																		0.0003
COEFFICIENT OF VARIATION (%)																		2

500 ml FERMENTATIONS - pH (DAY 12)

SOURCE	PUMP RETRIALS + FLOW TRIALS														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
1	4.05		0.00	4.11		0.01	4.13		0.00	4.30		0.00	4.27		-
2	4.04	4.05	0.00	4.32	4.22	0.01	4.04	4.09	0.00	4.17	4.24	0.00	-	-	-
3	4.05		0.00	4.20		0.00	4.19		0.00	4.25		0.00	4.29		0.00
4	4.08	4.07	0.00	4.24	4.22	0.00	4.16	4.18	0.00	4.31	4.28	0.00	4.28	4.28	0.00
5	4.09		0.00	4.10		0.00	4.13		0.00	4.25		0.00	4.33		0.00
6	4.06	4.08	0.00	4.23	4.17	0.00	4.16	4.15	0.00	4.16	4.21	0.00	4.27	4.30	0.00
7	4.09		0.00	4.22		0.00	4.15		0.00	4.32		0.00	4.34		0.00
8	4.10	4.10	0.00	4.14	4.18	0.00	4.15	4.15	0.00	4.29	4.31	0.00	4.34	4.34	0.00
9													4.35		0.00
10													4.37	4.36	0.00
11													4.37		0.00
12													4.36	4.37	0.00
TOTALS	32.56		0.00	33.56		0.03	33.11		0.00	34.05		0.01	43.28		0.00
TOTAL SQUARED ERRORS															0.06
NUMBER OF POINTS															42
NUMBER OF SUBSETS															21
POOLED STANDARD DEVIATION															0.05
COEFFICEINT OF VARIATION (%)															1

500 ml FERMENTATIONS - DIACETYL (DAY 12)

SOURCE	PUMP RETRIALS + FLOW TRIALS														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
1	89		32	-		-	84		195	156		425	134		-
2	77	83	32	177	-	-	56	70	195	115	136	425	-	-	-
3	76		1	132		22	88		0	142		203	144		1
4	78	77	1	142	137	22	88	88	0	171	156	203	146	145	1
5	79		81	-		-	75		50	139		285	156		70
6	97	88	81	131	-	-	89	82	50	105	122	285	140	148	70
7	98		0	125		83	89		0	175		15	156		41
8	99	99	0	107	116	83	89	89	0	167	171	15	168	162	41
9													158		28
10													168	163	28
11													162		0
12													162	162	0
TOTALS	694		227	505		210	658		490	1170		1857	1560		276
TOTAL SQUARED ERRORS															3061
NUMBER OF POINTS															36
NUMBER OF SUBSETS															19
POOLED STANDARD DEVIATION															13
COEFFICEINT OF VARIATION (%)															11

500 ml FERMENTATIONS - SO2 (DAY 12)

SOURCE	PUMP RETRIALS + FLOW TRIALS														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
1	12		0	14		0	13		1	12		1	13		-
2	12	12	0	14	14	0	11	12	1	14	13	1	-	-	-
3	14		-	16		0	12		0	14		0	14		0
4	-	-	-	18	17	0	13	12	0	13	14	0	13	14	0
5	15		0	14		2	12		1	16		1	13		0
6	15	15	0	17	16	2	14	13	1	14	15	1	14	14	0
7	11		1	22		3	13		0	14		0	15		0
8	13	12	1	19	21	3	13	13	0	15	14	0	13	14	0
9													14		0
10													13	13	0
11													15		0
12													14	14	0
TOTALS	73		3	134		10	101		4	112		5	138		2
TOTAL SQUARED ERRORS															24
NUMBER OF POINTS															40
NUMBER OF SUBSETS															20
POOLED STANDARD DEVIATION															1
COEFFICEINT OF VARIATION (%)															8

500 ml FERMENTATIONS - ACETALDEHYDE (DAY 12)

SOURCE	PUMP RETRIALS + FLOW TRIALS														
RUN	RUN 1			RUN 2			RUN 3			RUN 4			FLOW		
SAMPLE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE	VALUE	AVG	SE
1	23		12	27		1	23		32	18		8	23		-
2	30	26	12	29	28	1	34	28	32	23	20	8	-	-	-
3	31		12	37		8	25		13	19		2	22		76
4	24	27	12	43	40	8	18	22	13	22	21	2	39	30	76
5	15		49	41		7	22		0	21		8	21		1
6	29	22	49	36	38	7	22	22	0	27	24	8	23	22	1
7	29		67	46		2	27		13	18		0	22		2
8	13	21	67	43	44	2	20	24	13	17	17	0	19	21	2
9													25		4
10													21	23	4
11													18		0
12													18	18	0
TOTALS	194		281	302		34	192		115	164		38	224		166
TOTAL SQUARED ERRORS															635
NUMBER OF POINTS															42
NUMBER OF SUBSETS															21
POOLED STANDARD DEVIATION															5
COEFFICEINT OF VARIATION (%)															21

APPENDIX D

EVALUATION OF ROUTINE BREWERY OPERATION: RESULTS AND STATISTICAL ANALYSIS

CONTENTS

PAIRED-SAMPLE t TEST FOR YEAST QUALITY ASSAYS of ON-LINE SAMPLES (EQUIP TEST)

SAMPLE	PROTEASE	DIFF	AVG	STDS	t
Before Pump (A)	0.08				
Before Pump (B)	0.05				
After Line (A)	0.04	-0.04			
After Line (B)	0.06	0.01			
Before Chiller (A)	0.05	0.01			
Before Chiller (B)	0.06	0.00			
After Chiller (A)	0.09	0.04			
After Chiller (B)	0.05	-0.01	0.00	0.03	0.15

SAMPLE	METH BLUE	DIFF	AVG	STDS	t
Before Pump (A)	96				
Before Pump (B)	96				
After Line (A)	91	-5			
After Line (B)	91	-5			
Before Chiller (A)	89	-2			
Before Chiller (B)	91	0			
After Chiller (A)	93	4			
After Chiller (B)	94	3	-1	4	-0.53

SAMPLE	OUR B	DIFF	AVG	STDS	t
Before Pump (A)	0.006				
Before Pump (B)	0.006				
After Line (A)	0.007	0.001			
After Line (B)	0.006	0.000			
Before Chiller (A)	0.007	0.000			
Before Chiller (B)	0.005	-0.001			
After Chiller (A)	0.007	0.000			
After Chiller (B)	0.005	0.000	0.000	0.001	0.00

SAMPLE	GLYCOGEN	DIFF	AVG	STDS	t
Before Pump (A)	23.5				
Before Pump (B)	24.3				
After Line (A)	24.5	1.0			
After Line (B)	24.7	0.4			
Before Chiller (A)	24.3	-0.2			
Before Chiller (B)	25.6	0.9			
After Chiller (A)	24.7	0.4			
After Chiller (B)	25.0	-0.6	0.3	0.8	1.25

SAMPLE	TREHALOSE	DIFF	AVG	STDS	t
Before Pump (A)	4.1				
Before Pump (B)	4.7				
After Line (A)	4.6	0.5			
After Line (B)	4.0	-0.7			
Before Chiller (A)	5.0	0.4			
Before Chiller (B)	3.9	-0.1			
After Chiller (A)	4.2	-0.8			
After Chiller (B)	4.8	0.9	0.0	0.7	0.12

PAIRED-SAMPLE t TEST FOR YEAST QUALITY ASSAYS of ON-LINE SAMPLES (EQUIP TEST)

SAMPLE	PROTEASE	DIFF	AVG	STDS	t
Before Pump (A)	0.08				
Before Pump (B)	0.05				
After Line (A)	0.04	-0.04			
After Line (B)	0.06	0.01	-0.02	0.04	-0.60
Before Chiller (A)	0.05	0.01			
Before Chiller (B)	0.06	0.00	0.01	0.01	1.00
After Chiller (A)	0.09	0.04			
After Chiller (B)	0.05	-0.01	0.02	0.04	0.60
Overall (A)		0.01			
Overall (B)		0.00	0.00	0.01	1.00

SAMPLE	METH BLUE	DIFF	AVG	STDS	t
Before Pump (A)	96				
Before Pump (B)	96				
After Line (A)	91	-5			
After Line (B)	91	-5	-5	0	MUD
Before Chiller (A)	89	-2			
Before Chiller (B)	91	0	-1	1	-1.00
After Chiller (A)	93	4			
After Chiller (B)	94	3	4	1	7.00
Overall (A)		-3			
Overall (B)		-2	-3	1	-5.00

SAMPLE	OUR B	DIFF	AVG	STDS	t
Before Pump (A)	0.006				
Before Pump (B)	0.006				
After Line (A)	0.007	0.001			
After Line (B)	0.006	0.000	0.001	0.001	1.00
Before Chiller (A)	0.007	0.000			
Before Chiller (B)	0.005	-0.001	-0.001	0.001	-1.00
After Chiller (A)	0.007	0.000			
After Chiller (B)	0.005	0.000	0.000	0.000	MUD
Overall (A)		0.001			
Overall (B)		-0.001	0.000	0.001	0.00

SAMPLE	GLYCOGEN	DIFF	AVG	STDS	t
Before Pump (A)	23.5				
Before Pump (B)	24.3				
After Line (A)	24.5	1.0			
After Line (B)	24.7	0.4	0.7	0.4	2.33
Before Chiller (A)	24.3	-0.2			
Before Chiller (B)	25.6	0.9	0.4	0.8	0.64
After Chiller (A)	24.7	0.4			
After Chiller (B)	25.0	-0.6	-0.1	0.7	-0.20
Overall (A)		1.2			
Overall (B)		0.7	0.95	0.35	3.80

SAMPLE	TREHALOSE	DIFF	AVG	STDS	t
Before Pump (A)	4.1				
Before Pump (B)	4.7				
After Line (A)	4.6	0.5			
After Line (B)	4.0	-0.7	-0.1	0.8	-0.17
Before Chiller (A)	5.0	0.4			
Before Chiller (B)	3.9	-0.1	0.2	0.4	0.60
After Chiller (A)	4.2	-0.8			
After Chiller (B)	4.8	0.9	0.1	1.2	0.06
Overall (A)		0.1			
Overall (B)		0.1	0.1	0.0	MUD

PAIRED-SAMPLE t TEST FOR CURVE FIT CONSTANTS AND BEER QUALITY INDICATORS for FERMENTATIONS OF ON-LINE SAMPLES (EQUIP TEST)

SAMPLE	ALPHA	DIFF	AVG	STDS	t	SAMPLE	BETA	DIFF	AVG	STDS	t
Before Pump (A)	13.77					Before Pump (A)	-0.0108				
Before Pump (B)	14.04					Before Pump (B)	-0.0104				
After Line (A)	13.46	-0.31				After Line (A)	-0.0109	-0.0001			
After Line (B)	14.06	0.03				After Line (B)	-0.0118	-0.0014			
Before Chiller (A)	13.61	0.15				Before Chiller (A)	-0.0110	-0.0001			
Before Chiller (B)	14.05	-0.01				Before Chiller (B)	-0.0110	0.0008			
After Chiller (A)	14.02	0.41				After Chiller (A)	-0.0108	0.0002			
After Chiller (B)	13.51	-0.55	-0.05	0.34	-0.34	After Chiller (B)	-0.0104	0.0006	0.0000	0.0008	0.00
SAMPLE	pH	DIFF	AVG	STDS	t	SAMPLE	DIACETYL	DIFF	AVG	STDS	t
Before Pump (A)	4.19					Before Pump (A)	84				
Before Pump (B)	4.20					Before Pump (B)	90				
After Line (A)	4.20	0.01				After Line (A)	88	4			
After Line (B)	4.13	-0.07				After Line (B)	70	-20			
Before Chiller (A)	4.18	-0.02				Before Chiller (A)	75	-13			
Before Chiller (B)	4.22	0.09				Before Chiller (B)	88	18			
After Chiller (A)	4.24	0.06				After Chiller (A)	93	18			
After Chiller (B)	4.17	-0.05	0.00	0.06	0.13	After Chiller (B)	86	-2	1	16	0.13
SAMPLE	SO2	DIFF	AVG	STDS	t	SAMPLE	ACETAL	DIFF	AVG	STDS	t
Before Pump (A)	8					Before Pump (A)	21				
Before Pump (B)	9					Before Pump (B)	22				
After Line (A)	8	0				After Line (A)	23	2			
After Line (B)	8	-1				After Line (B)	23	1			
Before Chiller (A)	8	0				Before Chiller (A)	21	-2			
Before Chiller (B)	10	2				Before Chiller (B)	23	0			
After Chiller (A)	9	1				After Chiller (A)	23	2			
After Chiller (B)	8	-2	0	1	0.00	After Chiller (B)	22	-1	0	2	0.50

PAIRED-SAMPLE t TEST FOR CURVE FIT CONSTANTS AND BEER QUALITY INDICATORS for FERMENTATIONS OF ON-LINE SAMPLES (EQUIP TEST)

SAMPLE	ALPHA	DIFF	AVG	STDS	t	SAMPLE	BETA	DIFF	AVG	STDS	t
Before Pump (A)	13.77					Before Pump (A)	-0.0108				
Before Pump (B)	14.04					Before Pump (B)	-0.0104				
After Line (A)	13.46	-0.31				After Line (A)	-0.0109	-0.0001			
After Line (B)	14.06	0.03	-0.14	0.24	-0.83	After Line (B)	-0.0118	-0.0014	-0.0007	0.0009	-1.15
Before Chiller (A)	13.61	0.15				Before Chiller (A)	-0.0110	-0.0001			
Before Chiller (B)	14.05	-0.01	0.07	0.12	0.84	Before Chiller (B)	-0.0110	0.0008	0.0004	0.0006	0.78
After Chiller (A)	14.02	0.41				After Chiller (A)	-0.0108	0.0002			
After Chiller (B)	13.51	-0.55	-0.07	0.67	-0.14	After Chiller (B)	-0.0104	0.0006	0.0004	0.0003	2.00
Overall (A)		0.25				Overall (A)		0.0000			
Overall (B)		-0.53	-0.14	0.55	-0.36	Overall (B)		0.0000	0.0000	0.0000	NDV
SAMPLE	pH	DIFF	AVG	STDS	t	SAMPLE	DIACETYL	DIFF	AVG	STDS	t
Before Pump (A)	4.19					Before Pump (A)	84				
Before Pump (B)	4.20					Before Pump (B)	90				
After Line (A)	4.20	0.01				After Line (A)	88	4			
After Line (B)	4.13	-0.07	-0.03	0.06	-0.75	After Line (B)	70	-20	-8	17	-0.67
Before Chiller (A)	4.18	-0.02				Before Chiller (A)	75	-13			
Before Chiller (B)	4.22	0.09	0.03	0.08	0.64	Before Chiller (B)	88	18	3	22	0.16
After Chiller (A)	4.24	0.06				After Chiller (A)	93	18			
After Chiller (B)	4.17	-0.05	0.01	0.06	0.09	After Chiller (B)	86	-2	8	14	0.80
Overall (A)		0.05				Overall (A)		9			
Overall (B)		-0.03	0.01	0.06	0.25	Overall (B)		-4	3	9	0.38
SAMPLE	SO2	DIFF	AVG	STDS	t	SAMPLE	ACETAL	DIFF	AVG	STDS	t
Before Pump (A)	8					Before Pump (A)	21				
Before Pump (B)	9					Before Pump (B)	22				
After Line (A)	8	0				After Line (A)	23	2			
After Line (B)	8	-1	-1	1	-1.00	After Line (B)	23	1	2	1	3.00
Before Chiller (A)	8	0				Before Chiller (A)	21	-2			
Before Chiller (B)	10	2	1	1	1.00	Before Chiller (B)	23	0	-1	1	-1.00
After Chiller (A)	9	1				After Chiller (A)	23	2			
After Chiller (B)	8	-2	-1	2	-0.33	After Chiller (B)	22	-1	1	2	0.33
Overall (A)		1				Overall (A)		2			
Overall (B)		-1	0	1	0.00	Overall (B)		0	1	1	1.00

PAIRED-SAMPLE t TEST FOR YEAST QUALITY ASSAYS of ON-LINE SAMPLES (YQ TEST)

SAMPLE	PROTEASE	DIFF	AVG	STDS	t
Before Pump (A)	0.08				
Before Pump (B)	0.05	-0.03			
After Line (A)	0.04				
After Line (B)	0.06	0.02			
Before Chiller (A)	0.05				
Before Chiller (B)	0.06	0.01			
After Chiller (A)	0.09				
After Chiller (B)	0.05	-0.04	-0.01	0.03	-0.68

SAMPLE	METH BLUE	DIFF	AVG	STDS	t
Before Pump (A)	96				
Before Pump (B)	96	0.0			
After Line (A)	91				
After Line (B)	91	0.0			
Before Chiller (A)	89				
Before Chiller (B)	91	2.0			
After Chiller (A)	93				
After Chiller (B)	94	1.0	0.8	1.0	1.57

SAMPLE	OUR B	DIFF	AVG	STDS	t
Before Pump (A)	0.006				
Before Pump (B)	0.006	0.000			
After Line (A)	0.007				
After Line (B)	0.006	-0.001			
Before Chiller (A)	0.007				
Before Chiller (B)	0.005	-0.002			
After Chiller (A)	0.007				
After Chiller (B)	0.005	-0.002	-0.001	0.001	-2.61

SAMPLE	GLYCOGEN	DIFF	AVG	STDS	t
Before Pump (A)	23.5				
Before Pump (B)	24.3	0.8			
After Line (A)	24.5				
After Line (B)	24.7	0.2			
Before Chiller (A)	24.3				
Before Chiller (B)	25.6	1.3			
After Chiller (A)	24.7				
After Chiller (B)	25.0	0.3	0.7	0.5	2.57

SAMPLE	TREHALOSE	DIFF	AVG	STDS	t
Before Pump (A)	4.1				
Before Pump (B)	4.7	0.6			
After Line (A)	4.6				
After Line (B)	4.0	-0.6			
Before Chiller (A)	5.0				
Before Chiller (B)	3.9	-1.1			
After Chiller (A)	4.2				
After Chiller (B)	4.8	0.6	-0.1	0.9	-0.29

PAIRED-SAMPLE t TEST FOR CURVE FIT CONSTANTS AND BEER QUALITY INDICATORS for FERMENTATIONS OF ON-LINE SAMPLES (YQ TEST)

SAMPLE	ALPHA	DIFF	AVG	STDS	t
Before Pump (A)	13.77				
Before Pump (B)	14.04	0.27			
After Line (A)	13.46				
After Line (B)	14.06	0.61			
Before Chiller (A)	13.61				
Before Chiller (B)	14.05	0.44			
After Chiller (A)	14.02				
After Chiller (B)	13.51	-0.51	0.20	0.50	0.81

SAMPLE	BETA	DIFF	AVG	STDS	t
Before Pump (A)	-0.0108				
Before Pump (B)	-0.0104	0.0004			
After Line (A)	-0.0109				
After Line (B)	-0.0118	-0.0009			
Before Chiller (A)	-0.0110				
Before Chiller (B)	-0.0110	0.0000			
After Chiller (A)	-0.0108				
After Chiller (B)	-0.0104	0.0004	-0.0000	0.0006	-0.08

SAMPLE	pH	DIFF	AVG	STDS	t
Before Pump (A)	4.19				
Before Pump (B)	4.20	0.01			
After Line (A)	4.20				
After Line (B)	4.13	-0.07			
Before Chiller (A)	4.18				
Before Chiller (B)	4.22	0.04			
After Chiller (A)	4.24				
After Chiller (B)	4.17	-0.07	-0.02	0.06	-0.80

SAMPLE	DIACETYL	DIFF	AVG	STDS	t
Before Pump (A)	84				
Before Pump (B)	90	6			
After Line (A)	88				
After Line (B)	70	-18			
Before Chiller (A)	75				
Before Chiller (B)	88	13			
After Chiller (A)	93				
After Chiller (B)	86	-7	-2	14	-0.22

SAMPLE	SO2	DIFF	AVG	STDS	t
Before Pump (A)	8				
Before Pump (B)	9	1			
After Line (A)	8				
After Line (B)	8	0			
Before Chiller (A)	8				
Before Chiller (B)	10	2			
After Chiller (A)	9				
After Chiller (B)	8	-1	1	1	0.77

SAMPLE	ACETAL	DIFF	AVG	STDS	t
Before Pump (A)	21				
Before Pump (B)	22	1			
After Line (A)	23				
After Line (B)	23	0			
Before Chiller (A)	21				
Before Chiller (B)	23	2			
After Chiller (A)	23				
After Chiller (B)	22	-1	1	1	0.77

ANALYSIS OF VARIANCE FOR ON-LINE TRIALS - YEAST QUALITY ASSAYS

ASSAY	Sample	Replicates A	Replicates B	Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
PROTEASE	Before Pump	0.08	0.05	0.13	0.07	2	Between Samples	5.0E-04	3	1.7E-04	0.44
	After Line	0.04	0.06	0.10	0.05	2	Within Samples	1.5E-03	4	3.7E-04	
	Before Chiller	0.05	0.06	0.11	0.06	2	Total	2.0E-03	7		
	After Chiller	0.09	0.05	0.14	0.07	2					
MODIFIED	Before Pump	96	96	192	96	2	Between Samples	43	3	14	23.13
METHYLENE	After Line	91	91	182	91	2	Within Samples	3	4	1	
BLUE	Before Chiller	89	91	180	90	2	Total	46	7		
	After Chiller	93	94	187	94	2					
OXYGEN	Before Pump	0.006	0.006	0.012	0.006	2	Between Samples	3.7E-07	3	1.2E-07	0.11
UTILISATION	After Line	0.007	0.006	0.013	0.007	2	Within Samples	4.5E-06	4	1.1E-06	
RATE	Before Chiller	0.007	0.005	0.012	0.006	2	Total	4.9E-06	7		
	After Chiller	0.007	0.005	0.012	0.006	2					
GLYCOGEN	Before Pump	23.5	24.3	47.8	23.9	2	Between Samples	1.3	3	0.4	1.46
CONTENT	After Line	24.5	24.7	49.2	24.6	2	Within Samples	1.2	4	0.3	
	Before Chiller	24.3	25.6	49.9	25.0	2	Total	2.6	7		
	After Chiller	24.7	25.0	49.7	24.9	2					
TREHALOSE	Before Pump	4.1	4.7	8.8	4.4	2	Between Samples	0.0	3	0.0	0.05
CONTENT	After Line	4.6	4.0	8.6	4.3	2	Within Samples	1.1	4	0.3	
	Before Chiller	5.0	3.9	8.9	4.5	2	Total	1.2	7		
	After Chiller	4.2	4.8	9.0	4.5	2					

ANALYSIS OF VARIANCE FOR ON-LINE TRIALS - FERMENTATION AND BEER QUALITY

ASSAY	Sample	Replicates A	Replicates B	Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
ALPHA CONSTANTS	Before Pump	13.77	14.04	27.80	13.90	2	Between Samples	0.027	3.0	0.009	0.08
	After Line	13.46	14.06	27.52	13.76	2	Within Samples	0.450	4.0	0.112	
	Before Chiller	13.61	14.05	27.66	13.83	2	Total	0.477	7.0		
	After Chiller	14.02	13.51	27.52	13.76	2					
BETA CONSTANTS	Before Pump	-0.0108	-0.0104	-0.0212	-0.0106	2	Between Samples	7.8E-07	3	2.6E-07	1.85
	After Line	-0.0109	-0.0118	-0.0227	-0.0114	2	Within Samples	5.7E-07	4	1.4E-07	
	Before Chiller	-0.0110	-0.0110	-0.0220	-0.0110	2	Total	1.3E-06	7		
	After Chiller	-0.0108	-0.0104	-0.0212	-0.0106	2					
pH	Before Pump	4.19	4.20	8.39	4.20	2	Between Samples	0.0019	3	0.0006	0.45
	After Line	4.20	4.13	8.33	4.17	2	Within Samples	0.0057	4	0.0014	
	Before Chiller	4.18	4.22	8.40	4.20	2	Total	0.0077	7		
	After Chiller	4.24	4.17	8.41	4.21	2					
DIACETYL	Before Pump	84	90	174	87	2	Between Samples	140.5	3	46.8	0.65
	After Line	88	70	158	79	2	Within Samples	289.0	4	72.3	
	Before Chiller	75	88	163	82	2	Total	429.5	7		
	After Chiller	93	86	179	90	2					
SO2	Before Pump	8	9	17	9	2	Between Samples	1.0	3	0.3	0.44
	After Line	8	8	16	8	2	Within Samples	3.0	4	0.8	
	Before Chiller	8	10	18	9	2	Total	4.0	7		
	After Chiller	9	8	17	9	2					
ACETALDEHYDE	Before Pump	21	22	43	22	2	Between Samples	2.5	3	0.8	1.11
	After Line	23	23	46	23	2	Within Samples	3.0	4	0.8	
	Before Chiller	21	23	44	22	2	Total	5.5	7		
	After Chiller	23	22	45	23	2					

APPENDIX E

THE EFFECT OF PUMP DESIGN AND OPERATION: RESULTS AND STATISTICAL ANALYSIS

ABBREVIATIONS

E1 - E10 **RESULTS OF INITIAL PUMP TRIALS**

Before	Samples taken at sampling point before pump
After	Samples taken at sampling point after pump

E11 - E39 **ANOVA of RESULTS OF INITIAL PUMP TRIALS**

S1B	Samples taken at sampling point before pump at first pump speed
S1A	Samples taken at sampling point after pump at first pump speed
S2B	Samples taken at sampling point before pump at second pump speed
S2A	Samples taken at sampling point after pump at second pump speed

E40 - E51 **RESULTS OF PUMP RETRIALS**

Before 1	Sample taken at sampling point before pump at first instance at which the pump was stopped for sampling
After 1	Sample taken at sampling point after pump at first instance at which the pump was stopped for sampling
Before 2	Sample taken at sampling point before pump at second instance at which the pump was stopped for sampling
After 2	Sample taken at sampling point after pump at second instance at which the pump was stopped for sampling

E52 - E75 **PAIRED-SAMPLE t TESTS on RESULTS OF PUMP RETRIALS**

As for RESULTS OF PUMP RETRIALS

E76 - E86 **ANOVA of RESULTS OF PUMP RETRIALS**

As for ANOVA of RESULTS OF INITIAL PUMP TRIALS

RESULTS OF YEAST QUALITY ASSAYS FOR INITIAL PUMP TRIALS - SP 40 BREDEL PERISTALTIC PUMP

RUN	1
DATE	11/1/95
YEAST	Castle C9 ex FV112
WORT	Steinecker
PUMPS	1. Bredel peristaltic (Walter Becker SA)

PUMP 1. Bredel peristaltic (Walter Becker SA)

Pump Speed	Sampling Point	MeBI %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10 ^ 5)	AP (pH units)	CAP (x 10 ^ 5)
50 rpm	Before	91	58	0.120	1.58	1.9	2.15	8
(72 l/min)	After	91	62	***0.133***	1.80	2.6	2.26	11
25 rpm	Before	93	34	0.118	1.86	4.0	2.35	14
(33 l/min)	After	92	32	0.139	1.84	1.6	2.32	11

RUN	2
DATE	17/1/95
YEAST	Castle D9 ex FV93
WORT	Steinecker
PUMPS	1.Depa diaphragm (Alfa-Laval)
	2 Bredel peristaltic (Walter Becker SA)

PUMP 2.Bredel peristaltic (Walter Becker SA)

Pump Speed	Sampling Point	MeBI %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10 ^ 5)	AP (pH units)	CAP (x 10 ^ 5)	Trehalose (g/100g)	Glycogen (g/100g)
47 rpm	Before	94	52	-	1.88	2.5	2.43	16	5.5	24.2
(63 l/min)	After	92	32	***0.155***	1.98	2.6	2.48	17	3.9	23.6
25 rpm	Before	95	62	***0.138***	1.71	2.6	2.34	13	3.5	24.0
(33 l/min)	After	92	56	0.170	1.91	2.2	2.49	17	5.0	22.3

RESULTS OF YEAST QUALITY ASSAYS FOR INITIAL PUMP TRIALS - APV LOBE PUMP

RUN	5
DATE	08/02/95
YEAST	Castle H4 ex FV89
WORT	Steinecker (Pellet Trial)
PUMPS	1.Maso sine (Aeromix)
	2.APV lobe (APV)

PUMP 2. APV lobe (APV)

Pump Speed	Sampling Point	MeBI %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10^5)	AP (pH units)	CAP (x 10^5)	Trehalose (g/100g)	Glycogen (g/100g)
40 rpm	Before	95	-	0.150	2.05	4.5	2.45	16	5.0	26.0
(16 l/min)	After	91	-	0.162	2.04	3.6	2.48	18	4.2	25.9
85 rpm	Before	90	-	0.154	2.08	4.1	2.47	16	3.7	26.8
(35 l/min)	After	90	-	0.178	2.10	2.9	2.55	18	5.7	26.2

RUN	6
DATE	15\02\95
YEAST	Castle(export) I4 ex FV82
WORT	Steinecker (Pellet Trial)
PUMPS	1.APV lobe (APV)
	2.Johnson lobe (NDE)

PUMP 1.APV lobe (APV)

Pump Speed	Sampling Point	MeBI %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10^5)	AP (pH units)	CAP (x 10^5)	Trehalose (g/100g)	Glycogen (g/100g)
40 rpm	Before	90	-	0.114	2.10	6.1	2.49	20	4.8	28.2
(16 l/min)	After	92	-	0.114	2.17	2.7	2.55	19	5.8	24.4
85 rpm	Before	90	-	0.113	2.16	4.5	2.49	19	5.2	25.0
(35 l/min)	After	91	-	0.113	2.36	4.2	2.72	27	5.4	26.3

RESULTS OF YEAST QUALITY ASSAYS FOR INITIAL PUMP TRIALS - IBEX LOBE PUMP

RUN	3
DATE	25/01/95
YEAST	Castle C12 ex FV 98
WORT	Steinecker
PUMPS	1. Ibex lobe (Alfa-laval)
	2. Depa diaphragm (Alfa-laval)

PUMP 1. Ibex lobe (Alfa-laval)

Pump Speed	Sampling Point	MeBI %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10^5)	AP (pH units)	CAP (x 10^5)	Trehalose (g/100g)	Glycogen (g/100g)
256 rpm	Before	93	60	0.132	1.87	2.1	2.35	12	4.7	25.0
(42 l/min)	After	93	57	0.125	*1.84*	*1.2*	*2.40*	*13*	3.5	24.4
108 rpm	Before	94	50	0.137	2.17	4.8	2.62	26	1.8	25.0
(8 l/min)	After	96	72	0.134	1.93	3.2	2.41	14	4.8	25.3

RUN	4
DATE	31\01\95
YEAST	LION G5 EX FV79
WORT	Huppmann
PUMPS	1.Ibex rotary lobe(Alfa-Laval)
	2.Maso sine pump (Aeromix)

PUMP 1. Ibex lobe (Alfa-laval)

Pump Speed	Sampling Point	MeBI %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10^5)	AP (pH units)	CAP (x 10^5)
256 rpm	Before	93	72	0.114	1.95	5.6	2.42	18
(42 l/min)	After	92	*50*	0.123	1.99	3.1	2.41	15
108 rpm	Before	93	48	0.112	1.94	2.0	2.36	13
(8 l/min)	After	91	31	0.126	2.01	2.3	2.45	16

RESULTS OF YEAST QUALITY ASSAYS FOR INITIAL PUMP TRIALS - JOHNSON LOBE PUMP

RUN	6
DATE	15\02\95
YEAST	Castle(export) l4 ex FV82
WORT	Steinecker (Pellet Trial)
PUMPS	1.APV lobe (APV)
	2.Johnson lobe (NDE)

PUMP 2. Johnson lobe (NDE)

