# SYNTHESIS AND OPTIMISATION OF THE WITS MICRO-BREWERY PLANT

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# DECLARATION

I declare that this dissertation is my own unaided work. It is being submitted to the degree of Masters of Science to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination to any other University.

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**17 February 2010**

# ABSTRACT

The brewing industry is facing challenges on rising commodity prices and utility prices that rise by a high margin on a yearly basis. Effective methods are required to reduce other process variables which elevate the operational costs in the production of beer. The aim of this study was to design and investigate the optimal control philosophy for beer fermentation using the proportional integral derivative (PID) control algorithm in the Micro-Brewery at the University of the Witwatersrand. This will be achieved by controlling the cooling regimes of the fermenter jacket in order to compute the gain constant ($K_1$) that would minimise the dead time ($T_d$) and process reaction time ($T_i$) and produce a beer which will have desirable palatability attributes. All investigations were performed using a *Saccharomyces cerevisiae* yeast strain and wort of $14.3^0P$ apparent extract. The controller constant, the dead time and rise time were found to be 9.6 second ,2 seconds and 1second, respectively**.** The Isoamyl acetate, Ethyl acetate and Ethyl Caproate esters were found to be 6.8, 25 and 9 ppm, respectively and the vicinal diketones (VDK) level was found to 20 ppb at the end of the fermentation. This study showed that beer colour (EBD) was found to improve with fermentation temperature for the first 2 days then remain unchanged and showed that temperature is a possible process variable in the optimisation of colour. The study also showed that the fermentation rate and duration increase with increase in temperature. The flavour ratio (R) improves as the temperature increases. The developed simulation program helped in selecting optimal profiles to reduce dead time and improve the controller efficiency.

# DEDICATION

I would love to dedicate this work to God and my mother and grandparents: Beatrice Baloyi,
Mr and Mrs Nelson and Amanda Komape for their unlimited supports which words will fail
me to express. Thank you for everything and may God's hand continue to be over you. I love
you!!!

# ACKNOWLEDGEMENTS

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# LIST OF ABBREVIATIONS AND SYMBOLS

DMS                          Dimethyl Sulphide

MMS                          Methyl methionine

PID                          Proportional Integral Derivative controller

PI                           Proportional integral controller

P                            Proportional controller

GC                           Gas Chromatography

$T_j$                        The temperature of the cooling jacket

$t$                          Fermentation time

$U$                          Overall heat transfer coefficient

$V_p$                        The volume of the wort in the fermenter

$Q$                          Total heat lost in the fermenter

$A_p$                        The area of heat loss in the fermenter

$m$                          Total mass of the fermenting wort in the fermenter

$\rho$                       Density of the fermenting wort in the fermenting vessel

$C_p$                        The specific heat capacity of the fermenting wort

$T$                          The fermenting temperature

$\Delta H$                   Enthalpy change in the tank

$T_m$                          The measured temperature in the tank

$T_{sp}$                          The setpoint temperature in the tank

$T\text{-}VDK_{max}$                          The maximum total Vicinal diketones concentration

$FAN_{min}$                          The minimum free amino nitrogen concentration in beer

$F_{in}$                          The inlet flowrate of water into the cooling jacket

$F_{out}$                          The exit flow of water from the cooling jacket

$K_1$                          The optimised controller gain

$K_2$                          The control valve gain

$K_3$                          The thermocouple's thermal resistance

D                          Lag time

R                          reaction rate

# CHAPTER ONE

## 1. INTRODUCTION

### 1.1 Background and motivation

The brewing process can be divided into two subsections brewhouse and fermentation. Brewhouse operations are those involved with the production of fermentable extract from malt, recovering, and then stabilising it, which includes five processes: milling, mashing, lautering, boiling and trub separation (Lewis and Young, 1995). The second section is the alcoholic fermentation an important stage in the beer production. During this phase, fermentable sugars present in the brewing wort are transformed into ethanol and several aroma compounds that are important for the final beer flavour. The main factors influencing fermentation performance and beer quality are as follows (Hough et al, 1971).

- The choice of yeast strains.
- The condition of the yeast at the time of pitching.
- The amount of yeast added to the wort.
- The distribution of the yeast in the fermenting wort throughout the fermentation, and size and geometry of the fermentation vessel.
- Aeration (rousing or stirring).
- Fermentation temperature and pressure.

The main focus of this work is to utilise the Proportional Integral Derivative (PID) control procedures to optimise the fermentation temperature profile so as to produce beer with desirable specific gravity (SG), pH and alcohol concentration of the wort.

## 1.2 Research problems and Questions

The ultimate goal of this research is to utilise PID control approach to optimise the fermentation process. Reduction of fermentation duration without any alteration in the quality of the brew is the main issue affecting all brewery operations (Boulton,1991). This research therefore will attempt to address the following question:

> ➢ Is the control of temperature profiles the only way to optimise the fermentation process?
>
> ➢ Would PID control approach assists in the reduction of the fermentation duration in the Wits microbrewery?
>
> ➢ Does proper fermentation process control influence or affects the rate of maturation of the beer?

## 1.3 Hypothesis

Temperature control and optimisation can lead to faster fermentation with stable flavour profile.

## 1.4 Justification of the study

Various studies have been perfomed with the aim to validate which of the three control configurations are best to be used in industrial scale biotechnological processes. The three conventional control algorithms studies were proportional(P), proportional and integral(PI) and Proportional integral and derivative control(PID). This study attempts to use the PID controller performance on a macro scale in the Wits University microbrewery.

## 1.5 Purposes and aims

The aim of this research is to develop a simple model for the control of temperature and specific gravity of the wort during fermentation. This could be achieved by;

> Specification of the control valve to control the cooling water flowrate into the fermenter.

> Integrate PID control algorithm (Proportional Integral-Derivative control) to regulate the fermentation temperature during fermentation.

## 1.6 Dissertation outline

### Chapter 1

This chapter discusses the background knowledge and motivation of this study, justification of the study, scope of the project, research problem and questions, purpose and aims.

### Chapter 2

Literature is reviewed to provide background knowledge on the fermentation process and various process variables important to successfully optimise the fermentation process. Brewing process is highlighted with detailed information on process units that are relevant to the focus of this dissertation. Emphasis is placed on describing key points when looking at beer quality and its characteristics. The chapter also focuses on various important aspects of fermentation biochemistry and the formation of flavour compounds.

**Chapter 3**

This chapter explains the experimental procedure taken during fermentation, this includes sampling techniques, optimisation of temperature control regimes, and analysis of various objective functions that are important to the overall optimisation of the fermentation process.

**Chapter 4**

This chapter aims at discussing the results obtained and possible reasons for their behaviours.

**Chapter 5**

This chapter comprises of the conclusions and recommendations drawn from the finding in this work.

# CHAPTER TWO

## 2. LITERATURE REVIEW

### 2.1 Brief History of Brewing

Alcoholic fermentation has been an activity that dates back atleast 5000 years. According to Corran (1975) 40% of the cereal crops in ancient Mesopotamia were used for brewing. Beer is essentially a beverage produced by fermentation of an aqueous medium which contains sugars derived mainly from cereals catalysed principally by yeast. According to Quain et al (2001) Beer Brewing constitutes of three phases. Firstly, wort production in which raw materials such as barley, hops, are used to produce an aqueous medium rich in sugars and other yeast nutrients. Secondly, during fermentation, certain components of the wort is assimilated by the yeast cells to produce ethanol, carbon dioxide and a multitude of other metabolic substances, which collectively form beer. Thirdly, post-fermenting processing in which the immature beer is conditioned for consumption. Alcohol consumption is a custom through all civilization. Forget (1988) reports 60 native beers which were produced from cereals. The choice of the cereals depends mainly on the type of beer, colour and flavor profile the finished product must resemble. The evolving of biotechnology has been of great effect in the brewing industry. In the native beer preparation, there existed a step where the malt was masticated by the brewer. The human saliva contains amylase and ptyalin enzymes which assisted in degrading the starch content of the grains thereby increasing the fermentability of the wort. Due to the boiling step involved in wort preparation, beer was historically viewed as sanitized water (Quain et al., 2001).

## 2.2 Primary Fermentation

Primary fermentation is the initial fermentative phase whereby carbohydrates are utilized by the yeast cells and converted to ethanol, carbon dioxide and other flavor compounds. This phase consists of two metabolic pathways known as the oxidative (aerobic) and fermentative (anaerobic) state (Reed et al., 1991). During the oxidative phase, there is aerobic growth of yeast until most oxygen in the wort has been depleted, whereas during the anaerobic fermentative stage the wort carbohydrates, such as glucose, maltose, maltotriose are degraded by the yeast to produce ethanol, carbon dioxide ($CO_2$) and other flavour and aroma compounds. The mechanisms underlying the two biochemical reactions pathways can be broadly described as:

Oxidative:　　$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$　　　　　$\Delta G = 686 kcal$

Fermentative:　$C_6H_{12}O_6 \longrightarrow 2CO_2 + 2C_2H_5OH + Yeast$　　$\Delta G = 54 kcal$
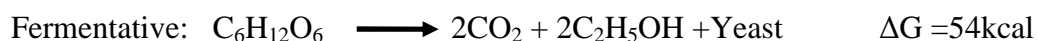
Where $\Delta G$ = free energy

As observed above the fermentative pathway releases approximately 8% of the total energy trapped in the sugars. The completion of the primary fermentation stages produces an immature beer with imbalanced aroma profile. This immaturely fermented beer is termed *green* beer. Process variables such as temperature, pressure and yeast cell structure must be monitored to ensure the success of the primary fermentation. The individual impact of these

variables on the fermentation performance will be discussed in the subsequent sections of this work.

## 2.3 Secondary Fermentation

During primary fermentation the important flavour and aroma compounds that characterise the quality of the beer are produced by yeast metabolism. These include higher alcohols, esters, sulphur compounds, carbonyl compounds and organic acids. Secondary fermentation is designed to improve the palatability of the green beer through flavour maturation, carbonation, standardisation, chill proofing, stabilisation, and clarification (Coors, 1977). Flavour maturation is the most important phase of all the aging process. Diacetyl, $H_2S$ and acetaldehyde are known to impart undesirable flavours to the beer and the main aim of the secondary fermentation is to ensure that their concentrations are kept below threshold (Reed et al, 1991).

## 2.3.1 Flavour Compounds in beer fermentations

Moncreiff (1967) describes Flavour as a complex sensation comprising of taste, odour, astringency, roughness or smoothness, hotness or coldness, pungency or blandness. The flavour attributes of beer are important in evaluating the feasibility of any beer fermentation process. Esters, higher alcohols, VDK(vicinal diketones), sulphur compounds, organic and fatty acids play a major role in the palatability of the beer. The following sections discuss their  individual or concomitant effects on the beer aroma and flavor.

9

## 2.3.1.1 Esters

Esters are flavour compounds that are generally characterized by their fruity-flowery aromas in beer. At extremely high concentrations they contribute to off-flavours thus making the beer unpalatable.100 different types of esters have been identified in beer which can be grouped into two categories (Drawert & Tressl, 1972; Meilgaard, 1975; Engan, 1981). Branyik et al (2008) identified these two categories as acetate and ethyl group of esters. The acetates group consists of compounds such as ethyl acetate(fruity, solvent), isoamyl acetates(banana) and phenylethyl acetate (roses, honey, apple) while ethyl or medium chained fatty acid contains compounds such as ethyl caproate and ethyl caprylate (apple like aromas). The ethyl esters are the most dominant presumably because ethanol is the most readily available substrate. Studies of the mechanisms governing the ester synthesis have been exhausted by many researchers, but no single author has clearly reported the complete mechanisms. Generally , it is accepted that Esters are synthesized via two biochemical routes. Firstly, by the decomposition of higher alcohols and acetyl/acyl CoA in the presence of the enzyme Alcohol Acyltransferase (Nordstrom, 1962, 1963, 1964). Secondly, by esterases working in a reverse direction(Soumalainen, 1981).

Traditionally, ester formation in batch processes is affected by the wort composition i:e. specific gravity(SG), Lipids content, zinc and free amino nitrogen(FAN) content and process conditions such as Temperature, pH, agitation rate, reactor design(hydrostatic pressure, dissolved $CO_2$ concentration) and pitching rate (Dufour, 2003, Knatchbull, 1987 and Verstrepen, 2003). The rate of ester formation depends mainly on the availability of higher

alcohols and acetyl/acyl-CoA substrates and the activity of the AaTase Enzymes. According to Fuji(1997) higher wort aeration affects ester synthesis through reduced availability of acyl-CoA (used for lipid synthesis and growth) and inhibition of AaTase . There's an overlap in many factors affecting their rate, but the distinguished factor is the Enzyme AaTase activity and its gene transcript regulation (Malcorps et al., 1991, Yoshioka et al .,1984). Several authors observed a reduced level of ester formation especially isoamyl acetate in immobilized bioreactors. Masschelein (1997) reported that elevated aeration levels affects ester formation in bioreactors. Several authors proposed that yeast strains physiology is another factor affecting ester synthesis in continuous systems. Careful consideration must be taken in selecting yeast strain for controlling the ester formation, since the average ester production and the proportion of the individual esters differs with various strains of yeast (Dufour, Malcorps and Silcock, 2003).The yeast strain employed by the authors was *Saccharomyces cerevisiae var uvarium.* Ester formation is a sensitive process, which is difficult to control due to various factors influencing it. Various authors proposed that the genetic modification of yeast strains can be lead to the control of ester synthesis.

## 2.3.1.2 VDK (Vicinal Diketones)

The VDK's are undesirable at high concentrations and the main purpose of fermentation management is to ensure that their concentration in the final product is less than its threshold levels. In brewing the two commonly measured VDK's in beer are 2,3 butanedione (diacetyl) and 2,3 pentadione. According to Mielgaard(1975) both have the flavour and aroma of butterscotch but the threshold concentration of diacetyl is almost ten times lower than 2,3-pentanedione, 0.15 ppm and 0.9 ppm respectively. Diacetyl and 2,3-pentanedione arise as an indirect result of yeast metabolism. According to Quain et al (2001) the occurrence of very

11

high diacetyl concentration in beer is indicative of microbial contamination. Historically this condition was referred to as "sarcina sickness" in which beers developed a characteristic sickly buttery aroma(Shimwell & Kirkpatrick,1939). Many Brewers in the past believed that diacetyl was a product of contamination, however, it is now accepted by the majority of the brewers that a proportion of the diacetyl is a product of yeast activity. Figure 2.1 presents the pathway for the formation of VDK's and their subsequent dissimilation.
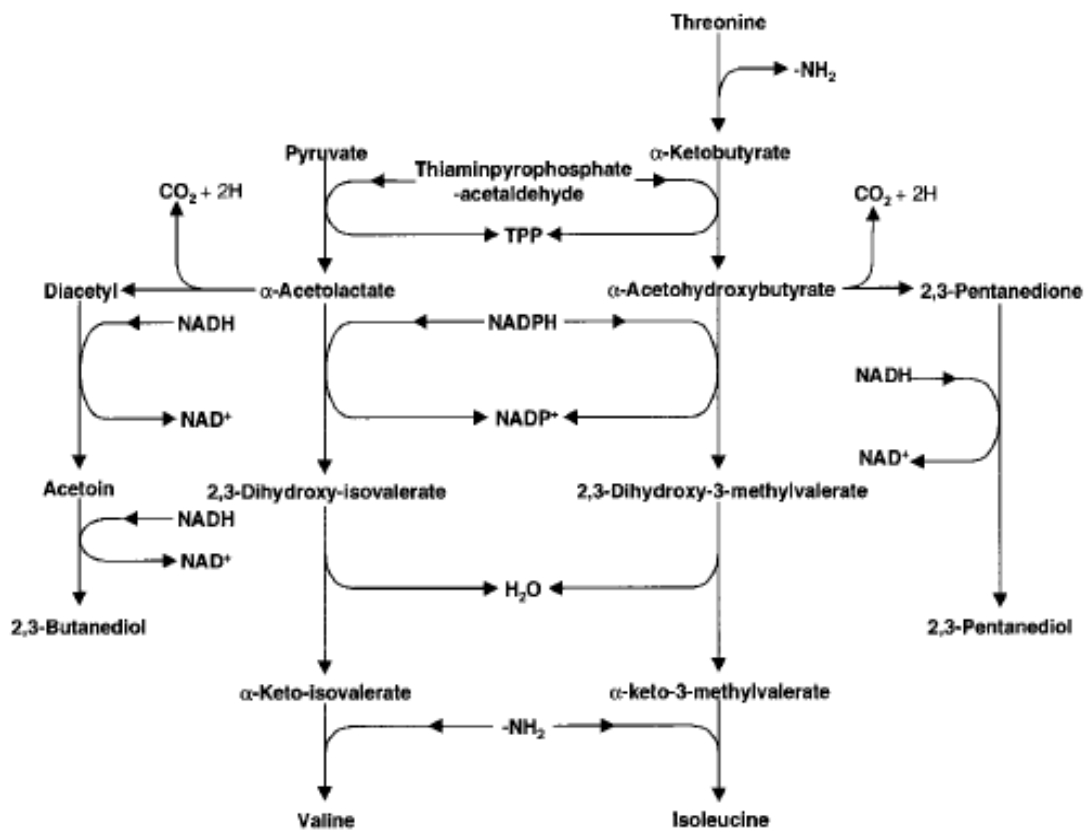


Figure 2.1: The pathway of VDK, 2.3 pentanedione formation and dissimilation in beer fermentations (Bolton et al, 1988).