Pump Speed	Sampling Point	MeBl %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10 ^ 5)	AP (pH units)	CAP (x 10 ^ 5)	Trehalose (g/100g)	Glycogen (g/100g)
80 rpm	Before	89	-	0.122	2.22	2.7	2.66	21	4.7	26.8
(18 l/min)	After	87	-	0.112	2.12	3.6	2.59	21	3.4	25.1
122 rpm	Before	93	-	0.113	2.25	2.4	2.63	22	5.9	24.5
(48 l/min)	After	89	-	0.115	2.24	3.0	2.66	24	5.1	27.0

RUN	7
DATE	08/03/95
YEAST	Castle J6 ex FV96
WORT	Huppmann
PUMPS	1.Wilden diaphragm (Flo-Mart)
	2.Johnson lobe (NDE)

PUMP 2.Johnson lobe (NDE)

Pump Speed	Sampling Point	MeBl %	Slides %	OUR (mg/min/g	Trehalose (g/100g)	Glycogen (g/100g)
80 rpm	Before	92	92	0.100	1.1	25.3
(18 l/min)	After	93	89	0.106	0.8	24.9
122 rpm	Before	91	93	0.120	1.2	25.7
(48 l/min)	After	94	90	0.121	1.0	25.1

RESULTS OF YEAST QUALITY ASSAYS FOR INITIAL PUMP TRIALS - WILFLO LOBE PUMP

RUN	9
DATE	04/04/95
YEAST	Castle M6 ex FV97
WORT	Steinecker
PUMPS	1.Scandibrew (Micro Matic) 2.Centrifugal (OCB) 3.Wilflo lobe (OCB)
PUMP	3.Wilflo lobe (OCB)

Pump Speed	Sampling Point	MeBI %	Slides %	OUR (mg/min/g cells)	Trehalose (g/100g)	Glycogen (g/100g)
243 rpm (66 l/min)	Before	93	90	0.124	2.4	21.9
	After	89	89	0.126	3.4	23.7

RESULTS OF YEAST QUALITY ASSAYS FOR INITIAL PUMP TRIALS - MASO SINE PUMP

RUN	4
DATE	31\01\95
YEAST	LION G5 EX FV79
WORT	Huppmann
PUMPS	1.Ibex rotary lobe(Alfa-Laval)
	2.Maso sine pump (Aeromix)

PUMP 2.Maso sine pump (Aeromix)

Pump Speed	Sampling Point	MeBI %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10^5)	AP (pH units)	CAP (x 10^5)
244 rpm	Before	89	55	0.119	1.98	2.8	2.38	14
(89 l/min)	After	92	52	0.117	2.03	2.8	2.46	15
713 rpm	Before	90	60	0.122	1.92	1.7	2.42	13
(289 l/min)	After	88	46	0.117	1.99	2.8	2.49	16

RUN	5
DATE	08/02/95
YEAST	Castle H4 ex FV89
WORT	Steinecker (Pellet Trial)
PUMPS	1.Maso sine (Aeromix)
	2.APV lobe (APV)

PUMP 1. Maso sine (Aeromix)

Pump Speed	Sampling Point	MeBI %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10^5)	AP (pH units)	CAP (x 10^5)	Trehalose (g/100g)	Glycogen (g/100g)
244 rpm	Before	90	-	*0.142*	2.15	4.1	2.53	18	6.9	26.5
(89 l/min)	After	90	-	0.154	1.91	2.5	2.43	15	5.4	25.4
400 rpm	Before	94	-	0.156	2.12	5.3	2.47	16	5.7	26.0
(162 l/min)	After	91	-	0.153	2.11	3.0	2.61	21	5.6	27.0

RESULTS OF YEAST QUALITY ASSAYS FOR INITIAL PUMP TRIALS - SCANDI BREW GEAR PUMP

RUN	8
DATE	22/03/95
YEAST	Castle K7 ex FV83
WORT	Huppmann
PUMPS	1.Scandibrew pump (Micro Matic) 2.Wilden diaphragm (Flo-Mart)

PUMP 1.Scandibrew pump (Micro Matic)

Pump Speed	Sampling Point	MeBI %	Slides %	OUR (mg/min/g	Trehalose (g/100g)	Glycogen (g/100g)
Setting 4	Before	94	95	0.090	-	-
(99 l/min)	After	95	94	0.090	-	-
Setting 8	Before	91	92	0.089	-	-
(193 l/min)	After	95	93	0.087	-	-

RUN	9
DATE	04/04/95
YEAST	Castle M6 ex FV97
WORT	Steinecker
PUMPS	1.Scandibrew (Micro Matic) 2.Centrifugal (OCB) 3.Wilflo lobe (OCB)

PUMP 1.Scandibrew pump (Micro Matic)

Pump Speed	Sampling Point	MeBI %	Slides %	OUR (mg/min/g	Trehalose (g/100g)	Glycogen (g/100g)
Setting 4	Before	94	92	*0.116*	2.5	23.8
(99 l/min)	After	91	91	-	2.1	24.1
Setting 8	Before	93	89	0.113	2.1	24.0
(193 l/min)	After	92	90	0.107	3.4	24.5

RESULTS OF YEAST QUALITY ASSAYS FOR INITIAL PUMP TRIALS - DEPA DIAPHRAGM PUMP

RUN	2
DATE	17/1/95
YEAST	Castle D9 ex FV93
WORT	Steinecker
PUMPS	1.Depa diaphragm (Alfa-Laval)
	2 Bredel peristaltic (Walter Becker SA)

PUMP 1.Depa diaphragm (Alfa-Laval)

Pump Speed	Sampling Point	MeBI %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10^5)	AP (pH units)	CAP (x 10^5)	Trehalose (g/100g)	Glycogen (g/100g)
400 kPa*	Before	94	32	*0.147*	1.79	2.5	2.47	16	5.5	27.4
(44 l/min)	After	94	37	0.159	1.79	1.7	2.45	15	3.4	22.9
600 kPa*	Before	95	35	*0.159*	1.88	2.0	2.48	16	6.3	25.3
(56 l/min)	After	90	58	*0.185*	1.89	1.7	2.47	17	4.5	24.4

RUN	3
DATE	25/01/95
YEAST	Castle C12 ex FV 98
WORT	Steinecker
PUMPS	1. Ibex lobe (Alfa-laval)
	2. Depa diaphragm (Alfa-laval)

PUMP 2. Depa diaphragm (Alfa-laval)

Pump Speed	Sampling Point	MeBI %	Plates %	OUR (mg/min/g	AP(10) (pH units)	CAP(10) (x 10^5)	AP (pH units)	CAP (x 10^5)	Trehalose (g/100g)	Glycogen (g/100g)
400 kPa*	Before	98	58	*0.136*	1.89	1.9	2.40	13	4.2	24.4
(44 l/min)	After	93	60	*0.121*	1.81	1.7	2.34	11	2.5	*25.2*
600 kPa*	Before	93	57	0.126	1.86	1.5	2.40	12	*2.7*	*23.3*
(56 l/min)	After	92	55	*0.135*	1.82	3.6	2.30	11	*2.8*	-

RESULTS OF YEAST QUALITY ASSAYS FOR INITIAL PUMP TRIALS - WILDEN DIAPHRAGM PUMP

RUN	7
DATE	08/03/95
YEAST	Castle J6 ex FV96
WORT	Huppmann
PUMPS	1.Wilden diaphragm (Flo-Mart) 2.Johnson lobe (NDE)

PUMP 1.Wilden diaphragm (Flo-Mart)

Pump Speed	Sampling Point	Statistical Parameter	MeBI %	Slides %	OUR (mg/min/g	Trehalose (g/100g)	Glycogen (g/100g)
400 kPa*	Before		94	92	0.090	3.8	25.2
(19 l/min)	After		94	91	0.100	5.1	23.5
550 kPa*	Before		93	91	0.096	2.7	24.9
(42 l/min)	After		91	93	0.097	2.0	24.7

RUN	8
DATE	22/03/95
YEAST	Castle K7 ex FV83
WORT	Huppmann
PUMPS	1.Scandibrew pump (Micro Matic) 2.Wilden diaphragm (Flo-Mart)

PUMP 2.Wilden diaphragm (Flo-Mart)

Pump Speed	Sampling Point	MeBI %	Slides %	OUR (mg/min/g	Trehalose (g/100g)	Glycogen (g/100g)
400 kPa*	Before	93	94	0.092	5.0	24.0
(19 l/min)	After	93	94	0.100	4.6	22.9
550 kPa*	Before	93	94	0.089	4.5	23.4
(42 l/min)	After	93	94	0.097	4.5	24.2

RESULTS OF YEAST QUALITY ASSAYS FOR INITIAL PUMP TRIALS - FRISTAM CENTRIFUGAL PUMP

RUN	9
DATE	04/04/95
YEAST	Castle M6 ex FV97
WORT	Steinecker
PUMPS	1.Scandibrew (Micro Matic) 2.Centrifugal (OCB) 3.Wilflo lobe (OCB)
PUMP	2.Centrifugal (OCB)

Pump Speed	Sampling Point	MeBI %	Slides %	OUR (mg/min/g cells)	Trehalose (g/100g)	Glycogen (g/100g)
3000 rpm	Before	93	87	0.100	2.0	23.6
	After	92	93	0.125	2.0	23.9

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - SP40 BREDEL PERISTALTIC PUMP (RUN 1)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	92	91	183	92	2	Between Samples	7.00	3	2.33	1.04
Blue	S1A	90	92	182	91	2	Within Samples	9.00	4	2.25	
	S2B	92	95	187	94	2	Total	16.00	7		
	S2A	91	93	184	92	2					
Plates	S1B	59	57	116	58	2	Between Samples	1394	3	465	6.38
	S1A	59	64	123	62	2	Within Samples	292	4	73	
	S2B	37	32	69	35	2	Total	1686	7		
	S2A	44	21	65	33	2					
Oxygen	S1B	0.118	0.122	0.240	0.120	2	Between Samples	0.00058	3	0.00019	15.59
Utilisation	S1A	0.133	-	0.133	0.133	1	Within Samples	0.00004	3	0.00001	
Rate	S2B	0.114	0.121	0.235	0.118	2	Total	0.00061	6		
	S2A	0.137	0.140	0.277	0.139	2					
Spontaneous	S1B	1.64	1.51	3.15	1.58	2	Between Samples	0.100	3	0.033	7.66
Acidification	S1A	1.81	1.78	3.59	1.80	2	Within Samples	0.017	4	0.004	
Power	S2B	1.86	1.85	3.71	1.86	2	Total	0.117	7		
	S2A	1.90	1.77	3.67	1.84	2					
Cumulative	S1B	1E-05	3E-05	4E-05	2E-05	2	Between Samples	7E-10	3	2E-10	1.03
Spontaneous	S1A	1E-05	4E-05	5E-05	3E-05	2	Within Samples	9E-10	4	2E-10	
Acidification	S2B	3E-05	5E-05	8E-05	4E-05	2	Total	2E-09	7		
Power	S2A	2E-05	8E-06	3E-05	2E-05	2					
Acidification	S1B	2.15	2.14	4.29	2.15	2	Between Samples	0.047	3	0.016	6.95
Power	S1A	2.26	2.26	4.52	2.26	2	Within Samples	0.009	4	0.002	
	S2B	2.36	2.33	4.69	2.35	2	Total	0.056	7		
	S2A	2.38	2.25	4.63	2.32	2					
Cumulative	S1B	8E-05	9E-05	2E-04	8E-05	2	Between Samples	4E-09	3	1E-09	3.40
Acidification	S1A	9E-05	1E-04	2E-04	1E-04	2	Within Samples	1E-09	4	4E-10	
Power	S2B	1E-04	1E-04	3E-04	1E-04	2	Total	5E-09	7		
	S2A	1E-04	9E-05	2E-04	1E-04	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - SP40 BREDEL PERISTALTIC PUMP (RUN 1) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Oxygen Utilisation Rate	S1B	0.118	0.122	0.240	0.120	2	Between Samples	0.00011	1	0.00011	14.08
	S1A	0.133	-	0.133	0.133	1	Within Samples	0.00001	1	0.00001	
							Total	0.00012	2		
	S2B	0.114	0.121	0.235	0.118	2	Between Samples	0.00044	1	0.00044	30.41
	S2A	0.137	0.140	0.277	0.139	2	Within Samples	0.00003	2	0.00001	
							Total	0.00047	3		
Spontaneous Acidification Power	S1B	1.64	1.51	3.15	1.58	2	Between Samples	0.048	1	0.048	10.88
	S1A	1.81	1.78	3.59	1.80	2	Within Samples	0.009	2	0.004	
							Total	0.057	3		
	S2B	1.86	1.85	3.71	1.86	2	Between Samples	0.000	1	0.000	0.09
	S2A	1.90	1.77	3.67	1.84	2	Within Samples	0.009	2	0.004	
							Total	0.009	3		
Acidification Power	S1B	2.15	2.14	4.29	2.15	2	Between Samples	0.013	1	0.013	529.00
	S1A	2.26	2.26	4.52	2.26	2	Within Samples	0.000	2	0.000	
							Total	0.013	3		
	S2B	2.36	2.33	4.69	2.35	2	Between Samples	0.001	1	0.001	0.20
	S2A	2.38	2.25	4.63	2.32	2	Within Samples	0.009	2	0.004	
							Total	0.010	3		

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - SP40 BREDEL PERISTALTIC PUMP (RUN 2)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	94	96	190	95	2	Between Samples	13.50	3	4.50	0.50
Blue	S1A	90	94	184	92	2	Within Samples	36.00	4	9.00	
	S2B	91	97	188	94	2	Total	49.50	7		
	S2A	94	90	184	92	2					
Plates	S1B	65	58	123	62	2	Between Samples	955	3	318	1.60
	S1A	37	75	112	56	2	Within Samples	795	4	199	
	S2B	54	50	104	52	2	Total	1750	7		
	S2A	28	37	65	33	2					
Oxygen	S1B	0.114	0.138	0.252	0.126	2	Between Samples	0.00194	1	0.00194	3.56
Utilisation	S1A	0.150	0.190	0.340	0.170	2	Within Samples	0.00109	2	0.00054	
Rate	S2B	-	-	-	-	-	Total	0.00302	3		
	S2A	-	0.155	-	-	-					
Spontaneous	S1B	1.54	1.88	3.42	1.71	2	Between Samples	0.078	3	0.026	1.69
Acidification	S1A	1.89	1.92	3.81	1.91	2	Within Samples	0.061	4	0.015	
Power	S2B	1.84	1.92	3.76	1.88	2	Total	0.139	7		
	S2A	1.98	1.98	3.96	1.98	2					
Cumulative	S1B	2E-05	3E-05	5E-05	3E-05	2	Between Samples	3E-11	3	9E-12	0.10
Spontaneous	S1A	2E-05	2E-05	4E-05	2E+00	2	Within Samples	3E-10	4	8E-11	
Acidification	S2B	2E-05	3E-05	5E-05	2E-05	2	Total	4E-10	7		
Power	S2A	1E-05	4E-05	5E-05	3E-05	2					
Acidification	S1B	2.29	2.39	4.68	2.34	2	Between Samples	0.027	3	0.009	3.18
Power	S1A	2.50	2.48	4.98	2.40	2	Within Samples	0.011	4	0.003	
	S2B	2.38	2.48	4.86	2.43	2	Total	0.039	7		
	S2A	2.45	2.50	4.95	2.48	2					
Cumulative	S1B	1E-04	2E-04	3E-04	1E-04	2	Between Samples	2E-09	3	7E-10	1.27
Acidification	S1A	2E-04	2E-04	3E-04	2E+00	2	Within Samples	2E-09	4	5E-10	
Power	S2B	1E-04	2E-04	3E-04	2E-04	2	Total	4E-09	7		
	S2A	2E-04	2E-04	3E-04	2E-04	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - SP40 BREDEL PERISTALTIC PUMP (RUN 2) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Glycogen Content	S1B	23.2	24.8	48.0	24.0	2	Between Samples	4.4	3	1.5	1.40
	S1A	23.1	21.5	44.6	22.3	2	Within Samples	4.2	4	1.0	
	S2B	24.7	23.7	48.4	24.2	2	Total	8.6	7		
	S2A	24.4	22.9	47.3	23.7	2					
Trehalose Content	S1B	3.3	3.8	7.1	3.6	2	Between Samples	4.72	3	1.57	1.61
	S1A	4.2	5.7	9.9	5.0	2	Within Samples	3.92	4	0.98	
	S2B	5.1	5.8	10.9	5.5	2	Total	8.64	7		
	S2A	5.0	2.8	7.8	3.9	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - APV LOBE PUMP (RUN 5)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	95	95	190	95	2	Between Samples	37.00	3	12.33	16.44
Blue	S1A	91	92	183	92	2	Within Samples	3.00	4	0.75	
	S2B	89	90	179	90	2	Total	40.00	7		
	S2A	89	91	180	90	2					
Oxygen	S1B	0.141	0.158	0.299	0.150	2	Between Samples	0.00092	3	0.00031	5.05
Utilisation	S1A	0.163	0.161	0.324	0.162	2	Within Samples	0.00024	4	0.00006	
Rate	S2B	0.147	0.160	0.307	0.154	2	Total	0.00117	7		
	S2A	0.180	0.175	0.355	0.178	2					
Spontaneous	S1B	2.11	1.99	4.10	2.05	2	Between Samples	0.004	3	0.001	0.22
Acidification	S1A	2.03	2.05	4.08	2.04	2	Within Samples	0.024	4	0.006	
Power	S2B	1.99	2.17	4.16	2.08	2	Total	0.028	7		
	S2A	2.11	2.08	4.19	2.10	2					
Cumulative	S1B	6E-05	3E-05	9E-05	4E-05	2	Between Samples	3E-10	3	9E-11	0.75
Spontaneous	S1A	4E-05	4E-05	7E-05	4E-05	2	Within Samples	5E-10	4	1E-10	
Acidification	S2B	5E-05	3E-05	8E-05	4E-05	2	Total	7E-10	7		
Power	S2A	3E-05	3E-05	6E-05	3E-05	2					
Acidification	S1B	2.46	2.43	4.89	2.45	2	Between Samples	0.013	3	0.004	1.72
Power	S1A	2.46	2.50	4.96	2.48	2	Within Samples	0.010	4	0.002	
	S2B	2.40	2.53	4.93	2.47	2	Total	0.022	7		
	S2A	2.55	2.55	5.10	2.55	2					
Cumulative	S1B	2E-04	2E-04	3E-04	2E-04	2	Between Samples	8E-10	3	3E-10	5.63
Acidification	S1A	2E-04	2E-04	4E-04	2E-04	2	Within Samples	2E-10	4	5E-11	
Power	S2B	2E-04	2E-04	3E-04	2E-04	2	Total	1E-09	7		
	S2A	2E-04	2E-04	4E-04	2E-04	2					
Glycogen	S1B	25.9	26.1	52.0	26.0	2	Between Samples	0.9	3	0.3	0.23
Content	S1A	25.5	26.3	51.8	25.9	2	Within Samples	5.0	4	1.2	
	S2B	27.2	26.3	53.5	26.8	2	Total	5.8	7		
	S2A	24.7	27.6	52.3	26.2	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - APV LOBE PUMP (RUN 5) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Trehalose Content	S1B	6.0	4.1	10.1	5.1	2	Between Samples	4.61	3	1.54	1.67
	S1A	3.3	5.0	8.3	4.2	2	Within Samples	3.68	4	0.92	
	S2B	4.1	3.2	7.3	3.7	2	Total	8.29	7		
	S2A	5.5	5.7	11.2	5.6	2					

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene Blue	S1B	95	95	190	95	2	Between Samples	12.25	1	12.25	49.00
	S1A	91	92	183	92	2	Within Samples	0.50	2	0.25	
							Total	12.75	3		
	S2B	89	90	179	90	2	Between Samples	0.25	1	0.25	0.20
	S2A	89	91	180	90	2	Within Samples	2.50	2	1.25	
							Total	2.75	3		

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - APV LOBE PUMP (RUN 6)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	92	89	181	91	2	Between Samples	3.00	3	1.00	0.24
Blue	S1A	92	92	184	92	2	Within Samples	17.00	4	4.25	
	S2B	92	89	181	91	2	Total	20.00	7		
	S2A	89	93	182	91	2					
Oxygen	S1B	0.110	0.118	0.228	0.114	2	Between Samples	0.00000	3	0.00000	0.07
Utilisation	S1A	0.111	0.117	0.228	0.114	2	Within Samples	0.00006	4	0.00002	
Rate	S2B	0.114	0.112	0.226	0.113	2	Total	0.00007	7		
	S2A	0.110	0.115	0.225	0.113	2					
Spontaneous	S1B	2.05	2.15	4.20	2.10	2	Between Samples	0.077	3	0.026	2.04
Acidification	S1A	2.15	2.18	4.33	2.17	2	Within Samples	0.051	4	0.013	
Power	S2B	2.10	2.21	4.31	2.16	2	Total	0.128	7		
	S2A	2.22	2.50	4.72	2.36	2					
Cumulative	S1B	5E-05	7E-05	1E-04	6E-05	2	Between Samples	1E-09	3	4E-10	3.70
Spontaneous	S1A	2E-05	3E-05	5E-05	3E-05	2	Within Samples	4E-10	4	1E-10	
Acidification	S2B	5E-05	4E-05	9E-05	4E-05	2	Total	2E-09	7		
Power	S2A	4E-05	4E-05	8E-05	4E-05	2					
Acidification	S1B	2.46	2.52	4.98	2.49	2	Between Samples	0.072	3	0.024	5.66
Power	S1A	2.50	2.60	5.10	2.55	2	Within Samples	0.017	4	0.004	
	S2B	2.47	2.50	4.97	2.49	2	Total	0.089	7		
	S2A	2.65	2.79	5.44	2.72	2					
Cumulative	S1B	2E-04	2E-04	4E-04	2E-04	2	Between Samples	8E-09	3	3E-09	3.01
Acidification	S1A	2E-04	2E-04	4E-04	2E-04	2	Within Samples	3E-09	4	8E-10	
Power	S2B	2E-04	2E-04	4E-04	2E-04	2	Total	1E-08	7		
	S2A	2E-04	3E-04	5E-04	3E-04	2					
Glycogen	S1B	28.4	27.9	56.3	28.2	2	Between Samples	16.6	3	5.5	9.30
Content	S1A	25.3	23.4	48.7	24.4	2	Within Samples	2.4	4	0.6	
	S2B	24.6	25.5	50.1	25.1	2	Total	19.0	7		
	S2A	26.4	26.1	52.5	26.3	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - APV LOBE PUMP (RUN 6) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Trehalose Content	S1B	4.6	5.1	9.7	4.9	2	Between Samples	1.03	3	0.34	0.75
	S1A	6.4	5.3	11.7	5.9	2	Within Samples	1.84	4	0.46	
	S2B	4.5	5.9	10.4	5.2	2	Total	2.87	7		
	S2A	5.1	5.6	10.7	5.4	2					

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Glycogen Content	S1B	28.4	27.9	56.3	28.2	2	Between Samples	14.4	1	14.4	14.96
	S1A	25.3	23.4	48.7	24.4	2	Within Samples	1.9	2	1.0	
							Total	16.4	3		
	S2B	24.6	25.5	50.1	25.1	2	Between Samples	1.4	1	1.4	6.40
	S2A	26.4	26.1	52.5	26.3	2	Within Samples	0.5	2	0.2	
							Total	1.9	3		

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - IBEX LOBE PUMP (RUN 3)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	90	96	186	93	2	Between Samples	12.00	3	4.00	0.44
Blue	S1A	95	91	186	93	2	Within Samples	36.00	4	9.00	
	S2B	93	95	188	94	2	Total	48.00	7		
	S2A	94	98	192	96	2					
Plates	S1B	80	40	120	60	2	Between Samples	481	3	160	0.25
	S1A	72	42	114	57	2	Within Samples	2555	4	639	
	S2B	60	40	100	50	2	Total	3036	7		
	S2A	48	95	143	72	2					
Oxygen	S1B	0.146	0.117	0.263	0.132	2	Between Samples	0.00015	3	0.00005	0.35
Utilisation	S1A	0.119	0.131	0.250	0.125	2	Within Samples	0.00055	4	0.00014	
Rate	S2B	0.132	0.141	0.273	0.137	2	Total	0.00070	7		
	S2A	0.137	0.131	0.268	0.134	2					
Spontaneous	S1B	1.80	1.94	3.74	1.87	2	Between Samples	0.120	3	0.040	2.01
Acidification	S1A	-	1.84	1.84	1.84	1	Within Samples	0.060	3	0.020	
Power	S2B	2.04	2.30	4.34	2.17	2	Total	0.180	6		
	S2A	1.84	2.02	3.86	1.93	2					
Cumulative	S1B	2E-05	2E-05	4E-05	2E-05	2	Between Samples	1E-09	3	4E-10	10.34
Spontaneous	S1A	-	1E-05	1E-05	1E-05	1	Within Samples	1E-10	3	4E-11	
Acidification	S2B	5E-05	4E-05	1E-04	5E-05	2	Total	1E-09	6		
Power	S2A	3E-05	3E-05	6E-05	3E-05	2					
Acidification	S1B	2.34	2.35	4.69	2.35	2	Between Samples	0.082	3	0.027	1.15
Power	S1A	-	2.40	2.40	2.40	1	Within Samples	0.072	3	0.024	
	S2B	2.43	2.80	5.23	2.62	2	Total	0.154	6		
	S2A	2.37	2.45	4.82	2.41	2					
Cumulative	S1B	1E-04	1E-04	2E-04	1E-04	2	Between Samples	2E-08	3	8E-09	1.73
Acidification	S1A	-	1E-04	1E-04	1E-04	1	Within Samples	1E-08	3	4E-09	
Power	S2B	2E-04	3E-04	5E-04	3E-04	2	Total	4E-08	6		
	S2A	1E-04	1E-04	3E-04	1E-04	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - IBEX LOBE PUMP (RUN 3) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Glycogen Content	S1B	24.1	25.9	50.0	25.0	2	Between Samples	1.0	3	0.3	0.42
	S1A	24.2	24.5	48.7	24.4	2	Within Samples	3.3	4	0.8	
	S2B	24.1	25.9	50.0	25.0	2	Total	4.4	7		
	S2A	25.5	25.2	50.7	25.4	2					
Trehalose Content	S1B	3.8	5.7	9.5	4.8	2	Between Samples	11.71	3	3.90	2.84
	S1A	3.9	3.1	7.0	3.5	2	Within Samples	5.49	4	1.37	
	S2B	1.2	2.4	3.6	1.8	2	Total	17.20	7		
	S2A	5.9	3.6	9.5	4.8	2					

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Cumulative Spontaneous Acidification Power	S1B	2E-05	2E-05	4E-05	2E-05	2	Between Samples	5E-11	1	5E-11	3.00
	S1A	-	1E-05	1E-05	1E-05	1	Within Samples	2E-11	1	2E-11	
							Total	7E-11	2		
	S2B	5E-05	4E-05	1E-04	5E-05	2	Between Samples	2E-10	1	2E-10	5.31
	S2A	3E-05	3E-05	6E-05	3E-05	2	Within Samples	9E-11	2	4E-11	
							Total	3E-10	3		

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - IBEX LOBE PUMP (RUN 4)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	93	93	186	93	2	Between Samples	7.00	3	2.33	1.04
Blue	S1A	90	94	184	92	2	Within Samples	9.00	4	2.25	
	S2B	92	93	185	93	2	Total	16.00	7		
	S2A	91	90	181	91	2					
Plates	S1B	100	43	143	72	2	Between Samples	1659	3	553	0.76
	S1A	-	50	50	50	1	Within Samples	2177	3	726	
	S2B	64	31	95	48	2	Total	3836	6		
	S2A	33	29	62	31	2					
Oxygen	S1B	0.109	0.118	0.227	0.114	2	Between Samples	0.00026	3	0.00009	0.77
Utilisation	S1A	0.121	0.124	0.245	0.123	2	Within Samples	0.00046	4	0.00011	
Rate	S2B	0.107	0.117	0.224	0.112	2	Total	0.00072	7		
	S2A	0.112	0.139	0.251	0.126	2					
Spontaneous	S1B	1.90	2.00	3.90	1.95	2	Between Samples	0.006	3	0.002	0.37
Acidification	S1A	1.96	2.01	3.97	1.99	2	Within Samples	0.023	4	0.006	
Power	S2B	1.85	2.03	3.88	1.94	2	Total	0.029	7		
	S2A	2.02	2.00	4.02	2.01	2					
Cumulative	S1B	5E-05	6E-05	1E-04	6E-05	2	Between Samples	2E-09	3	5E-10	9.02
Spontaneous	S1A	2E-05	4E-05	6E-05	3E-05	2	Within Samples	2E-10	4	6E-11	
Acidification	S2B	1E-05	3E-05	4E-05	2E-05	2	Total	2E-09	7		
Power	S2A	3E-05	2E-05	5E-05	2E-05	2					
Acidification	S1B	2.43	2.40	4.83	2.42	2	Between Samples	0.007	3	0.002	0.84
Power	S1A	2.40	2.41	4.81	2.41	2	Within Samples	0.012	4	0.003	
	S2B	2.30	2.42	4.72	2.36	2	Total	0.019	7		
	S2A	2.40	2.49	4.89	2.45	2					
Cumulative	S1B	2E-04	2E-04	4E-04	2E-04	2	Between Samples	3E-09	3	9E-10	3.14
Acidification	S1A	1E-04	2E-04	3E-04	2E-04	2	Within Samples	1E-09	4	3E-10	
Power	S2B	1E-04	1E-04	3E-04	1E-04	2	Total	4E-09	7		
	S2A	1E-04	2E-04	3E-04	2E-04	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - IBEX LOBE PUMP (RUN 4) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Cumulative Spontaneous Acidification Power	S1B	5E-05	6E-05	1E-04	6E-05	2	Between Samples	6E-10	1	6E-10	10.54
	S1A	2E-05	4E-05	6E-05	3E-05	2	Within Samples	1E-10	2	6E-11	
							Total	7E-10	3		
	S2B	1E-05	3E-05	4E-05	2E-05	2	Between Samples	9E-12	1	9E-12	0.15
	S2A	3E-05	2E-05	5E-05	2E-05	2	Within Samples	1E-10	2	6E-11	
							Total	1E-10	3		

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - JOHNSON LOBE PUMP (RUN 6)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	94	92	186	93	2	Between Samples	34.38	3	11.46	1.67
Blue	S1A	89	90	179	90	2	Within Samples	27.50	4	6.88	
	S2B	88	89	177	89	2	Total	61.88	7		
	S2A	91	84	175	88	2					
Oxygen	S1B	0.110	0.116	0.226	0.113	2	Between Samples	0.00012	3	0.00004	2.55
Utilisation	S1A	0.117	0.112	0.229	0.115	2	Within Samples	0.00006	4	0.00002	
Rate	S2B	0.118	0.126	0.244	0.122	2	Total	0.00019	7		
	S2A	0.111	0.113	0.224	0.112	2					
Spontaneous	S1B	2.25	2.24	4.49	2.25	2	Between Samples	0.021	3	0.007	2.26
Acidification	S1A	2.18	2.29	4.47	2.24	2	Within Samples	0.013	4	0.003	
Power	S2B	2.23	2.20	4.43	2.22	2	Total	0.034	7		
	S2A	2.17	2.06	4.23	2.12	2					
Cumulative	S1B	3E-05	2E-05	5E-05	2E-05	2	Between Samples	1E-10	3	5E-11	0.38
Spontaneous	S1A	4E-05	2E-05	6E-05	3E-05	2	Within Samples	5E-10	4	1E-10	
Acidification	S2B	3E-05	2E-05	5E-05	3E-05	2	Total	7E-10	7		
Power	S2A	4E-05	3E-05	7E-05	4E-05	2					
Acidification	S1B	2.60	2.66	5.26	2.63	2	Between Samples	0.007	3	0.002	1.69
Power	S1A	2.64	2.68	5.32	2.66	2	Within Samples	0.005	4	0.001	
	S2B	2.68	2.64	5.32	2.66	2	Total	0.012	7		
	S2A	2.62	2.56	5.18	2.59	2					
Cumulative	S1B	2E-04	2E-04	4E-04	2E-04	2	Between Samples	1E-09	3	5E-10	1.86
Acidification	S1A	2E-04	2E-04	5E-04	2E-04	2	Within Samples	1E-09	4	2E-10	
Power	S2B	2E-04	2E-04	4E-04	2E-04	2	Total	2E-09	7		
	S2A	2E-04	2E-04	4E-04	2E-04	2					
Glycogen	S1B	22.6	26.3	48.9	24.5	2	Between Samples	9.3	3	3.1	1.41
Content	S1A	27.3	26.7	54.0	27.0	2	Within Samples	8.8	4	2.2	
	S2B	27.0	26.5	53.5	26.8	2	Total	18.1	7		
	S2A	24.2	26.0	50.2	25.1	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - JOHNSON LOBE PUMP (RUN 6) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Trehalose	S1B	6.4	5.5	11.9	6.0	2	Between Samples	7.04	3	2.35	0.90
Content	S1A	5.5	4.7	10.2	5.1	2	Within Samples	10.45	4	2.61	
	S2B	3.5	5.9	9.4	4.7	2	Total	17.49	7		
	S2A	5.2	1.5	6.7	3.4	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - JOHNSON LOBE PUMP (RUN 7)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	93	92	185	93	2	Between Samples	12.50	3	4.17	2.38
Blue	S1A	93	93	186	93	2	Within Samples	7.00	4	1.75	
	S2B	92	90	182	91	2	Total	19.50	7		
	S2A	96	93	189	95	2					
Slide	S1B	94	91	185	93	2	Between Samples	24.38	3	8.13	1.27
Counts	S1A	91	87	178	89	2	Within Samples	25.50	4	6.38	
	S2B	93	94	187	94	2	Total	49.88	7		
	S2A	88	93	181	91	2					
Oxygen	S1B	0.098	0.102	0.200	0.100	2	Between Samples	0.00063	3	0.00021	3.53
Utilisation	S1A	0.109	0.103	0.212	0.106	2	Within Samples	0.00024	4	0.00006	
Rate	S2B	0.110	0.130	0.240	0.120	2	Total	0.00087	7		
	S2A	0.123	0.118	0.241	0.121	2					
Glycogen	S1B	25.1	25.6	50.7	25.4	2	Between Samples	0.9	3	0.3	0.52
Content	S1A	25.3	24.6	49.9	25.0	2	Within Samples	2.2	4	0.6	
	S2B	25.7	26.0	51.7	25.8	2	Total	3.1	7		
	S2A	24.2	26.1	50.3	25.2	2					
Trehalose	S1B	1.7	0.5	2.2	1.1	2	Between Samples	0.93	3	0.31	0.46
Content	S1A	1.5	2.2	3.7	1.9	2	Within Samples	2.69	4	0.67	
	S2B	0.5	2.0	2.5	1.3	2	Total	3.63	7		
	S2A	0.4	1.5	1.9	1.0	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - WILFLO LOBE PUMP (RUN 9)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene Blue	S1B	95	92	187	94	2	Between Samples	20.25	1	20.25	3.24
	S1A	87	91	178	89	2	Within Samples	12.50	2	6.25	
							Total	32.75	3		
Slide Counts	S1B	88	92	180	90	2	Between Samples	2.25	1	2.25	0.53
	S1A	89	88	177	89	2	Within Samples	8.50	2	4.25	
							Total	10.75	3		
Oxygen Utilisation Rate	S1B	0.139	0.109	0.248	0.124	2	Between Samples	0.00000	1	0.00000	0.01
	S1A	0.127	0.124	0.251	0.126	2	Within Samples	0.00045	2	0.00023	
							Total	0.00046	3		
Glycogen Content	S1B	21.6	22.2	43.8	21.9	2	Between Samples	3.1	1	3.1	33.11
	S1A	23.7	23.6	47.3	23.7	2	Within Samples	0.2	2	0.1	
							Total	3.2	3		
Trehalose Content	S1B	1.3	3.4	4.7	2.4	2	Between Samples	1.2	1	1.2	0.86
	S1A	4.0	2.9	6.9	3.5	2	Within Samples	2.8	2	1.4	
							Total	4.0	3		

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - MASO SINE PUMP (RUN 4)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	90	88	178	89	2	Between Samples	18.38	3	6.13	2.13
Blue	S1A	91	92	183	92	2	Within Samples	11.50	4	2.88	
	S2B	92	89	181	91	2	Total	29.88	7		
	S2A	89	86	175	88	2					
Plates	S1B	53	57	110	55	2	Between Samples	191	3	64	0.21
	S1A	38	67	105	53	2	Within Samples	1191	4	298	
	S2B	47	72	119	60	2	Total	1382	7		
	S2A	61	31	92	46	2					
Oxygen	S1B	0.114	0.124	0.238	0.119	2	Between Samples	0.00003	3	0.00001	0.18
Utilisation	S1A	0.110	0.124	0.234	0.117	2	Within Samples	0.00017	3	0.00006	
Rate	S2B	0.125	0.119	0.244	0.122	2	Total	0.00020	6		
	S2A	0.117	-	0.117	0.117	1					
Spontaneous	S1B	1.96	1.99	3.95	1.98	2	Between Samples	0.012	3	0.004	1.69
Acidification	S1A	2.04	2.01	4.05	2.03	2	Within Samples	0.010	4	0.002	
Power	S2B	1.90	1.93	3.83	1.92	2	Total	0.022	7		
	S2A	2.05	1.92	3.97	1.99	2					
Cumulative	S1B	3E-05	3E-05	6E-05	3E-05	2	Between Samples	2E-10	3	6E-11	2.58
Spontaneous	S1A	3E-05	3E-05	6E-05	3E-05	2	Within Samples	1E-10	4	3E-11	
Acidification	S2B	2E-05	2E-05	3E-05	2E-05	2	Total	3E-10	7		
Power	S2A	3E-05	2E-05	6E-05	3E-05	2					
Acidification	S1B	2.35	2.40	4.75	2.38	2	Between Samples	0.015	3	0.005	6.71
Power	S1A	2.47	2.44	4.91	2.46	2	Within Samples	0.003	4	0.001	
	S2B	2.40	2.43	4.83	2.42	2	Total	0.018	7		
	S2A	2.51	2.47	4.98	2.49	2					
Cumulative	S1B	1E-04	1E-04	3E-04	1E-04	2	Between Samples	9E-10	3	3E-10	3.63
Acidification	S1A	2E-04	2E-04	3E-04	2E-04	2	Within Samples	3E-10	4	9E-11	
Power	S2B	1E-04	1E-04	3E-04	1E-04	2	Total	1E-09	7		
	S2A	2E-04	1E-04	3E-04	2E-04	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - MASO SINE PUMP (RUN 4) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Acidification Power	S1B	2.35	2.40	4.75	2.38	2	Between Samples	0.006	1	0.006	7.53
	S1A	2.47	2.44	4.91	2.46	2	Within Samples	0.002	2	0.001	
							Total	0.008	3		
	S2B	2.40	2.43	4.83	2.42	2	Between Samples	0.006	1	0.006	9.00
	S2A	2.51	2.47	4.98	2.49	2	Within Samples	0.001	2	0.001	
							Total	0.007	3		

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - MASO SINE PUMP (RUN 5)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	93	87	180	90	2	Between Samples	27.38	3	9.13	1.28
Blue	S1A	88	92	180	90	2	Within Samples	28.50	4	7.13	
	S2B	95	94	189	95	2	Total	55.88	7		
	S2A	90	92	182	91	2					
Oxygen	S1B	-	0.142	0.142	0.142	1	Between Samples	0.00014	3	0.00005	2.23
Utilisation	S1A	0.159	0.149	0.308	0.154	2	Within Samples	0.00006	3	0.00002	
Rate	S2B	0.156	0.156	0.312	0.156	2	Total	0.00020	6		
	S2A	0.150	0.155	0.305	0.153	2					
Spontaneous	S1B	2.20	2.10	4.30	2.15	2	Between Samples	0.075	3	0.025	3.72
Acidification	S1A	1.85	1.96	3.81	1.91	2	Within Samples	0.027	4	0.007	
Power	S2B	2.20	2.04	4.24	2.12	2	Total	0.102	7		
	S2A	2.15	2.07	4.22	2.11	2					
Cumulative	S1B	3E-05	5E-05	8E-05	4E-05	2	Between Samples	9E-10	3	3E-10	7.03
Spontaneous	S1A	3E-05	2E-05	5E-05	3E-05	2	Within Samples	2E-10	4	4E-11	
Acidification	S2B	5E-05	5E-05	1E-04	5E-05	2	Total	1E-09	7		
Power	S2A	3E-05	3E-05	6E-05	3E-05	2					
Acidification	S1B	2.54	2.52	5.06	2.53	2	Between Samples	0.038	3	0.013	16.45
Power	S1A	2.41	2.45	4.86	2.43	2	Within Samples	0.003	4	0.001	
	S2B	2.49	2.44	4.93	2.47	2	Total	0.041	7		
	S2A	2.59	2.63	5.22	2.61	2					
Cumulative	S1B	2E-04	2E-04	4E-04	2E-04	2	Between Samples	4E-09	3	1E-09	7.03
Acidification	S1A	1E-04	1E-04	3E-04	1E-04	2	Within Samples	8E-10	4	2E-10	
Power	S2B	2E-04	2E-04	3E-04	2E-04	2	Total	5E-09	7		
	S2A	2E-04	2E-04	4E-04	2E-04	2					
Glycogen	S1B	25.4	27.6	53.0	26.5	2	Between Samples	2.7	3	0.9	1.22
Content	S1A	25.2	25.6	50.8	25.4	2	Within Samples	3.0	4	0.7	
	S2B	25.5	26.4	51.9	26.0	2	Total	5.7	7		
	S2A	26.8	27.1	53.9	27.0	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - MASO SINE PUMP (RUN 5) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Trehalose Content	S1B	7.7	6.2	13.9	7.0	2	Between Samples	3.01	3	1.00	2.08
	S1A	5.5	5.3	10.8	5.4	2	Within Samples	1.93	4	0.48	
	S2B	6.2	5.1	11.3	5.7	2	Total	4.94	7		
	S2A	5.9	5.3	11.2	5.6	2					
Cumulative Spontaneous Acidification Power	S1B	3E-05	5E-05	8E-05	4E-05	2	Between Samples	3E-10	1	3E-10	3.18
	S1A	3E-05	2E-05	5E-05	3E-05	2	Within Samples	2E-10	2	8E-11	
							Total	4E-10	3		
	S2B	5E-05	5E-05	1E-04	5E-05	2	Between Samples	5E-10	1	5E-10	86.84
	S2A	3E-05	3E-05	6E-05	3E-05	2	Within Samples	1E-11	2	6E-12	
							Total	5E-10	3		
Acidification Power	S1B	2.54	2.52	5.06	2.53	2	Between Samples	0.010	1	0.010	20.00
	S1A	2.41	2.45	4.86	2.43	2	Within Samples	0.001	2	0.000	
							Total	0.011	3		
	S2B	2.49	2.44	4.93	2.47	2	Between Samples	0.021	1	0.021	20.51
	S2A	2.59	2.63	5.22	2.61	2	Within Samples	0.002	2	0.001	
							Total	0.023	3		
Cumulative Acidification Power	S1B	2E-04	2E-04	4E-04	2E-04	2	Between Samples	8E-10	1	8E-10	3.23
	S1A	1E-04	1E-04	3E-04	1E-04	2	Within Samples	5E-10	2	2E-10	
							Total	1E-09	3		
	S2B	2E-04	2E-04	3E-04	2E-04	2	Between Samples	2E-09	1	2E-09	14.31
	S2A	2E-04	2E-04	4E-04	2E-04	2	Within Samples	3E-10	2	2E-10	
							Total	3E-09	3		

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - SCANDI BREW GREAR PUMP (RUN 8)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	95	93	188	94	2	Between Samples	16.38	3	5.46	3.36
Blue	S1A	95	95	190	95	2	Within Samples	6.50	4	1.63	
	S2B	90	93	183	92	2	Total	22.88	7		
	S2A	95	95	190	95	2					
Slide	S1B	94	96	190	95	2	Between Samples	13.38	3	4.46	2.10
Counts	S1A	94	94	188	94	2	Within Samples	8.50	4	2.13	
	S2B	90	93	183	92	2	Total	21.88	7		
	S2A	94	92	186	93	2					
Oxygen	S1B	0.097	0.082	0.179	0.090	2	Between Samples	0.00001	3	0.00000	0.07
Utilisation	S1A	0.094	0.085	0.179	0.090	2	Within Samples	0.00017	4	0.00004	
Rate	S2B	0.086	0.091	0.177	0.089	2	Total	0.00018	7		
	S2A	0.088	0.086	0.174	0.087	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - SCANDI BREW GREAR PUMP (RUN 9)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	93	96	189	95	2	Between Samples	13.38	3	4.46	1.70
Blue	S1A	92	90	182	91	2	Within Samples	10.50	4	2.63	
	S2B	94	92	186	93	2	Total	23.88	7		
	S2A	91	93	184	92	2					
Slide	S1B	93	90	183	92	2	Between Samples	7.00	3	2.33	0.12
Counts	S1A	90	92	182	91	2	Within Samples	81.00	4	20.25	
	S2B	94	84	178	89	2	Total	88.00	7		
	S2A	94	87	181	91	2					
Oxygen	S1B	-	0.116	0.116	0.116	-	Between Samples	0.00004	1	0.00004	1.90
Utilisation	S1A	-	-	-	-	-	Within Samples	0.00004	2	0.00002	
Rate	S2B	0.109	0.117	0.226	0.113	2	Total	0.00009	3		
	S2A	0.104	0.109	0.213	0.107	2					
Glycogen	S1B	24.5	23.2	47.7	23.9	2	Between Samples	0.5	3	0.2	0.07
Content	S1A	25.6	22.6	48.2	24.1	2	Within Samples	9.2	4	2.3	
	S2B	22.7	25.3	48.0	24.0	2	Total	9.7	7		
	S2A	24.0	25.0	49.0	24.5	2					
Trehalose	S1B	1.1	4.0	5.1	2.6	2	Between Samples	2.3	3	0.8	0.65
Content	S1A	2.6	1.7	4.3	2.2	2	Within Samples	4.6	4	1.2	
	S2B	2.0	2.1	4.1	2.1	2	Total	6.9	7		
	S2A	3.5	3.3	6.8	3.4	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - DEPA DIAPHRAGM PUMP (RUN 2)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	94	94	188	94	2	Between Samples	33.00	3	11.00	3.38
Blue	S1A	94	94	188	94	2	Within Samples	13.00	4	3.25	
	S2B	94	95	189	95	2	Total	46.00	7		
	S2A	92	87	179	90	2					
Plates	S1B	30	35	65	33	2	Between Samples	815	3	272	10.15
	S1A	38	36	74	37	2	Within Samples	107	4	27	
	S2B	30	41	71	36	2	Total	922	7		
	S2A	54	62	116	58	2					
Oxygen	S1B	-	0.147	0.147	0.147	1	Between Samples	0.00078	3	0.00026	0.51
Utilisation	S1A	0.175	0.143	0.318	0.159	2	Within Samples	0.00051	1	0.00051	
Rate	S2B	-	0.159	0.159	0.159	1	Total	0.00129	4		
	S2A	0.185	-	0.185	0.185	1					
Spontaneous	S1B	1.74	1.84	3.58	1.79	2	Between Samples	0.018	3	0.006	1.64
Acidification	S1A	1.85	1.73	3.58	1.79	2	Within Samples	0.015	4	0.004	
Power	S2B	1.85	1.91	3.76	1.88	2	Total	0.033	7		
	S2A	1.87	1.91	3.78	1.89	2					
Cumulative	S1B	2E-05	3E-05	5E-05	2E-05	2	Between Samples	8E-11	3	3E-11	6.37
Spontaneous	S1A	2E-05	1E-05	3E-05	2E-05	2	Within Samples	2E-11	4	4E-12	
Acidification	S2B	2E-05	2E-05	4E-05	2E-05	2	Total	9E-11	7		
Power	S2A	2E-05	2E-05	3E-05	2E-05	2					
Acidification	S1B	2.47	2.46	4.93	2.47	2	Between Samples	0.001	3	0.000	0.13
Power	S1A	2.48	2.42	4.90	2.45	2	Within Samples	0.006	4	0.002	
	S2B	2.46	2.49	4.95	2.48	2	Total	0.007	7		
	S2A	2.42	2.51	4.93	2.47	2					
Cumulative	S1B	2E-04	2E-04	3E-04	2E-04	2	Between Samples	4E-10	3	1E-10	0.60
Acidification	S1A	2E-04	1E-04	3E-04	1E-04	2	Within Samples	8E-10	4	2E-10	
Power	S2B	2E-04	2E-04	3E-04	2E-04	2	Total	1E-09	7		
	S2A	2E-04	2E-04	3E-04	2E-04	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - DEPA DIAPHRAGM PUMP (RUN 2) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Glycogen Content	S1B	27.7	27.2	54.9	27.5	2	Between Samples	21.6	3	7.2	7.11
	S1A	22.0	23.8	45.8	22.9	2	Within Samples	4.0	4	1.0	
	S2B	25.8	24.8	50.6	25.3	2	Total	25.7	7		
	S2A	23.5	25.4	48.9	24.5	2					
Trehalose Content	S1B	6.2	4.7	10.9	5.5	2	Between Samples	9.71	3	3.24	1.95
	S1A	3.1	3.7	6.8	3.4	2	Within Samples	6.63	4	1.66	
	S2B	7.8	4.9	12.7	6.4	2	Total	16.35	7		
	S2A	5.2	3.7	8.9	4.5	2					

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Plates	S1B	30	35	65	33	2	Between Samples	20.25	1	20.25	2.79
	S1A	38	36	74	37	2	Within Samples	14.50	2	7.25	
							Total	34.75	3		
	S2B	30	41	71	36	2	Between Samples	506.25	1	506.25	10.95
	S2A	54	62	116	58	2	Within Samples	92.50	2	46.25	
							Total	598.75	3		
Glycogen Content	S1B	27.7	27.2	54.9	27.5	2	Between Samples	20.7	1	20.7	23.73
	S1A	22.0	23.8	45.8	22.9	2	Within Samples	1.7	2	0.9	
							Total	22.4	3		
	S2B	25.8	24.8	50.6	25.3	2	Between Samples	0.7	1	0.7	0.63
	S2A	23.5	25.4	48.9	24.5	2	Within Samples	2.3	2	1.2	
							Total	3.0	3		

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - DEPA DIAPHRAGM PUMP (RUN 3)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	96	100	196	98	2	Between Samples	50.50	3	16.83	3.21
Blue	S1A	95	91	186	93	2	Within Samples	21.00	4	5.25	
	S2B	93	92	185	93	2	Total	71.50	7		
	S2A	93	90	183	92	2					
Plates	S1B	70	45	115	58	2	Between Samples	21	3	7	0.03
	S1A	52	68	120	60	2	Within Samples	929	4	232	
	S2B	59	55	114	57	2	Total	950	7		
	S2A	71	40	111	56	2					
Oxygen	S1B	-	0.136	0.136	0.136	1	Between Samples	0.00017	3	0.00006	1.74
Utilisation	S1A	-	0.121	0.121	0.121	1	Within Samples	0.00003	1	0.00003	
Rate	S2B	0.130	0.122	0.252	0.126	2	Total	0.00020	4		
	S2A	-	0.135	0.135	0.135	1					
Spontaneous	S1B	1.85	1.93	3.78	1.89	2	Between Samples	0.008	3	0.003	0.89
Acidification	S1A	1.76	1.86	3.62	1.81	2	Within Samples	0.013	4	0.003	
Power	S2B	1.87	1.84	3.71	1.86	2	Total	0.021	7		
	S2A	1.77	1.86	3.63	1.82	2					
Cumulative	S1B	1E-05	2E-05	4E-05	2E-05	2	Between Samples	6E-10	3	2E-10	1.02
Spontaneous	S1A	2E-05	1E-05	3E-05	2E-05	2	Within Samples	7E-10	4	2E-10	
Acidification	S2B	2E-05	1E-05	3E-05	2E-05	2	Total	1E-09	7		
Power	S2A	2E-05	5E-05	7E-05	4E-05	2					
Acidification	S1B	2.37	2.43	4.80	2.40	2	Between Samples	0.014	3	0.005	2.58
Power	S1A	2.29	2.39	4.68	2.34	2	Within Samples	0.007	4	0.002	
	S2B	2.40	2.39	4.79	2.40	2	Total	0.021	7		
	S2A	2.29	2.31	4.60	2.30	2					
Cumulative	S1B	1E-04	1E-04	3E-04	1E-04	2	Between Samples	5E-10	3	2E-10	1.70
Acidification	S1A	1E-04	1E-04	2E-04	1E-04	2	Within Samples	4E-10	4	1E-10	
Power	S2B	1E-04	1E-04	2E-04	1E-04	2	Total	9E-10	7		
	S2A	1E-04	1E-04	2E-04	1E-04	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - DEPA DIAPHRAGM PUMP (RUN 3) (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Glycogen	S1B	24.3	24.4	48.7	24.4	2	Between Samples	0.5	1	0.5	96.33
Content	S1A	-	25.2	25.2	25.2	1	Within Samples	0.0	1	0.0	
	S2B	23.3	-	-	-	-	Total	0.5	2		
	S2A	-	-	-	-	-					
Trehalose	S1B	5.5	3.0	8.5	4.3	2	Between Samples	3.24	1	3.24	2.07
Content	S1A	2.4	2.5	4.9	2.5	2	Within Samples	3.13	2	1.56	
	S2B	2.7	-	-	-	-	Total	6.37	3		
	S2A	-	2.8	-	-	-					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - WILDEN DIAPHRAGM PUMP (RUN 7)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	94	95	189	95	2	Between Samples	14.50	3	4.83	6.44
Blue	S1A	93	95	188	94	2	Within Samples	3.00	4	0.75	
	S2B	94	93	187	94	2	Total	17.50	7		
	S2A	91	91	182	91	2					
Slide	S1B	90	95	185	93	2	Between Samples	5.00	3	1.67	0.17
Counts	S1A	92	91	183	92	2	Within Samples	39.00	4	9.75	
	S2B	93	89	182	91	2	Total	44.00	7		
	S2A	90	96	186	93	2					
Oxygen	S1B	0.087	0.093	0.180	0.090	2	Between Samples	0.00011	3	0.00004	1.46
Utilisation	S1A	0.094	0.106	0.200	0.100	2	Within Samples	0.00010	4	0.00002	
Rate	S2B	0.094	0.097	0.191	0.096	2	Total	0.00020	7		
	S2A	0.096	0.098	0.194	0.097	2					
Glycogen	S1B	24.3	26.1	50.4	25.2	2	Between Samples	3.6	3	1.2	0.67
Content	S1A	22.2	24.7	46.9	23.5	2	Within Samples	7.2	4	1.8	
	S2B	25.6	24.3	49.9	25.0	2	Total	10.8	7		
	S2A	25.6	23.8	49.4	24.7	2					
Trehalose	S1B	3.3	4.4	7.7	3.9	2	Between Samples	11.03	3	3.68	4.52
Content	S1A	4.0	6.1	10.1	5.1	2	Within Samples	3.25	4	0.81	
	S2B	3.1	2.3	5.4	2.7	2	Total	14.29	7		
	S2A	1.7	2.2	3.9	2.0	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - WILDEN DIAPHRAGM PUMP (RUN 8)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene	S1B	94	93	187	94	2	Between Samples	1.38	3	0.46	0.19
Blue	S1A	94	93	187	94	2	Within Samples	9.50	4	2.38	
	S2B	92	93	185	93	2	Total	10.88	7		
	S2A	95	91	186	93	2					
Slide	S1B	93	94	187	94	2	Between Samples	0.38	3	0.13	0.14
Counts	S1A	93	95	188	94	2	Within Samples	3.50	4	0.88	
	S2B	93	94	187	94	2	Total	3.88	7		
	S2A	94	93	187	94	2					
Oxygen	S1B	0.087	0.097	0.184	0.092	2	Between Samples	0.00015	3	0.00005	0.76
Utilisation	S1A	0.093	0.106	0.199	0.100	2	Within Samples	0.00026	4	0.00006	
Rate	S2B	0.085	0.092	0.177	0.089	2	Total	0.00040	7		
	S2A	0.090	0.104	0.194	0.097	2					
Glycogen	S1B	24.0	23.9	47.9	24.0	2	Between Samples	2.0	3	0.7	1.72
Content	S1A	22.7	23.1	45.8	22.9	2	Within Samples	1.6	4	0.4	
	S2B	22.7	24.1	46.8	23.4	2	Total	3.6	7		
	S2A	23.7	24.7	48.4	24.2	2					
Trehalose	S1B	4.2	5.8	10.0	5.0	2	Between Samples	0.33	3	0.11	0.15
Content	S1A	3.7	5.4	9.1	4.6	2	Within Samples	2.85	4	0.71	
	S2B	4.8	4.3	9.1	4.6	2	Total	3.18	7		
	S2A	4.5	4.5	9.0	4.5	2					

ANALYSIS OF VARIANCE FOR INITIAL PUMP TRIALS - FRISTAM CENTRIFUGAL PUMP (RUN 9)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Methylene Blue	S1B	92	94	186	93	2	Between Samples	1.00	1	1.00	0.50
	S1A	93	91	184	92	2	Within Samples	4.00	2	2.00	
							Total	5.00	3		
Slide Counts	S1B	85	89	174	87	2	Between Samples	36.00	1	36.00	9.00
	S1A	93	93	186	93	2	Within Samples	8.00	2	4.00	
							Total	44.00	3		
Oxygen Utilisation Rate	S1B	0.096	0.103	0.199	0.100	2	Between Samples	0.00062	1	0.00062	50.00
	S1A	0.125	0.124	0.249	0.125	2	Within Samples	0.00002	2	0.00001	
							Total	0.00065	3		
Glycogen Content	S1B	24.1	23.2	47.3	23.7	2	Between Samples	0.0	1	0.0	0.05
	S1A	23.1	24.6	47.7	23.9	2	Within Samples	1.5	2	0.8	
							Total	1.6	3		
Trehalose Content	S1B	2.7	1.4	4.1	2.1	2	Between Samples	0.0	1	0.0	0.00
	S1A	2.3	1.8	4.1	2.1	2	Within Samples	1.0	2	0.5	
							Total	1.0	3		

RESULTS OF PUMP RETRIALS - MASO SINE PUMP (RETRIAL 1)

RETRIAL	1
DATE	31/8/95
YEAST	CASTLE Y5 EX FV 112

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY Protease (absorbance)	VIABILITY Mod MeBl (% viability)	VITALITY OUR (mg/l/min/10^8 viable cells)		
240 rpm (89 l/min)	before 1	0.05	92	0.015		
	before 2	0.06	89	0.008		
	after 1	0.08	93	0.009		
	after 2	0.07	90	0.008		
404 rpm (164 l/min)	before 1	0.04	92	0.015		
	before 2	0.10	94	0.011		
	after 1	0.10	93	0.007		
	after 2	0.10	93	0.008		

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH Inc in Biomass (factor)	ATTENUATION Final Gravity (Plato)	CURVE FIT CONSTANTS Alpha Constants (Plato)	Beta Constants (1/hr)	R2 Values
240 rpm (89 l/min)	before 1	3.0	2.55	16.27	-0.0101	0.9777
	before 2	3.0	2.51	15.96	-0.0103	0.9777
	after 1	3.3	2.52	16.20	-0.0103	0.9788
	after 2	3.0	2.43	15.97	-0.0105	0.9788
404 rpm (164 l/min)	before 1	3.4	2.48	16.40	-0.0104	0.9749
	before 2	3.3	2.41	15.98	-0.0106	0.9767
	after 1	3.3	2.36	16.11	-0.0105	0.9794
	after 2	3.5	2.51	15.81	-0.0103	0.9803

BEER QUALITY (DAY 8)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
240 rpm (89 l/min)	before 1	3.94	121	15	23
	before 2	3.92	141	12	23
	after 1	3.93	139	14	24
	after 2	3.94	156	15	24
404 rpm (164 l/min)	before 1	3.95	-	16	21
	before 2	3.92	152	15	24
	after 1	3.94	148	20	24
	after 2	3.95	169	13	22

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
240 rpm (89 l/min)	before 1	3.97	81	14	42
	before 2	3.94	76	13	38
	after 1	3.95	76	13	45
	after 2	3.95	85	14	34
404 rpm (164 l/min)	before 1	3.98	68	10	36
	before 2	3.96	76	13	41
	after 1	3.97	62	14	48
	after 2	3.98	80	16	34

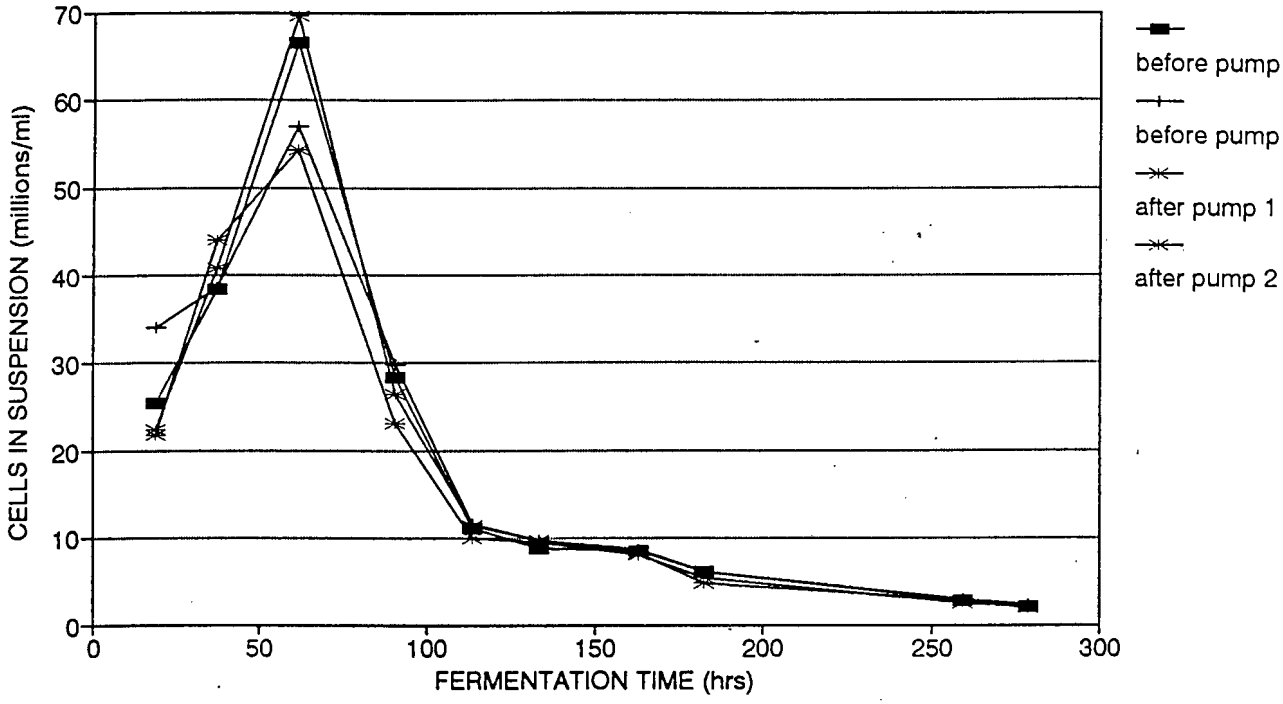

Figure E1 Cell count profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Maso sine pump on yeast quality (Pump speed = 244 rpm ≈ 89 L/min)