Chuang &Collins (1968) suggested an alternative route to figure 2.1. These authors perfomed their investigations with radiolabelled substrate. These authors concluded that in *S. Cerevisiae*, acetoin and diacetyl arose from condensation reactions between hydroxyethylamine pyrophosphate and acetaldehyde and hydroxyethylamine pyrophosphate and acetyl-CoA, respectively. Although the evidence supported the existence of this pathway

the majority of work performed by others suggests that it is likely to be of minor importance and that most diacetyl arises via the route depicted by Figure 2.1. Quain et al(2001) reports that the  biochemistry of VDK's and the influence of fermentation conditions can be considered in three phases: steps leading to the formation of α-acetohydroxy acids, factors affecting the spontaneous decarboxylation of α-acetohydroxy acids and the subsequent reduction of VDK. According to Quain et al(2001) the major rate-determining step for VDK formation is the non-enzymic decarboxylation of α-acetohydroxy acids. Furthermore, the subsequent yeast catalysed reduction of VDK is usually very rapid. Therefore, analysis of fermenting worts is usually referred to as 'total VDK' and represents the sum of α-acetohydroxyacids and diacetyl. This is normally done under high temperature ensuring that all α-acetohydroxy acids are converted to VDk prior to analysis.

The formation of α-acetohydroxy acids is related to amino acids metabolism. In this respect the total wort amino acid content and amino acid spectrum are influential, as are parameters which regulate yeast growth and by inference affects amino acid utilization(Quain et al.,2001). During intermediate parts of the fermentation α-acetohydroxy acids are passed to the wort for oxidative decarboxylation for the formation of diacetyl and 2,3-pentanedione.This process occurs spontaneously. Towards the end of the fermentation process diacetyl and 2,3-pentanedione form acetoin and 2.3 butanediol by means of yeast cells reductases through metabolisms. These metabolites persist in beers but since they are much less flavour-active than VDK, their presence can be tolerated. Nakatani et al (1984a) investigated the behavior of VDK's under various conditions. He observed that at high levels of wort free amino nitrogen (FAN) the VDK profile took the form of an extended peak, which appeared early in fermentation. The authors demonstrated that a relationship exists

between the minimum concentration of the FAN in the wort and the maximum evolved total (T-VDK) max. Equation 1 describes the relationship as:

$$\text{T-VDK}_{max} = \frac{0.161}{\text{FAN}_{min} - 3.87} + 0.415$$

(1)

From the above equation, it was concluded that in order to maximise the VDK in the wort, the FAN level has to be kept at its minimum possible threshold at all times. The author concluded that since FAN utilisation and yeast growth are positively correlated, thus, high wort oxygen concentrations, trub levels, pitching rates and fermentation temperature, favour rapid and extensive yeast growth, also promoted high VDK levels, presumably due to increased FAN utilisation. Nakatani et al (1948b) confirmed the work of Wanwright (1973), that amino acids valine and isoeuline contents of the wort has suppressive effects on the formation of the α-acetohydroxy acid. These amino acids are assimilated in the early stages of the fermentation process and this repressive effects accounts for the delayed onset of the VDK formation. Other amino acids (valine, leucine, alanine, threonine, glutamate) and ammonium chloride, all showed a decrease in the VDK concentration during fermentation (Barton and Slaughter.,1992). The reduction of VDK occurs in the later stages of the fermentation process, specifically in the conditioning stage of the fermentation process.

### 2.3.1.3  Higher Alcohol

According to Engan (1981) there are more than 40 higher alcohols in beer. Among these are n-propanol, iso-butanol and isoamyl alcohols (2 methyl and 3-methyl butanol) which have organoleptic importance because they occur at concentrations above flavour threshold. Isoamyl alcohols are the most abundant in beer. According to Meilgard (1974), the aromatic phenylethanol has a distinct rosy/floral ordour in beer which is desirable characteristic of this

compound. Higher alcohols contribute to beer by intensifying the alcoholic taste, aroma and imparting a warmth mouthfeel. In addition, they also serve as a precursor as for ester synthesis. The formation of higher alcohols takes place via two metabolic routes, namely: the anabolic and catabolic routes. The anabolic route is achieved by synthesis from wort carbohydrates via pyruvare, while the catabolic route is synthesised as byproducts of amino acid assimilation(Ayarapa.,1965). Higher alcohols achieve maximum concentrations in batch fermentations coincident with cell growth arrest and minimal FAN concentrations. Figure 2.2 represents the metabolic pathway of higher alcohols which are of important for the beer flavour.
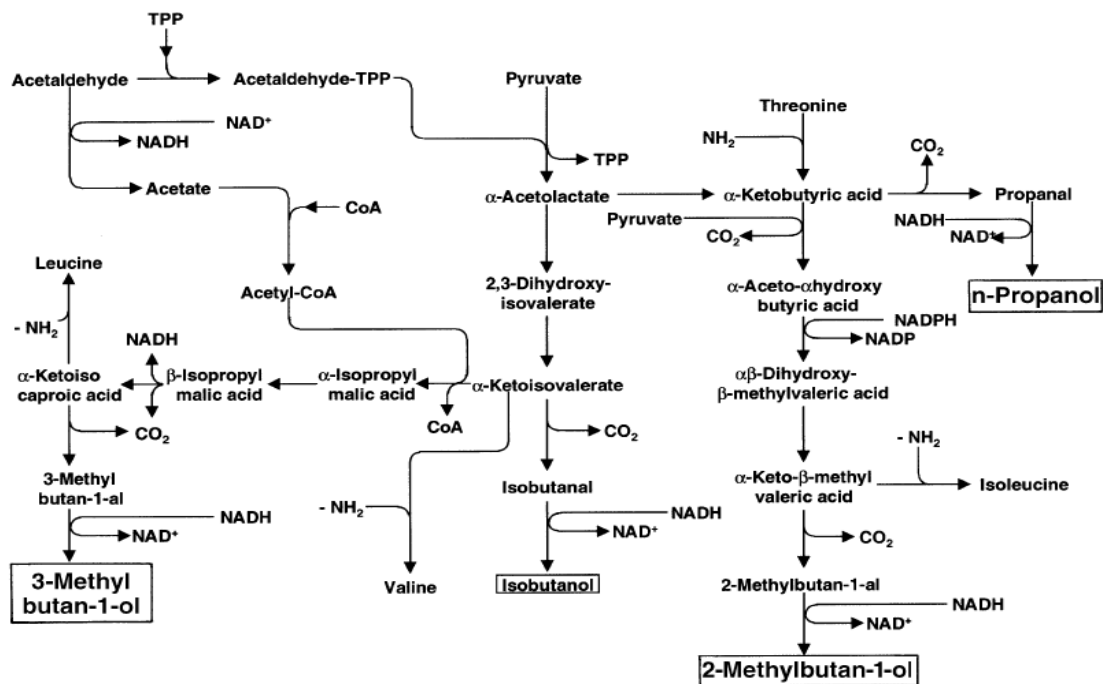


Figure 2.2: Biosynthesis of some alcohols of importance to beer aroma and flavour((Bolton et al,1988)

In both cases, the immediate precursors are 2-oxo (α-keto) acids. In the anabolic route the 2-oxo acids, arising from carbohydrate metabolism, are decarboxylated to form aldehydes,

which are reduced to the corresponding alcohols. Simultaneously, 2-oxo acids also derive from amino acid utilization, which is termed the catabolic (Ehrlich) route to higher alcohol formation (Chen.,1978, Oshita et al.,1995). The synthesis pathway is governed by amino nitrogen concentration in the wort while lower levels of amino acid concentrations favour the anabolic route. Initially, the catabolic route is predominant due to high amino acid concentrations and overlaps with the anabolic route due to reduced amino acid concentrations.

Control of higher alcohol formation during fermentation can be accomplished in three ways. Firstly, by choice of an appropriate yeast strain, followed, by modification to wort composition, and, lastly, by manipulation of fermentation operating conditions. Szlavko (1974), Engan (1978) and Romano et al. (1992) investigated the dominant factor influencing the concentrations of higher alcohols employing various yeast strains under identical operating conditions, these authors concluded that the choice of the yeast strain is the dominant factor in the production of higher alcohols. According to Hudson and Stevens(1960) ale strains (*S cerevisiae var diastaticus*) produce a greater variety of higher alcohols compared to lager strains(*S cerevisiae var uvarium)*. The concentration of higher alcohols can be manipulated by the genetic modification of the yeast strains(Rous and Snow,1983).

Higher wort oxygen and temperature enhanced yeast growth thus favouring the higher alcohol production. According to Quain et al (2001) temperature affects the rate of yeast growth not the growth extent and therefore it should not affect the yields of metabolites which are growth related. Peddie (1990) suggested that altering cell membrane fluidity and

diffusion rates can have good implications on the growth metabolites. An inverse correlation exists between higher alcohols, other volatiles, yeast growth extent and applied pressure. According to Rice et al (1976) in order to control higher alcohol spectra, pressure should be applied to the wort during fermentation.

Comparisons of higher alcohols were made to beers fermented using a free and immobilised systems. a trend was observed towards increased propanol yields accompanied by lower 1-butanol and isoamyl alcohol formation. This can be attributed to yeast growth rates, levels of amino acids utilisation and mass transfer limitations. Different immobilization techniques yields differing results. For cells immobilized by entrapment (e.g alginate, carragenaan, calcium pectate) the diminution of higher alcohols is proportional to FAN utilization ( Dömény et al.,1998; Ryder et al.,1985; Šmogrovičová et al.,1999). Control of higher alcohols formation in continous systems can be achieved by choice of appropriate yeast strain, wort composition, fermentation conditions, immobilisation method and reactor design.

## 2.3.1.4 Organic and Fatty acids

According to Meilgaard (1975) there exists 110 organic or short-chain fatty acids in beer. This includes pyruvate (100-200 ppm), citrate (100-150 ppm), malate (30-50 ppm), acetate (10-50ppm), succinate (50-150 ppm) lactate (50-300 ppm) and 2-oxoglutarate (0-60 ppm) (Coote&Kirsop.,1974; Whiting,1976; Klopper et al., 1986). Organic acids contributes to the sour flavours experienced in beer and are responsible for the decreased pH during the fermentative phase and impaires the head retention in the final beer. For example, succinate has a bitter/sour taste (Whiting, 1976). Short chained fatty acids such as caproic and caprylic

acids impaires "goat like" aromas to the beer during fermentation. They are synthesised anaerobically through pyruvate or TCA cycle and excreted into the beer as by-products of yeast metabolism(Wales et al., 1980). The spectrum of organic acids in various beers is influenced by the wort compositions but the dominant factor influencing their range is due to the yeast strain employed.

According to the findings by Chen (1980), 90% of free fatty acids in the wort consists of palmitic (16:0), linoleic (18:2), stearic (18:0) and oleic (18:l) and (70-80)% of fatty acids which consists of caprylic (8:0), caproic (6:0) and capric (10:0) acids. The author observed an increase in the concentration of the total fatty acids and attributed this to the fact that the longer chain fatty acids are assimilated by the growing yeast and impounded into the structural lipids. The shorter chains are fatty acids that are excreted into the fermenting wort as byproducts longer chain lipids. It can be deduced that any process variable favouring the yeast growth will also favour the formation of shorter chain lipids thereby increasing their concentration in the wort. Thus higher temperature, high pitching rate and high initial wort oxygenation will favour the short chain lipid synthesis reactions. Short chain fatty especially $C_8$-$C_{14}$ acids synthesis must be discouraged because they are toxic to yeast cells (Nordstrom., 1964). This toxins are disruptive to the cell membrane and its excretion to the beer is improbable.


No Literature has been found on the behaviour of organic acids in immobilised systems. Minor differences were observed in the total organic acid and pH in continuously fermented beers and traditionally fermented beers (Šmogrovičová et al.,1999; Yamauchi et al.,1999). However different acids within the total organic acid spectrum responded differently to fermentation system and process variables. The organic acids spectrum is highly dependent

on the fermentation intensity, thus it can be manipulated by changing the intensity and duration of the respire-fermentative and fermentative process stages (Šmogrovičová et al,1999, Bolton et al,2006).

## 2.3.1.5 Sulphur Compounds

The majority of sulphur containing compounds ,both organic and inorganic, contribute both directly and indirectly to beer flavour. Most of these sulphur compounds are derived from the wort and remain unutilised, while others are altered during yeast metabolism. Particularly $H_2S$ (hydrogen sulphide) and $SO_2$ (sulphur dioxide) are influenced by yeast metabolism. Both compounds are undesirable in higher concentrations in beer due to their ability to impart undesirable aromas and flavours. $SO_2$ can form reversible adducts with carbonyl compounds thus promoting off flavour formations. In this way, for example, high sulphite concentrations, which may arise during fermentation, can stabilise beer flavour by binding compounds associated with beer flavour staling such as acetaldehyde and trans-2-nonenal. In addition, sulphite acts as a natural antioxidant. Dufour et al. (1989) demonstrated that a positive correlation existed between sulphite concentration and wort OG(original gravity). Conversely, increase in wort oxygenation, or elevated wort lipid (in the form of trub) was associated with reduced $SO_2$. Dilute wort or partial alcoholic beers have undesirable staling potential due to the reduced sulphite concentration. This shortfall is normally accounted for during packaging, but this is an expensive procedure and requires additional labeling in various countries (Quain et al,2001). Dufour (1991) discussed the significance of carbonyl-sulphite adduct formation during fermentation. This author highlighted that the degree of binding of each type of carbonyl would be a function of the magnitude of the adduct's equilibrium constants. In this regard, the highest affinities would be shown for acetaldehyde, whereas the VDK's would have medium affinities. Thus the sulphite formation rate regulates

the proportion of carbonyls bound as adducts and that fraction reduced by yeast. Carbonyl adducts persisting in beer are flavour negative; however, under some circumstances they may be released to exert their staling effects.

$H_2S$ accumulates during the early parts of the fermentation process and then declines in later stages (Clarke et al.,1991). During faulty fermentations the later declining phases might not occur and instead increase further resulting in off flavours. The Quantity of $H_2S$ formed is much influenced by the yeast strain (Romano & Suzzi, 1992). Therefore, deficiencies in wort composition, yeast strain physiology and inappropriate control of fermentation process may contribute to high concentrations of $H_2S$. King et al (1990) reported that supplementation of worts with pantothenate (0.01 ppm) suppressed $H_2S$ formation and decreases the concentrations of SO2 and acetaldehyde (Lodolo et al., 1995). These authors suggested that this improves flavour stability by binding acetaldehyde to $SO_2$ in preference to more flavour staling longer chained aldehydes. Addition of amino acid supplements Serine effectively reduces sulphury odours in cask beers during secondary fermentation.

Investigations on sulphite formation revealed that a correlation between sulphite formation and wort carbohydrates concentration (Korch et al., 1991). These authors noticed a concomitant increase in ethanol and acetaldehydes, and proposed that these observations implicated an intermediate of glucose catabolism. They suggested that pyruvate and acetaldehyde formed adducts with sulphides and this deprived the methionine synthetic pathway of sulphite. As a result the sulphite synthetic pathway becomes increasingly derepressed. According to dufour (1991) there are four stages to sulphite formation during fermentation. Firstly, during initial stages of the process when the methionone and threonine

levels repress the sulphite synthetic pathway. Secondly, the active growth phase during which the methione and threonine concentrations decline from the yeast utilisation. This derepresses the sulphite synthetic pathway. Thirdly, during mid to late fermentations, during this phase the yeast growth halts and the reductase enzyme activity declines. However the availability of carbohydrates in the medium promotes sulphite synthesis. The last phase entails the depletion of the sulphite accumulation due to carbohydrates reduction in the wort. Diverse attempts have been made to genetically modify yeast cells that are unaffected by the presence of amino acid suppressants (Dufour et al., 1989; Korch et al., 1991; Hansen & Kielland-Brandt, 1995).