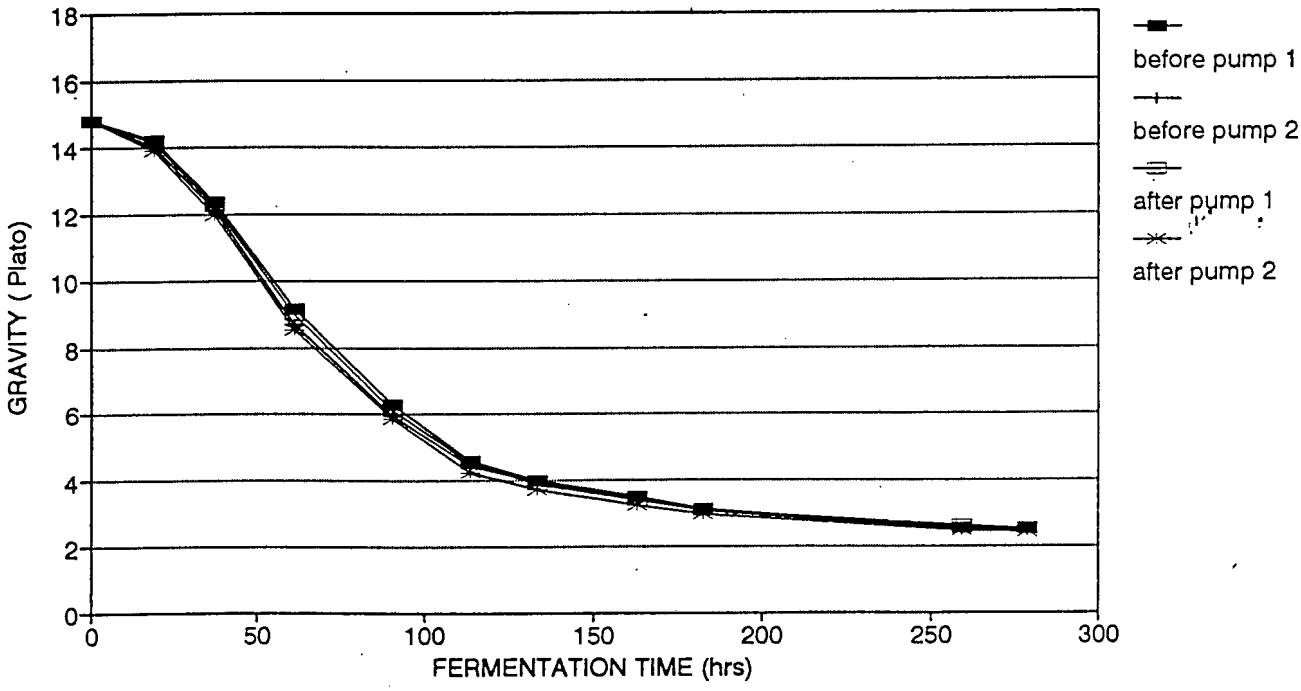

Figure E2 Attenuation profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Maso sine pump on yeast quality (Pump speed = 244 rpm ≈ 89 L/min)

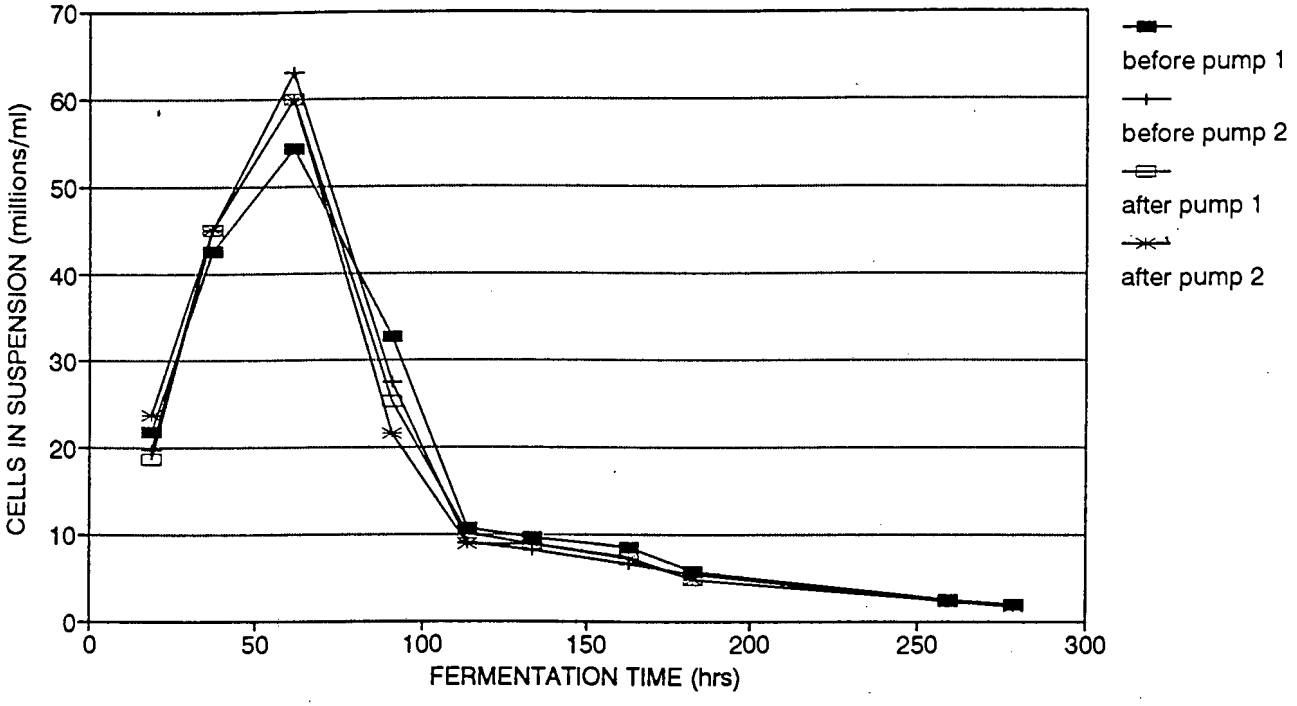

Figure E3 Cell count profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Maso sine pump on yeast quality (Pump speed = 404 rpm ≈ 164 L/min)

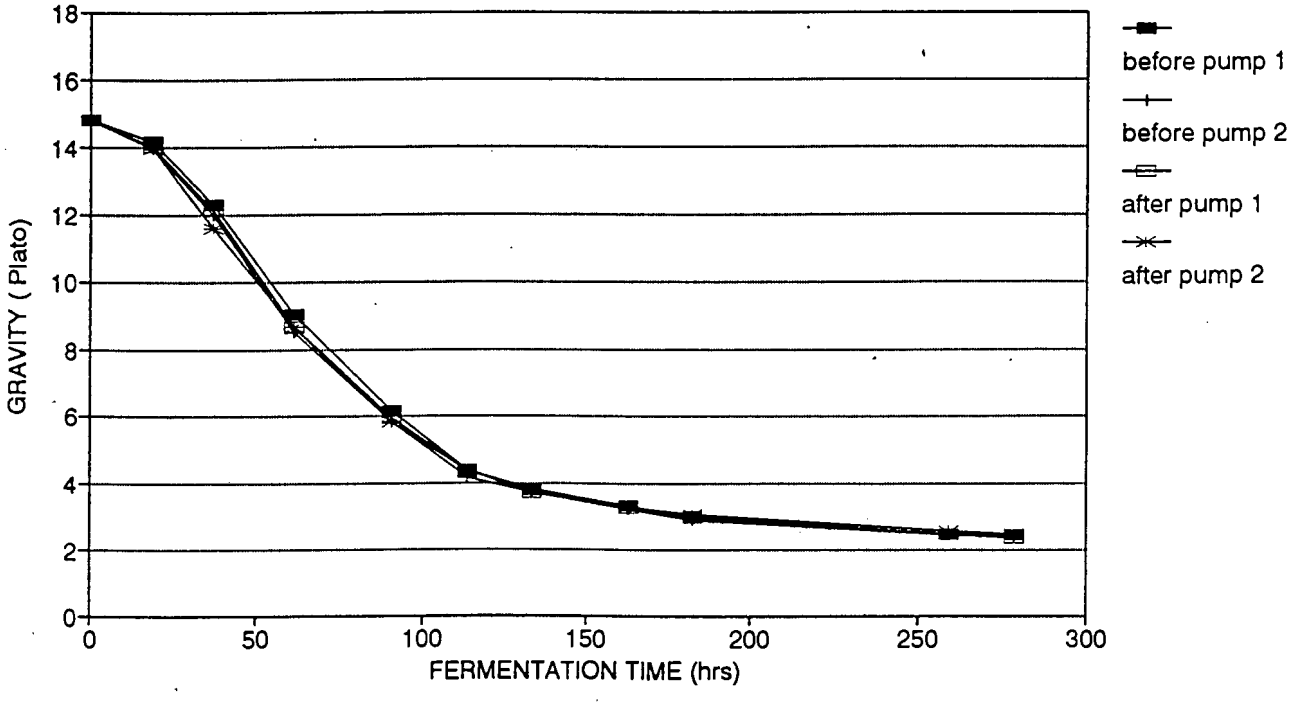

Figure E4 Attenuation profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Maso sine pump on yeast quality (Pump speed = 404 rpm ≈ 164 L/min)

RESULTS OF PUMP RETRIALS - APV LOBE PUMP (RETRIAL 2)

RETRIAL	2
DATE	14/9/95
YEAST	CASTLE C2 EX FV 97

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY Protease (absorbance)	VIABILITY Mod MeBI (% viability)	VITALITY OUR (mg/l/min/10 ^ 8 viable cells)		
40 rpm (16 l/min)	before 1	0.08	94	0.010		
	before 2	0.07	89	0.012		
	after 1	0.08	86	0.009		
	after 2	0.08	92	0.008		
86 rpm (35 l/min)	before 1	0.08	87	0.009		
	before 2	0.07	93	0.012		
	after 1	0.09	88	0.013		
	after 2	0.08	93	0.009		

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH Inc in Biomass (factor)	ATTENUATION Final Gravity (Plato)	CURVE FIT CONSTANTS Alpha Constants (Plato)	Beta Constants (1/hr)	R2 Values
40 rpm (16 l/min)	before 1	2.9	2.75	14.72	-0.0098	0.9560
	before 2	2.8	2.72	14.68	-0.0101	0.9560
	after 1	2.8	2.71	14.47	-0.0101	0.9569
	after 2	2.8	2.74	14.64	-0.0103	0.9560
86 rpm (35 l/min)	before 1	2.5	2.71	14.12	-0.0103	0.9277
	before 2	2.8	2.74	14.78	-0.0103	0.9277
	after 1	2.8	2.71	14.57	-0.0104	0.9277
	after 2	3.0	2.58	14.53	-0.0106	0.9491

BEER QUALITY (DAY 8)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
40 rpm (16 l/min)	before 1	4.03	125	15	21
	before 2	4.06	123	14	21
	after 1	4.05	116	12	21
	after 2	4.06	107	20	21
86 rpm (35 l/min)	before 1	4.04	118	11	19
	before 2	4.02	109	14	20
	after 1	4.04	108	11	20
	after 2	4.05	110	13	20

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
40 rpm (16 l/min)	before 1	4.05	67	9	26
	before 2	4.09	63	9	24
	after 1	4.08	63	9	24
	after 2	4.10	62	11	27
86 rpm (35 l/min)	before 1	4.07	61	9	23
	before 2	4.06	57	13	24
	after 1	4.08	62	15	25
	after 2	4.09	68	8	24

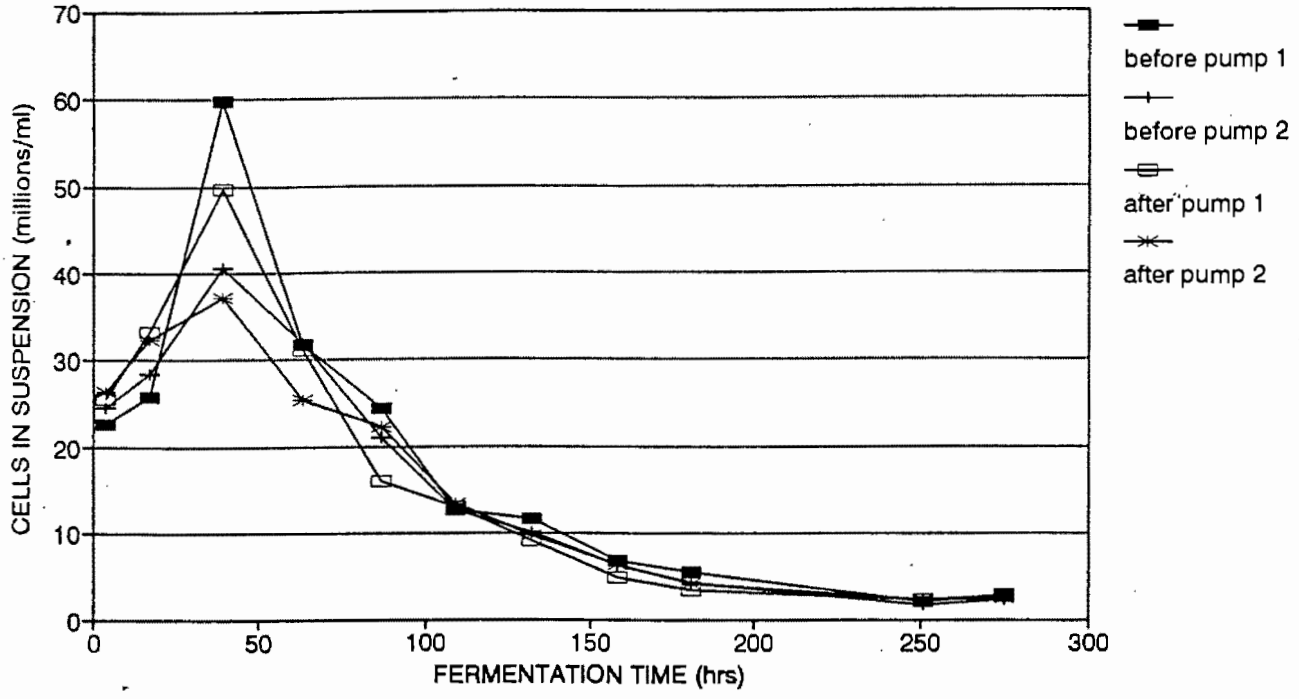

Figure E5 Cell count profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the APV lobe pump on yeast quality (Pump speed = 40 rpm ≈ 16 L/min)

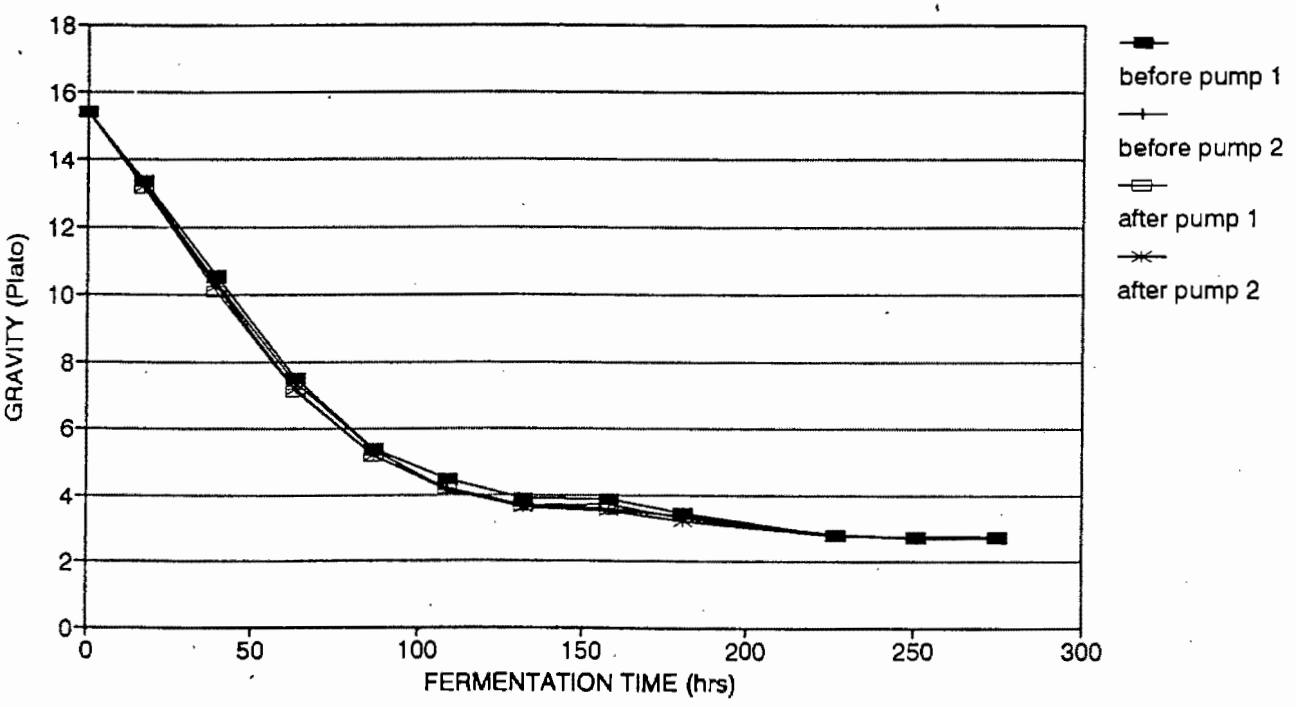

Figure E6 Attenuation profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the APV lobe pump on yeast quality (Pump speed = 40 rpm ≈ 16 L/min)

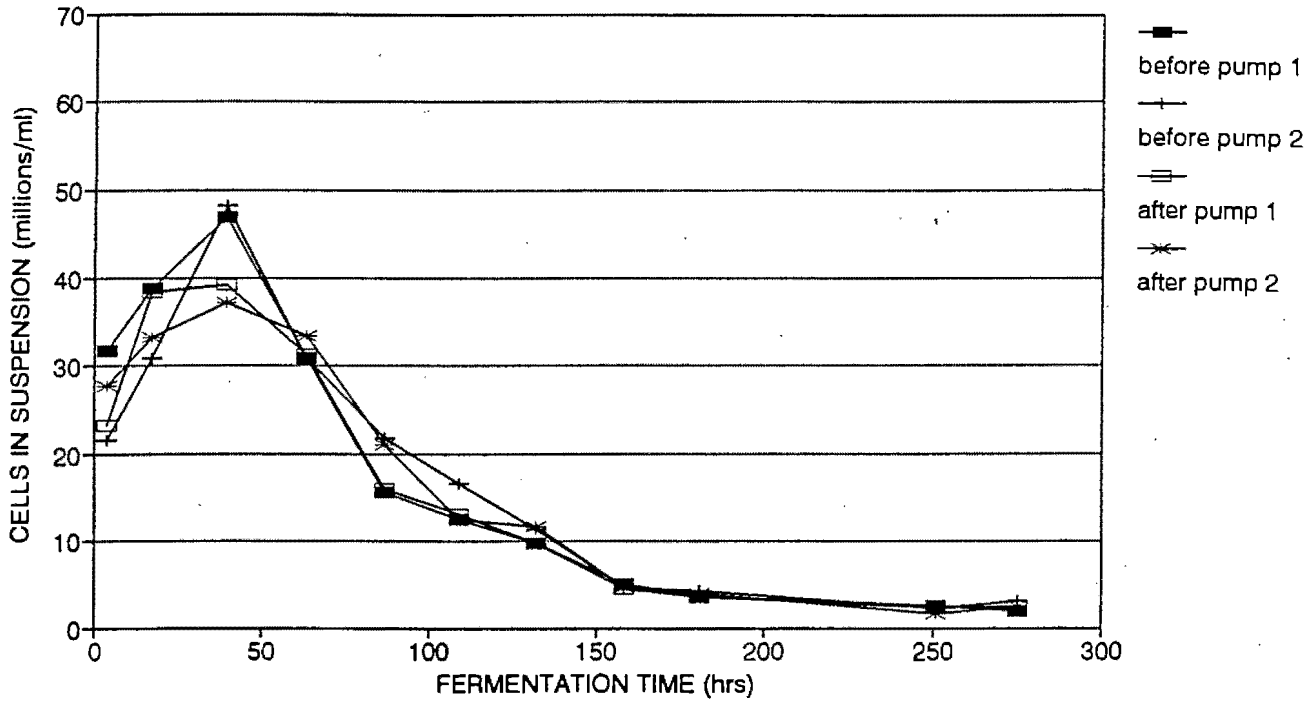

Figure E7 Cell count profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the APV lobe pump on yeast quality (Pump speed = 86 rpm ≈ 35 L/min)

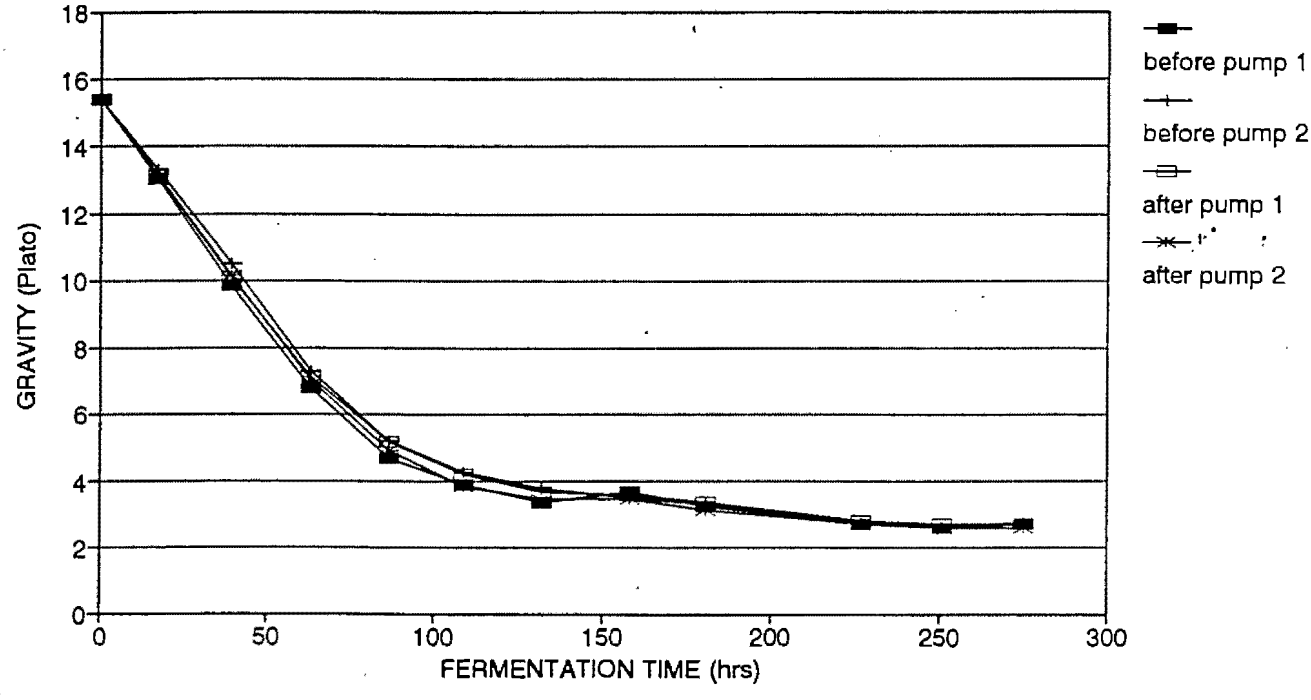

Figure E8 Attenuation profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the APV lobe pump on yeast quality (Pump speed = 86 rpm ≈ 35 L/min)

RESULTS OF PUMP RETRIALS - BREDEL PERISTALTIC PUMP (RETRIAL 3)

RETRIAL	3
DATE	28/9/95
YEAST	CASTLE A6 EX FV 81

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY Protease (absorbance)	VIABILITY Mod MeBl (% viability)	VITALITY OUR (mg/l/min/10^8 viable cells)		
23 rpm (59 l/min)	before 1	0.16	96	0.011		
	before 2	0.17	94	0.010		
	after 1	0.16	94	0.011		
	after 2	0.18	94	0.011		
42 rpm (110 l/min)	before 1	0.23	95	0.010		
	before 2	0.21	96	0.009		
	after 1	0.24	96	0.008		
	after 2	0.21	96	0.008		

BEER QUALITY (DAY 8)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
23 rpm (59 l/min)	before 1	3.93	88	9	15
	before 2	3.93	75	9	16
	after 1	3.94	83	10	19
	after 2	3.94	77	9	21
42 rpm (110 l/min)	before 1	3.86	72	9	16
	before 2	3.92	86	11	19
	after 1	4.02	91	11	20
	after 2	3.91	78	9	18

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH Inc in Biomass (factor)	ATTENUATION Final Gravity (Plato)	CURVE FIT CONSTANTS Alpha Constants (Plato)	Beta Constants (1/hr)	R2 Values
23 rpm (59 l/min)	before 1	2.3	2.82	14.89	-0.0093	0.9592
	before 2	2.6	2.75	14.75	-0.0093	0.9525
	after 1	3.0	2.64	14.95	-0.0096	0.9550
	after 2	2.9	2.62	14.92	-0.0096	0.9569
42 rpm (110 l/min)	before 1	2.3	2.30	15.47	-0.0108	0.9544
	before 2	2.9	2.80	14.93	-0.0093	0.9629
	after 1	2.8	2.86	14.88	-0.0095	0.9637
	after 2	2.3	2.70	14.54	-0.0097	0.9456

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
23 rpm (59 l/min)	before 1	3.97	79	9	18
	before 2	3.95	77	8	17
	after 1	3.97	77	10	19
	after 2	3.98	81	8	19
42 rpm (110 l/min)	before 1	3.90	63	8	20
	before 2	4.00	92	10	20
	after 1	4.08	101	15	24
	after 2	3.95	68	8	18

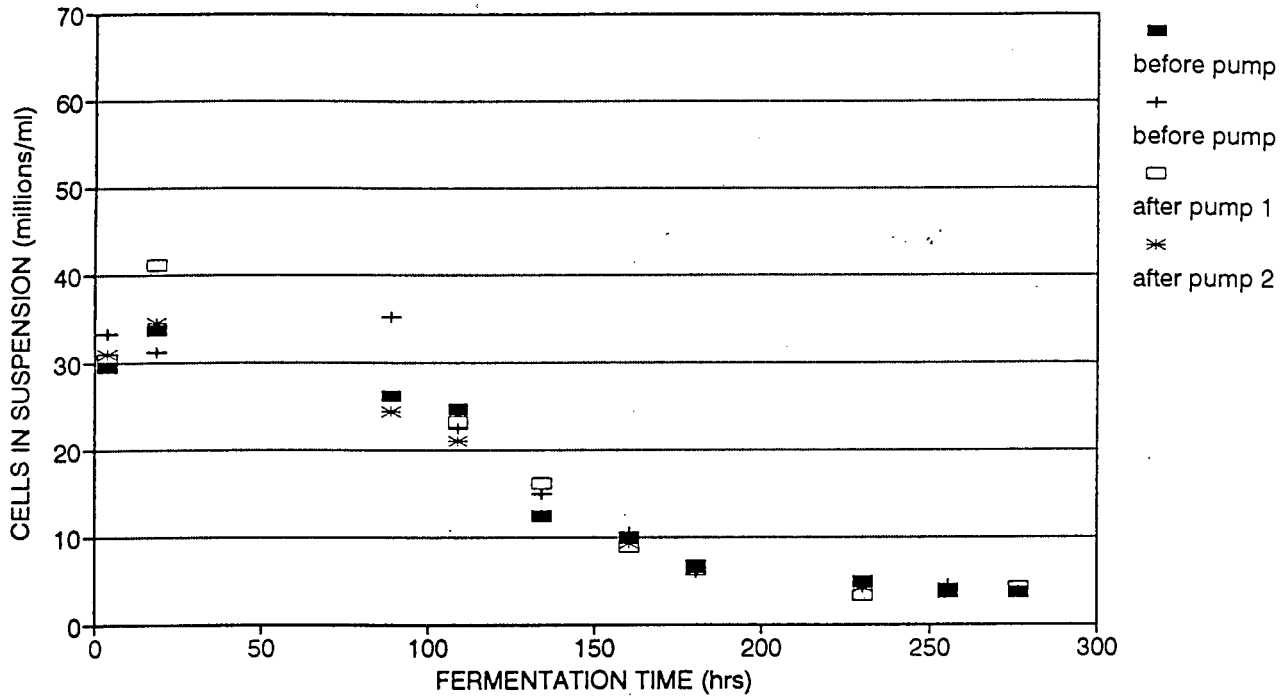

Figure E9 Cell count profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Bredel SP/50 peristaltic pump on yeast quality (Pump speed = 23 rpm ≈ 59 L/min)

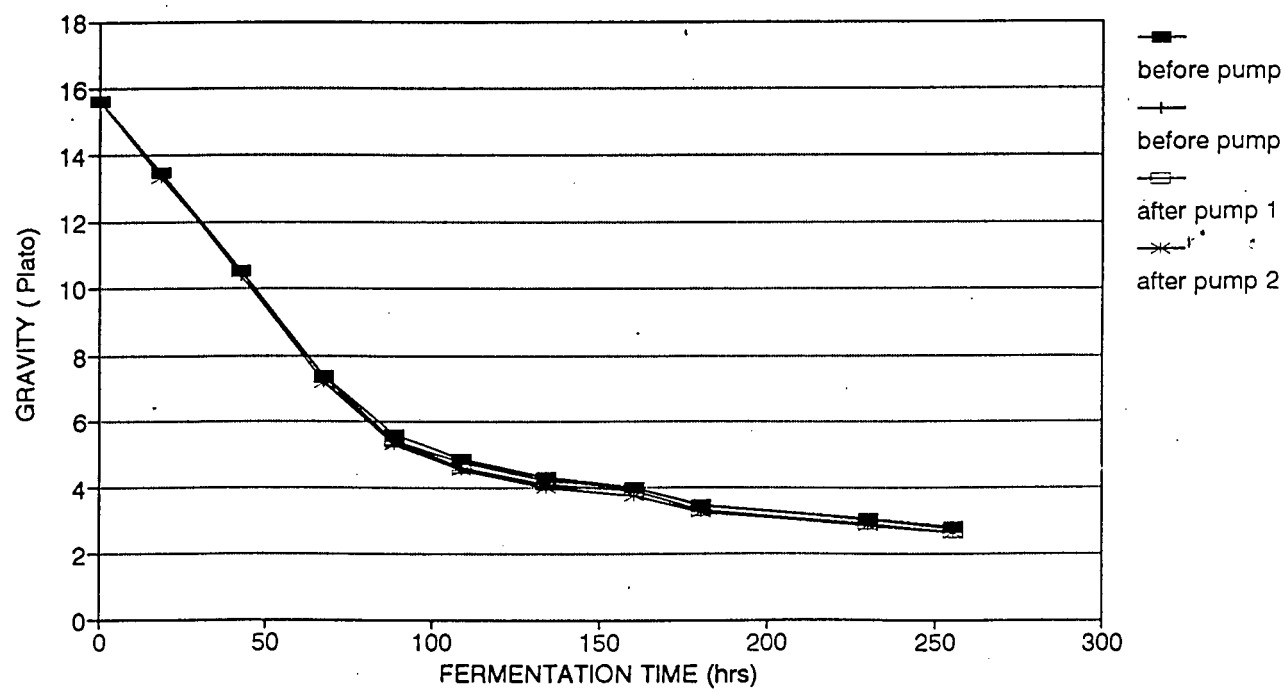

Figure E10 Attenuation profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Bredel SP/50 peristaltic pump on yeast quality (Pump speed = 23 rpm ≈ 59 L/min)

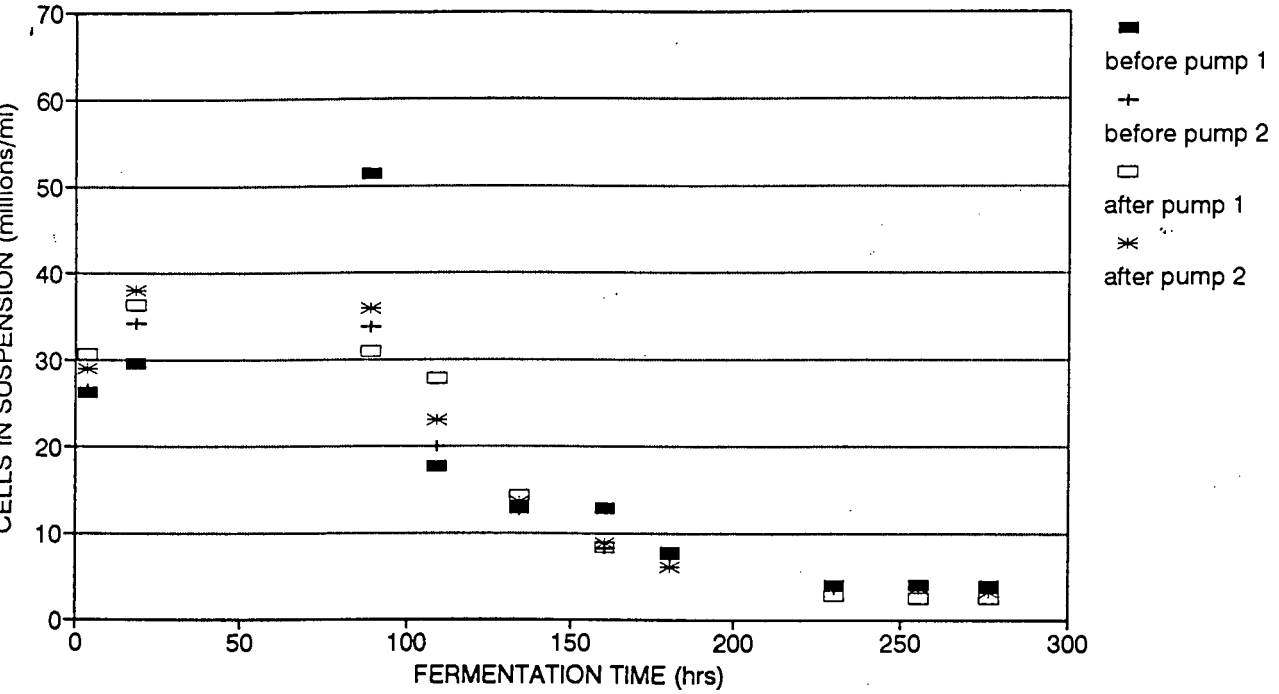

Figure E11 Cell count profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Bredel SP/50 peristaltic pump on yeast quality (Pump speed = 42 rpm ≈ 110 L/min)

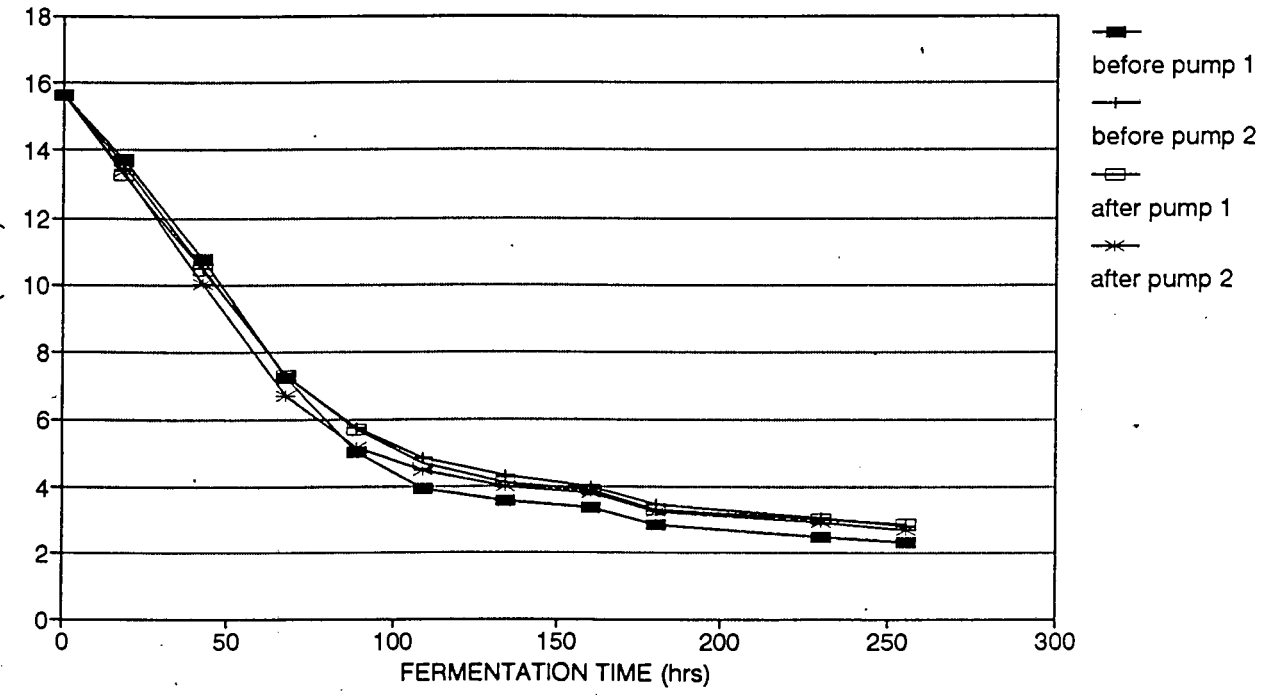

Figure E12 Attenuation profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Bredel SP/50 peristaltic pump on yeast quality (Pump speed = 42 rpm ≈ 110 L/min)

RESULTS OF PUMP RETRIALS - BREDEL PERISTALTIC PUMP (RETRIAL 4)

RETRIAL	4
DATE	11/10/95
YEAST	CASTLE D4 EX FV 98

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY	VIABILITY	VITALITY		
		Protease (absorbance)	Mod MeBl (% viability)	OUR (mg/l/min/10 ^ 8 viable cells)		
25 rpm (33 l/min)	before 1	0.01	93	0.008		
	before 2	0.00	93	0.007		
	after 1	0.02	92	0.010		
	after 2	0.02	92	0.011		
40 rpm (57 l/min)	before 1	0.02	93	0.008		
	before 2	0.02	92	0.008		
	after 1	0.03	96	0.009		
	after 2	0.03	95	0.010		

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH	ATTENUATION	CURVE FIT CONSTANTS		
		Inc in Biomass (factor)	Final Gravity (Plato)	Alpha Constants (Plato)	Beta Constants (1/hr)	R2 Values
25 rpm (33 l/min)	before 1	2.7	2.43	15.24	-0.0110	0.9683
	before 2	2.9	2.55	15.18	-0.0110	0.9683
	after 1	2.9	2.52	15.04	-0.0105	0.9667
	after 2	2.7	2.39	15.04	-0.0111	0.9650
40 rpm (57 l/min)	before 1	2.7	2.52	15.24	-0.0109	0.9682
	before 2	2.9	2.42	15.10	-0.0107	0.9724
	after 1	2.8	2.38	15.27	-0.0106	0.9746
	after 2	2.4	2.11	15.09	-0.0117	0.9627

BEER QUALITY (DAY 8)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
25 rpm (33 l/min)	before 1	4.04	120	10	19
	before 2	4.07	119	10	20
	after 1	4.01	111	9	19
	after 2	4.07	119	11	21
40 rpm (57 l/min)	before 1	4.09	118	11	21
	before 2	4.05	113	11	20
	after 1	4.07	124	11	20
	after 2	3.96	98	8	16

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
25 rpm (33 l/min)	before 1	4.07	78	10	21
	before 2	4.09	86	10	22
	after 1	4.02	72	9	21
	after 2	4.10	74	10	24
40 rpm (57 l/min)	before 1	4.11	76	11	23
	before 2	4.07	80	11	21
	after 1	4.04	81	10	22
	after 2	3.98	61	8	18

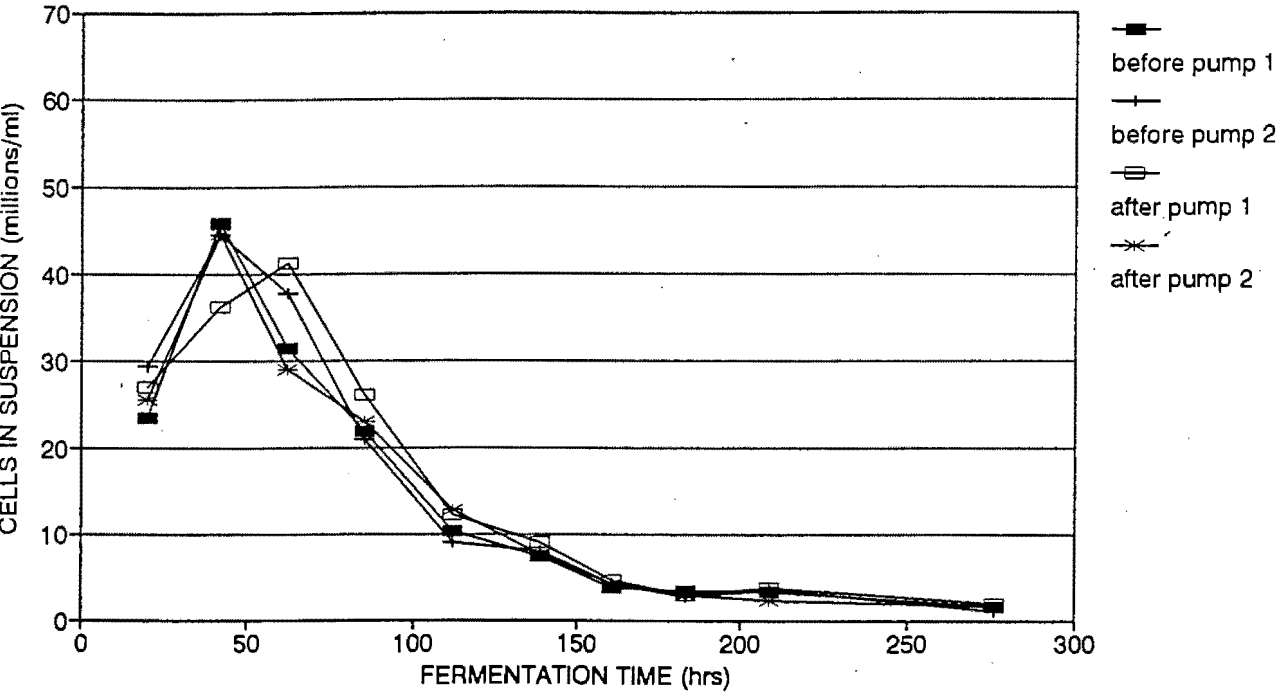

Figure E13 Cell count profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Bredel SP/40 peristaltic pump on yeast quality (Pump speed = 25 rpm ≈ 33 L/min)

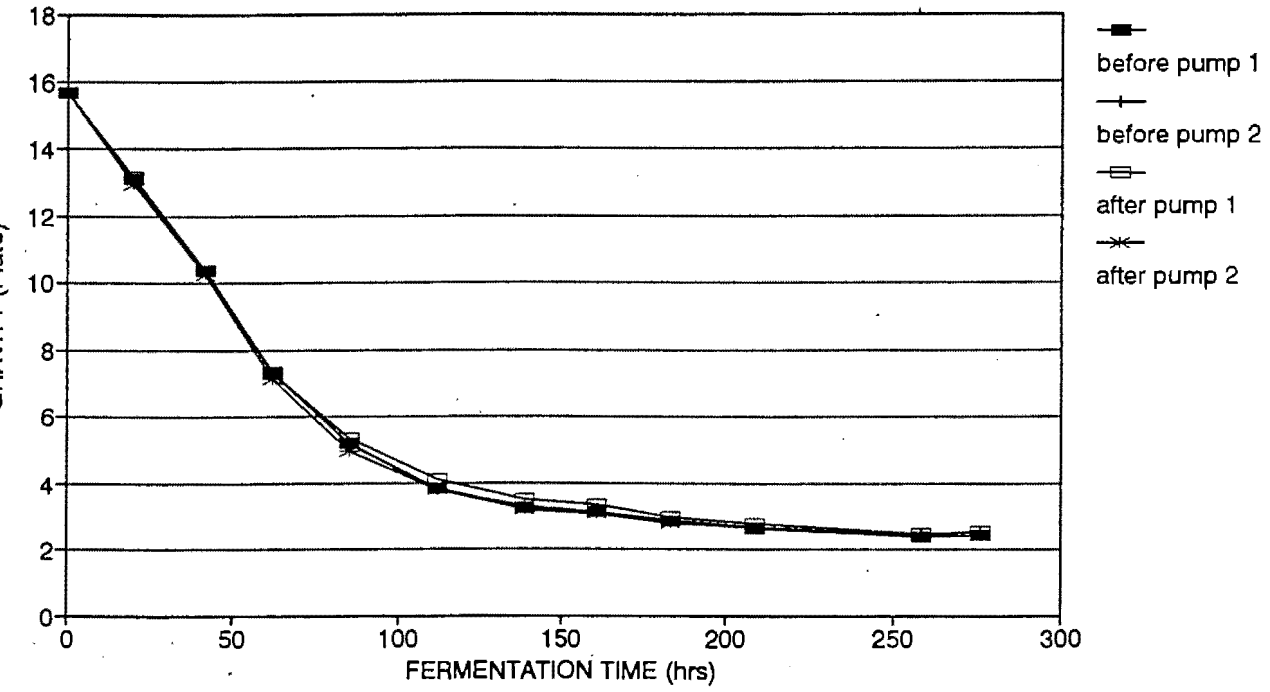

Figure E14 Attenuation profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Bredel SP/40 peristaltic pump on yeast quality (Pump speed = 25 rpm ≈ 33 L/min)

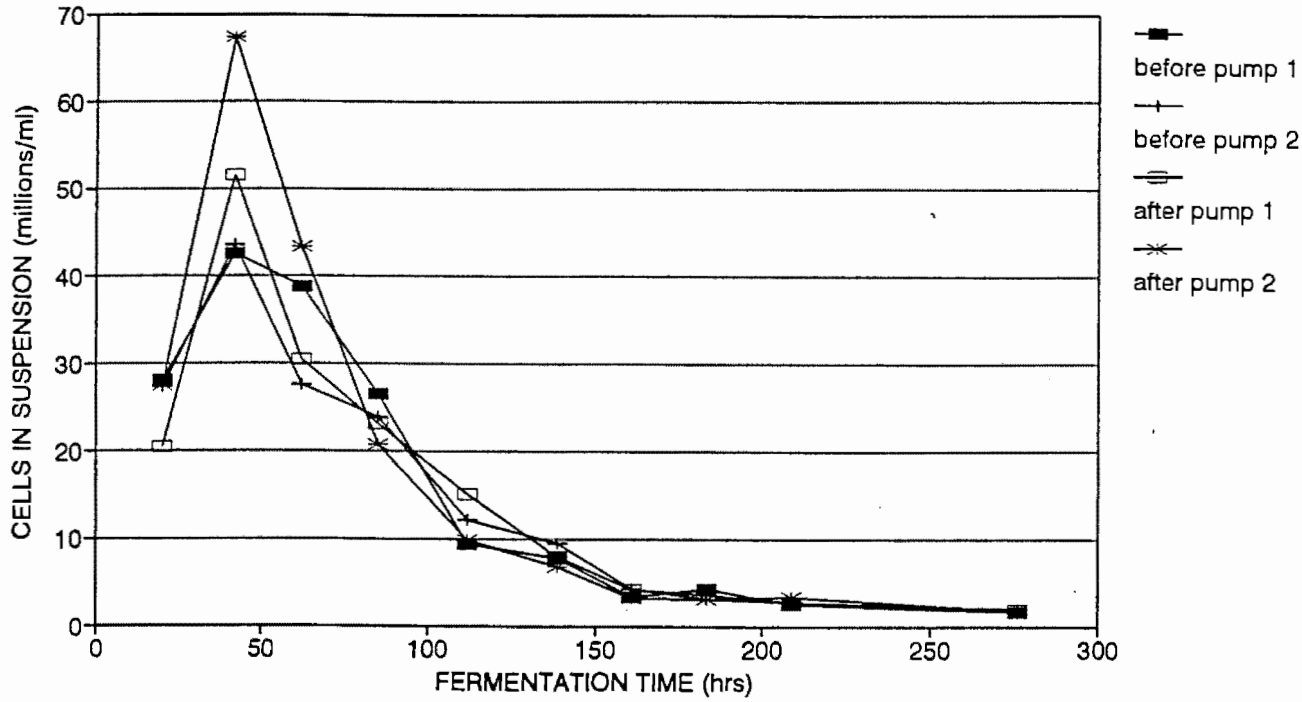

Figure E15 Cell count profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Bredel SP/40 peristaltic pump on yeast quality (Pump speed = 40 rpm ≈ 57 L/min)

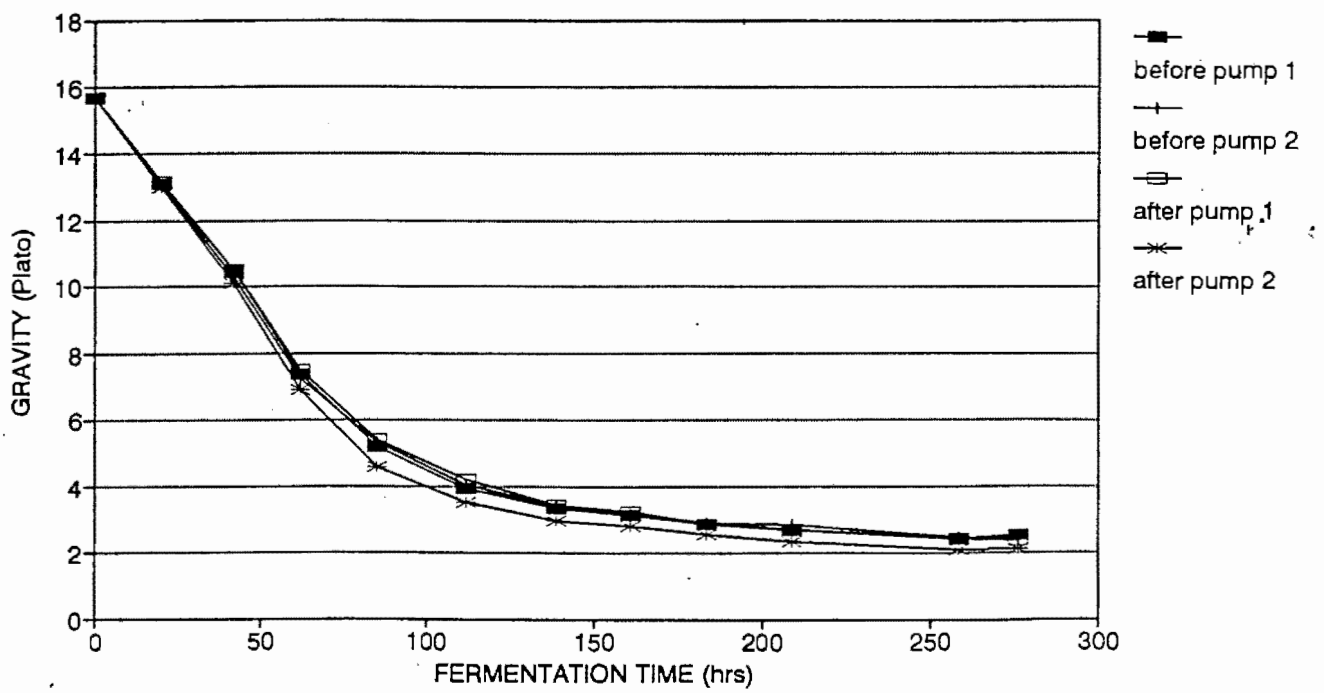

Figure E16 Attenuation profiles for 2 L EBC tube fermentations of samples taken to evaluate the effect of the Bredel SP/40 peristaltic pump on yeast quality (Pump speed = 40 rpm ≈ 57 L/min)

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - MASO SINE PUMP (RETRIAL 1) YEAST QUALITY AND FERMENTATION INDICATORS

RETRIAL	1
DATE	31/8/95
YEAST	CASTLE Y5 EX FV 112

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY						VIABILITY						VITALITY					
		Protease	Statistical Parameters					Mod MeBl	Statistical Parameters					OUR	Statistical Parameters				
		(absorbance)	Diff	Avg	Stds	t	SS?	(% viability)	Diff	Avg	Stds	t	SS?	(mg/l/min/10^8 viable cells)	Diff	Avg	Stds	t	SS?
240 rpm	before 1	0.05						92						0.015					
(89 l/min)	before 2	0.06						89						0.008					
	after 1	0.08	0.03					93	1					0.009	-0.006				
	after 2	0.07	0.01					90	1					0.008	0.000				
404 rpm	before 1	0.04						92						0.015					
(164 l/min)	before 2	0.10						94						0.011					
	after 1	0.10	0.06					93	1					0.007	-0.008				
	after 2	0.10	0.00	0.03	0.03	1.89	NSS	93	-1	1	t	1.00	NSS	0.008	-0.003	-0.004	0.004	-2.43	NSS

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH						ATTENUATION					
		Inc in Biomass	Statistical Parameters					Final Gravity	Statistical Parameters				
		(factor)	Diff	Avg	Stds	t	SS?	(Plato)	Diff	Avg	Stds	t	SS?
240 rpm	before 1	3.0						2.55					
(89 l/min)	before 2	3.0						2.51					
	after 1	3.3	0.3					2.52	-0.03				
	after 2	3.0	0.0					2.43	-0.08				
404 rpm	before 1	3.4						2.48					
(164 l/min)	before 2	3.3						2.41					
	after 1	3.3	-0.1					2.36	-0.12				
	after 2	3.5	0.2	0.1	0.2	1.08	NSS	2.51	0.10	-0.0	0.1	-0.68	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - MASO SINE PUMP (RETRIAL 1) BEER QUALITY (DAY 8) AND FERMENTATION INDICATORS

RETRIAL	1
DATE	31/8/95
YEAST	CASTLE Y5 EX FV 112

BEER QUALITY (DAY 8)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters Diff	Avg	Stds	t	SS?	Diacetyl (ppb)	Statistical Parameters Diff	Avg	Stds	t	SS?
240 rpm	before 1	3.94						121					
(89 l/min)	before 2	3.92						141					
	after 1	3.93	-0.01					139	18				
	after 2	3.94	0.02					156	15				
404 rpm	before 1	3.95						-					
(164 l/min)	before 2	3.92						152					
	after 1	3.94	-0.01					148	-				
	after 2	3.95	0.03	0.01	0.02	0.73	NSS	169	17	16.67	1.53	18.90	SS

FERMENTATION

PUMP SPEED	SAMPLE	Alpha Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
240 rpm	before 1	16.27					
(89 l/min)	before 2	15.96					
	after 1	16.20	-0.06				
	after 2	15.97	0.01				
404 rpm	before 1	16.40					
(164 l/min)	before 2	15.98					
	after 1	16.11	-0.29				
	after 2	15.81	-0.18	-0.13	0.13	-1.95	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?	Acetaldehyde (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?
240 rpm	before 1	15						23					
(89 l/min)	before 2	12						23					
	after 1	14	-1					24	1				
	after 2	15	3					24	1				
404 rpm	before 1	16						21					
(164 l/min)	before 2	15						24					
	after 1	20	4					24	3				
	after 2	13	-2	1	3	0.68	NSS	22	-2	1	2	0.73	NSS

PUMP SPEED	SAMPLE	Beta Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
240 rpm	before 1	-0.0101					
(89 l/min)	before 2	-0.0103					
	after 1	-0.0103	-2E-04				
	after 2	-0.0105	-2E-04				
404 rpm	before 1	-0.0104					
(164 l/min)	before 2	-0.0106					
	after 1	-0.0105	-1E-04				
	after 2	-0.0103	3E-04	-5E-05	2E-04	-0.42	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - MASO SINE PUMP (RETRIAL 1) BEER QUALITY (DAY 12)

RETRIAL	1
DATE	31/8/95
YEAST	CASTLE Y5 EX FV 112

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
240 rpm	before 1	3.97						81					
(89 l/min)	before 2	3.94						76					
	after 1	3.95	-0.02					76	-5				
	after 2	3.95	0.01					85	9				
404 rpm	before 1	3.98						68					
(164 l/min)	before 2	3.96						76					
	after 1	3.97	-0.01					62	-6				
	after 2	3.98	0.02	0.00	0.02	0.00	NSS	80	4	1	7	0.14	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
240 rpm	before 1	14						42					
(89 l/min)	before 2	13						38					
	after 1	13	-1					45	3				
	after 2	14	1					34	-4				
404 rpm	before 1	10						36					
(164 l/min)	before 2	13						41					
	after 1	14	4					48	12				
	after 2	18	3	2	2	1.58	NSS	34	-7	1	8	0.24	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - APV LOBE PUMP (RETRIAL 2) YEAST QUALITY AND FERMENTATION INDICATORS

RETRIAL	2
DATE	14/9/95
YEAST	CASTLE C2 EX FV 97

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY						VIABILITY						VITALITY					
		Protease	Statistical Parameters					Mod MeBl	Statistical Parameters					OUR	Statistical Parameters				
		(absorbance)	Diff	Avg	Stds	t	SS?	(% viability)	Diff	Avg	Stds	t	SS?	(mg/l/min/10^8 viable cells)	Diff	Avg	Stds	t	SS?
40 rpm	before 1	0.08						94						0.010					
(16 l/min)	before 2	0.07						89						0.012					
	after 1	0.08	0.00					86	-8					0.009	-0.001				
	after 2	0.08	0.01					92	3					0.008	-0.004				
86 rpm	before 1	0.08						87						0.009					
(35 l/min)	before 2	0.07						93						0.012					
	after 1	0.09	0.01					86	1					0.013	0.004				
	after 2	0.08	0.01	0.01	0.00	3.00	NSS	93	0	-1	5	-0.41	NSS	0.009	-0.003	-0.001	0.004	-0.56	NSS

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH						ATTENUATION					
		Inc in Biomass	Statistical Parameters					Final Gravity	Statistical Parameters				
		(factor)	Diff	Avg	Stds	t	SS?	(Plato)	Diff	Avg	Stds	t	SS?
40 rpm	before 1	2.9						2.75					
(16 l/min)	before 2	2.8						2.72					
	after 1	2.8	-0.1					2.71	-0.04				
	after 2	2.8	0.0					2.74	0.02				
86 rpm	before 1	2.5						2.71					
(35 l/min)	before 2	2.8						2.74					
	after 1	2.8	0.3					2.71	0.00				
	after 2	3.0	0.2	0.1	0.2	1.10	NSS	2.58	-0.16	-0.05	0.08	-1.12	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - APV LOBE PUMP (RETRIAL 2) BEER QUALITY (DAY 8) AND FERMENTATION INDICATORS

RETRIAL	2
DATE	14/9/95
YEAST	CASTLE C2 EX FV 97

BEER QUALITY (DAY 8)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters Diff	Avg	Stds	t	SS?	Diacetyl (ppb)	Statistical Parameters Diff	Avg	Stds	t	SS?
40 rpm	before 1	4.03						125					
(16 l/min)	before 2	4.06						123					
	after 1	4.05	0.02					116	-9				
	after 2	4.06	0.00					107	-16				
86 rpm	before 1	4.04						118					
(35 l/min)	before 2	4.02						109					
	after 1	4.04	0.00					108	-10				
	after 2	4.05	0.03	0.01	0.02	1.67	NSS	110	1	-9	7	-2.41	NSS

FERMENTATION

SAMPLE	Alpha Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
before 1	14.72					
before 2	14.68					
after 1	14.47	-0.24				
after 2	14.64	-0.04				
before 1	14.12					
before 2	14.78					
after 1	14.57	0.45				
after 2	14.53	-0.25	-0.02	0.33	-0.13	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?	Acetaldehyde (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?
40 rpm	before 1	15						21					
(16 l/min)	before 2	14						21					
	after 1	12	0					21	0				
	after 2	20	6					21	0				
86 rpm	before 1	11						19					
(35 l/min)	before 2	14						20					
	after 1	11	0					20	1				
	after 2	13	-1	1	3	0.78	NSS	20	0	0	1	1.00	NSS

SAMPLE	Beta Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
before 1	-0.0098					
before 2	-0.0101					
after 1	-0.0101	0E+00				
after 2	-0.0103	-2E-04				
before 1	-0.0103					
before 2	-0.0103					
after 1	-0.0104	-1E-04				
after 2	-0.0106	-3E-04	-1E-04	1E-04	-2.32	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - APV LOBE PUMP (RETRIAL 2) BEER QUALITY (DAY 12)

RETRIAL	2
DATE	14/9/95
YEAST	CASTLE C2 EX FV 97

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
40 rpm	before 1	4.05						67					
(16 l/min)	before 2	4.09						63					
	after 1	4.08	0.03					63	-4				
	after 2	4.10	0.01					62	-1				
86 rpm	before 1	4.07						61					
(35 l/min)	before 2	4.06						57					
	after 1	4.08	0.01					62	1				
	after 2	4.09	0.03	0.02	0.01	3.46	SS	68	11	2	7	0.54	NSS

PUMP SPEED	SAMPLE -	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
40 rpm	before 1	9						26					
(16 l/min)	before 2	9						24					
	after 1	9	0					24	-2				
	after 2	11	2					27	3				
86 rpm	before 1	9						23					
(35 l/min)	before 2	13						24					
	after 1	15	6					25	2				
	after 2	8	-5	1	5	0.33	NSS	24	0	1	2	0.66	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 50 BREDEL PERISTALTIC PUMP (RETRIAL 3) YEAST QUALITY AND FERMENTATION INDICATORS

RETRIAL	3
DATE	28/9/95
YEAST	CASTLE A6 EX FV 81

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY						VIABILITY						VITALITY					
		Protease	Statistical Parameters					Mod MeBl	Statistical Parameters					OUR	Statistical Parameters				
		(absorbance)	Diff	Avg	Stds	t	SS?	(% viability)	Diff	Avg	Stds	t	SS?	(mg/l/min/10^8 viable cells)	Diff	Avg	Stds	t	SS?
23 rpm	before 1	0.16						96						0.011					
(59 l/min)	before 2	0.17						94						0.010					
	after 1	0.16	0.00					94	-2					0.011	0.000				
	after 2	0.18	0.01					94	0					0.011	0.001				
42 rpm	before 1	0.23						95						0.010					
(110 l/min)	before 2	0.21						96						0.009					
	after 1	0.24	0.01					96	1					0.008	-0.002				
	after 2	0.21	0.00	0.00	0.01	1.73	NSS	96	0	-0	1	-0.40	NSS	0.008	-0.001	-0.001	0.001	-0.77	NSS

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH						ATTENUATION					
		Inc in Biomass	Statistical Parameters					Final Gravity	Statistical Parameters				
		(factor)	Diff	Avg	Stds	t	SS?	(Plato)	Diff	Avg	Stds	t	SS?
23 rpm	before 1	2.3						2.82					
(59 l/min)	before 2	2.6						2.75					
	after 1	3.0	0.7					2.64	-0.18				
	after 2	2.9	0.3					2.62	-0.13				
42 rpm	before 1	2.3						2.30					
(110 l/min)	before 2	2.9						2.80					
	after 1	2.8	0.5					2.86	0.56				
	after 2	2.3	-0.6	0.2	0.6	0.78	NSS	2.70	-0.10	0.04	0.35	0.21	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 50 BREDEL PERISTALTC PUMP (RETRIAL 3) BEER QUALITY (DAY 8) AND FERMENTATION INDICATORS