Dimethyl sulphide (DMS) is another sulphur compounds responsible for flavour attributes of most beers. Its presence in beers is detected by its distinct "cooked sweet corn" or "cooked cabbage" aroma and flavours (Quain et al,2001). DMS rarely exceeds its 30ppb threshold in ale beer(Harrison & Collins, 1968). However, it is very prominent in lager beers with the threshold range of 30-100 ppb. It is objectionable at concentrations greater than 100ppb(Anness & Bamforth, 1982). It is known that DMS is produced in two phases, firstly by the decomposition of S-methylmethionine(SMM) during kilning, secondly by the reduction of DMSO. Kilning is the final phase of malting, it reduces the moisture content of malt in order to increase its storage time and reduce potential for fungal infection and growth. DMSO is formed above 60°C kilning temperatures (Anness et al., 1979; Parsons et al., 1977). Both DMS and DMSO are produced during malting process. The proportions produced are modulated by malting conditions, wort quality and fermentation conditions (Anness & Bamforth, 1982; Dickenson, 1983). SMM is converted to DMS during wort boiling. DMS is heat unstable and the majority of it is lost during wort boiling . Conversely DMSO is heat stable and continues unaltered throughout mashing and wort boiling. The conversion of SMM

to DMS is temperature sensitive and halts during fermentation due to low temperatures. During fermentation SMM is assimilated and converted to methionine by yeast cells. DMSO in wort is reduced to DMS by yeast during fermentation (Anness et al., 1979; Gibson et al., 1985). Gibson et al (1985b) demonstrated that the specific activity of dimethyl sulphoxide reductase increased when yeast growth ceased. The increase in specific activity was associated with nitrogen limitation. Investigations were undertaken to validate this proposition. in defined media, DMSO reduction was found to be rapid in mediums where methionine was the limiting source. From these results it was concluded that DMSO reductase was subject to nitrogen catabolite repression and that it was involved in the uptake of methionine. Annes and Bramford (1982) summarized the effects of fermentation variables other than wort amino nitrogen on DMSO reduction by yeast. These authors observed that *S. cerevisiae* were more effective than *S. uvarum (*lager strains*).* Low temperatures favour formation of DMS by yeast and this may partly explain why levels in lagers are generally higher than ales. Wort concentration and DMS formation by yeast are positively correlated. The relationship is not linear and at very high gravities there is a disproportionate increase in the yield of DMS. Formation of DMS during fermentation is favoured by high pH. High capacity deep fermenting vessels are associated with high DMS levels.

## 2.3.2 Assimilation of Wort Nutrients

### 2.3.2.1 Sugar uptake

Different brewing yeasts strains consume a variable spectrum of the carbohydrates in the wort. These carbohydrates includes glucose, sucrose, fructose, maltose, galactose, raffinose, maltotriose and dextrins. According to Quain et al(2001) sucrose is always utilized first and the resultant hydrolyses is followed by an increase in fructose. Fructose and glucose are more

or less assimilated simultaneously. The disappearance of glucose is followed by maltose uptake ,which is the major carbohydrate in brewing worts. Maltotriose is utilised after all maltose has been assimilated. Dextrins are higher polysaccharides that are not utilised by brewing yeasts. They contribute to beer flavour by imparting palate fullness. Investigations have been carried out to utilise dextrins, firstly by yeast strain genetic modifications (Tubb et al., 1981; Goodey & Tubb, 1982;Vakeria & Hinchliffe, 1989; Lancashire et al., 1989; Hansen et al., 1990). Secondly, hydrolysation of dextrins is achieved through addition of commercialised dextrinase enzymes to the wort. The sequential uptake of wort sugars reflects the genotype of the yeast and ways in which this is expressed by repression and induction and by carbon catabolite inactivation. There exists multiple carriers for individual sugars, their activities are regulated by the spectrum and concentrations of sugars in the wort . According to Langus (1993) glucose is the most preferred substrate, its existence represses carriers for subsequent sugar uptake. There are two mechanics for glucose uptake: low affirnity and high affinity types, which operate by facilitated diffusion (Bisson & Fraenkel,1983a). Both mechanisms are active with glucose and fructose. The high affinity carrier requires the presence of a kinase although phosphorylation of glucose during uptake has been discounted in view of the evidence that the non-phosphorylable analogue, 6-deoxyglucose, had similar uptake kinetics to glucose (Bisson & Fraenkel, 1983b; Kruckenberg & Bisson, 1990). The low affinity system is constitutive, whereas the high affinity transporter is repressed in the presence of high glucose concentrations (Bisson & Fraenkel, 1984; Neigeborn et al., 1986). The role of the high affinity system is to provide scavenging mechanisms in an event of competition of glucose at low concentrations. According to Does and Bisson (1989), the repression of the high affinity system occur only in fermentative stages and it occurs as a result of catabolite repression phenomenon. It has been suggested that the low affinity system is merely passive diffusion. However, Gamo et al (1995) refuted this on the basis that actual

uptake rates using this transporter were 2-3 orders of magnitude higher than could be accounted for simply by passive diffusion. The glucose carriers are influenced by components of the medium other than glucose itself. Thus, it is reported that exhaustion of nitrogen sources during batch growth brings about an irreversible inactivation of the glucose (and other) sugar transporters (Lagunas et al., 1982). This inactivation is apparently due to proteolysis of the carrier molecules (Busturia & Lagunas, 1986; Lucero et al., 1993). The consequences of these effects to brewery fermentation are unclear.

## 2.3.2.2 Uptake of Wort Nitrogenous Components

Wort nitrogenous content is heterogeneous in nature (Quain et al.,2001). According to Ingledew (1975) nitrogen spectrum consists of protein, 20%; polypeptides, 30-40%, amino acids, 30-40% and nucleotides, 10%. The amino acid fraction is of most significance to fermentation performance and beer quality. In order to assimilate wort nitrogen various permeases are utilised, some vary with individual acids and general amino acids permease (GAP) with various substrate specificities. Similarly to sugar uptake, the presence of an exogenous supply of certain nitrogenous nutrients abolishes the utilisation of others by repressing the enzymes responsible for their assimilation.

According to Hinnebusch (1987) nitrogen assimilation is an active process that is energy intensive. Olivera et al. (1993) investigated the activity of amino acid permeases in chemostat cultures of *S. cerevisiae*, a technique which compares various nutrient limitations. These authors concluded that the specific permeases were likely to be involved in uptake of amino acids during anabolic pathways, notably protein synthesis, whereas the GAP permease and the others, which are subject to nitrogen catabolite repression, had catabolic roles.

The Nature of specific permeases has been investigated by the use of mutant strain in which only the carrier of interest is active. Garcia and Kotyk (1988) studied L-lysine uptake in a double mutant of *S. cerevisiae*. They concluded that, in the strain used, a specific L-lysine permease was present, which was not active with any other naturally occurring amino acid. Tullin et al. (1991) classified specific permeases as either high capacity, low affinity or low capacity, high-affinity. Investigations into nature of the specific permeases have relied on the use of mutant strains in which only the carrier of interest is functional. The activity of these permeases is regulated by environmental conditions. Slaughter et al. (1987) showed that increased pressure and carbon dioxide concentration resulted in altered patterns of amino acid uptake and that this could be related to changes in the formation of flavour volatiles. In general, any brewing wort made according to a given method will have the same amino acids spectrum (O'Connor-Cox & Ingledew, 1989). Contrary to Quain et al (2001) infers that the use of low nitrogen adjuncts can distract this balance.Table 2.1 shows the typical amino acids spectrum available in brewing wort.

Table 2.1: The amino acids spectrum in brewing wort by order of assimilation during fermentation (Pierce,1987).

| Class A | Class B | Class C | Class D |
|---|---|---|---|
| Arginine | Histidine | Alanine | Proline |
| Asparagine | Isoleucine | Ammonia | |
| Aspartate | Leucine | Glycerine | |
| Glutamate | Methionine | Phenylalanine | |
| Glutamine | Valine | Tyrosine | |
| Lysine | | Tryptophan | |
| Serine | | | |
| Threonine | | | |

Class A amino acids are assimilated immediately after yeast pitching. Class B are assimilated more slowly than Class A, whereas Class C are repressed by Class A existence and are

25

unutilized until class A acids are completely assimilated. Proline is the sole member of class D and its dissimilation requires the presence of a mitochondrial oxidase not present under the repressed and anaerobic conditions of fermentation (Wang & Brandriss, 1987).

## 2.3.2.3 Uptake of Lipids

Lipids consist of a diverse group of molecules linked only by their properties of sparing solubility in water but being readily soluble in organic solvents such as chloroform (Bolton et al,2001). Lipids uptake in brewing can reduce the oxygen requirements in worts. Lentini et al (1994) investigated the Lipid fraction of wort Trub, and discovered that 18:2 unsaturated fatty acids were the most dorminant. Additionally, these authors concluded that rich trub worts have higher fermentation rates and higher yeast growth compared to clear worts.

*S cerevisiae var diastaticus* yeast strains employ facilitated diffusion to absorb fatty acids at low concentrations and simple diffusion at high concentrations (Finnerty,1989). According to van der Rest et al (1995), it is assumed that the diffusion of lipids into yeast cells is dependent on the lipid content of the cell membranes. Sterols are passively assimilated by yeast under aerobic conditions (Saleno and sparks.,1983,Lorenz et al.,1986), however results show no uptake in stationery phase under anaerobic respiration. This phenomenon is termed aerobic sterol exclusion (Lewis et al.,1988).

## 2.3.2.4 Metal Ion Uptake

Assimilation of metal ions is important to yeast cells firstly because, they are essential to yeast cells as nutrients and secondly, they serve as cofactor of various enzymes in the yeast cell. Zinc, magnesium and calcium are the most dominant in brewing (Lie & Jacobsen, 1983,Lentini et al., 1990; Walker et al., 1996; Bromberg et al., 1997; Walker & Maynard(1997). The absence of metal ions can cause metabolic deficiencies in the yeast cells. As with microbial cells, yeast cells can concentrate metal ions that are non beneficial to its physiological function. Thus creating a toxic environment for the yeast cells. This is normally evident when mixtures of metals are represented to the yeast cells. Yeast cells are skilled at removing metal ion from external environment. Avery and Tobin (1992) proposed that they can be employed to recover metals of economic value in contaminated water.

Metal ion uptake processes are classified into two namely, biosorption and bioaccumulation (Quain et al.,2001). During biosorption the metal ions rapidly attached themselves to the cell wall. This process is fast, insensitive to temperature variations, independent of metabolic energy and unaffected by inhibitors. Several mechanism for cell binding have been proposed, this includes ion exchange, adsorption, precipitation and complexation (Blackwell et al., 1995). Comparatively, bioaccumulation is a slower process, affected by process variables such as metabolic inhibitors, temperature etc. Genes are particularly important in the transport of metal ions and individual genes are responsible for regulating different uptake systems, For example, gene FREl codes for a protein linked to reduction and uptake of ferric ions (Anderson et al., 1992). The high affirnity copper uptake system is regulated by the gene CRT1 (Dancis et al.,1994). Bioaccumulation methods include Lipid peroxidation,

permeation, involvement of specific carriers, endocytosis and ion channels. All these methods utilise ions concentrated within the cell. Once in the cells, ions are incorporated into proteins or alternatively, they are sequestered within the cells. From the latter option, cells serves as a temporary store of useful ions and makes the cytotoxic effect of metal ions amenable (Blackwell et al., 1995). According to Quain et al (2001), metal ions can bind to yeast cells and other wort components rendering useful metal ions unavailable for assimilation. Lentini et al (1994) reported that the worts with high trub content were associated with reduced zinc concentration levels due to non specific binding. However, kreder (1999) demonstrated that yeast assimilate zinc bound to the yeast during fermentation.

## 2.4 Factors influencing fermentation in brewery applications

Various factors influence the fermentation performance of beers, this includes wort clarity, wort lipid content, wort oxygenation, pitching rate, temperature and Pressure.

### 2.4.1 Temperature

Primarily, temperature has an effect on the metabolic rate and yeast growth during fermentations. Many authors have embarked on investigating the effect of temperature on the fermentation process. The overall conclusion was that the higher the temperature the faster the fermentation time, but with compromised flavor and ethanol concentration. The maximum temperature range that can be used for fermentation should be between $30\text{-}35^0C$, as many yeast species deactivate at high temperature range resulting in faulty fermentations. Several factors account for the unacceptability of high temperature operations, including loss in flavour volatiles and ethanol caused by gas stripping. Generally Ales ($18\text{-}25^0C$) ferments at higher temperatures compared to lagers ($6\text{-}15^0C$). Ales tend to be more highly flavoured than

lagers, whereas lagers contain greater concentrations of more subtle flavour components. The greater flavour intensity of ales is partly due to the malts and hops used in their preparation. In addition, the beers also usually contain greater concentrations of higher alcohols and esters. Some of these effects can be attributed to the yeast strain used for the fermentation. Ales strains produce higher concentration of esters and higher alcohols. However, increased temperature also produces elevated levels of higher alcohols and possibly esters (Stevens, 1960; Mandl et al., 1975; Kumada et al., 1975; Posada et al, 1977; Miedener, 1978). Higher alcohols are implicative of higher fermentation temperature. Clarity has not been established on the extent of the effects temperature has on esters, various factors can be implicative, including wort oxygen concentration (Quain et al,2001). The temperature at which fermentation is conducted may influence oxygen requirement. As the temperature at which yeast is grown is reduced, the cells incorporate a progressively greater proportion of unsaturated fatty acid residues into the plasma membrane in order to maintain fluidity (Boulton & Ratledge, 1985). There is a negative correlation between ester synthesis and wort oxygenation levels.

Another aspect of high temperature might be its influence on the VDK. Elevated temperatures increases the peak value of the VDK's, not only its value but also the peak occurs ealier as compared to other temperature levels (Quain et al,2001). In addition, the rates of formation and declining are more rapid.

## 2.4.2 Wort oxygenation

Oxygenation concentration is an important regulator of yeast growth. Oxygen is required by yeast for sterol and unsaturated fatty acids synthesis which are both necessary for cell membrane functions. Attempts have been made to classify brewing yeast according to the minimum oxygen requirement for fermentation (Kirsop., 1974; Jacobsen & Thorne., 1980). It was proposed that Ale strains have a higher demand for dissolved oxygen as compared to lager strains. However this proposition has not been thoroughly validated. The oxygen requirements in worts ranges from 4-35ppm. Concentrations above this can result in sluggish fermentations. Oxygen availability, promotes yeast growth. The increase of the oxygen concentration in worts will favour further increase in yeast metabolites therefore reducing the ester concentrations in the beer. The effects of oxygen on fermentation rate, yeast growth, ethanol yield and formation of beer flavour components related to yeast growth are also modulated by the pitching rate (Quain et al,2001). Figure 2.4 shows the results obtained by Bramforth et al (1988), Which gave Pitching rate of $15 \times 10^6$ viable cells and temperature was $11^0$C.
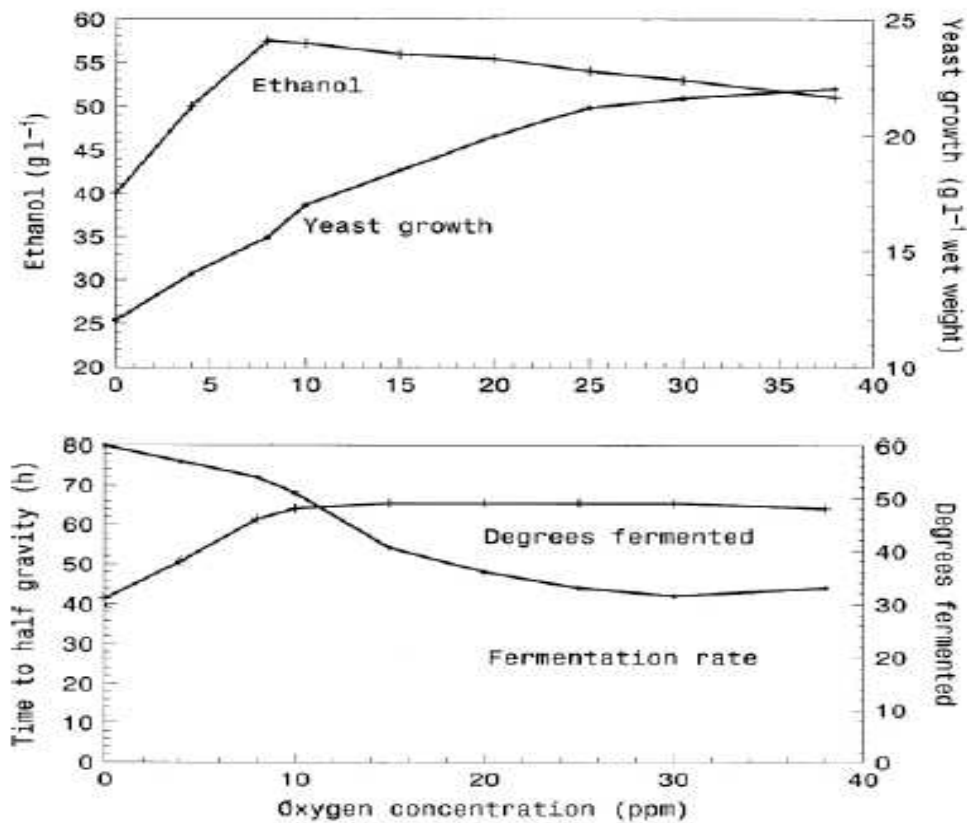
Figure 2.3: The effect of initial wort oxygen concentration on ethanol, yeast growth and fermentation rate in a stirred tank laboratory fermenter using high gravity wort 1.060 (Bolton et al,1988).

## 2.4.3 Pressure

Pressurizing the wort is a strategy to manipulate ester formation, but if unmonitored has potential to render deleterious effects to the fermentation process through the manipulation of the yeast cell growth. Pressure presents itself in three ways during fermentation. firstly, hydrostatic pressure due to the height of the fermentation vessel. Secondly, osmotic pressure of the yeast cells, or variable water activity which is related to the composition of the fermenting wort. Thirdly, in closed vessels it is possible to restrict the outflow of carbon dioxide and allow the fermenter to pressurise. The magnitude of osmotic pressure is a

function of the wort concentration, hence very high gravity worts records osmotic pressures of 40atm(owades.,1981). According to Gervais et al (1992) yeast cells can withstand osmotic pressure of up to 100atm, provided they are in their appropriate physiological condition. Water activity and pressure characterise the osmotic effect of the yeast cells. Normally in solutions, solids and water activities are inversely related. According to Hocking (1988) few yeast cells can withstand low water activities.  In brewing, water activities limits the maximum gravity that can be used, without altering both beer quality and yeast conditions. Pressure causes ultrastructural changes in yeast cells, resulting in leakage between intracellular compartments (Shimada et al., 1993). Investigations by Kamihira et al  (1987), shows that metabolic processes are disturbed by high pressures (100MPa) and it is used as a method of sterilisation.

Hydrostatic pressures is generated by the height of the brewing wort in the fermenter. During primary fermentation, agitation is important to accelerate the fermentation process by continuously circulating the yeast cells throughout the vessel. The circulatory process subjects the yeast cells to fluctuating pressure altering the metabolic pathway of the yeast cells and flavour profile of the beer. Pressurisation of vessels exposes the yeast cell to elevated pressures and high concentration of carbon dioxide. The effect of these two factors have not been studied in context of fusel alcohols but in fuel alcohols production (Thibault et al., 1987; L'Italien et al., 1989). These authors discovered that hyperbaric conditions suppresses the ethanol formation. Inhibition by carbon dioxide appears to be more significant than effects simply due to pressure. Hyperbaric conditions are only necessary to modulate yeasts growth and beer flavour influenced by the alterations of other  control parameters. However, conflicting results were observed by Rice et al (1976) and Arcay et al (1984). Rice et al(1976) investigated the effects of carbon dioxide top pressure of  0.16 MPa on 100litres

of lager fermentations. These authors observed that The extent of yeast growth and concentrations of beer volatiles produced at 22°C were the same as those observed at 15°C with no top pressure. While, Arcay-Ledzema & Slaughter (1984) observed reduced fermentation rate, yeast growth extent and higher alcohols at 2atm. Additionally, an elevated concentration of the VDK was evident towards the end of the fermentation with ale yeast strain in use. Commercially, pressure fermentation compensates for deleterious effects of high temperature on beer flavour (Nielsen et al., 1986, 1987). The relationship between pressure on higher alcohols and ester synthesis is unclear. Miedener (1978) reported a number of analytical differences between beers from normal and pressurised fermentations. The latter pH was higher, this is a consequence of increased yeast shock excretion of nucleotides and amino acids in response to higher pressure. Additionally, reduced head retention was also observed. The observed results are as a result of yeast autolysis due to increased content of short chain fatty acids($C_6$-$C_{10}$). In terms of flavour, normal fermented beers offer no significant changes to higher alcohols except for 2-phenylethanol. In pressurizes fermenters the concentration of 2-phenyethanol doubled. Esters such as ethyl and isoamyl acetate concentrations were not altered in normal beers but esters of hexanoic, octanoic and decanoid acids were doubled in concentration in pressurised fermenters. Also, DMS (dimethyl sulphides) and VDK's were reduced in pressure fermentation of wort. Posada (1978) confirmed the hypothesis of pressure on yeast growth. However, in his report he suggested that the effect of pressure on flavour components is strain specific. He discovered that with two different yeast strains, elevated pressures had contrary effects on 2-phenylehanol. Strain dependent variations are the only reason to merge conflicting observations of pressure application on beer fermentation.

## 2.4.4 Yeast Physiology and Pitching Rate

The health of the yeast cell is important in brewing because it dictates the quality and character of the final product obtained. According to Heggart et al (2000), Yeast is known to some extent to affect organic acids, esters, higher alcohols, aldehydes and diacetyl throughout fermentation and maturation, and consequently contribute to the overall organoleptic properties of the final beer. Therefore it is important to assess the quality of the yeast before and after each fermentation stage. The quality of yeast is described in terms of its "viability" or its "vitality" (Lentini, 1993). Yeast viability is described as the ability of yeast cells to grow, reproduce and interact with their environment (Smart et al,1999), whereas Yeast vitality has been variously described as a measure of activity (D_Amore, 1992), fermentation performance (Boulton, 1991), or the capacity to overcome and recover from physiological stress (Smart,1996). Heggart et al (2000) reported in their work various methods of assessing brewer's yeast viability and vitality. According to Jones (1987) major component of viability measurements involves cell replication, methods using vital stains and measurements of cellular products resulting from metabolic activities. Yeast vitality methods usually involve the measurement of specific yeast cell components critical to fermentation activity, or the determination of a metabolic activity that may be related to fermentation performance (Axcell & O_Connor-Cox, 1996). Guido et al (2003) investigated the effects of physiological conditions of the yeast on various fermentation. These authors used yeasts with different viability and vitality to pitch their worts in various fermentation tests. The pitching rate used was similar for subsequent batches of fermenting wort. Figure 2.4 shows the conditions of the yeasts used for various fermentations.
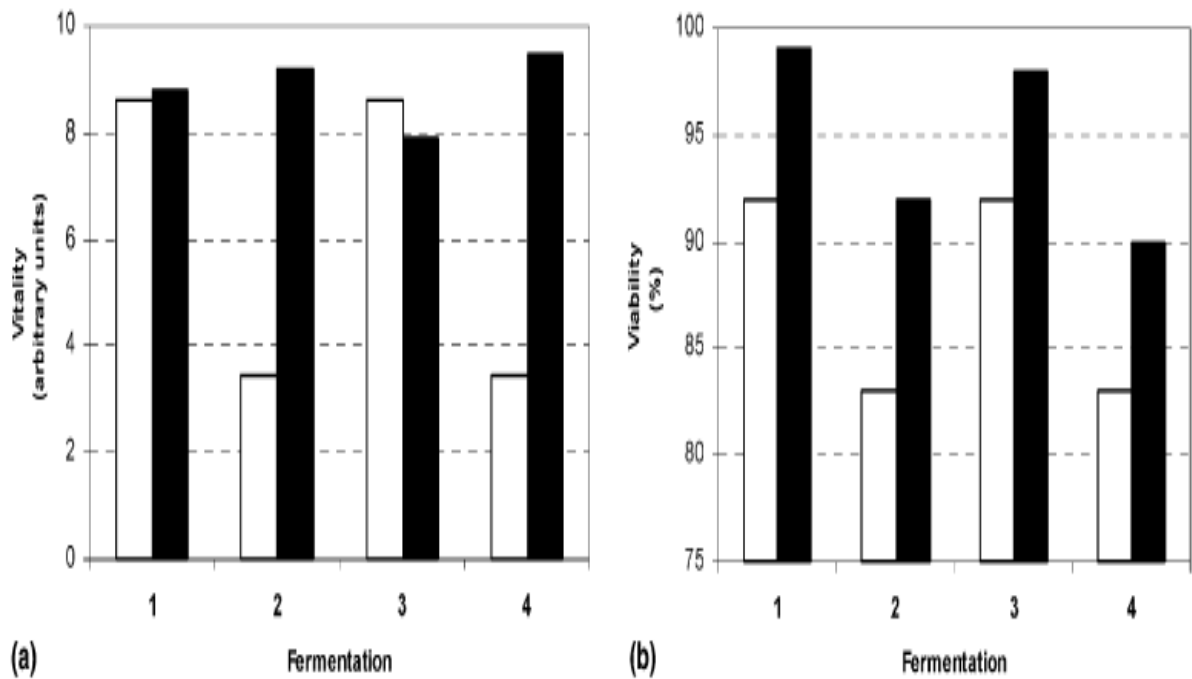
Figure 2.4: Viability and vitality of yeasts used for various fermentations, (a) fermentations 1 & 3: higher vitality; (b) fermentations 2 & 4 lower vitality (Guido et al., 2003).

The carbohydrates profile obtained was similar for all subsequent fermentations. In the first two days, there was increase in fermentation vigour and this was displaced by the drop in the specific gravity of the wort. Clearly the vigour was not due to the high replication rates of the yeats cells, since there was no noticeable extent of the yeast growth. The vigour of the fermentation was attributed to the intense metabolic activity catalysed by the yeast vitality levels. These authors also indicated a high levels of sulphur dioxide and acetaldehyde for high vitality fermentations. The higher alcohols and ester were also indicative for higher vitality cells. Table 2.2 represents the higher alcohols and ester found for these fermentation tests.

Table 2.2: The effects of yeast physiological conditions on beer alcohols and volatile ester contents (mg/L). Beers 1&3 represents high vital pitching yeasts whereas beers 2&4 represents lower vital yeasts.

|  | Beer 1 | Beer 2 | Beer 3 | Beer 4 |
|---|---|---|---|---|
| Isopropanol | 17.7 | 17.1 | 18.8 | 17.4 |
| Isobutanol | 11.6 | 13.3 | 11.9 | 13.2 |
| Amyl alcohol | 68.9 | 72.8 | 68.8 | 74.4 |
| Ethyl acetate | 17 | 17.3 | 17.1 | 16.7 |
| Isoamyl acetate | 1.2 | 1.21 | 1.14 | 1.17 |
| Ratio alcohols/esters | 5.4 | 5.6 | 5.4 | 5.9 |

Figure 2.5 shows the effects of the same fermentations conditions on the sulphur dioxide and acetaldehyde concentrations.
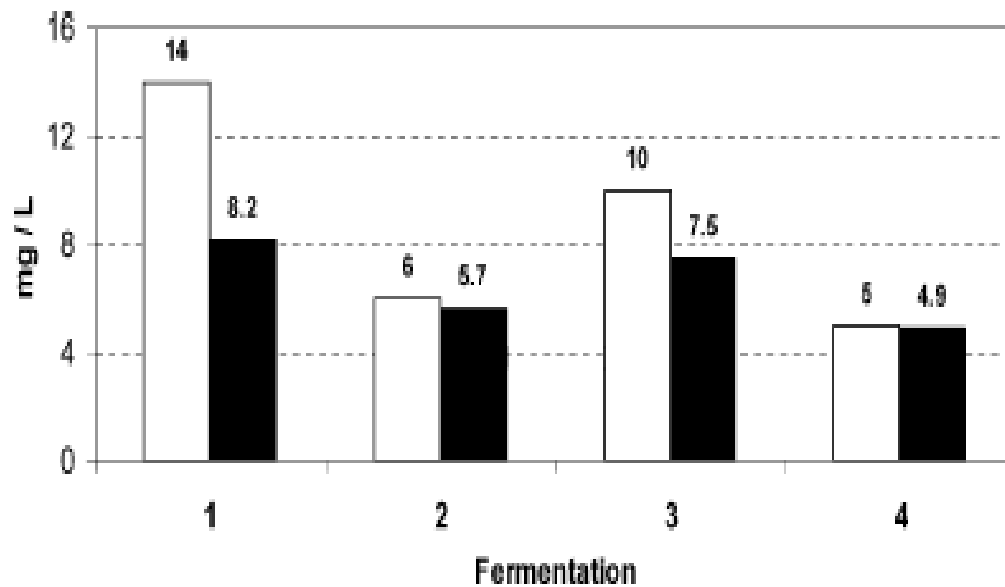


Figure 2.5: The effects of yeast physiological conditions on the total $SO_2$ (▢) and acetaldehyde (■) contents at the end of the fermentations. Beers 1&3 represent high vital pitching yeasts whereas beers 2&4 represent lower vital yeasts.

## 2.5 Process Control strategy

A Proportional–Integral–Derivative (PID) is a three-term feedback controller that has a long history in the automatic control field, dating from the beginning of the last century (Bennet, 1993). PID is the dominant form of feedback control used today and it has vast industrial applications such as motor drives, magnetic and optic membranes, flight control, instrumentation etc. it comes in a variety forms such as standard standard single loop controllers, as software package in a programmable logic controllers and distributed control systems, as a built in controller in robots and CD players. The PID controller is the bread and butter of automatic control, it is the first solution to be tried and tested before any other form of feedback is used.

The PID controller utilises a variety of turning mechanisms to optimise its operational efficiency. The most widely used turning algorithm is the Ziegler Nichols algorithm which makes use of the process reaction curves (PRC). Most of the PID rules developed in the last 50yrs use frequency response methods. Examples include, Ziegler–Nichols rule (Ziegler & Nichols, 1942), symmetric optimum rule (Kessler, 1958; Voda & Landau, 1995), Ziegler–Nichols' complementary rule (Mantz & Tacconi, 1989), some-overshoot rule (Seborg, Edgar, & Mellichamp, 1989), no-overshoot rule (Seborg et al., 1989), refined Ziegler–Nichols rule (Hang, Astrom, & Ho, 1991), integral of squared time weighted error rule ( Zhuang & Atherton, 1993), and integral of absolute error rule (Pessen, 1994). These methods are straightforward to apply since they provide simple tuning formulae to determine the PID controller parameters. However, only since a small amount information on the dynamic behaviour of the process is used, in many situations they do not provide good enough tuning or produce a satisfactory closed-loop response. For example, in practice, the Ziegler–Nichols

rule often leads to a rather oscillatory response to setpoint changes. Figure 2.6 presents the general loop configuration of the PID controller.
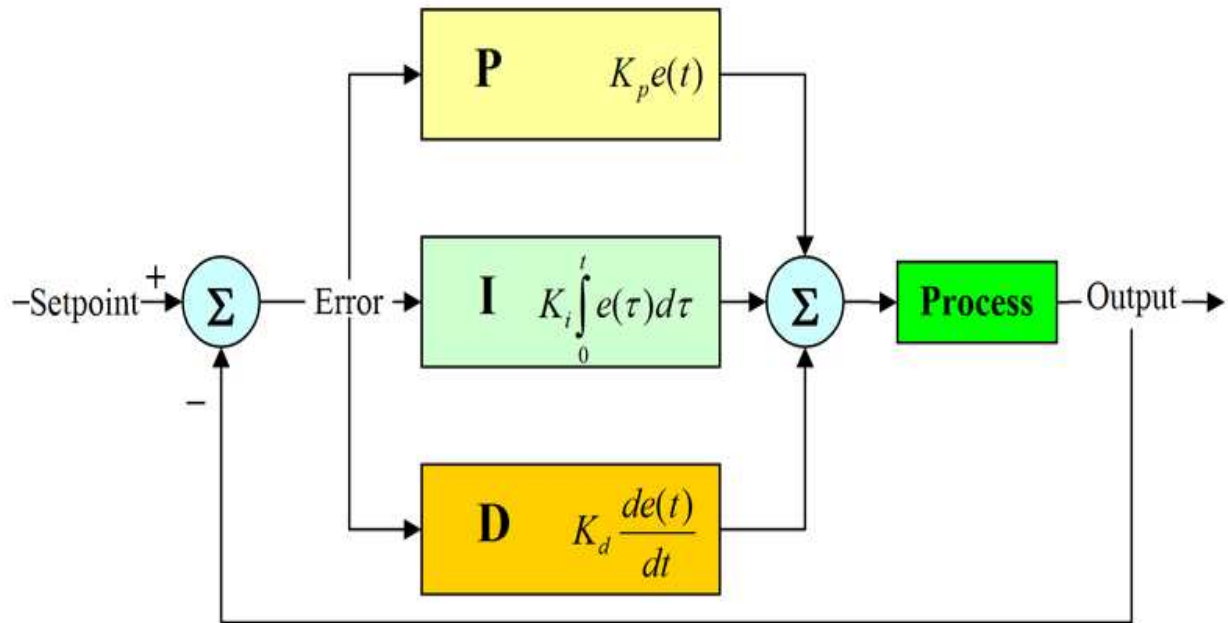


Figure 2.6: The mathematical representation of a typical PID control loop

The P, I and the D are the Proportional, Integral and Derivative, respectively. The complete representation of the controller is given below

$$\mathrm{u(t)} = \mathrm{MV(t)} = K_p e(t) + K_i \int_0^t e(\tau)\,d\tau + K_d \frac{d}{dt} e(t) \tag{3}$$

Where $K_d$, $K_i$, $K_p$ is the derivative gain, integral gain and the proportional gains, respectively

The development of Ziegler Nichols was achieved by the simulation of various processes and correlating the controller parameters with features of the step response. The key design criterion is the quarter amplitude damping. Another key issue is to characterise a general turning method for second order processes. The general representation of the first order and the second order transfer function is given in equation (4).

$$P(s) = \frac{K_v}{s(1 + sT_1)} \quad \text{and} \quad P(s) = \frac{K_p}{(1 + sT_1)(1 + sT_2)} \tag{4}$$

The above equations are used to calculate the process reaction curves to estimate the reaction rates and time delay.