RETRIAL	3
DATE	28/9/95
YEAST	CASTLE A6 EX FV 81

BEER QUALITY (DAY 8)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters Diff	Avg	Stds	t	SS?	Diacetyl (ppb)	Statistical Parameters Diff	Avg	Stds	t	SS?
23 rpm	before 1	3.93						88					
(59 l/min)	before 2	3.93						75					
	after 1	3.94	0.01					83	-5				
	after 2	3.94	0.01					77	2				
42 rpm	before 1	3.86						72					
(110 l/min)	before 2	3.92						86					
	after 1	4.02	0.16					91	19				
	after 2	3.91	-0.01	0.04	0.08	1.08	NSS	78	-8	2	12	0.33	NSS

FERMENTATION

PUMP SPEED	SAMPLE	Alpha Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
23 rpm	before 1	14.89					
(59 l/min)	before 2	14.75					
	after 1	14.95	0.06				
	after 2	14.92	0.17				
42 rpm	before 1	15.47					
(110 l/min)	before 2	14.93					
	after 1	14.88	-0.59				
	after 2	14.54	-0.39	-0.19	0.36	-1.04	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?	Acetaldehyde (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?
23 rpm	before 1	9						15					
(59 l/min)	before 2	9						16					
	after 1	10	1					19	4				
	after 2	9	0					21	5				
42 rpm	before 1	9						16					
(110 l/min)	before 2	11						19					
	after 1	11	2					20	4				
	after 2	9	-2	0	2	0.29	NSS	18	-1	3	3	2.22	NSS

PUMP SPEED	SAMPLE	Beta Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
23 rpm	before 1	-0.0093					
(59 l/min)	before 2	-0.0093					
	after 1	-0.0096	-3E-04				
	after 2	-0.0096	-3E-04				
42 rpm	before 1	-0.0108					
(110 l/min)	before 2	-0.0093					
	after 1	-0.0095	1E-03				
	after 2	-0.0097	-4E-04	7E-05	8E-04	0.18	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 50 BREDEL PERISTALTIC PUMP (RETRIAL 3) BEER QUALITY (DAY 12)

RETRIAL	3
DATE	28/9/95
YEAST	CASTLE A6 EX FV 81
BEER QUALITY (	

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
23 rpm	before 1	3.97						79					
(59 l/min)	before 2	3.95						77					
	after 1	3.97	0.00					77	-2				
	after 2	3.98	0.03					81	4				
42 rpm	before 1	3.90						63					
(110 l/min)	before 2	4.00						92					
	after 1	4.08	0.18					101	38				
	after 2	3.95	-0.05	0.04	0.10	0.81	NSS	68	-24	4	28	0.31	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
23 rpm	before 1	9						18					
(59 l/min)	before 2	8						17					
	after 1	10	1					19	1				
	after 2	8	0					19	2				
42 rpm	before 1	8						20					
(110 l/min)	before 2	10						20					
	after 1	15	7					24	4				
	after 2	8	-2	2	4	0.77	NSS	18	-2	1	3	1.00	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 40 BREDEL PERISTALTIC PUMP (RETRIAL 4) YEAST QUALITY AND FERMENTATION INDICATORS

RETRIAL	4
DATE	11/10/95
YEAST	CASTLE D4 EX FV 98

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY						VIABILITY						VITALITY					
		Protease	Statistical Parameters					Mod MeBl	Statistical Parameters					OUR	Statistical Parameters				
		(absorbance)	Diff	Avg	Stds	t	SS?	(% viability)	Diff	Avg	Stds	t	SS?	(mg/l/min/10^8 viable cells)	Diff	Avg	Stds	t	SS?
25 rpm	before 1	0.01						93						0.008					
(33 l/min)	before 2	0.00						93						0.007					
	after 1	0.02	0.01					92	-1					0.010	0.002				
	after 2	0.02	0.02					92	-1					0.011	0.004				
40 rpm	before 1	0.02						93						0.008					
(57 l/min)	before 2	0.02						92						0.008					
	after 1	0.03	0.01					96	3					0.009	0.001				
	after 2	0.03	0.01	0.01	0.01	5.00	SS	95	3	1	2	0.87	NSS	0.010	0.002	0.002	0.001	3.58	SS

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH						ATTENUATION					
		Inc in Biomass	Statistical Parameters					Final Gravity	Statistical Parameters				
		(factor)	Diff	Avg	Stds	t	SS?	(Plato)	Diff	Avg	Stds	t	SS?
25 rpm	before 1	2.7						2.43					
(33 l/min)	before 2	2.9						2.55					
	after 1	2.9	0.2					2.52	0.09				
	after 2	2.7	-0.2					2.39	-0.16				
40 rpm	before 1	2.7						2.52					
(57 l/min)	before 2	2.9						2.42					
	after 1	2.8	0.1					2.38	-0.14				
	after 2	2.4	-0.5	-0.1	0.3	-0.63	NSS	2.11	-0.31	-0.13	0.17	-1.57	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 40 BREDEL PERISTALTC PUMP (RETRIAL 4) BEER QUALITY (DAY 8) AND FERMENTATION INDICATORS

RETRIAL	4
DATE	11/10/95
YEAST	CASTLE D4 EX FV 98

BEER QUALITY (DAY 8) | FERMENTATION

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters Diff	Avg	Stds	t	SS?	Diacetyl (ppb)	Statistical Parameters Diff	Avg	Stds	t	SS?	Alpha Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
25 rpm	before 1	4.04						120						15.24					
(33 l/min)	before 2	4.07						119						15.18					
	after 1	4.01	-0.03					111	-9					15.04	-0.20				
	after 2	4.07	0.00					119	0					15.04	-0.15				
40 rpm	before 1	4.09						118						15.24					
(57 l/min)	before 2	4.05						113						15.10					
	after 1	4.07	-0.02					124	6					15.27	0.03				
	after 2	3.96	-0.09	-0.03	0.04	-1.81	NSS	98	-15	-5	9	-0.96	NSS	15.09	-0.01	-0.08	0.11	-1.54	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?	Acetaldehyde (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?	Beta Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
25 rpm	before 1	10						19						-0.0110					
(33 l/min)	before 2	10						20						-0.0110					
	after 1	9	-1					19	0					-0.0105	0.0005				
	after 2	11	1					21	1					-0.0111	-0.0001				
40 rpm	before 1	11						21						-0.0109					
(57 l/min)	before 2	11						20						-0.0107					
	after 1	11	0					20	-1					-0.0106	0.0003				
	after 2	8	-3	-1	2	-0.88	NSS	16	-4	-1	2	-0.93	NSS	-0.0117	-0.0010	-0.0001	0.0007	-0.23	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 40 BREDEL PERISTALTIC PUMP (RETRIAL 4] BEER QUALITY (DAY 12)

RETRIAL	4
DATE	11/10/95
YEAST	CASTLE D4 EX FV 98

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
25 rpm	before 1	4.07						78					
(33 l/min)	before 2	4.09						86					
	after 1	4.02	-0.05					72	-6				
	after 2	4.10	0.01					74	-12				
40 rpm	before 1	4.11						76					
(57 l/min)	before 2	4.07						80					
	after 1	4.04	-0.07					81	5				
	after 2	3.98	-0.09	-0.05	0.04	-2.31	NSS	61	-19	-8	10	-1.57	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
25 rpm	before 1	10						21					
(33 l/min)	before 2	10						22					
	after 1	9	-1					21	0				
	after 2	10	0					24	2				
40 rpm	before 1	11						23					
(57 l/min)	before 2	11						21					
	after 1	10	-1					22	-1				
	after 2	8	-3	-1	1	-1.99	NSS	18	-3	-1	2	-0.48	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - MASO SINE PUMP (RETRIAL 1) YEAST QUALITY AND FERMENTATION INDICATORS

RETRIAL	1
DATE	31/8/95
YEAST	CASTLE Y5 EX FV 112

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY						VIABILITY						VITALITY					
		Protease	Statistical Parameters					Mod MeBl	Statistical Parameters					OUR	Statistical Parameters				
		(absorbance)	Diff	Avg	Stda	t	SS?	(% viability)	Diff	Avg	Stda	t	SS?	(mg/l/min/10^8 viable cells)	Diff	Avg	Stda	t	SS?
240 rpm	before 1	0.05						92						0.015					
(89 l/min)	before 2	0.06						89						0.008					
	after 1	0.08	0.03					93	1					0.009	-0.006				
	after 2	0.07	0.01	0.02	0.01	2.00	NSS	90	1	1	0	-	SDZ	0.008	0.000	-0.003	0.004	-1.00	NSS
404 rpm	before 1	0.04						92						0.015					
(164 l/min)	before 2	0.10						94						0.011					
	after 1	0.10	0.06					93	1					0.007	-0.008				
	after 2	0.10	0.00	0.03	0.04	1.00	NSS	93	-1	0	1	0.00	NSS	0.008	-0.003	-0.006	0.004	-2.20	NSS

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH						ATTENUATION					
		Inc in Biomass	Statistical Parameters					Final Gravity	Statistical Parameters				
		(factor)	Diff	Avg	Stda	t	SS?	(Plato)	Diff	Avg	Stda	t	SS?
240 rpm	before 1	3.0						2.55					
(89 l/min)	before 2	3.0						2.51					
	after 1	3.3	0.3					2.52	-0.03				
	after 2	3.0	0.0	0.2	0.2	1.06	NSS	2.43	-0.08	-0.05	0.04	-2.20	NSS
404 rpm	before 1	3.4						2.48					
(164 l/min)	before 2	3.3						2.41					
	after 1	3.3	-0.1					2.38	-0.12				
	after 2	3.5	0.2	0.0	0.2	0.27	NSS	2.51	0.10	-0.01	0.16	-0.09	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - MASO SINE PUMP (RETRIAL 1) BEER QUALITY (DAY 8) AND FERMENTATION INDICATORS

RETRIAL	1
DATE	31/8/95
YEAST	CASTLE YS EX FV 112

BEER QUALITY (DAY 8) | FERMENTATION

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters Diff	Avg	Stds	t	SS?	Diacetyl (ppb)	Statistical Parameters Diff	Avg	Stds	t	SS?	Alpha Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
240 rpm	before 1	3.94						121						16.27					
(89 l/min)	before 2	3.92						141						15.96					
	after 1	3.93	-0.01					139	18					16.20	-0.06				
	after 2	3.94	0.02	0.01	0.02	0.33	NSS	156	15	17	2	11.00	NSS	15.97	0.01	-0.02	0.06	-0.64	NSS
404 rpm	before 1	3.95						-						16.40					
(164 l/min)	before 2	3.92						152						15.98					
	after 1	3.94	-0.01					148	-					16.11	-0.29				
	after 2	3.95	0.03	0.01	0.03	0.50	NSS	169	17	-	-	-	-	15.81	-0.18	-0.23	0.08	-4.19	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?	Acetaldehyde (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?	Beta Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
240 rpm	before 1	15						23						-0.0101					
(89 l/min)	before 2	12						23						-0.0103					
	after 1	14	-1					24	1					-0.0103	-2E-04				
	after 2	15	3	1	3	0.50	NSS	24	1	1	0	-	SDZ	-0.0105	-2E-04	-2E-04	0E+00	-	SDZ
404 rpm	before 1	16						21						-0.0104					
(164 l/min)	before 2	15						24						-0.0106					
	after 1	20	4					24	3					-0.0105	-1E-04				
	after 2	13	-2	1	4	0.33	NSS	22	-2	1	4	0.20	NSS	-0.0103	3E-04	1E-04	3E-04	0.50	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - MASO SINE PUMP (RETRIAL 1) BEER QUALITY (DAY 12)

RETRIAL	1
DATE	31/8/95
YEAST	CASTLE Y5 EX FV 112

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
240 rpm (89 l/min)	before 1	3.97						81					
	before 2	3.94						78					
	after 1	3.95	-0.02					76	-5				
	after 2	3.95	0.01	-0.00	0.02	-0.33	NSS	85	9	2	10	0.29	NSS
404 rpm (164 l/min)	before 1	3.98						68					
	before 2	3.96						76					
	after 1	3.97	-0.01					82	-6				
	after 2	3.98	0.02	0.01	0.02	0.33	NSS	80	4	-1	7	-0.20	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
240 rpm (89 l/min)	before 1	14						42					
	before 2	13						38					
	after 1	13	-1					45	3				
	after 2	14	1	0	1	0.00	NSS	34	-4	-1	5	-0.14	NSS
404 rpm (164 l/min)	before 1	10						36					
	before 2	13						41					
	after 1	14	4					48	12				
	after 2	16	3	4	1	7.00	NSS	34	-7	3	13	0.26	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - APV LOBE PUMP (RETRIAL 2) YEAST QUALITY AND FERMENTATION INDICATORS

RETRIAL	2
DATE	14/9/95
YEAST	CASTLE C2 EX FV 97

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY						VIABILITY						VITALITY					
		Protease	Statistical Parameters					Mod MeBl	Statistical Parameters					OUR	Statistical Parameters				
		(absorbance)	Diff	Avg	Stds	t	SS?	(% viability)	Diff	Avg	Stds	t	SS?	(mg/l/min/10^8 viable cells)	Diff	Avg	Stds	t	SS?
40 rpm	before 1	0.08						94						0.010					
(16 l/min)	before 2	0.07						89						0.012					
	after 1	0.08	0.00					86	-8					0.009	-0.001				
	after 2	0.08	0.01	0.00	0.01	1.00	NSS	92	3	-3	8	-0.45	NSS	0.008	-0.004	-0.003	0.002	-1.67	NSS
86 rpm	before 1	0.08						87						0.009					
(35 l/min)	before 2	0.07						93						0.012					
	after 1	0.09	0.01					88	1					0.013	0.004				
	after 2	0.08	0.01	0.01	0.00	-	SDZ	93	0	1	1	1.00	NSS	0.009	-0.003	0.000	0.005	0.14	NSS

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH						ATTENUATION					
		Inc in Biomass	Statistical Parameters					Final Gravity	Statistical Parameters				
		(factor)	Diff	Avg	Stds	t	SS?	(Plato)	Diff	Avg	Stds	t	SS?
40 rpm	before 1	2.9						2.75					
(16 l/min)	before 2	2.8						2.72					
	after 1	2.8	-0.1					2.71	-0.04				
	after 2	2.8	0.0	-0.1	0.1	-1.00	NSS	2.74	0.02	-0.01	0.04	-0.33	NSS
86 rpm	before 1	2.5						2.71					
(35 l/min)	before 2	2.8						2.74					
	after 1	2.8	0.3					2.71	0.00				
	after 2	3.0	0.2	0.3	0.1	5.00	NSS	2.58	-0.16	-0.08	0.11	-1.00	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - APV LOBE PUMP (RETRIAL 2) BEER QUALITY (DAY 8) AND FERMENTATION INDICATORS

RETRIAL	2
DATE	14/9/95
YEAST	CASTLE C2 EX FV 97

BEER QUALITY (DAY 8)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
40 rpm	before 1	4.03						125					
(16 l/min)	before 2	4.06						123					
	after 1	4.05	0.02					116	-9				
	after 2	4.06	0.00	0.01	0.01	1.00	NSS	107	-16	-13	5	-3.57	NSS
86 rpm	before 1	4.04						118					
(35 l/min)	before 2	4.02						109					
	after 1	4.04	0.00					108	-10				
	after 2	4.05	0.03	0.02	0.02	1.00	NSS	110	1	-5	8	-0.82	NSS

FERMENTATION

Alpha Constants	Statistical Parameters				
	Diff	Avg	Stds	t	SS?
14.72					
14.68					
14.47	-0.24				
14.64	-0.04	-0.14	0.14	-1.38	NSS
14.12					
14.78					
14.57	0.45				
14.53	-0.25	0.10	0.49	0.28	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
40 rpm	before 1	15						21					
(16 l/min)	before 2	14						21					
	after 1	12	-3					21	0				
	after 2	20	6	2	6	0.33	NSS	21	0	0	0	-	VI
86 rpm	before 1	11						19					
(35 l/min)	before 2	14						20					
	after 1	11	0					20	1				
	after 2	13	-1	-1	1	-1.00	NSS	20	0	1	1	1.00	NSS

Beta Constants	Statistical Parameters				
	Diff	Avg	Stds	t	SS?
-0.0098					
-0.0101					
-0.0101	-3E-04				
-0.0103	-2E-04	-3E-04	7E-05	-5.00	NSS
-0.0103					
-0.0103					
-0.0104	-1E-04				
-0.0106	-3E-04	-2E-04	1E-04	-2.00	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - APV LOBE PUMP (RETRIAL 2) BEER QUALITY (DAY 12)

RETRIAL	2
DATE	14/9/95
YEAST	CASTLE C2 EX FV 87

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
40 rpm	before 1	4.05						67					
(16 l/min)	before 2	4.09						63					
	after 1	4.08	0.03					63	-4				
	after 2	4.10	0.01	0.02	0.01	2.00	NSS	62	-1	-3	2	-1.67	NSS
86 rpm	before 1	4.07						61					
(35 l/min)	before 2	4.06						57					
	after 1	4.08	0.01					62	1				
	after 2	4.09	0.03	0.02	0.01	2.00	NSS	68	11	6	7	1.20	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
40 rpm	before 1	9						26					
(16 l/min)	before 2	9						24					
	after 1	9	0					24	-2				
	after 2	11	2	1	1	1.00	NSS	27	3	1	4	0.20	NSS
86 rpm	before 1	9						23					
(35 l/min)	before 2	13						24					
	after 1	15	6					25	2				
	after 2	8	-5	1	8	0.09	NSS	24	0	1	1	1.00	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 50 BREDEL PERISTALTIC PUMP (RETRIAL 3) YEAST QUALITY AND FERMENTATION INDICATORS

RETRIAL	3
DATE	28/9/95
YEAST	CASTLE A6 EX FV 81

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY						VIABILITY						VITALITY					
		Protease	Statistical Parameters					Mod MeBl	Statistical Parameters					OUR	Statistical Parameters				
		(absorbance)	Diff	Avg	Stda	t	SS?	(% viability)	Diff	Avg	Stda	t	SS?	(mg/l/min/10^8 viable cells)	Diff	Avg	Stda	t	SS?
23 rpm	before 1	0.16						96						0.011					
(59 l/min)	before 2	0.17						94						0.010					
	after 1	0.16	0.00					94	-2					0.011	0.000				
	after 2	0.18	0.01	0.00	0.01	1.00	NSS	94	0	-1	1	-1.00	NSS	0.011	0.001	0.000	0.001	1.00	NSS
42 rpm	before 1	0.23						95						0.010					
(110 l/min)	before 2	0.21						96						0.009					
	after 1	0.24	0.01					96	1					0.008	-0.002				
	after 2	0.21	0.00	0.00	0.01	1.00	NSS	96	0	1	1	1.00	NSS	0.008	-0.001	-0.001	0.001	-3.00	NSS

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH						ATTENUATION					
		Inc in Biomass	Statistical Parameters					Final Gravity	Statistical Parameters				
		(factor)	Diff	Avg	Stda	t	SS?	(Plato)	Diff	Avg	Stda	t	SS?
23 rpm	before 1	2.3						2.82					
(59 l/min)	before 2	2.6						2.75					
	after 1	3.0	0.7					2.64	-0.18				
	after 2	2.9	0.3	0.5	0.3	2.50	NSS	2.82	-0.13	-0.15	0.04	-6.20	NSS
42 rpm	before 1	2.3						2.30					
(110 l/min)	before 2	2.9						2.80					
	after 1	2.8	0.5					2.86	0.56				
	after 2	2.3	-0.6	-0.1	0.8	-0.09	NSS	2.70	-0.10	0.23	0.47	0.70	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 50 BREDEL PERISTALTC PUMP (RETRIAL 3) BEER QUALITY (DAY 8) AND FERMENTATION INDICATORS

RETRIAL 3
DATE 28/9/95

YEAST CASTLE A6 EX FV 81

BEER QUALITY (DAY 8)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters Diff	Avg	Stds	t	SS?	Diacetyl (ppb)	Statistical Parameters Diff	Avg	Stds	t	SS?
23 rpm	before 1	3.93						88					
(59 l/min)	before 2	3.93						75					
	after 1	3.94	0.01					83	-5				
	after 2	3.94	0.01	0.01	0.00	-	SDZ	77	2	-2	5	-0.43	NSS
42 rpm	before 1	3.86						72					
(110 l/min)	before 2	3.92						86					
	after 1	4.02	0.16					91	19				
	after 2	3.91	-0.01	0.07	0.12	0.88	NSS	78	-8	6	19	0.41	NSS

FERMENTATION

Alpha Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
14.89					
14.75					
14.95	0.06				
14.92	0.17	0.11	0.07	2.22	NSS
15.47					
14.93					
14.88	-0.59				
14.54	-0.39	-0.49	0.14	-4.81	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?	Acetaldehyde (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?
23 rpm	before 1	9						15					
(59 l/min)	before 2	9						16					
	after 1	10	1					19	4				
	after 2	9	0	1	1	1.00	NSS	21	5	5	1	9.00	NSS
42 rpm	before 1	9						16					
(110 l/min)	before 2	11						19					
	after 1	11	2					20	4				
	after 2	9	-2	0	3	0.00	NSS	18	-1	2	4	0.60	NSS

Beta Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
-0.0093					
-0.0093					
-0.0096	-3E-04				
-0.0096	-3E-04	-3E-04	0E+00	-	SDZ
-0.0108					
-0.0093					
-0.0095	1E-03				
-0.0097	-4E-04	4E-04	1E-03	0.53	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 50 BREDEL PERISTALTIC PUMP (RETRIAL 3) BEER QUALITY (DAY 12)

RETRIAL	3
DATE	28/9/95
YEAST	CASTLE A6 EX FV 81

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
23 rpm (59 l/min)	before 1	3.97						79					
	before 2	3.95						77					
	after 1	3.97	0.00					77	-2				
	after 2	3.98	0.03	0.01	0.02	1.00	NSS	81	4	1	4	0.33	NSS
42 rpm (110 l/min)	before 1	3.90						63					
	before 2	4.00						92					
	after 1	4.08	0.18					101	38				
	after 2	3.95	-0.05	0.07	0.16	0.57	NSS	68	-24	7	44	0.23	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
23 rpm (59 l/min)	before 1	9						18					
	before 2	8						17					
	after 1	10	1					19	1				
	after 2	6	0	1	1	1.00	NSS	19	2	2	1	3.00	NSS
42 rpm (110 l/min)	before 1	8						20					
	before 2	10						20					
	after 1	15	7					24	4				
	after 2	8	-2	3	6	0.56	NSS	18	-2	1	4	0.33	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 40 BREDEL PERISTALTIC PUMP (RETRIAL 4) YEAST QUALITY AND FERMENTATION INDICATORS

RETRIAL	4
DATE	11/10/95
YEAST	CASTLE D4 EX FV 98

YEAST QUALITY

PUMP SPEED	SAMPLE	INTEGRITY						VIABILITY						VITALITY					
		Protease (absorbance)	Statistical Parameters					Mod MeBl (% viability)	Statistical Parameters					OUR (mg/l/min/10^6 viable cells)	Statistical Parameters				
			Diff	Avg	Stda	t	SS?		Diff	Avg	Stda	t	SS?		Diff	Avg	Stda	t	SS?
25 rpm	before 1	0.01						93						0.008					
(33 l/min)	before 2	0.00						93						0.007					
	after 1	0.02	0.01					92	-1					0.010	0.002				
	after 2	0.02	0.02	0.02	0.01	3.00	NSS	92	-1	-1	0	-	SDZ	0.011	0.004	0.003	0.001	3.00	NSS
40 rpm	before 1	0.02						93						0.008					
(57 l/min)	before 2	0.02						92						0.008					
	after 1	0.03	0.01					96	3					0.009	0.001				
	after 2	0.03	0.01	0.01	0.00	-	SDZ	95	3	3	0	-	SDZ	0.010	0.002	0.001	0.001	3.00	NSS

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH						ATTENUATION					
		Inc in Biomass (factor)	Statistical Parameters					Final Gravity (Plato)	Statistical Parameters				
			Diff	Avg	Stda	t	SS?		Diff	Avg	Stda	t	SS?
25 rpm	before 1	2.7						2.43					
(33 l/min)	before 2	2.9						2.55					
	after 1	2.9	0.2					2.52	0.09				
	after 2	2.7	-0.2	0.0	0.3	0.00	NSS	2.39	-0.16	-0.03	0.18	-0.28	NSS
40 rpm	before 1	2.7						2.52					
(57 l/min)	before 2	2.9						2.42					
	after 1	2.8	0.1					2.38	-0.14				
	after 2	2.4	-0.5	-0.2	0.4	-0.67	NSS	2.11	-0.31	-0.23	0.12	-2.65	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 40 BREDEL PERISTALTC PUMP (RETRIAL 4] BEER QUALITY (DAY 8) AND FERMENTATION INDICATORS

RETRIAL	4
DATE	11/10/95
YEAST	CASTLE D4 EX FV 98

BEER QUALITY (DAY 8)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters Diff	Avg	Stds	t	SS?	Diacetyl (ppb)	Statistical Parameters Diff	Avg	Stds	t	SS?
25 rpm (33 l/min)	before 1	4.04						120					
	before 2	4.07						119					
	after 1	4.01	-0.03					111	-9				
	after 2	4.07	0.00	-0.02	0.02	-1.00	NSS	119	0	-5	6	-1.00	NSS
40 rpm (57 l/min)	before 1	4.09						118					
	before 2	4.05						113					
	after 1	4.07	-0.02					124	6				
	after 2	3.96	-0.09	-0.05	0.05	-1.57	NSS	98	-15	-5	15	-0.43	NSS

FERMENTATION

Alpha Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
15.24					
15.18					
15.04	-0.20				
15.04	-0.15	-0.17	0.04	-6.73	NSS
15.24					
15.10					
15.27	0.03				
15.09	-0.01	0.01	0.03	0.38	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?	Acetaldehyde (ppm)	Statistical Parameters Diff	Avg	Stds	t	SS?
25 rpm (33 l/min)	before 1	10						19					
	before 2	10						20					
	after 1	9	-1					19	0				
	after 2	11	1	0	1	0.00	NSS	21	1	1	1	1.00	NSS
40 rpm (57 l/min)	before 1	11						21					
	before 2	11						20					
	after 1	11	0					20	-1				
	after 2	8	-3	-2	2	-1.00	NSS	16	-4	-3	2	-1.67	NSS

Beta Constants	Statistical Parameters Diff	Avg	Stds	t	SS?
-0.0110					
-0.0110					
-0.0105	5E-04				
-0.0111	-1E-04	2E-04	4E-04	0.67	NSS
-0.0109					
-0.0107					
-0.0106	3E-04				
-0.0117	-1E-03	-4E-04	9E-04	-0.54	NSS

PAIRED-SAMPLE t TESTS FOR PUMP RETRIALS - SP 40 BREDEL PERISTALTIC PUMP (RETRIAL 4] BEER QUALITY (DAY 12)

RETRIAL	4
DATE	11/10/95
YEAST	CASTLE D4 EX FV 98

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Statistical Parameters					Diacetyl (ppb)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
25 rpm	before 1	4.07						78					
(33 l/min)	before 2	4.09						86					
	after 1	4.02	-0.05					72	-8				
	after 2	4.10	0.01	-0.02	0.04	-0.67	NSS	74	-12	-9	4	-3.00	NSS
40 rpm	before 1	4.11						76					
(57 l/min)	before 2	4.07						80					
	after 1	4.04	-0.07					81	5				
	after 2	3.98	-0.09	-0.08	0.01	-8.00	NSS	61	-19	-7	17	-0.58	NSS

PUMP SPEED	SAMPLE	SO2 (ppm)	Statistical Parameters					Acetaldehyde (ppm)	Statistical Parameters				
			Diff	Avg	Stds	t	SS?		Diff	Avg	Stds	t	SS?
25 rpm	before 1	10						21					
(33 l/min)	before 2	10						22					
	after 1	9	-1					21	0				
	after 2	10	0	-1	1	-1.00	NSS	24	2	1	1	1.00	NSS
40 rpm	before 1	11						23					
(57 l/min)	before 2	11						21					
	after 1	10	-1					22	-1				
	after 2	8	-3	-2	1	-2.00	NSS	18	-3	-2	1	-2.00	NSS

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - MASO SINE PUMP (RETRIAL 1) - YEAST QUALITY AND FERMENTATION INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Protease	S1B	0.05	0.06	0.11	0.06	2	Between Samples	0.0021	3	0.0007	1.47
	S1A	0.08	0.07	0.15	0.08	2	Within Samples	0.0019	4	0.0005	
	S2B	0.04	0.10	0.14	0.07	2	Total	0.0040	7		
	S2A	0.10	0.10	0.20	0.10	2					
Modified Methylene Blue	S1B	92	89	181	91	2	Between Samples	9.00	3	3.00	1.09
	S1A	93	90	183	92	2	Within Samples	11.00	4	2.75	
	S2B	92	94	186	93	2	Total	20.00	7		
	S2A	93	93	186	93	2					
Oxygen Utilisation Rate	S1B	0.015	0.008	0.023	0.012	2	Between Samples	0.000039	3	0.000013	1.57
	S1A	0.009	0.008	0.017	0.009	2	Within Samples	0.000033	4	0.000008	
	S2B	0.015	0.011	0.026	0.013	2	Total	0.000073	7		
	S2A	0.007	0.008	0.015	0.008	2					
Growth	S1B	3.0	3.0	6.0	3.0	2	Between Samples	0.21	3	0.07	3.90
	S1A	3.3	3.0	6.3	3.2	2	Within Samples	0.07	4	0.02	
	S2B	3.4	3.3	6.7	3.4	2	Total	0.28	7		
	S2A	3.3	3.5	6.8	3.4	2					
Final Attenuation	S1B	2.55	2.51	5.06	2.53	2	Between Samples	0.0109	3	0.0036	0.79
	S1A	2.52	2.43	4.95	2.48	2	Within Samples	0.0185	4	0.0046	
	S2B	2.48	2.41	4.89	2.45	2	Total	0.0295	7		
	S2A	2.36	2.51	4.87	2.44	2					
Alpha Constant	S1B	16.27	15.96	32.22	16.11	2	Between Samples	0.0560	3.0	0.0187	0.36
	S1A	16.20	15.97	32.17	16.09	2	Within Samples	0.2051	4.0	0.0513	
	S2B	16.40	15.98	32.38	16.19	2	Total	0.2611	7.0		
	S2A	16.11	15.81	31.92	15.96	2					
Beta Constant	S1B	-0.0101	-0.0103	-0.0204	-0.0102	2	Between Samples	9.5E-08	3	3.2E-08	1.58
	S1A	-0.0103	-0.0105	-0.0208	-0.0104	2	Within Samples	8.0E-08	4	2.0E-08	
	S2B	-0.0104	-0.0106	-0.0210	-0.0105	2	Total	1.8E-07	7		
	S2A	-0.0105	-0.0103	-0.0208	-0.0104	2					