# CHAPTER THREE

## EXPERIMENTAL PROCEDURE

### 3.1 Materials and Methods

**Fermentation**

All fermentations were performed in a 100litre cylindroconical fermenter supplied by Falcon Engineering (SA) (Pty) Ltd. All samples were produced from the lager malt supplied by SABMiller Alrode. All fermentations were maintained at 70 litre to ensure efficient control of the temperature and reduce potential high pressures that can affect the yeast metabolism negatively and produce flavour. Low oxygen level at the start of pitching the oxygen in the tank is replenished by supplying additional air from an air cylinder until sufficient air levels are reached before the pitching of yeast is commissioned. Fermentations were all performed at various temperatures from 14-23 $^{o}$C, while all other process variables remain almost the same for all fermentations performed. The wort was maintained at original gravity (OG) of 1.058 and apparent extract of 14.3$^{0}$P for all experiments to ensure consistency in all the investigations.

The fermentation tank was sterilized with water at 100$^{o}$C for 30min prior to fermentations. This is done in order to ensure that no bacterial growth exists in the tank during fermentation that can contaminate the water. During fermentation samples were taken manually every 12hrs for analysis.

**Yeast Cell Propagation**

Prior to pitching the yeast cells were cultivated and the propagated yeast strain (*S. cerevisiae var uvarium*) was monitored for vitality(intracellular PH by ICP method) . Viable cells were separated and propagated once again until the desired pitching rate was reached. The yeast was stored at -2$^o$C for 7 days.

**Analysis of volatiles**

Samples of the beer were recorded at every 12 hour period and filtered using a 30microns filter with an installed vacuum pump and stored in 350ml bottles and stored at -3$^0$C in a refrigerator. The filtering was to ensure that all yeast is removed from the beer to prevent continued fermentation during storage and alter the quality of the investigation. The storage bottles were first sterilized by using the autoclave at 100$^0$C for 30 min to ensure that no bacterium persists in beers which might alter the flavor profile of the beer. All samples collected were sent to SABMiller Alrode for analysis. Esters concentrations were determined by using the headspace gas chromatography (GC-MS). The Diacetyl concentration is measured by using the Hewlett Packard 5890A gas chromatography fitted with a HP6890 series injector .

**3.2 Procedure**

**Modelling and Control Philosophy**

Temperature was monitored and controlled using a PID control loop designed and commissioned at The University of the Witwatersrand. The control loop employs the feed forward control criteria. The PID controller (REX-F4) supplied by RKC INSTRUMENTS INC, Control Valve(1inch ID control) with a 4-20mA actuating signal externally powered electronic actuator which was supplied by SPIRAX SARCO (PTY) LTD and Temperature

Transmitter utilizing a K-type thermocouple inserted on the side of the cylindro-cornical fermentation tank. The Temperature control loop controls the cooling water at inlet temperature of $3^0C$ circulating in the fermenter cooling jacket. The controller inputs are the derivatives, integral and temperature setpoints and it actuates the valve positioning. Figure 3.1 presents the schematic diagram of the fermentation tank with temperature control loop.
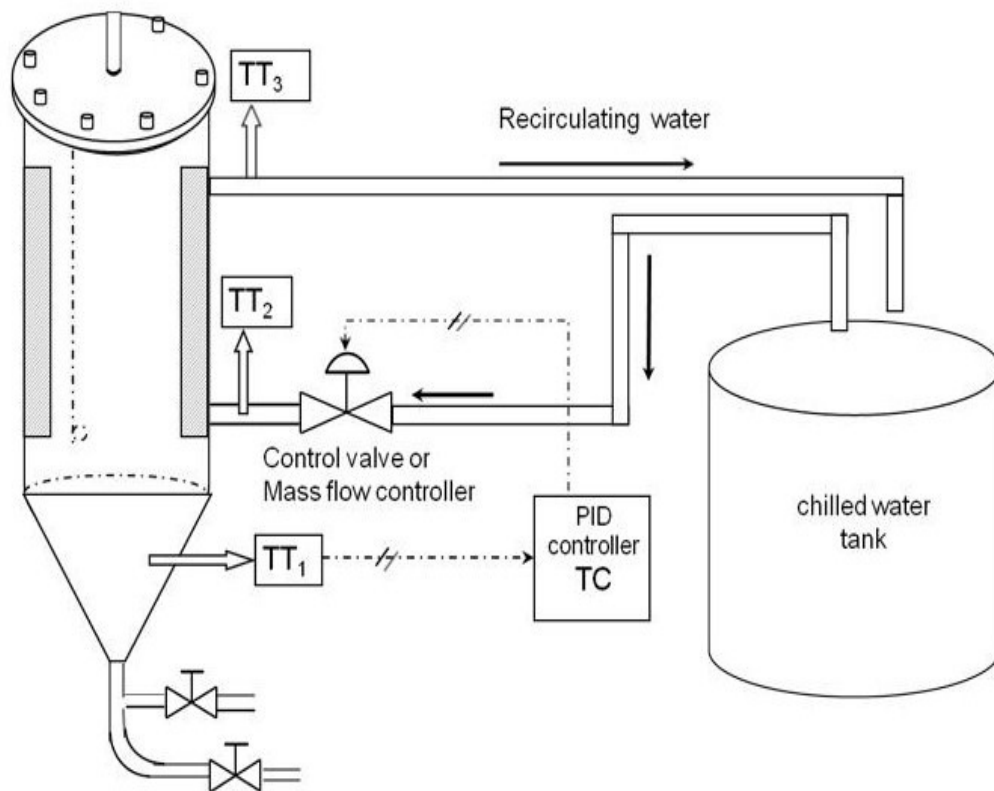


Figure 3.1: The schematic of the cylindro-conical fermentation tank and the temperature control loop.

The derivation below is the modeling of the tank energy balance which is the basis of the control equation used to calculate other control variables which are of importance for optimization.

Assumptions

1.  The Heat transfer in the fermenter occurs radially and all axial heat loss is negligible due to the batch fermentation.

2.  The only reaction occurring is the conversion of carbohydrates to alcohol and flavor compounds.

3.  The film coefficient at the fermenter wall is negligible and heat loss or gained is due to the cooling water in the fermenter jacket

The energy balance in the fermenter can be written as follows

Heat generated during fermentation= heat lost due to the cooling water effects

$$\Delta H = Q \tag{3.1}$$

$$Q = UA_p(T - T_j) = F_{in}C_{p_w}T_{in} - F_{out}C_{p_w}T_{out} = F_{in}C_{p_w}(T_{in} - T_{out}) \tag{3.2}$$

U= Overall heat transfer coefficient of the tank

$A_p$ = Area of the cooling jacket pipe

$C_{pw}$ = specific heat capacity of the water

$F_{in}$= water inlet flow rate

$T_{in}$= inlet temperature of the water into the tank

$T_{out}$= outlet temperature of the water in the tank

Since the process is batch, so the heat of reaction changes with time as expressed by equation 3.3.

$$\Delta H = m_b cp_b \frac{dT_b}{dt} = \rho_b V_b C_{p_b} \frac{dT_b}{dt}$$

(3.3)

Combining equation 3.2and 3.3 will give:

$$\frac{dT_b}{dt} = \frac{F_{in}C_{p_w}(T_{in}-T_{out})}{\rho_b V_b C_{p_b}}$$

(3.4)

Where:

$T_b=$ The beer temperature

$V_b=$ volume of the beer in the tank

$C_{pb}=$ specific heat capacity of the beer

$t =$ time

**Process Control Philosophy**

The controller used in these experiments is the PID or the proportional integral derivative controller. This controller was chosen due to its ability to minimize errors and process offsets. Since the process capacity is large and the dead time and offsets required should be minimized therefore the PID controller is the suitable controller used in this investigation

**Mathematical Expression**

The PID controller design equation for parameters estimation $K_1$, $T_i$ and $T_d$ which will be computed using the process reaction curves.

$$U(s) = K_1(1+\frac{1}{T_i s}+T_d s)(T_d s - T_m s)$$

(3.5)

Equation 3.5 below shows the control equation governing the control valve in Laplace form

$$\frac{V}{U}(s) = \frac{K_2}{1+T_1}(s) \qquad\qquad (3.6)$$

Where:

U(s) = controller signal to the valve

V(s)= exit flowrate of the water in the valve

$K_{2(s)}$= the valve constant or gain

**Controller Turning**

Determining the constants in the PID controller is done by generating process reaction curves. The process reaction curves were generated using *Matlab 7.1*. The process variable is then used to calculate the controller gain, integral time and derivative time. These methods are performed in an open loop, so no control action occurs and the process response can be isolated. The $K_1$ to be used is the optimised value which reduced the response time and dead time to enable quality control of the temperature during fermentation. The controller optimisation values are calculated using the Ziegler-Nichols method. The parameters are calculated using the formulas 3.7, 3.8 and 3.9. the R and D are computed from the process reaction curve given in the following section 4, with $R(s^{-1})$ being the slope of the tangent touching the point of inflection and D(s) being the time it takes for the curve to resemble a logarithmic behaviour.

$$K_1 = \frac{1.2}{RD} \qquad\qquad (3.7)$$

$$T_i = 2D \qquad\qquad (3.8)$$

$$T_d = 0.5D \qquad\qquad (3.9)$$

# CHAPTER FOUR

## 4. RESULTS AND DISCUSSION

**4**.1 Parameter prediction and estimation for cooling water control
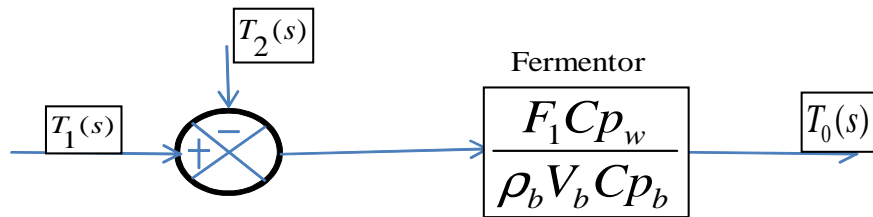


Figure 4.1: The open loop response diagram of the fermentation tank.
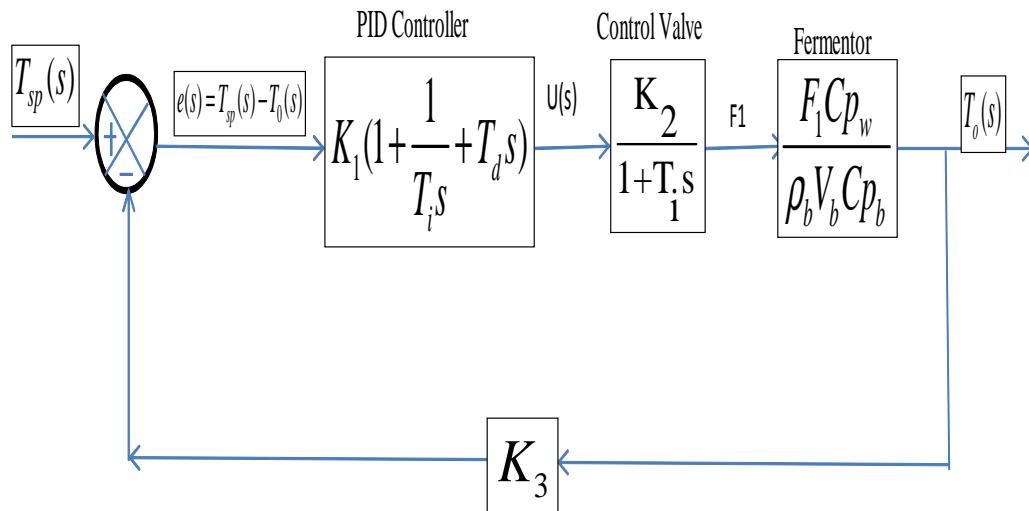


Figure 4.2: The Block Diagram of the temperature control loop in the fermentor.

According to Figure 4.2, the setpoint temperature or desired temperature is specified as an input into the system. The controller checks whether there's a difference in the setpoint temperature of the tank and the actual temperature as read by the temperature transmitter. The difference is registered as an error (e(s)) which activates the controller action. The controller sends a signal (U(s)) to the control valve's actuator which actuates the valve position and thus regulates the flow of water out of the valve which will be set to the desired temperature. The steady state values of the temperature will be reached when the controller error (e(s)) becomes zero, thereby deactivating any signal to the controller. $K_1$, $K_2$ and $K_3$ represent the controller gain, control valve gain and the thermocouple's resistance gain respectively. $K_2$ and $K_3$ are known but $K_1$ is computed using the Ziegler Nichols turning from the process reaction curves as shown in Figure 4.3.
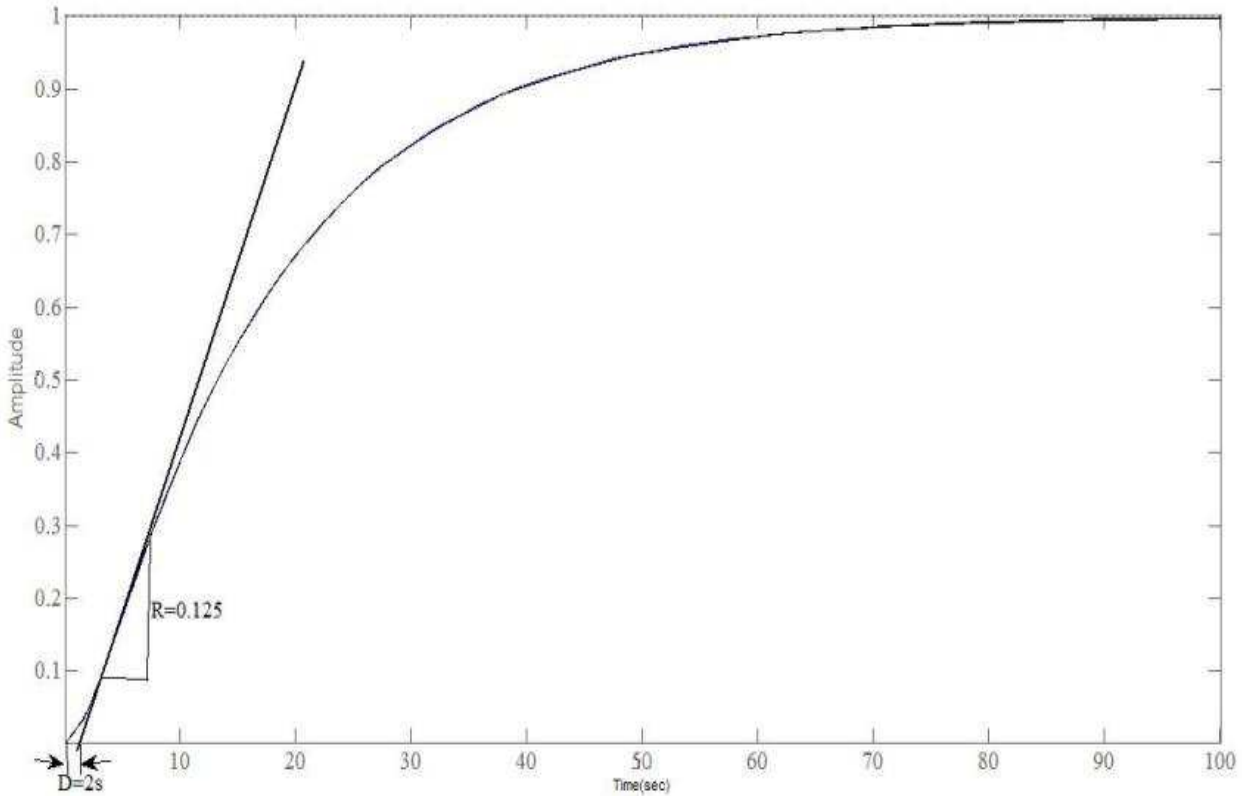
Figure 4.3: The process reaction curve computed from figure 4.2.

The process reaction curve (Figure 4.3) above was computed using the matlab software and simulated to obtain the controller constants namely the controller gain ($K_1$),the dead time ($T_d$) and rise time ($T_i$). The equations used for the computation of these values is discussed in detail in the previous section section 3. The controller constant ($K_1$),the dead time ($T_d$) and rise time($T_i$) were found to be 9.6 second ,2 seconds and 1 second, respectively. The obtained controller gain is used as an input to the controller to determine the behaviour of the controller for various temperature setpoints in the tank. Figure 4.4 shows the open loop behaviour of the tank temperature without the temperature controller.
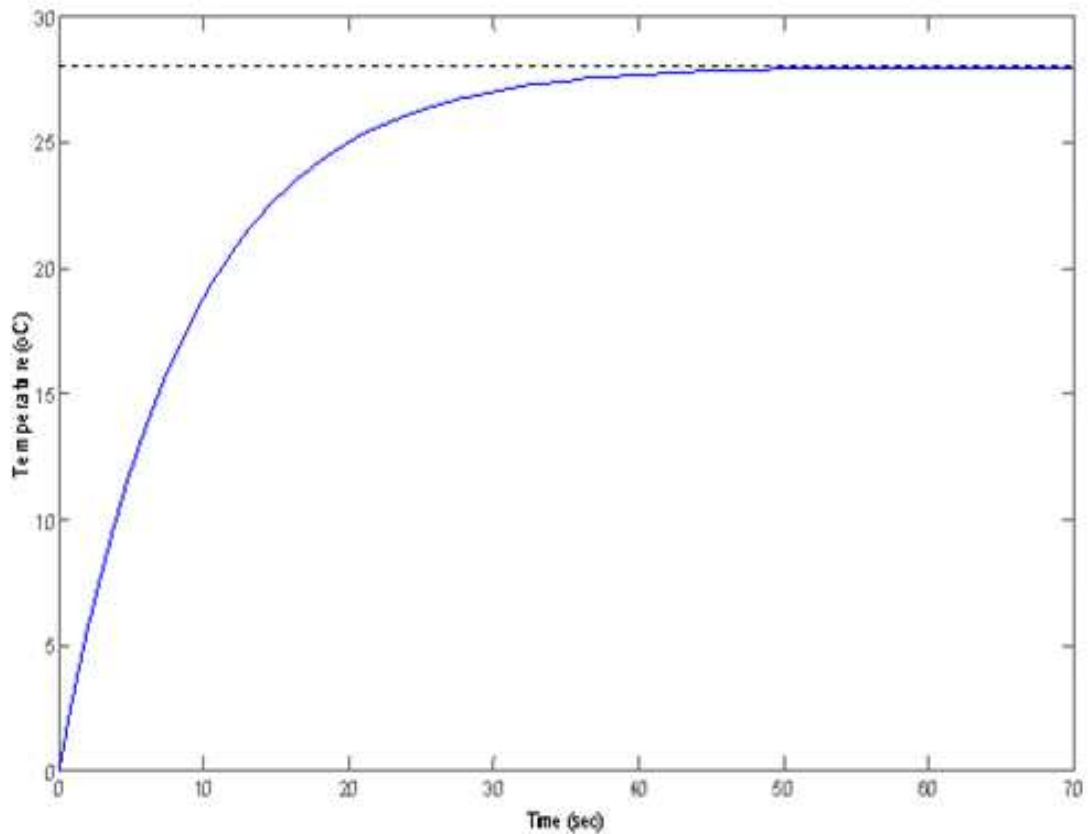
Figure 4.4: The step response of the open loop behaviour of the beer tank temperature

Figure 4.5 shows the closed loop response of the system including the controller and the controller performance for the setpoint represented. The setpoint chosen is $20^0$C and the amplitude is represented as a ratio of the setpoint temperature. According to Ziegler-Nichols the design criteria should be that the ratio of the successive peaks which are computed from the reference point of 1 as indicated on Figure 4.5 is 3.2. The optimum design criterion by Ziegler Nichols is 4. The designed controller will be 80% efficient as relative to the ideal controller envisioned by Ziegler Nichols.
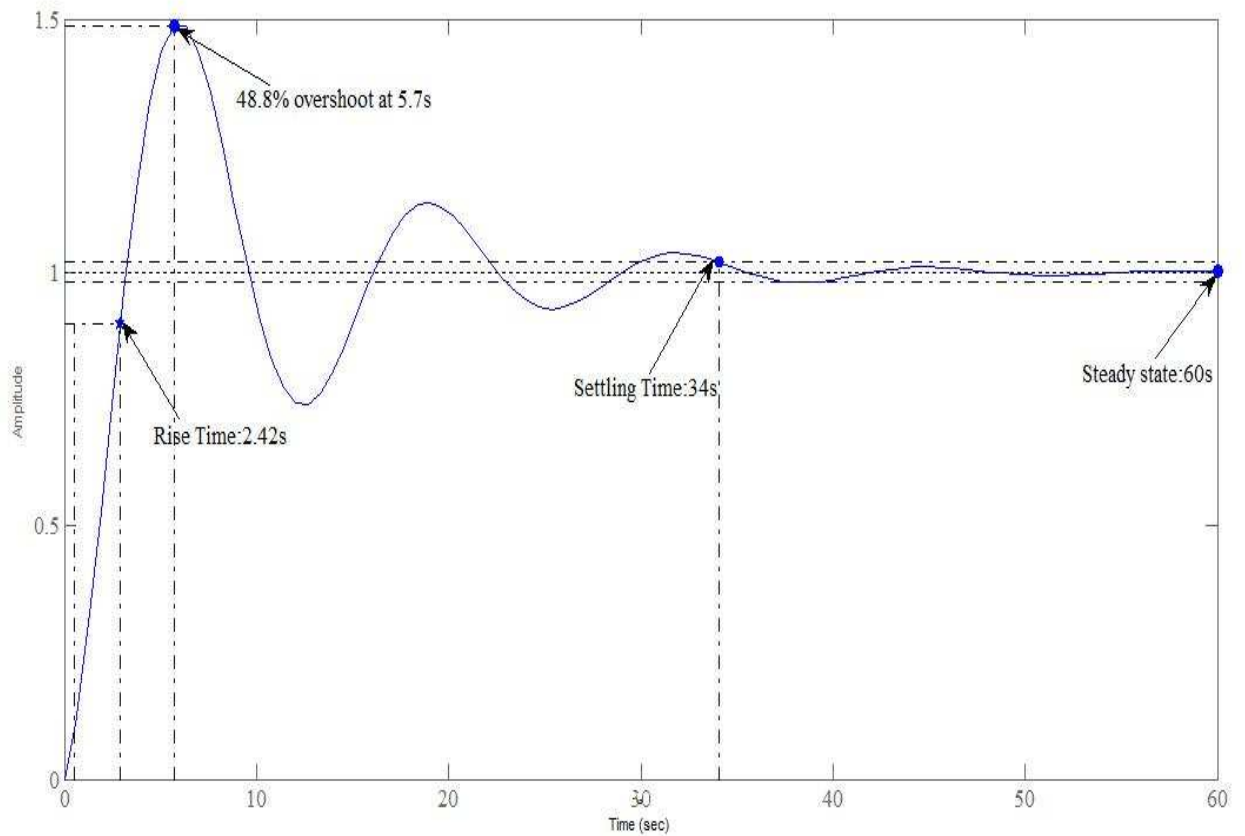
Figure 4.5: The closed loop temperature response of step response of temperature control system using PID controller tuned using Ziegler-Nichols process reaction method.

From Figure 4.5 the controller variables such as the rise time, settling time, percentage overshoot and the steady state value are represented on the figure. The rise time, settling time, percentage overshoot and steady state time are 2.42s, 34s, 48.8% and 60s respectively. It can be deduced from the figure that it takes precisely 60 seconds for the controller to maintain a steady state valve position to regulate the temperature setpoint in the tank.

## 4.2. Temperature on the fermentation rates and time

A study was conducted in order to investigate the effect of temperature on the fermentation rates. Figure 4.6 represents the results of the fermentation rates at constant original gravity (OG) while the experimental results are correlated with predicted data in Figure 4.7.
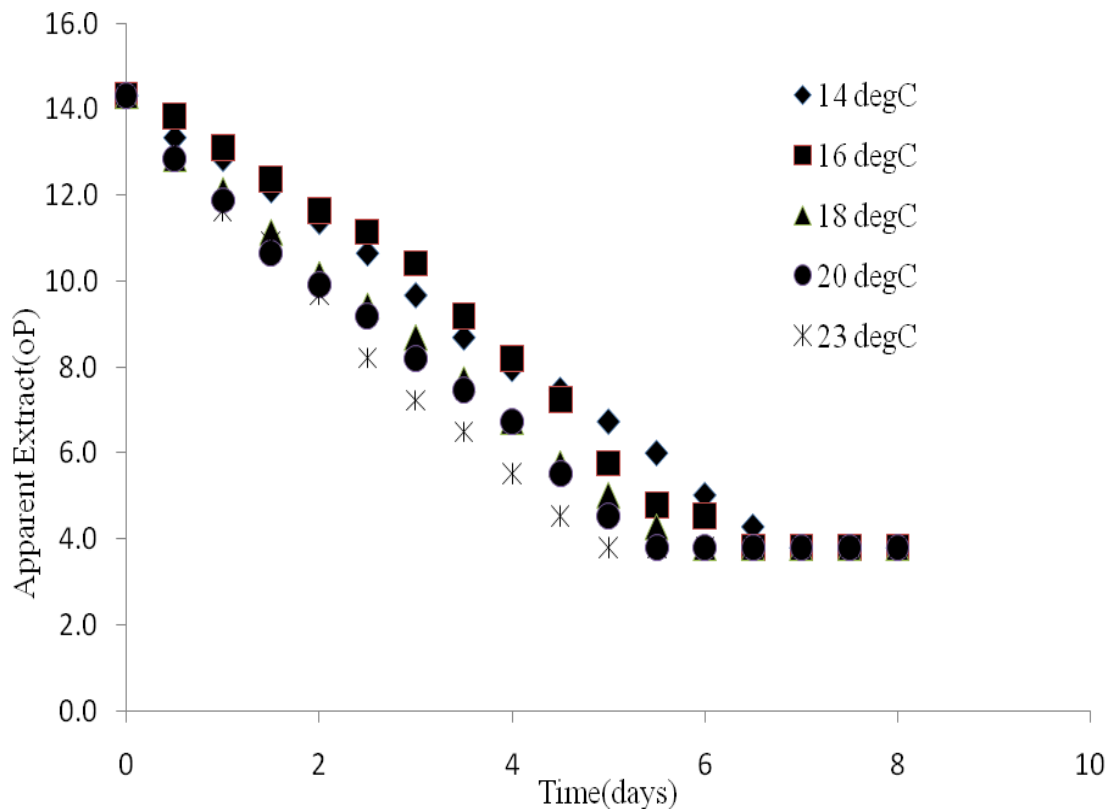


Figure 4.6: The attenuation profiles of lager fermentation performed at temperatures between 14-23$^0$C

All of the fermentations were perfomed with the apparent extract of 14.3$^O$P in a 100litres unstirred fermentor with a *Saccharomyces Cerevisea* yeast strain. As observed from Figure 4.6 the higher temperature favours high fermentation rates. The rate will be interpreted as the rate of decrease of the apparent extract per time. From the figure, 4.6 the highest rate will be credited to the 23$^o$C fermentation temperature, which takes 5 days to reach the maximum attenuation level followed by the 20$^0$C which completes its fermentation in 6 days. There is

insignificant change in the duration of the fermentation as well as the attenuation profiles at low temperatures between 14-18$^0$C which might be accounted to the reduced activity of the yeast cells at low temperatures. Since the same yeast strain is employed for all the fermentation it is expected that the final extract be at 3.8$^o$P for all fermentations. According to the results obtained, it will be reasonable to deduce that higher temperatures are beneficial for fermentations. Towards the end of the fermentation the extract point all seem to breakway from the smooth profile predicted. According to the results obtained by Lucero et al (2000) high temperatures reduced the yeast viability and promoted high toxicity which is detrimental to the functionality of the yeast cells and leads to faulty fermentations.
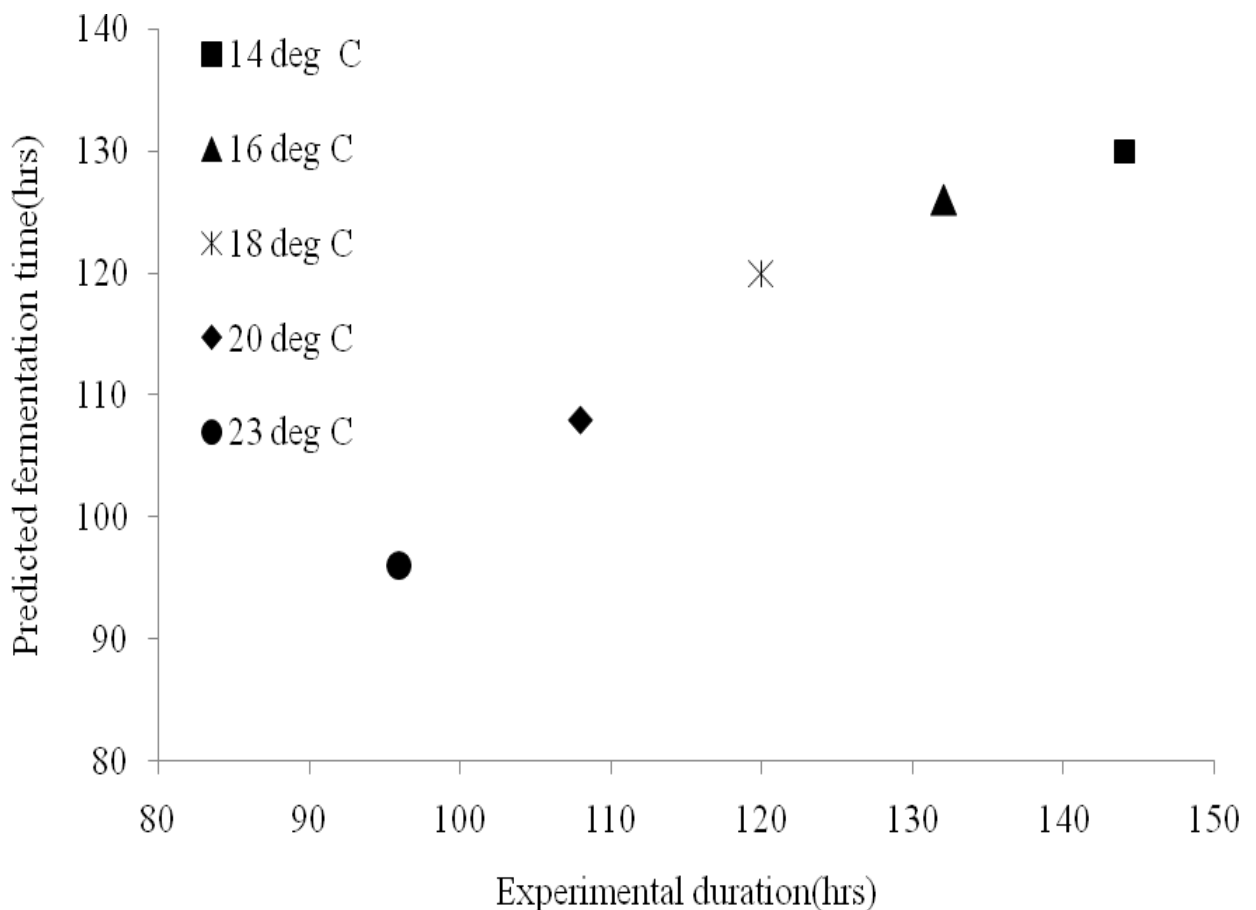


Figure 4.7: The correlation adequacy of the predicted values as compared with the experimental ones.

Figure 4.7 represents the correlation between the expected fermentation and experimental fermentation times at various temperatures. The expected fermentation times were estimated using the correlation presented by Brown and Hammond(2003) which is represented in section 3.5. This figure was plotted with various temperatures while other process variables remain the same for all fermentations. as observed the graph is linear at high temperatures which is represented by the low experimental duration. At low temperatures the model deviates greatly from the trend as compared to the trend at high temperatures. These results are in agreement with the findings of Brown et al (2003) which also concluded that the fermentation rates at high temperatures are lower as compared to lower temperatures with all the other process variables remaining the same throughout for all fermentations. In Addition to these findings, Brown et al (2000) also noticed an increase in fermentation rates at higher dissolved oxygen concentration, yeast pitching rate and stirrer agitation speeds. The shortfall of the above model is that it does not account for the toxicity of the beer due to ethanol concentration increasing towards the end of the fermentation.