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - MASO SINE PUMP (RETRIAL 1) - BEER QUALITY INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
pH	S1B	3.94	3.92	7.86	3.93	2	Between Samples	0.0002	3	0.0001	0.42
(Day 8)	S1A	3.93	3.94	7.87	3.94	2	Within Samples	0.0008	4	0.0002	
	S2B	3.95	3.92	7.87	3.94	2	Total	0.0010	7		
	S2A	3.94	3.95	7.89	3.95	2					
Diacetyl	S1B	121	141	262	131	2	Between Samples	800.71	3	266.90	1.42
(Day 8)	S1A	139	156	295	148	2	Within Samples	565.00	3	188.33	
	S2B	-	152	152	152	1	Total	1365.71	6		
	S2A	148	169	317	159	2					
SO2	S1B	15	12	27	14	2	Between Samples	10.00	3	3.33	0.44
(Day 8)	S1A	14	15	29	15	2	Within Samples	30.00	4	7.50	
	S2B	16	15	31	16	2	Total	40.00	7		
	S2A	20	13	33	17	2					
Acetaldehyde	S1B	23	23	46	23	2	Between Samples	2.38	3	0.79	0.49
(Day 8)	S1A	24	24	48	24	2	Within Samples	6.50	4	1.63	
	S2B	21	24	45	23	2	Total	8.88	7		
	S2A	24	22	46	23	2					
pH	S1B	3.97	3.94	7.91	3.96	2	Between Samples	0.0008	3	0.0003	1.62
(Day 12)	S1A	3.95	3.95	7.90	3.95	2	Within Samples	0.0007	4	0.0002	
	S2B	3.98	3.96	7.94	3.97	2	Total	0.0016	7		
	S2A	3.97	3.98	7.95	3.98	2					
Diacetyl	S1B	81	76	157	79	2	Between Samples	133.00	3	44.33	0.72
(Day 12)	S1A	76	85	161	81	2	Within Samples	247.00	4	61.75	
	S2B	68	76	144	72	2	Total	380.00	7		
	S2A	62	80	142	71	2					
SO2	S1B	14	13	27	14	2	Between Samples	12.38	3	4.13	2.20
(Day 12)	S1A	13	14	27	14	2	Within Samples	7.50	4	1.88	
	S2B	10	13	23	12	2	Total	19.88	7		
	S2A	14	16	30	15	2					
Acetaldehyde	S1B	42	38	80	40	2	Between Samples	6.50	3	2.17	0.05
(Day 12)	S1A	45	34	79	40	2	Within Samples	179.00	4	44.75	
	S2B	36	41	77	39	2	Total	185.50	7		
	S2A	48	34	82	41	2					

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - APV LOBE PUMP (RETRIAL 2) - BEER QUALITY INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
pH	S1B	4.03	4.06	8.09	4.05	2	Between Samples	0.0006	3	0.0002	1.13
(Day 8)	S1A	4.05	4.06	8.11	4.06	2	Within Samples	0.0008	4	0.0002	
	S2B	4.04	4.02	8.06	4.03	2	Total	0.0014	7		
	S2A	4.04	4.05	8.09	4.05	2					
Diacetyl	S1B	125	123	248	124	2	Between Samples	261.00	3	87.00	4.09
(Day 8)	S1A	116	107	223	112	2	Within Samples	85.00	4	21.25	
	S2B	118	109	227	114	2	Total	346.00	7		
	S2A	108	110	218	109	2					
SO2	S1B	15	14	29	15	2	Between Samples	20.50	3	6.83	0.70
(Day 8)	S1A	12	20	32	16	2	Within Samples	39.00	4	9.75	
	S2B	11	14	25	13	2	Total	59.50	7		
	S2A	11	13	24	12	2					
Acetaldehyde	S1B	21	21	42	21	2	Between Samples	3.38	3	1.13	9.00
(Day 8)	S1A	21	21	42	21	2	Within Samples	0.50	4	0.13	
	S2B	19	20	39	20	2	Total	3.88	7		
	S2A	20	20	40	20	2					
pH	S1B	4.05	4.09	8.14	4.07	2	Between Samples	0.0009	3	0.0003	1.03
(Day 12)	S1A	4.08	4.10	8.18	4.09	2	Within Samples	0.0011	4	0.0003	
	S2B	4.07	4.06	8.13	4.07	2	Total	0.0020	7		
	S2A	4.08	4.09	8.17	4.09	2					
Diacetyl	S1B	67	63	130	65	2	Between Samples	48.38	3	16.13	1.87
(Day 12)	S1A	63	62	125	63	2	Within Samples	34.50	4	8.63	
	S2B	61	57	118	59	2	Total	82.88	7		
	S2A	62	68	130	65	2					
SO2	S1B	9	9	18	9	2	Between Samples	7.38	3	2.46	0.29
(Day 12)	S1A	9	11	20	10	2	Within Samples	34.50	4	8.63	
	S2B	9	13	22	11	2	Total	41.88	7		
	S2A	15	8	23	12	2					
Acetaldehyde	S1B	26	24	50	25	2	Between Samples	4.38	3	1.46	0.78
(Day 12)	S1A	24	27	51	26	2	Within Samples	7.50	4	1.88	
	S2B	23	24	47	24	2	Total	11.88	7		
	S2A	25	24	49	25	2					

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - APV LOBE PUMP (RETRIAL 2) - BEER QUALITY INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
pH	S1B	4.03	4.06	8.09	4.05	2	Between Samples	0.0006	3	0.0002	1.13
(Day 8)	S1A	4.05	4.06	8.11	4.06	2	Within Samples	0.0008	4	0.0002	
	S2B	4.04	4.02	8.06	4.03	2	Total	0.0014	7		
	S2A	4.04	4.05	8.09	4.05	2					
Diacetyl	S1B	125	123	248	124	2	Between Samples	261.00	3	87.00	4.09
(Day 8)	S1A	116	107	223	112	2	Within Samples	85.00	4	21.25	
	S2B	118	109	227	114	2	Total	346.00	7		
	S2A	108	110	218	109	2					
SO2	S1B	15	14	29	15	2	Between Samples	20.50	3	6.83	0.70
(Day 8)	S1A	12	20	32	16	2	Within Samples	39.00	4	9.75	
	S2B	11	14	25	13	2	Total	59.50	7		
	S2A	11	13	24	12	2					
Acetaldehyde	S1B	21	21	42	21	2	Between Samples	3.38	3	1.13	9.00
(Day 8)	S1A	21	21	42	21	2	Within Samples	0.50	4	0.13	
	S2B	19	20	39	20	2	Total	3.88	7		
	S2A	20	20	40	20	2					
pH	S1B	4.05	4.09	8.14	4.07	2	Between Samples	0.0009	3	0.0003	1.03
(Day 12)	S1A	4.08	4.10	8.18	4.09	2	Within Samples	0.0011	4	0.0003	
	S2B	4.07	4.06	8.13	4.07	2	Total	0.0020	7		
	S2A	4.08	4.09	8.17	4.09	2					
Diacetyl	S1B	67	63	130	65	2	Between Samples	48.38	3	16.13	1.87
(Day 12)	S1A	63	62	125	63	2	Within Samples	34.50	4	8.63	
	S2B	61	57	118	59	2	Total	82.88	7		
	S2A	62	68	130	65	2					
SO2	S1B	9	9	18	9	2	Between Samples	7.38	3	2.46	0.29
(Day 12)	S1A	9	11	20	10	2	Within Samples	34.50	4	8.63	
	S2B	9	13	22	11	2	Total	41.88	7		
	S2A	15	8	23	12	2					
Acetaldehyde	S1B	26	24	50	25	2	Between Samples	4.38	3	1.46	0.78
(Day 12)	S1A	24	27	51	26	2	Within Samples	7.50	4	1.88	
	S2B	23	24	47	24	2	Total	11.88	7		
	S2A	25	24	49	25	2					

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - APV LOBE PUMP (RETRIAL 2) - BEER QUALITY INDICATORS (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Acetaldehyde (Day 8)	S1B	21	21	42	21	2	Between Samples	0.00	1	0.00	VI
	S1A	21	21	42	21	2	Within Samples	0.00	2	0.00	
							Total	0.00	3		
	S2B	19	20	39	20	2	Between Samples	0.25	1	0.25	1.00
	S2A	20	20	40	20	2	Within Samples	0.50	2	0.25	
							Total	0.75	3		

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - SP50 BREDEL PERISTALTIC PUMP (RETRIAL 3) - YEAST QUALITY/FERMENTATION INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Protease	S1B	0.16	0.17	0.33	0.17	2	Between Samples	0.0061	3	0.0020	9.04
	S1A	0.16	0.18	0.34	0.17	2	Within Samples	0.0009	4	0.0002	
	S2B	0.23	0.21	0.44	0.22	2	Total	0.0070	7		
	S2A	0.24	0.21	0.45	0.23	2					
Modified Methylene Blue	S1B	96	94	190	95	2	Between Samples	4.38	3	1.46	2.33
	S1A	94	94	188	94	2	Within Samples	2.50	4	0.63	
	S2B	95	96	191	96	2	Total	6.88	7		
	S2A	96	96	192	96	2					
Oxygen Utilisation Rate	S1B	0.011	0.010	0.021	0.011	2	Between Samples	0.000010	3	0.000003	14.00
	S1A	0.011	0.011	0.022	0.011	2	Within Samples	0.000001	4	0.000000	
	S2B	0.010	0.009	0.019	0.010	2	Total	0.000011	7		
	S2A	0.008	0.008	0.016	0.008	2					
Growth	S1B	2.3	2.6	4.9	2.5	2	Between Samples	0.28	3	0.09	1.07
	S1A	3.0	2.9	5.9	3.0	2	Within Samples	0.35	4	0.09	
	S2B	2.3	2.9	5.2	2.6	2	Total	0.64	7		
	S2A	2.8	2.3	5.1	2.6	2					
Final Attenuation	S1B	2.82	2.75	5.57	2.79	2	Between Samples	0.0805	3	0.0268	0.76
	S1A	2.64	2.62	5.26	2.63	2	Within Samples	0.1404	4	0.0351	
	S2B	2.30	2.80	5.10	2.55	2	Total	0.2210	7		
	S2A	2.86	2.70	5.56	2.78	2					
Alpha Constant	S1B	14.89	14.75	29.64	14.82	2	Between Samples	0.2650	3	0.0883	1.62
	S1A	14.95	14.92	29.86	14.93	2	Within Samples	0.2184	4	0.0546	
	S2B	15.47	14.93	30.40	15.20	2	Total	0.4834	7		
	S2A	14.88	14.54	29.42	14.71	2					
Beta Constant	S1B	-0.0093	-0.0093	-0.0186	-0.0093	2	Between Samples	5.7E-07	3	1.9E-07	0.67
	S1A	-0.0096	-0.0096	-0.0192	-0.0096	2	Within Samples	1.1E-06	4	2.9E-07	
	S2B	-0.0108	-0.0093	-0.0201	-0.0101	2	Total	1.7E-06	7		
	S2A	-0.0095	-0.0097	-0.0192	-0.0096	2					

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - SP 50 BREDEL PERISTALTIC PUMP (RETRIAL 3) - YEAST QUALITY INDICATORS (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Protease	S1B	0.16	0.17	0.33	0.17	2	Between Samples	0.0000	1	0.0000	0.20
	S1A	0.16	0.18	0.34	0.17	2	Within Samples	0.0003	2	0.0001	
							Total	0.0003	3		
	S2B	0.23	0.21	0.44	0.22	2	Between Samples	0.0000	1	0.0000	0.08
	S2A	0.24	0.21	0.45	0.23	2	Within Samples	0.0007	2	0.0003	
							Total	0.0007	3		
Oxygen Utilisation Rate	S1B	0.011	0.010	0.021	0.011	2	Between Samples	0.000000	1	0.000000	1.00
	S1A	0.011	0.011	0.022	0.011	2	Within Samples	0.000001	2	0.000000	
							Total	0.000001	3		
	S2B	0.010	0.009	0.019	0.010	2	Between Samples	0.000002	1	0.000002	9.00
	S2A	0.008	0.008	0.016	0.008	2	Within Samples	0.000000	2	0.000000	
							Total	0.000003	3		

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - SP 50 BREDEL PERISTALTIC PUMP (RETRIAL 3) - BEER QUALITY INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
pH	S1B	3.93	3.93	7.86	3.93	2	Between Samples	0.0058	3	0.0019	0.99
(Day 8)	S1A	3.94	3.94	7.88	3.94	2	Within Samples	0.0079	4	0.0020	
	S2B	3.86	3.92	7.78	3.89	2	Total	0.0137	7		
	S2A	4.02	3.91	7.93	3.97	2					
Diacetyl	S1B	88	75	163	82	2	Between Samples	34.50	3	11.50	0.16
(Day 8)	S1A	83	77	160	80	2	Within Samples	285.00	4	71.25	
	S2B	72	86	158	79	2	Total	319.50	7		
	S2A	91	78	169	85	2					
SO2	S1B	9	9	18	9	2	Between Samples	1.38	3	0.46	0.41
(Day 8)	S1A	10	9	19	10	2	Within Samples	4.50	4	1.13	
	S2B	9	11	20	10	2	Total	5.88	7		
	S2A	11	9	20	10	2					
Acetaldehyde	S1B	15	16	31	16	2	Between Samples	25.38	3	8.46	4.51
(Day 8)	S1A	19	21	40	20	2	Within Samples	7.50	4	1.88	
	S2B	16	19	35	18	2	Total	32.88	7		
	S2A	20	19	39	20	2					
pH	S1B	3.97	3.95	7.92	3.96	2	Between Samples	0.0049	3	0.0016	0.48
(Day 12)	S1A	3.97	3.98	7.95	3.98	2	Within Samples	0.0137	4	0.0034	
	S2B	3.90	4.00	7.90	3.95	2	Total	0.0186	7		
	S2A	4.08	3.95	8.03	4.02	2					
Diacetyl	S1B	79	77	156	78	2	Between Samples	62.50	3	20.83	0.09
(Day 12)	S1A	77	81	158	79	2	Within Samples	975.00	4	243.75	
	S2B	63	92	155	78	2	Total	1037.50	7		
	S2A	101	68	169	85	2					
SO2	S1B	9	8	17	9	2	Between Samples	11.00	3	3.67	0.51
(Day 12)	S1A	10	8	18	9	2	Within Samples	29.00	4	7.25	
	S2B	8	10	18	9	2	Total	40.00	7		
	S2A	15	8	23	12	2					
Acetaldehyde	S1B	18	17	35	18	2	Between Samples	13.38	3	4.46	0.96
(Day 12)	S1A	19	19	38	19	2	Within Samples	18.50	4	4.63	
	S2B	20	20	40	20	2	Total	31.88	7		
	S2A	24	18	42	21	2					

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - SP 40 BREDEL PERISTALTIC PUMP (RETRIAL 4) - YEAST QUALITY/ FERMENTATION INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Protease	S1B	0.01	0.00	0.01	0.01	2	Between Samples	0.0007	3	0.0002	9.33
	S1A	0.02	0.02	0.04	0.02	2	Within Samples	0.0001	4	0.0000	
	S2B	0.02	0.03	0.05	0.03	2	Total	0.0008	7		
	S2A	0.03	0.03	0.06	0.03	2					
Modified	S1B	93	93	186	93	2	Between Samples	14.50	3	4.83	19.33
Methylene	S1A	92	92	184	92	2	Within Samples	1.00	4	0.25	
Blue	S2B	93	92	185	93	2	Total	15.50	7		
	S2A	96	95	191	96	2					
Oxygen	S1B	0.008	0.007	0.015	0.008	2	Between Samples	0.000011	3	0.000004	10.11
Utilisation	S1A	0.010	0.011	0.021	0.011	2	Within Samples	0.000002	4	0.000000	
Rate	S2B	0.008	0.008	0.016	0.008	2	Total	0.000013	7		
	S2A	0.009	0.010	0.019	0.010	2					
Growth	S1B	2.7	2.9	5.6	2.8	2	Between Samples	0.06	3	0.02	0.57
	S1A	2.9	2.7	5.6	2.8	2	Within Samples	0.14	4	0.04	
	S2B	2.7	2.9	5.6	2.8	2	Total	0.20	7		
	S2A	2.8	2.4	5.2	2.6	2					
Final	S1B	2.43	2.55	4.98	2.49	2	Between Samples	0.0783	3	0.0261	1.83
Attenuation	S1A	2.52	2.39	4.91	2.46	2	Within Samples	0.0571	4	0.0143	
	S2B	2.52	2.42	4.94	2.47	2	Total	0.1354	7		
	S2A	2.38	2.11	4.49	2.25	2					
Alpha	S1B	15.24	15.18	30.42	15.21	2	Between Samples	0.0346	3.0	0.0115	1.71
Constant	S1A	15.04	15.04	30.08	15.04	2	Within Samples	0.0270	4.0	0.0068	
	S2B	15.24	15.10	30.34	15.17	2	Total	0.0616	7.0		
	S2A	15.27	15.09	30.36	15.18	2					
Beta	S1B	-0.0110	-0.0110	-0.0220	-0.0110	2	Between Samples	1.7E-07	3	5.8E-08	0.29
Constant	S1A	-0.0105	-0.0111	-0.0216	-0.0108	2	Within Samples	8.0E-07	4	2.0E-07	
	S2B	-0.0109	-0.0107	-0.0216	-0.0108	2	Total	9.8E-07	7		
	S2A	-0.0106	-0.0117	-0.0223	-0.0112	2					

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - SP 40 BREDEL PERISTALTIC PUMP (RETRIAL 4) - YEAST QUALITY INDICATORS (continued)

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Protease	S1B	0.01	0.00	0.01	0.01	2	Between Samples	0.0002	1	0.0002	9.00
	S1A	0.02	0.02	0.04	0.02	2	Within Samples	0.0001	2	0.0000	
							Total	0.0003	3		
	S2B	0.02	0.03	0.05	0.03	2	Between Samples	0.0000	1	0.0000	1.00
	S2A	0.03	0.03	0.06	0.03	2	Within Samples	0.0000	2	0.0000	
							Total	0.0001	3		
Modified Methylene Blue	S1B	93	93	186	93	2	Between Samples	1.00	1	1.00	WSVZ
	S1A	92	92	184	92	2	Within Samples	0.00	2	0.00	
							Total	1.00	3		
	S2B	93	92	185	93	2	Between Samples	9.00	1	9.00	18.00
	S2A	96	95	191	96	2	Within Samples	1.00	2	0.50	
							Total	10.00	3		
Oxygen Utilisation Rate	S1B	0.008	0.007	0.015	0.008	2	Between Samples	0.000009	1	0.000009	18.00
	S1A	0.010	0.011	0.021	0.011	2	Within Samples	0.000001	2	0.000001	
							Total	0.000010	3		
	S2B	0.008	0.008	0.016	0.008	2	Between Samples	0.000002	1	0.000002	9.00
	S2A	0.009	0.010	0.019	0.010	2	Within Samples	0.000000	2	0.000000	
							Total	0.000003	3		

ANALYSIS OF VARIANCE FOR PUMP RETRIALS - SP 40 BREDEL PERISTALTIC PUMP (RETRIAL 4) - BEER QUALITY INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
pH	S1B	4.04	4.07	8.11	4.06	2	Between Samples	0.0033	3	0.0011	0.48
(Day 8)	S1A	4.01	4.07	8.08	4.04	2	Within Samples	0.0091	4	0.0023	
	S2B	4.09	4.05	8.14	4.07	2	Total	0.0124	7		
	S2A	4.07	3.96	8.03	4.02	2					
Diacetyl	S1B	120	119	239	120	2	Between Samples	72.50	3	24.17	0.25
(Day 8)	S1A	111	119	230	115	2	Within Samples	383.00	4	95.75	
	S2B	118	113	231	116	2	Total	455.50	7		
	S2A	124	98	222	111	2					
SO2	S1B	10	10	20	10	2	Between Samples	2.38	3	0.79	0.49
(Day 8)	S1A	9	11	20	10	2	Within Samples	6.50	4	1.63	
	S2B	11	11	22	11	2	Total	8.88	7		
	S2A	11	8	19	10	2					
Acetaldehyde	S1B	19	20	39	20	2	Between Samples	7.00	3	2.33	0.85
(Day 8)	S1A	19	21	40	20	2	Within Samples	11.00	4	2.75	
	S2B	21	20	41	21	2	Total	18.00	7		
	S2A	20	16	36	18	2					
pH	S1B	4.07	4.09	8.16	4.08	2	Between Samples	0.0076	3	0.0025	1.69
(Day 12)	S1A	4.02	4.10	8.12	4.06	2	Within Samples	0.0060	4	0.0015	
	S2B	4.11	4.07	8.18	4.09	2	Total	0.0136	7		
	S2A	4.04	3.98	8.02	4.01	2					
Diacetyl	S1B	78	86	164	82	2	Between Samples	148.00	3	49.33	0.82
(Day 12)	S1A	72	74	146	73	2	Within Samples	242.00	4	60.50	
	S2B	76	80	156	78	2	Total	390.00	7		
	S2A	81	61	142	71	2					
SO2	S1B	10	10	20	10	2	Between Samples	4.38	3	1.46	2.33
(Day 12)	S1A	9	10	19	10	2	Within Samples	2.50	4	0.63	
	S2B	11	11	22	11	2	Total	6.88	7		
	S2A	10	8	18	9	2					
Acetaldehyde	S1B	21	22	43	22	2	Between Samples	7.00	3	2.33	0.62
(Day 12)	S1A	21	24	45	23	2	Within Samples	15.00	4	3.75	
	S2B	23	21	44	22	2	Total	22.00	7		
	S2A	22	18	40	20	2					

APPENDIX F

THE EFFECT OF FLOW CONDITIONS: RESULTS AND STATISTICAL ANALYSIS

ABBREVIATIONS

F1 - F7	**RESULTS OF FLOW TRIALS**
Before 1	Sample taken at sampling point before pump at first instance at which the pump was stopped for sampling
After 1	Sample taken at sampling point after pump at first instance at which the pump was stopped for sampling
Before 2	Sample taken at sampling point before pump at second instance at which the pump was stopped for sampling
After 2	Sample taken at sampling point after pump at second instance at which the pump was stopped for sampling
Before 3	Sample taken at sampling point before pump at third instance at which the pump was stopped for sampling
After 3	Sample taken at sampling point after pump at third instance at which the pump was stopped for sampling
F8 - F13	**ANOVA of RESULTS OF FLOW TRIALS**
S1B	Samples taken at sampling point before pump at first pump speed
S1A	Samples taken at sampling point after pump at first pump speed
S2B	Samples taken at sampling point before pump at second pump speed
S2A	Samples taken at sampling point after pump at second pump speed
S3B	Samples taken at sampling point before pump at third pump speed
S3A	Samples taken at sampling point after pump at third pump speed

RESULTS OF FLOW TRIALS - SP 50 BREDEL PERISTALTIC PUMP (TRIAL 1)

TRIAL	1
DATE	28/9/95
YEAST	CASTLE A6 EX FV 81

YEAST QUALITY

FLOW CONDITIONS	SAMPLE	INTEGRITY Protease (absorbance)	VIABILITY Mod MeBl (% viability)	VITALITY OUR (mg/l/min/10^8 viable cells)
v = 2.0 m/s	before 1	0.16	94	0.011
Re = 598	before 2	0.18	93	0.011
	after 1	0.17	94	0.009
	after 2	0.15	92	0.011
v = 3.7 m/s	before 1	0.24	96	0.008
Re = 1114	before 2	0.20	96	0.008
	after 1	0.29	94	0.008
	after 2	0.25	94	0.009

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH Inc in Biomass (factor)	ATTENUATION Final Gravity (Plato)	CURVE FIT CONSTANTS Alpha Constants (Plato)	Beta Constants (1/hr)	R2 Values
v = 2.0 m/s	before 1	3.1	2.08	14.86	-0.0105	0.9707
Re = 598	before 2	3.1	2.08	14.93	-0.0110	0.9742
	after 1	3.0	2.10	14.71	-0.0106	0.9734
	after 2	2.8	2.19	14.82	-0.0103	0.9790
v = 3.7 m/s	before 1	3.0	2.08	15.00	-0.0101	0.9720
Re = 1114	before 2	3.0	2.14	14.99	-0.0105	0.9720
	after 1	3.0	2.09	15.16	-0.0103	0.9792
	after 2	3.1	2.09	14.88	-0.0104	0.9782

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
v = 2.0 m/s	before 1	4.13	84	13	23
Re = 598	before 2	4.04	56	11	34
	after 1	4.13	75	12	22
	after 2	4.16	89	14	22
v = 3.7 m/s	before 1	4.19	88	12	25
Re = 1114	before 2	4.16	88	13	18
	after 1	4.15	89	13	27
	after 2	4.15	89	13	20

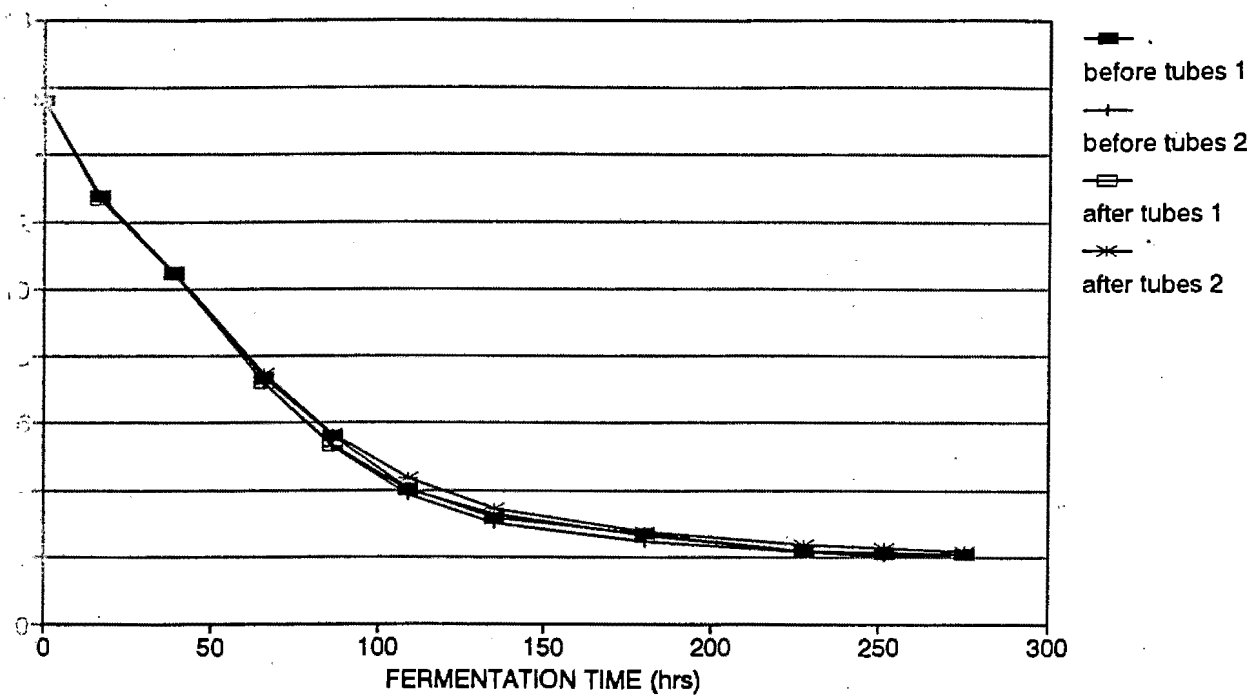

Figure F1 Attenuation profiles for the 500 mL fermentations of samples taken during operation at v = 2 m/s and Re = 598

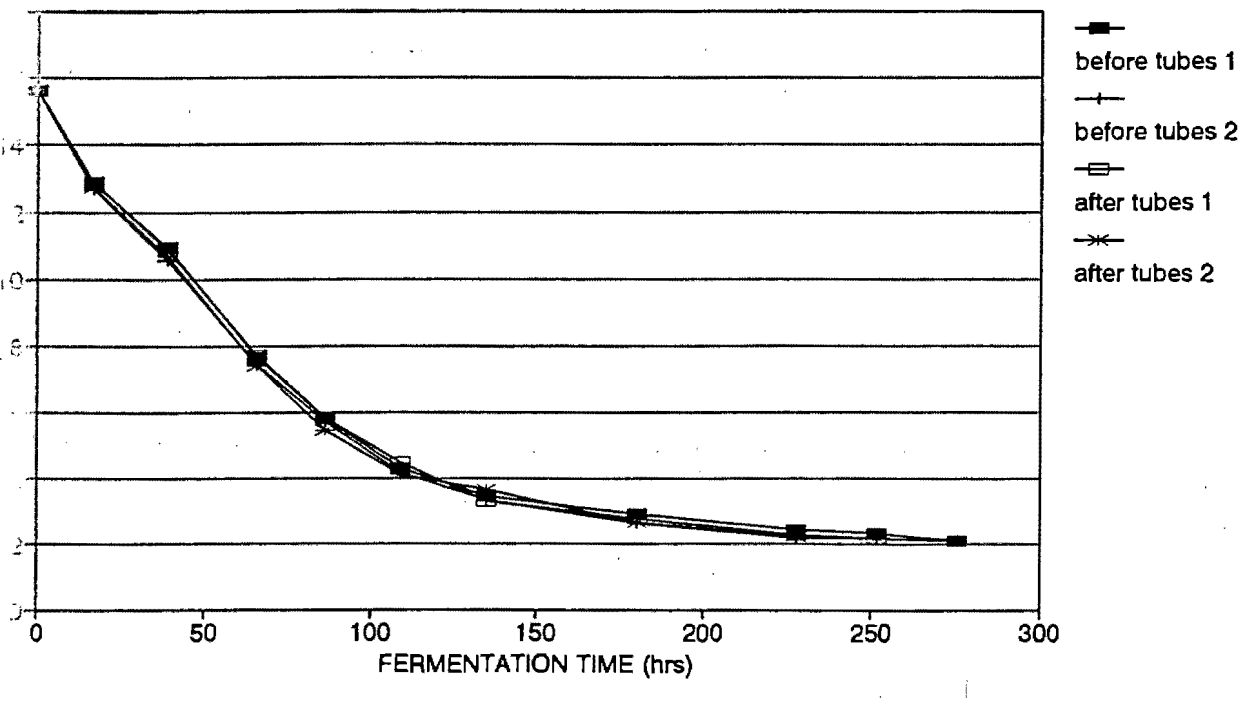

Figure F2 Attenuation profiles for the 500 mL fermentations of samples taken during operation at v = 3.7 m/s and Re = 1114

RESULTS OF FLOW TRIALS - SP 40 BREDEL PERISTALTIC PUMP (TRIAL 2)

TRIAL	2
DATE	11/10/95
YEAST	CASTLE D4 EX FV 98

YEAST QUALITY

FLOW CONDITIONS	SAMPLE	INTEGRITY Protease (absorbance)	VIABILITY Mod MeBl (% viability)	VITALITY OUR (mg/l/min/10^8 viable cells)
v = 0.3 m/s	before 1	0.02	92	0.010
Re = 152	before 2	0.02	92	0.011
	after 1	0.03	94	0.011
	after 2	0.04	96	0.009
v = 0.5 m/s	before 1	0.03	96	0.009
Re = 289	before 2	0.03	95	0.009
	after 1	0.03	94	0.008
	after 2	0.04	95	0.013

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH Inc in Biomass (factor)	ATTENUATION Final Gravity (Plato)	CURVE FIT CONSTANTS Alpha Constants	Beta Constants	R2 Values
v = 0.3 m/s	before 1	2.7	1.94	16.24	-0.0119	0.9907
Re = 152	before 2	2.8	1.83	16.29	-0.0122	0.9950
	after 1	2.9	1.87	16.52	-0.0121	0.9966
	after 2	2.7	1.82	16.25	-0.0126	0.9947
v = 0.5 m/s	before 1	2.7	1.92	16.69	-0.0119	0.9954
Re = 289	before 2	2.8	1.90	16.52	-0.0116	0.9956
	after 1	2.8	1.97	16.45	-0.0115	0.9960
	after 2	3.1	1.98	16.40	-0.0117	0.9957

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
v = 0.3 m/s	before 1	4.30	156	12	18
Re = 152	before 2	4.17	115	14	23
	after 1	4.25	139	16	21
	after 2	4.31	105	14	27
v = 0.5 m/s	before 1	4.25	142	14	19
Re = 289	before 2	4.16	171	13	22
	after 1	4.32	175	14	18
	after 2	4.29	167	15	17