## 4.3 Effect of temperature on flavour components in beer.

Beer Flavour is known as the overall integrated impression of taste, aroma and mouth feel. The mouth feel of every beer is the quality of the beer and every brewer's goal is to ensure a consistent product and avoid any changes in beer profile. Figures 4.8-4.10 presents flavour components of esters and total vicinal diketones which are some of the major flavour variables in the beer.
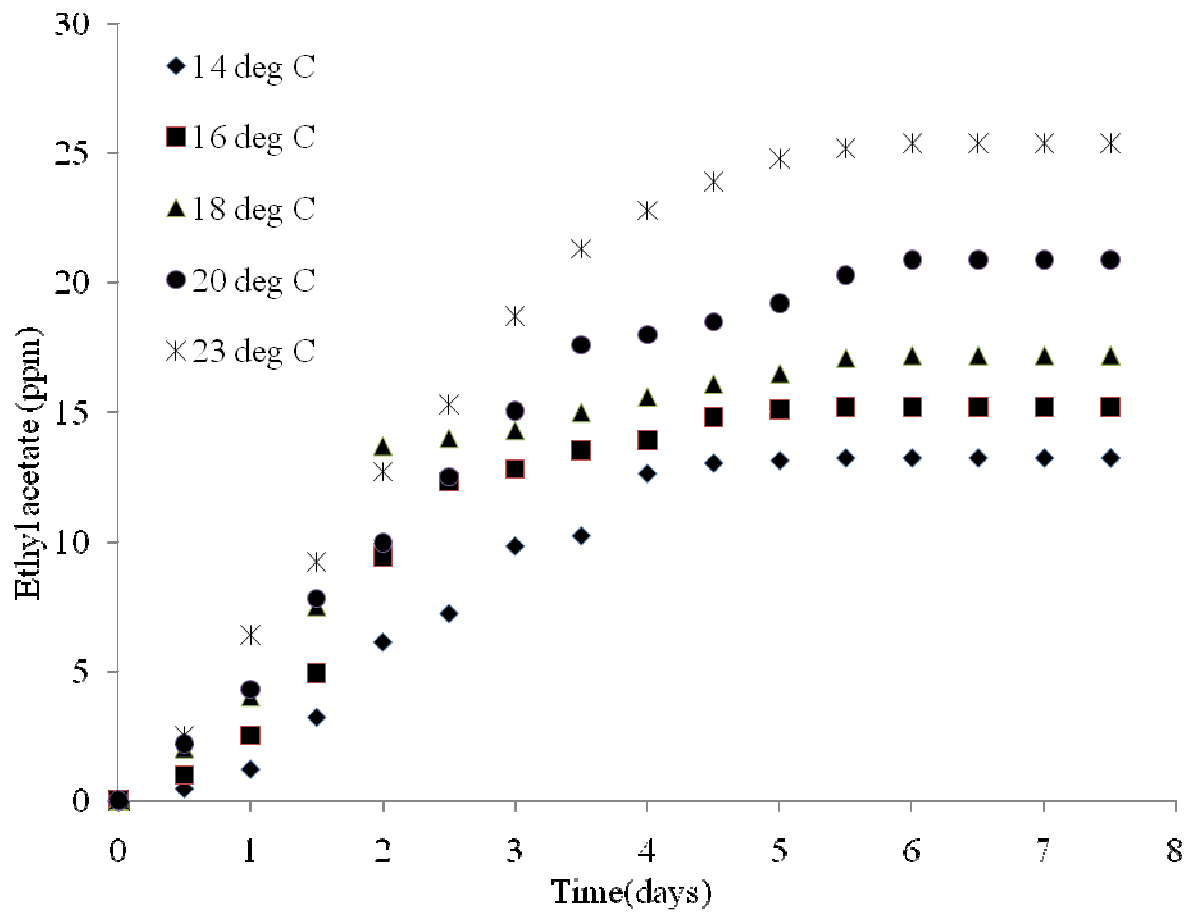
Figure 4.8: The profile of ethyl acetate ester with time during fermentation
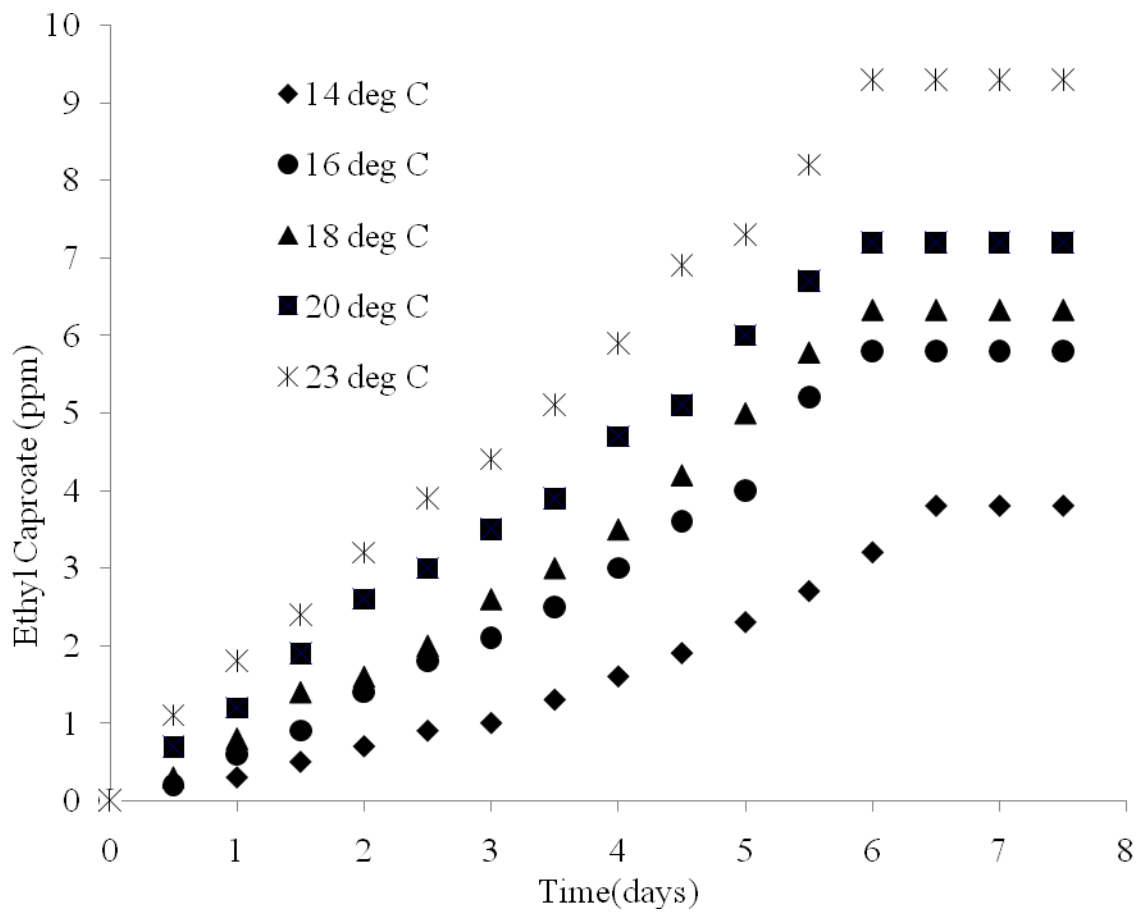
Figure 4.9: The concentration profile of ethyl caproate with time during fermentation.
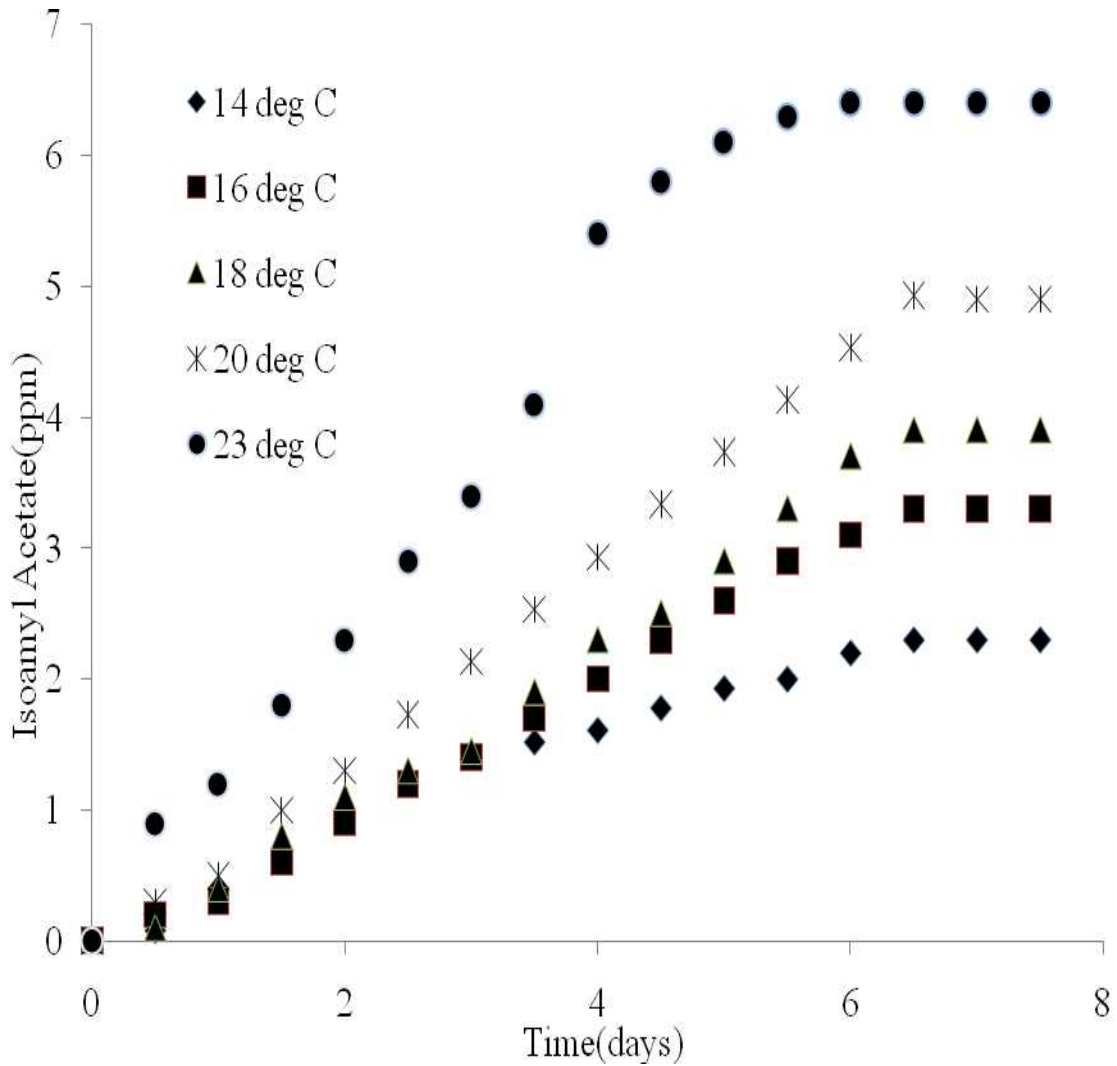
Figure 4.10: The concentration profile of Isoamyl Acetate esters with time during fermentation.

Figures 4.8, 4.9 and 4.10 show the various concentration changes of esters with temperature during fermentation. The esters investigated are Isoamyl Acetate, Ethyl Acetate and Ethyl Caproate which are known to exist in significant amounts in lager beers. Esters play an important role in the beer as they regulate the palatability of the beer. Thus they were investigated as the important attribute of beers and optimised. As observed from figure 4.8-4.10 all the esters investigated show an increase in concentration with time at various fermentation temperatures. These results are in accordance with the ones reported by Peddie

(1990). The increase in concentrations with temperature is due to the yeast cell membrane fluidity at high temperatures which modulates the activity of the membrane bound alcohol acetyl transferase (Peddie, 1990). In general, high temperatures favour high mass transfer rates, hence at high temperatures the diffusion rates of esters from the cells into the beer also increase causing high concentrations of the flavour components.

It is evident from Figure 4.7 that the ethyl acetate(fruity) has higher concentrations as compared to Ethyl Caproate and Isoamyl acetate (banana/Apple flavoured) esters at all temperatures investigated. According to the study conducted by Boultox et al (2001) with wort of $7.9^oP$ apparent extract and $13^0C$ fermentation temperature obtained the ethyl acetate and Isoamyl Acetate concentrations were of 12ppm and 1.5ppm respectively. The experimental results obtained from this study with the wort of apparent extract of $14.3^oP$ and the final concentration at highest temperature investigated was found to be 25ppm, which is reasonable this is because the original apparent extract used is almost double as compared to the one utilised by Boultox et al (2001). These results were also validated by Anderson et al (1974) who also concluded from their findings that ester levels increase exponentially with an increase in wort original gravity. Ethyl Acetate ester exists in higher concentrations due to the availability of ethanol as a substrate for its formation. Their exponential increase with time is a strong result of the ethanol concentration increasing during fermentation as indicated by a drop in apparent extract in Figure 4.5. For all the fermentation performed above it is evident that the ester concentration in the beer increases exponentially with an increase in fermentation temperature. Therefore, the monitoring of ester concentrations during fermentation is an important tool to check whether all flavour compounds are within their expected concentration limits as set by industrial breweries. The control of fermentation

temperature is therefore important in order to predict the end product ester concentration prior to the brew.
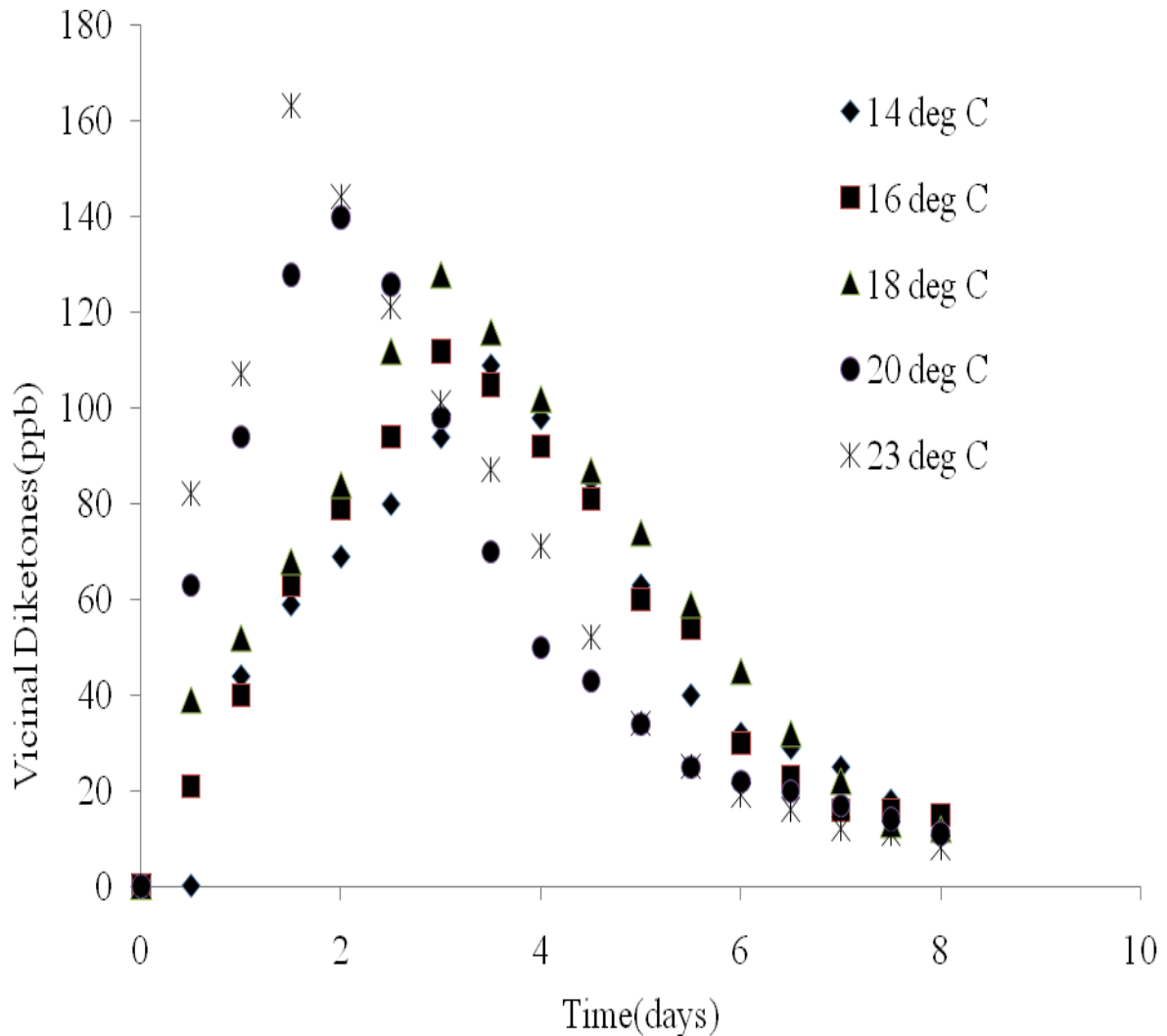


Figure 4.11: The total vicinal diketones(VDK's) concentration with time at various fermentation temperature.