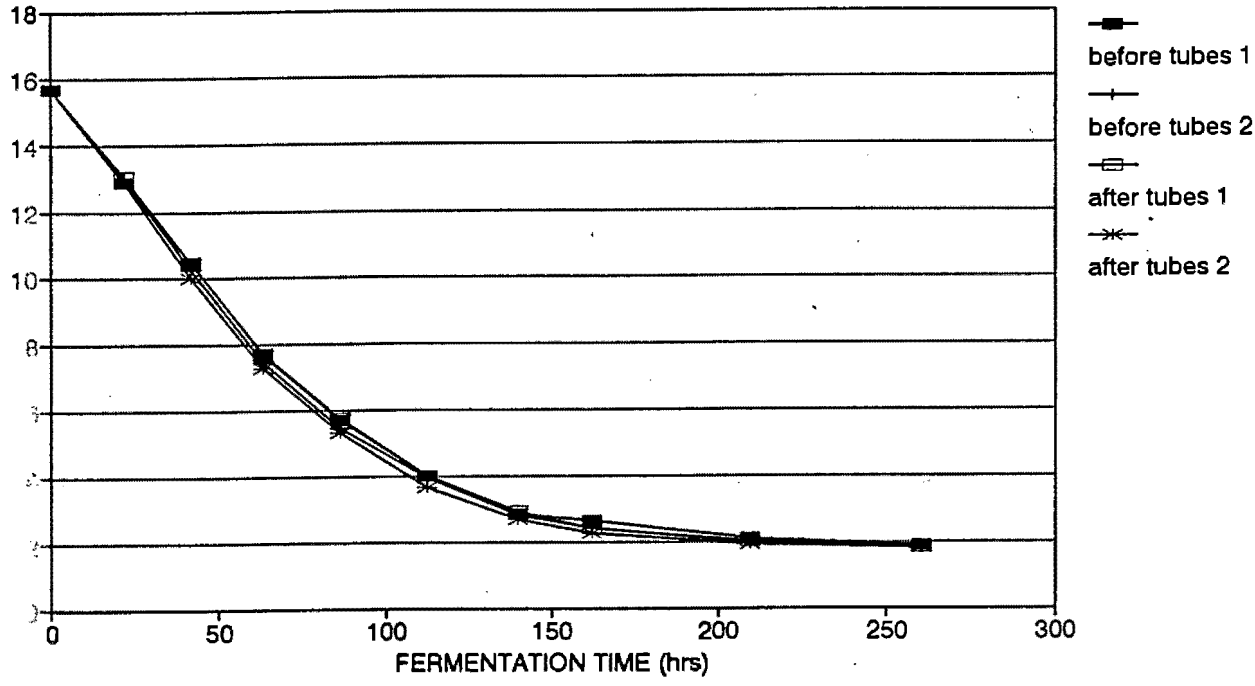

Figure F3 Attenuation profiles for the 500 mL fermentations of samples taken during operation at v = 0.3 m/s and Re = 152

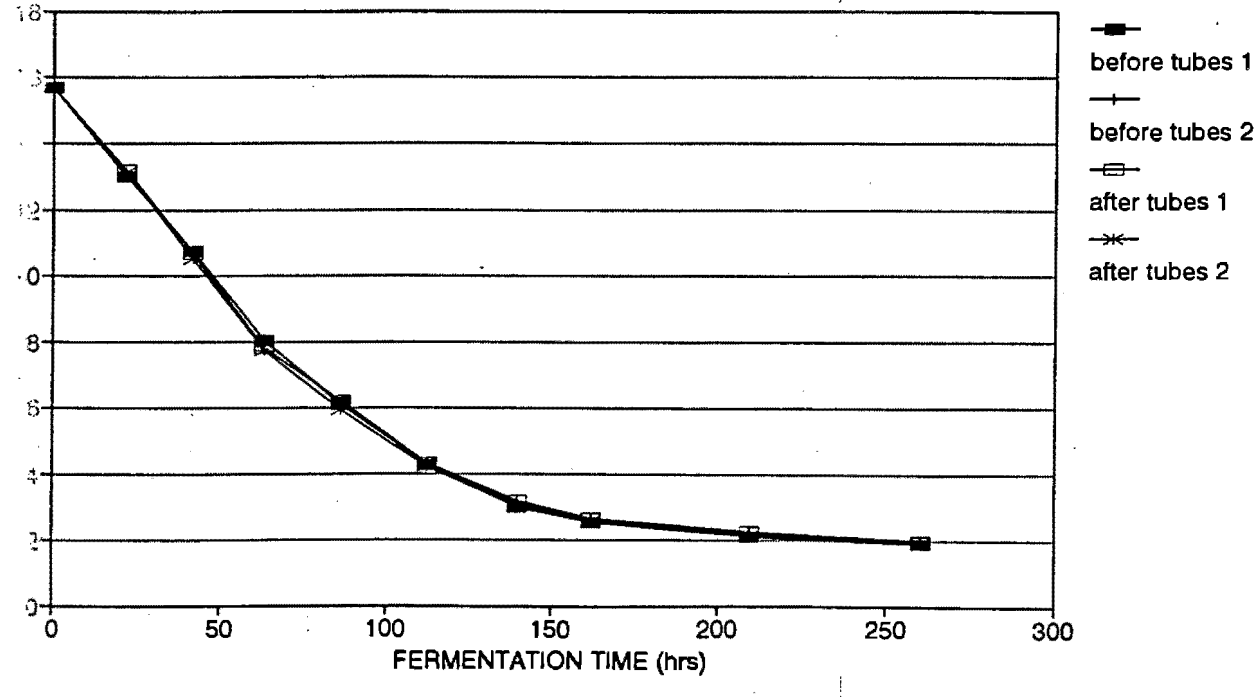

Figure F4 Attenuation profiles for the 500 mL fermentations of samples taken during operation at v = 0.5 m/s and Re = 289

RESULTS OF FLOW TRIALS - APV LOBE PUMP (TRIAL 3)

TRIAL	3
DATE	15/11/95
YEAST	CASTLE (BICPAP) F5 EX FV 108

YEAST QUALITY

FLOW CONDITIONS	SAMPLE	INTEGRITY Protease (absorbance)	VIABILITY Mod MeBl (% viability)	VITALITY OUR (mg/l/min/10^8 viable cells)
v = 0.1 m/s	before 1	0.06	94	0.007
Re = 86	before 2	0.09	93	0.006
	after 1	0.11	93	0.006
	after 2	0.06	92	0.006
v = 0.6 m/s	before 1	0.07	88	0.007
Re = 339	before 2	0.12	90	0.008
	after 1	0.11	91	0.005
	after 2	0.11	90	0.008
v = 2.5 m/s	before 1	0.08	92	0.005
Re = 760	before 2	0.14	91	0.006
	after 1	0.07	94	0.006
	after 2	0.07	91	0.007

FERMENTATION

PUMP SPEED	SAMPLE	GROWTH Inc in Biomass (factor)	ATTENUATION Final Gravity (Plato)	CURVE FIT CONSTANTS Alpha Constants (Plato)	Beta Constants (1/hr)	R2 Values
v = 0.1 m/s	before 1	2.8	1.73	14.46	-0.0111	0.9716
Re = 86	before 2	2.7	1.73	14.40	-0.0114	0.9716
	after 1	2.7	1.72	14.62	-0.0115	0.9709
	after 2	2.7	1.76	14.62	-0.0115	0.9752
v = 0.6 m/s	before 1	2.7	1.73	14.38	-0.0113	0.9698
Re = 339	before 2	2.7	1.76	14.40	-0.0110	0.9715
	after 1	2.8	1.74	14.28	-0.0113	0.9689
	after 2	2.6	1.69	14.54	-0.0115	0.9710
v = 2.5 m/s	before 1	2.9	1.76	14.25	-0.0115	0.9592
Re = 760	before 2	-	-	-	-	0.9592
	after 1	2.9	1.73	14.42	-0.0115	0.9666
	after 2	2.9	1.71	14.29	-0.0114	0.9836

BEER QUALITY (DAY 12)

PUMP SPEED	SAMPLE	pH (pH units)	Diacetyl (ppb)	SO2 (ppm)	Acetaldehyde (ppm)
v = 0.1 m/s	before 1	4.33	156	13	21
Re = 86	before 2	4.27	140	14	23
	after 1	4.34	156	15	22
	after 2	4.34	168	13	19
v = 0.6 m/s	before 1	4.35	158	14	25
Re = 339	before 2	4.37	168	13	21
	after 1	4.37	162	15	16
	after 2	4.36	162	14	16
v = 2.5 m/s	before 1	4.27	134	13	23
Re = 760	before 2	-	-	-	-
	after 1	4.29	144	14	22
	after 2	4.26	146	13	39

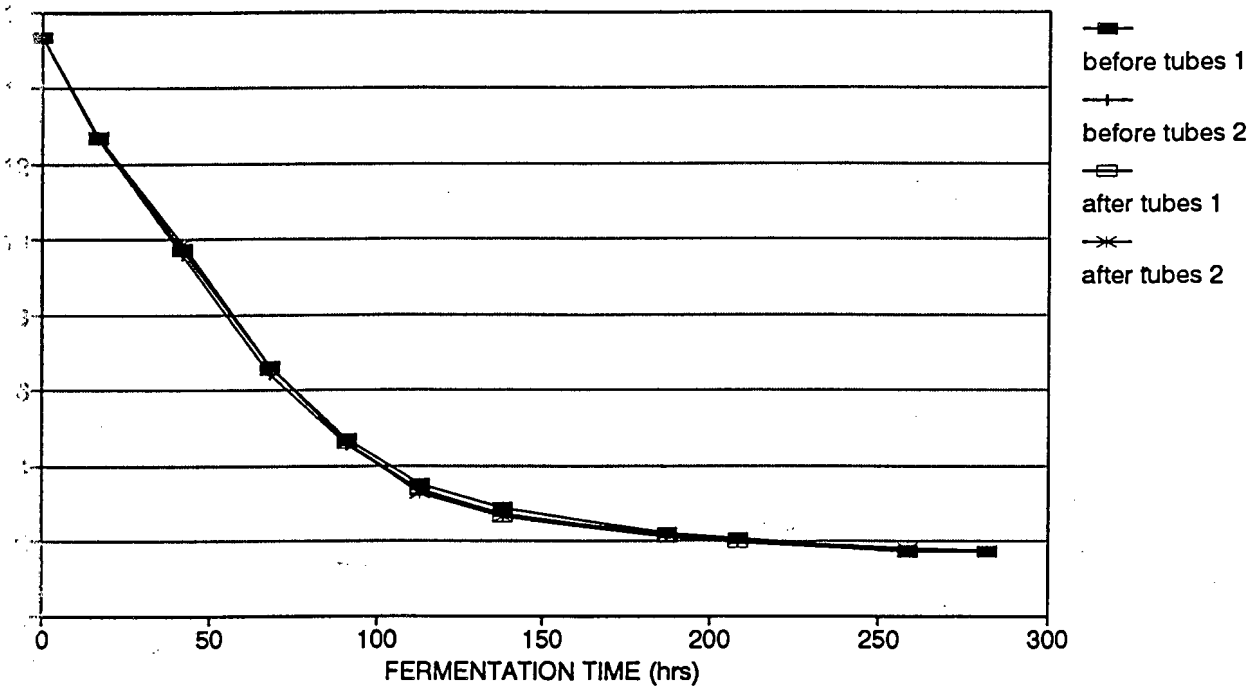

Figure F5 Attenuation profiles for the 500 mL fermentations of samples taken during operation at v = 0.1 m/s and Re = 86

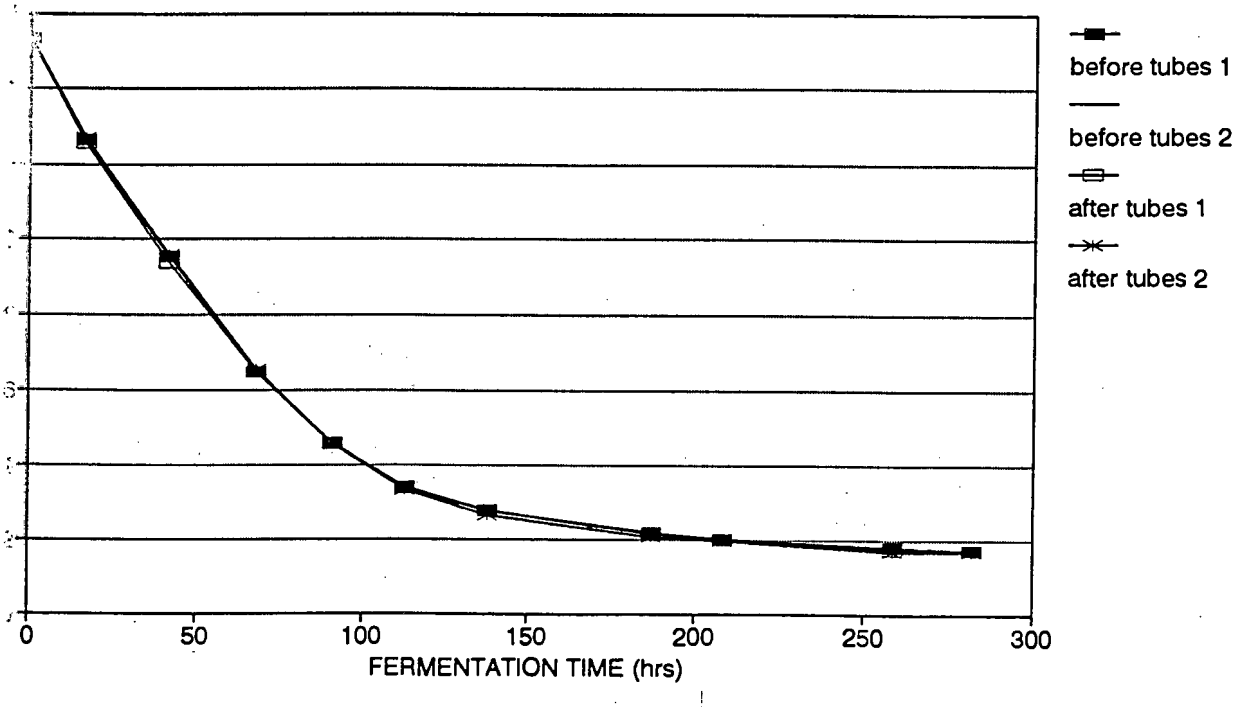

Figure F6 Attenuation profiles for the 500 mL fermentations of samples taken during operation at v = 0.6 m/s and Re = 339

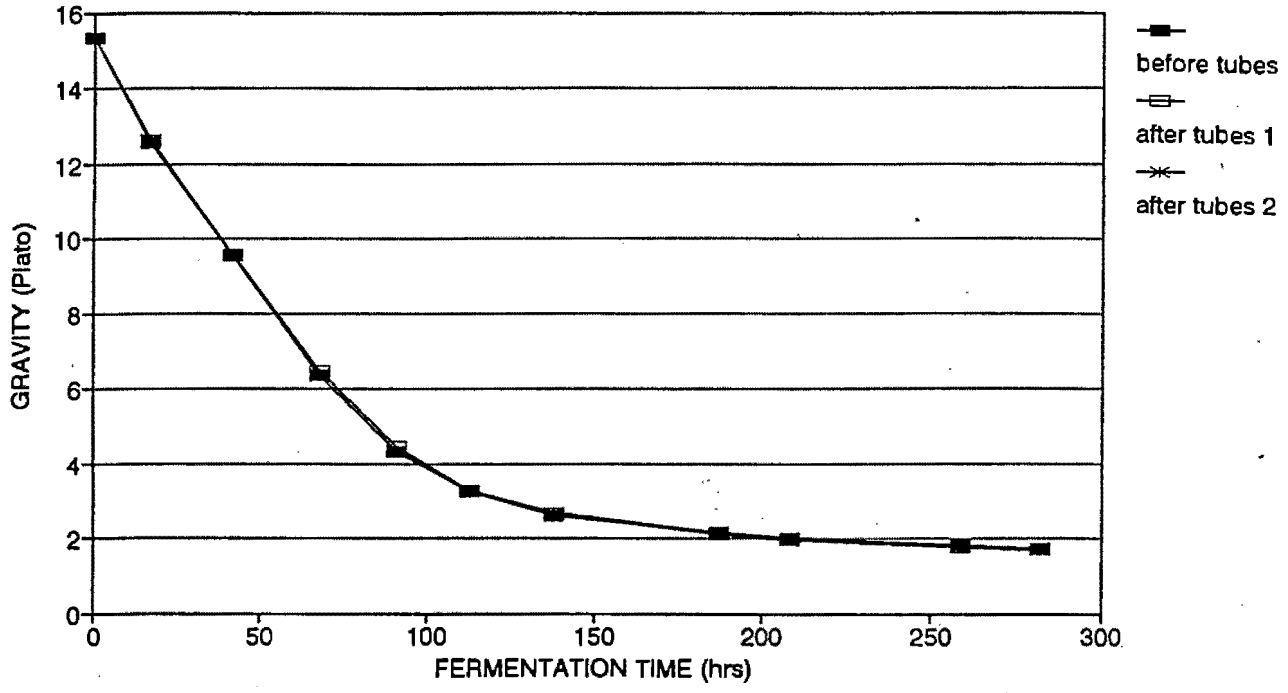

Figure F7 Attenuation profiles for the 500 mL fermentations of samples taken during operation at v = 2.5 m/s and Re = 760

ANALYSIS OF VARIANCE FOR FLOW TRIALS - SP 50 BREDEL PERISTALTIC PUMP (TRIAL 1) - YEAST QUALITY AND FERMENTATION INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Protease	S1B	0.16	0.18	0.34	0.17	2	Between Samples	0.0154	3	0.0051	10.27
	S1A	0.17	0.15	0.32	0.16	2	Within Samples	0.0020	4	0.0005	
	S2B	0.24	0.20	0.44	0.22	2	Total	0.0174	7		
	S2A	0.29	0.25	0.54	0.27	2					
Modified	S1B	94	93	187	94	2	Between Samples	10.38	3	3.46	5.53
Methylene	S1A	94	92	186	93	2	Within Samples	2.50	4	0.63	
Blue	S2B	96	96	192	96	2	Total	12.88	7		
	S2A	94	94	188	94	2					
Oxygen	S1B	0.011	0.011	0.022	0.011	2	Between Samples	0.000011	3	0.000004	6.07
Utilisation	S1A	0.009	0.011	0.020	0.010	2	Within Samples	0.000003	4	0.000001	
Rate	S2B	0.008	0.008	0.016	0.008	2	Total	0.000014	7		
	S2A	0.008	0.009	0.017	0.009	2					
Growth	S1B	3.1	3.1	6.2	3.1	2	Between Samples	0.04	3	0.01	2.67
	S1A	3.0	2.8	5.8	2.9	2	Within Samples	0.02	4	0.00	
	S2B	3.0	3.0	6.0	3.0	2	Total	0.06	7		
	S2A	3.0	3.0	6.0	3.0	2					
Final	S1B	2.08	2.08	4.16	2.08	2	Between Samples	0.0049	3	0.0016	1.13
Attenuation	S1A	2.10	2.19	4.29	2.15	2	Within Samples	0.0058	4	0.0015	
	S2B	2.08	2.14	4.22	2.11	2	Total	0.0108	7		
	S2A	2.09	2.09	4.18	2.09	2					
Alpha	S1B	14.86	14.93	29.79	14.89	2	Between Samples	0.08	3	0.03	2.10
Constant	S1A	14.71	14.82	29.54	14.77	2	Within Samples	0.05	4	0.01	
	S2B	15.00	14.99	30.00	15.00	2	Total	0.13	7		
	S2A	15.16	14.88	30.04	15.02	2					
Beta	S1B	-0.0105	-0.0110	-0.0215	-0.0108	2	Between Samples	1.7E-04	3	5.6E-05	1.04
Constant	S1A	-0.0106	-0.0103	-0.0209	-0.0105	2	Within Samples	2.1E-04	4	5.4E-05	
	S2B	-0.0101	-0.0105	-0.0206	-0.0103	2	Total	3.8E-04	7		
	S2A	-0.0103	0.0104	0.0001	0.0000	2					

ANALYSIS OF VARIANCE FOR FLOW TRIALS - SP 50 BREDEL PERISTALTIC PUMP (TRIAL 1) - BEER QUALITY INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
pH	S1B	4.13	4.04	8.17	4.09	2	Between Samples	0.0087	3	0.0029	2.35
(Day 12)	S1A	4.13	4.16	8.29	4.15	2	Within Samples	0.0050	4	0.0012	
	S2B	4.19	4.16	8.35	4.18	2	Total	0.0137	7		
	S2A	4.15	4.15	8.30	4.15	2					
Diacetyl	S1B	84	56	140	70	2	Between Samples	457.50	3	152.50	1.24
(Day 12)	S1A	75	89	164	82	2	Within Samples	490.00	4	122.50	
	S2B	88	88	176	88	2	Total	947.50	7		
	S2A	89	89	178	89	2					
SO2	S1B	13	11	24	12	2	Between Samples	1.38	3	0.46	0.41
(Day 12)	S1A	12	14	26	13	2	Within Samples	4.50	4	1.13	
	S2B	12	13	25	13	2	Total	5.88	7		
	S2A	13	13	26	13	2					
Acetaldehyde	S1B	23	34	57	29	2	Between Samples	61.38	3	20.46	0.75
(Day 12)	S1A	22	22	44	22	2	Within Samples	109.50	4	27.38	
	S2B	25	18	43	22	2	Total	170.88	7		
	S2A	27	20	47	24	2					

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Protease	S1B	0.16	0.18	0.34	0.17	2	Between Samples	0.0001	1	0.0001	0.50
	S1A	0.17	0.15	0.32	0.16	2	Within Samples	0.0004	2	0.0002	
							Total	0.0005	3		
	S2B	0.24	0.20	0.44	0.22	2	Between Samples	0.0025	1	0.0025	3.13
	S2A	0.29	0.25	0.54	0.27	2	Within Samples	0.0016	2	0.0008	
							Total	0.0041	3		

ANALYSIS OF VARIANCE FOR FLOW TRIALS - SP 40 BREDEL PERISTALTIC PUMP (TRIAL 2) - YEAST QUALITY AND FERMENTATION INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Protease	S1B	0.02	0.02	0.04	0.02	2	Between Samples	0.0003	3	0.0001	4.00
	S1A	0.03	0.04	0.07	0.04	2	Within Samples	0.0001	4	0.0000	
	S2B	0.03	0.03	0.06	0.03	2	Total	0.0004	7		
	S2A	0.03	0.04	0.07	0.04	2					
Modified	S1B	92	92	184	92	2	Between Samples	14.50	3	4.83	6.44
Methylene	S1A	94	96	190	95	2	Within Samples	3.00	4	0.75	
Blue	S2B	96	95	191	96	2	Total	17.50	7		
	S2A	94	95	189	95	2					
Oxygen	S1B	0.010	0.011	0.021	0.011	2	Between Samples	0.000003	3	0.000001	0.27
Utilisation	S1A	0.011	0.009	0.020	0.010	2	Within Samples	0.000015	4	0.000004	
Rate	S2B	0.009	0.009	0.018	0.009	2	Total	0.000018	7		
	S2A	0.008	0.013	0.021	0.011	2					
Growth	S1B	2.7	2.8	5.5	2.8	2	Between Samples	0.05	3	0.02	0.96
	S1A	2.9	2.7	5.6	2.8	2	Within Samples	0.08	4	0.02	
	S2B	2.7	2.8	5.5	2.8	2	Total	0.13	7		
	S2A	2.8	3.1	5.9	3.0	2					
Final	S1B	1.94	1.83	3.77	1.89	2	Between Samples	0.0151	3	0.0050	2.67
Attenuation	S1A	1.87	1.82	3.69	1.85	2	Within Samples	0.0076	4	0.0019	
	S2B	1.92	1.90	3.82	1.91	2	Total	0.0227	7		
	S2A	1.97	1.96	3.93	1.97	2					
Alpha	S1B	16.24	16.29	32.53	16.26	2	Between Samples	0.1231	3	0.0410	3.08
Constant	S1A	16.52	16.25	32.76	16.38	2	Within Samples	0.0533	4	0.0133	
	S2B	16.69	16.52	33.22	16.61	2	Total	0.1763	7		
	S2A	16.45	16.40	32.85	16.42	2					
Beta	S1B	-0.0119	-0.0122	-0.0241	-0.0121	2	Between Samples	6.6E-07	3	2.2E-07	3.77
Constant	S1A	-0.0121	-0.0126	-0.0247	-0.0124	2	Within Samples	2.4E-07	4	5.9E-08	
	S2B	-0.0119	-0.0116	-0.0235	-0.0118	2	Total	9.0E-07	7		
	S2A	-0.0115	-0.0117	-0.0232	-0.0116	2					

ANALYSIS OF VARIANCE FOR FLOW TRIALS - SP 40 BREDEL PERISTALTIC PUMP (TRIAL 2) - BEER QUALITY INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
pH	S1B	4.30	4.17	8.47	4.24	2	Between Samples	0.0120	3	0.0040	1.09
(Day 12)	S1A	4.25	4.31	8.56	4.28	2	Within Samples	0.0148	4	0.0037	
	S2B	4.25	4.16	8.41	4.21	2	Total	0.0268	7		
	S2A	4.32	4.29	8.61	4.31	2					
Diacetyl	S1B	156	115	271	136	2	Between Samples	2842.50	3	947.50	2.03
(Day 12)	S1A	139	105	244	122	2	Within Samples	1871.00	4	467.75	
	S2B	142	171	313	157	2	Total	4713.50	7		
	S2A	175	167	342	171	2					
SO2	S1B	12	14	26	13	2	Between Samples	5.00	3	1.67	1.33
(Day 12)	S1A	16	14	30	15	2	Within Samples	5.00	4	1.25	
	S2B	14	13	27	14	2	Total	10.00	7		
	S2A	14	15	29	15	2					
Acetaldehyde	S1B	18	23	41	21	2	Between Samples	42.38	3	14.13	1.59
(Day 12)	S1A	21	27	48	24	2	Within Samples	35.50	4	8.88	
	S2B	19	22	41	21	2	Total	77.88	7		
	S2A	18	17	35	18	2					

ANALYSIS OF VARIANCE FOR FLOW TRIALS - APV LOBE PUMP (TRIAL 3) - YEAST QUALITY AND FERMENTATION INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
Protease	S1B	0.06	0.09	0.15	0.08	2	Between Samples	0.0029	5	0.0006	0.74
	S1A	0.11	0.06	0.17	0.09	2	Within Samples	0.0047	6	0.0008	
	S2B	0.07	0.12	0.19	0.10	2	Total	0.0077	11		
	S2A	0.11	0.11	0.22	0.11	2					
	S3B	0.08	0.14	0.22	0.11	2					
	S3A	0.07	0.07	0.14	0.07	2					
Modified Methylene Blue	S1B	94	93	187	94	2	Between Samples	26.42	5	5.28	3.73
	S1A	93	92	185	93	2	Within Samples	8.50	6	1.42	
	S2B	88	90	178	89	2	Total	34.92	11		
	S2A	91	90	181	91	2					
	S3B	92	91	183	92	2					
	S3A	94	91	185	93	2					
Oxygen Utilisation Rate	S1B	0.007	0.006	0.013	0.007	2	Between Samples	0.000004	5	0.000001	0.82
	S1A	0.006	0.006	0.012	0.006	2	Within Samples	0.000006	6	0.000001	
	S2B	0.007	0.008	0.015	0.008	2	Total	0.000011	11		
	S2A	0.005	0.008	0.013	0.007	2					
	S3B	0.005	0.006	0.011	0.006	2					
	S3A	0.006	0.007	0.013	0.007	2					
Growth	S1B	2.8	2.7	5.5	2.8	2	Between Samples	0.08	5	0.02	3.22
	S1A	2.7	2.7	5.4	2.7	2	Within Samples	0.02	5	0.00	
	S2B	2.7	2.7	5.4	2.7	2	Total	0.11	10		
	S2A	2.8	2.6	5.4	2.7	2					
	S3B	2.9	-	2.9	2.9	1					
	S3A	2.9	2.9	5.8	2.9	2					
Final Attenuation	S1B	1.73	1.73	3.46	1.73	2	Between Samples	0.0021	5	0.0004	0.78
	S1A	1.72	1.76	3.48	1.74	2	Within Samples	0.0027	5	0.0005	
	S2B	1.73	1.76	3.49	1.75	2	Total	0.0048	10		
	S2A	1.74	1.69	3.43	1.72	2					
	S3B	1.76	-	1.76	1.76	1					
	S3A	1.73	1.71	3.44	1.72	2					
Alpha Constant	S1B	14.48	14.40	28.86	14.43	2	Between Samples	0.1231	5	0.0246	2.70
	S1A	14.62	14.62	29.25	14.62	2	Within Samples	0.0455	5	0.0091	
	S2B	14.38	14.40	28.78	14.39	2	Total	0.1687	10		
	S2A	14.28	14.54	28.82	14.41	2					
	S3B	14.25	-	14.25	14.25	1					
	S3A	14.42	14.29	28.71	14.35	2					
Beta Constant	S1B	-0.0111	-0.0114	-0.0225	-0.0113	2	Between Samples	1.9E-07	5	3.8E-08	1.66
	S1A	-0.0115	-0.0115	-0.0230	-0.0115	2	Within Samples	1.1E-07	5	2.3E-08	
	S2B	-0.0113	-0.0110	-0.0223	-0.0112	2	Total	3.1E-07	10		
	S2A	-0.0113	-0.0115	-0.0228	-0.0114	2					
	S3B	-0.0115	-	-0.0115	-0.0115	1					
	S3A	-0.0115	-0.0114	-0.0229	-0.0115	2					

ANALYSIS OF VARIANCE FOR FLOW TRIALS - APV LOBE PUMP (TRIAL 3) - BEER QUALITY INDICATORS

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
pH	S1B	4.33	4.27	8.60	4.30	2	Between Samples	0.0153	5	0.0031	6.13
(Day 12)	S1A	4.34	4.34	8.68	4.34	2	Within Samples	0.0025	5	0.0005	
	S2B	4.35	4.37	8.72	4.36	2	Total	0.0178	10		
	S2A	4.37	4.36	8.73	4.37	2					
	S3B	4.27	-	4.27	4.27	1					
	S3A	4.29	4.26	8.55	4.28	2					
Diacetyl	S1B	156	140	296	148	2	Between Samples	1052.00	5	210.40	4.17
(Day 12)	S1A	156	168	324	162	2	Within Samples	252.00	5	50.40	
	S2B	158	168	326	163	2	Total	1304.00	10		
	S2A	162	162	324	162	2					
	S3B	134	-	134	134	1					
	S3A	144	148	290	145	2					
SO2	S1B	13	14	27	14	2	Between Samples	2.18	5	0.44	0.55
(Day 12)	S1A	15	13	28	14	2	Within Samples	4.00	5	0.80	
	S2B	14	13	27	14	2	Total	6.18	10		
	S2A	15	14	29	15	2					
	S3B	13	-	13	13	1					
	S3A	14	13	27	14	2					
Acetaldehyde	S1B	21	23	44	22	2	Between Samples	221.73	5	44.35	1.39
(Day 12)	S1A	22	19	41	21	2	Within Samples	159.00	5	31.80	
	S2B	25	21	46	23	2	Total	380.73	10		
	S2A	16	16	32	16	2					
	S3B	23	-	23	23	1					
	S3A	22	39	61	31	2					

Assay	Sample	Replicates		Total	Mean	No of Replicates	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
pH	S1B	4.33	4.27	8.60	4.30	2	Between Samples	0.0016	1	0.0016	1.78
(Day 12)	S1A	4.34	4.34	8.68	4.34	2	Within Samples	0.0018	2	0.0009	
							Total	0.0034	3		
	S2B	4.35	4.37	8.72	4.36	2	Between Samples	0.0000	1	0.0000	0.20
	S2A	4.37	4.36	8.73	4.37	2	Within Samples	0.0003	2	0.0001	
							Total	0.0003	3		
	S3B	4.27	-	4.27	4.27	1	Between Samples	0.0000	1	0.0000	0.04
	S3A	4.29	4.26	8.55	4.28	2	Within Samples	0.0004	1	0.0004	
							Total	0.0005	2		