Figure 4.11 presents the results obtained for total vicinal diketones concentration in lager beers. As described in Chapter 2, VDK's imparts a butterscotch flavour and are unpleasant in lager beers at high concentrations and they can also serve as a tool for optimisation. According to Wainwright (1973) the acceptable level of VDK's in beer is 0.6 ppm. The

concentration above 0.6 ppm is only noticeable during the first few days of the fermentation and reduces at a greater level towards the end of all fermentations reported. As seen in Figure 4.11 the profile of VDK's is parabolic in nature for all temperatures investigated. VDK's concentrations increase with temperature in the first few days and drop exponentially as the fermentation progresses. The results obtained resemble a similar trend to the ones reported by Boultox and Box (2001). Although the direct impact of temperature on the VDK concentration is during fermentation is unclear, But conditions that produce rapid yeast growth also promotes the formation of VDK's (Inoue et al., 1991). High temperature facilitates the diffusion of glucose and other fermentable sugars into the cell and thus favour rapid yeast growth (Quain et al., 2001). Hence it can be deduced that temperature can also affect VDK formation in lager beers. As observed from the figure 4.11 the maximum concentration of VDK's is reported at $23^oC$ at 0.14 ppm, and the maximum VDK formed drops as a function of temperatures with $14^0C$ having the lowest maximum concentration achieved towards the end of the fermentation on the $4^{th}$ day. The drop in concentrations of the VDK after its peak is ascribed to the activation of yeast reductases, However, the reduction in VDK production is unclear and their activity mechanisms is outside the scope of this study.

At lower temperatures the maximum VDK is reached near the end of the fermentation which is very disadvantageous as it will require prolonged secondary fermentation and maturation period in order to reduce the concentration of the Diketones to acceptable levels and improve the palatability of the beer. On the other hand, for secondary fermentation and maturation the fermentation temperature will have to be reduced to $6^0C$ in order to deactivate the yeast cell metabolism. The cooling effect at the end of the fermentation can incur great expenditure on the utilities thus increasing the total cost of the brew. Due to high utility expenditure of approximately 7kW/litre of beer, it will be reasonable to operate the fermentation at low to

moderate temperatures in order to reduce the utility costs and reduce the secondary fermentation period by a significant difference..
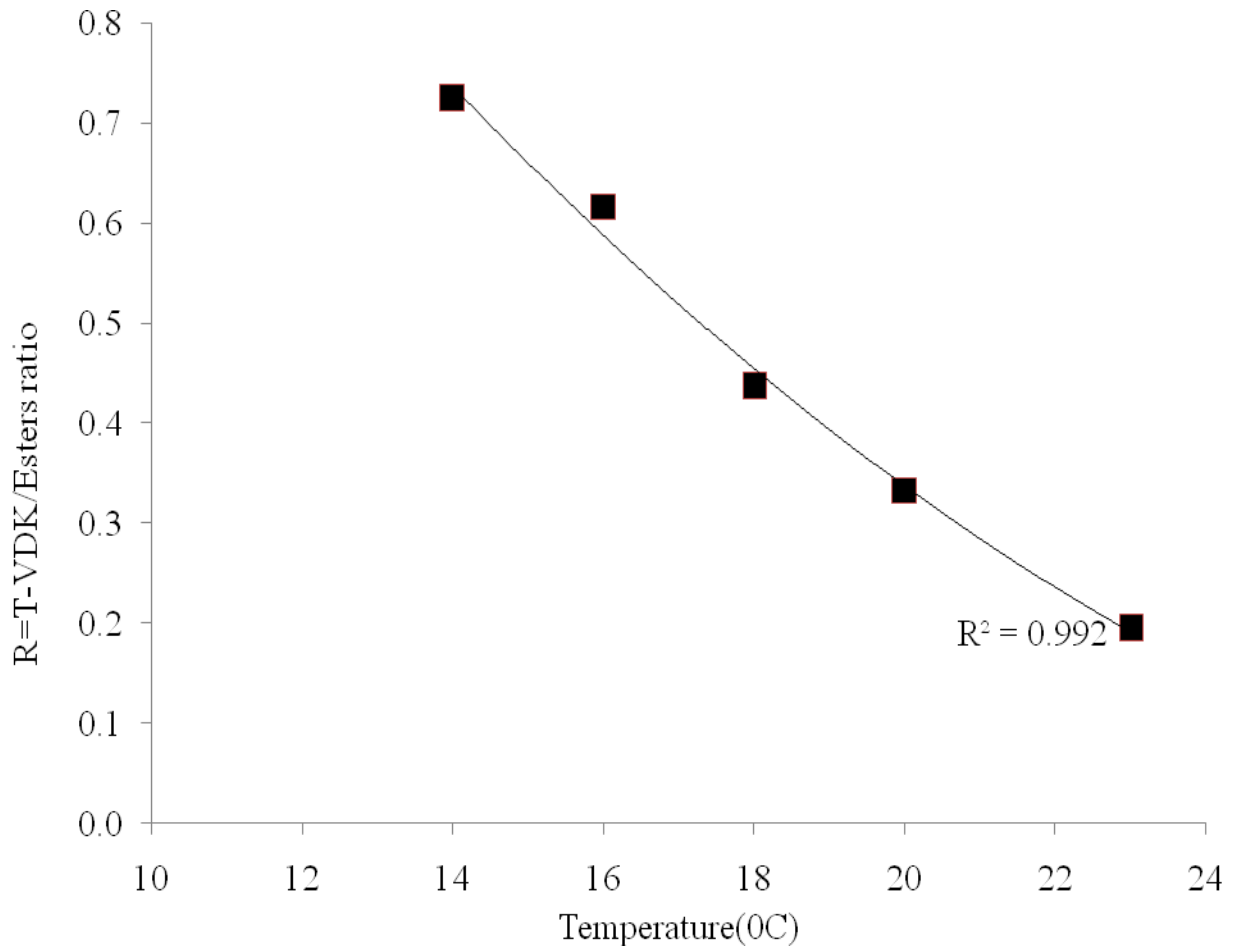


Figure 4.12: The variation of the total vicinal diketones (VDK's) to total esters ratio(R)with temperature.

The ratios of VDK/esters were calculated from the final concentrations of esters obtained and the VDK's at the end points of the fermentation process. These were computed in order to check the flavour profiles of the final beer. From Figure 4.11 a model can be devised that will help to determine the quality of the beer to be produced prior to the fermentation knowing the yeast strain and other process variables that can be set prior to the fermentation by only

knowing temperature as a process variable. The flavour ratio (R) reduces as the temperature increases. At higher temperatures it is evident from the figure that esters increase in concentration more rapidly than VDK's. *Saccharomycs cerevisiae* yeast strain function optimally at temperatures between 18-25$^{o}$C, it is generally known that between these temperature range the yeast viability and vitality is high enough to help facilitates the diffusion of nutrients and metabolites from and into the beer by the yeast cells.
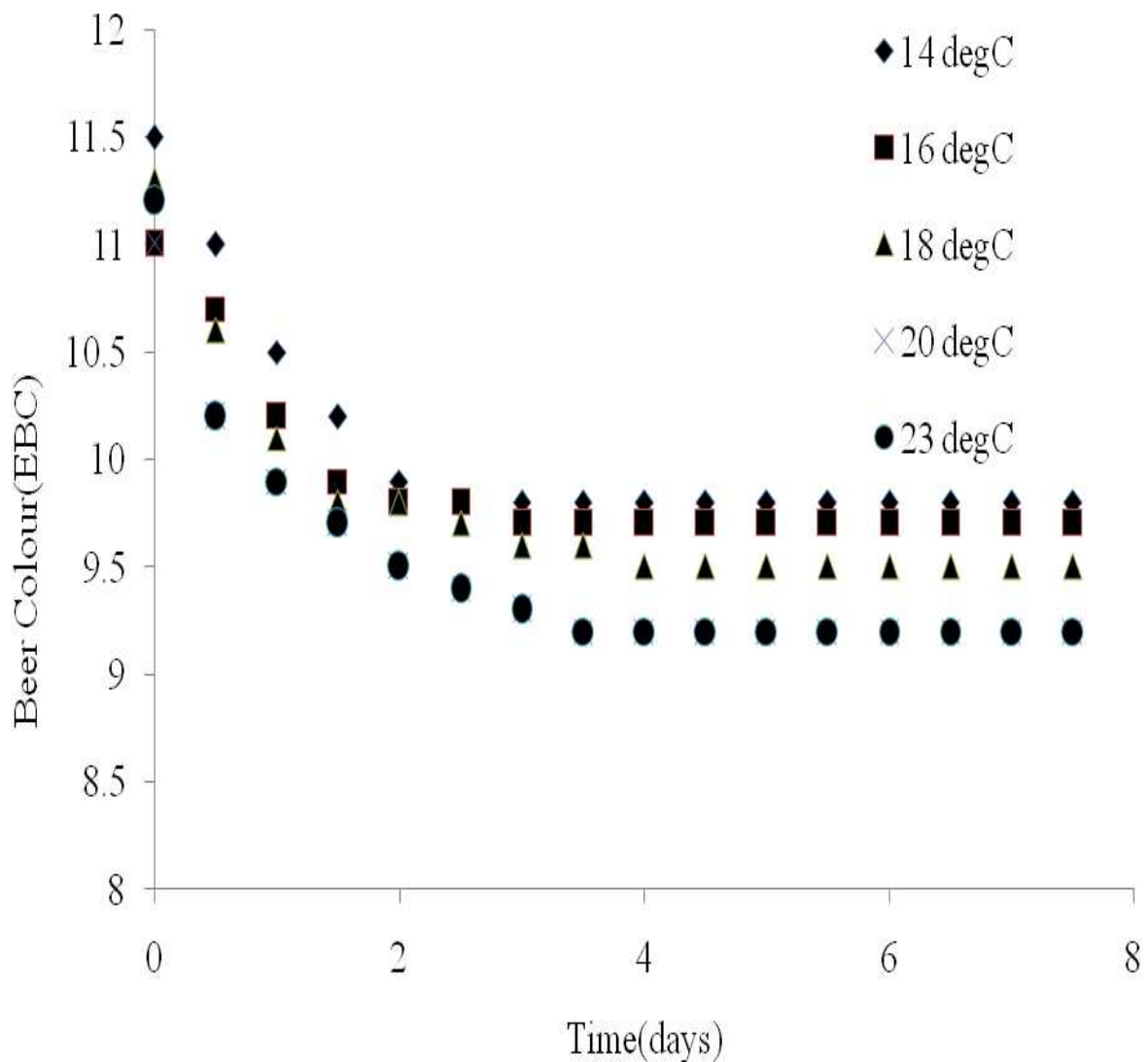


Figure 4.13: Variation of beer colour at various fermentation temperature.

Figure 4.13 presents the changes occurring to the colour of the beer during fermentation at temperatures investigated. The results obtained show that there's a correlation between the fermentation temperature and the final beer colour. According to the results beer colour improves as the temperature increases. The highest colour quality that can be obtained is at $23^{o}$C with the EBD of $9.2^{0}$. No consistent literature has found the impact of colour during fermentation. The results obtained can be due to the Maillard reactions between reducing sugars and amines form melanoidins, which contribute to beer colour. These reactions continue in fermentation and the rate is proportional to temperature and reducing sugars (Quain et al, 2001). The results for $20^{0}$C and $23^{0}$C degrees temperatures seem to have overlapping from the first day of the fermentation. This can suggest that the optimal temperature needed to promote the maillard reactions for the colour change is reached at $20^{o}$C. The colour reaches minimum level at $9.2^{0}$ EBC which is the point at which the reducing sugars reaches their optimum level to promote the maillard reactions. Various investigations should be conducted to investigate the effect of colour at various starting original gravity and fermentation temperatures above $23^{0}$C utilising a different yeast strain.

**CHAPTER FIVE**

## 5.1 CONCLUSIONS

Control scheme of temperature during fermentation experiments in the Wits Microbrewery has been synthesized and used to optimise beer quality. Proportional integral Derivative (PID) control algorithm has been used to regulate the fermentation temperature by controlling the cooling water flowrate with a control valve. It is observed that the increase in temperature affects the rate of fermentation of the lager beer investigated and esters and diketones flavour compounds. Positive correlation exists between the ester compounds formed and the concentrations at increasing temperatures. The maximum obtained concentrations of ethyl acetate, ethyl caproate and isoamyl acetate are 25.1, 9.7 and 6.4 ppm respectively. VDK's concentration increase in concentration as temperature increases for the first few days of the fermentation and reaches a maximum concentration before being reduced by other competitive reactions for the substrates. The maximum concentration of VDK's reached at 14,16,18,20 and $23^0$C are 120,132,148,156 and 168ppb respectively. The maximum concentration to be reached is positively correlated with fermentation temperature and the ultimately the beer taste and flavour threshold. The final concentration of VDK's can be regulated by increasing the storage time of the beer during maturation as the concentration drops to lower levels during maturation. The flavour ratio(R) improves with increase in temperature showing that longer fermentation improves the flavour threshold of the beer at high temperatures. The improved flavour ratio is a desirable attribute of the beer. From these findings, it can be concluded that temperature is one of the important parameters that can be optimised in order to ensure a consistent beer flavour in all brews conducted at the Wits micro scale brewery plant.

The controller design and parameter estimation has been perfomed and important input data has been optimised showing the PID controller used which has an efficiency of 80%. The controller optimisation was out carried using Ziegler-Nichols turning method. The optimised controller gain ($K_1$), the dead time ($T_d$) and rise time (Ti) was found to be 9.6, 2 and 1s respectively. The optimised controller gain reduces the disturbances in the system and errors in the fermentation profile by removing outlying points from the expected.

## 5.2 RECOMMENDATIONS

It is recommended that various test be performed to find the following.

➢ The impact of flavour and fermentation rates at higher temperatures above $25^0$C.

➢ The impact of the magnetic agitator or stirrer or circulating the wort semi continuously on the flavour compounds will determine the flavour ratio(R) and the final quality of the beer. This will ensure rapid mass transfer within the beer during the fermentation process. Due to high dispersion of yeast cells within the beer during fermentation it will increase the rate of diffusion of the flavour compounds from the yeast cells to the entire beer so that the concentration of flavour compounds is uniform throughout the entire batch.

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## Appendix A

## TABLES OF RESULTS

**TABLE A1: Apparent extract ($^o$P) with time**

| Time(days) | 14$^0$C | 16$^0$C | 18$^0$C | 20$^0$C | 23$^0$C |
|---|---|---|---|---|---|
| 0 | 14.3 | 14.3 | 14.3 | 14.3 | 14.3 |
| 0.5 | 13.3 | 13.8 | 12.9 | 12.9 | 13.1 |
| 1 | 12.9 | 13.1 | 12.1 | 11.9 | 11.6 |
| 1.5 | 12.1 | 12.4 | 11.1 | 10.7 | 10.9 |
| 2 | 11.4 | 11.6 | 10.2 | 9.9 | 9.7 |
| 2.5 | 10.7 | 11.1 | 9.4 | 9.2 | 8.2 |
| 3 | 9.7 | 10.4 | 8.7 | 8.2 | 7.2 |
| 3.5 | 8.7 | 9.2 | 7.7 | 7.5 | 6.5 |
| 4 | 8.0 | 8.2 | 6.7 | 6.7 | 5.5 |
| 4.5 | 7.5 | 7.2 | 5.8 | 5.5 | 4.5 |
| 5 | 6.7 | 5.8 | 5.0 | 4.5 | 3.8 |
| 5.5 | 6.0 | 4.8 | 4.3 | 3.8 | 3.8 |
| 6 | 5.0 | 4.5 | 3.8 | 3.8 | 3.8 |
| 6.5 | 4.3 | 3.8 | 3.8 | 3.8 | 3.8 |
| 7 | 3.8 | 3.8 | 3.8 | 3.8 | 3.8 |
| 7.5 | 3.8 | 3.8 | 3.8 | 3.8 | 3.8 |
| 8 | 3.8 | 3.8 | 3.8 | 3.8 | 3.8 |

**Table A2: Ethyl acetate concentrations (ppm) with time**

| Time(days) | 14$^o$C | 16$^o$C | 18$^o$C | 20$^o$C | 23$^o$C |
|---:|---:|---:|---:|---:|---:|
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.5 | 1.0 | 2.0 | 2.2 | 2.5 |
| 1 | 1.2 | 2.5 | 4.0 | 4.3 | 6.4 |
| 1.5 | 3.2 | 4.9 | 7.5 | 7.8 | 9.2 |
| 2 | 6.1 | 9.4 | 13.7 | 10.0 | 12.7 |
| 2.5 | 7.2 | 12.3 | 14.0 | 12.5 | 15.3 |
| 3 | 9.8 | 12.8 | 14.3 | 15.1 | 18.7 |
| 3.5 | 10.2 | 13.5 | 15.0 | 17.6 | 21.3 |
| 4 | 12.6 | 13.9 | 15.6 | 18.0 | 22.8 |
| 4.5 | 13.0 | 14.8 | 16.1 | 18.5 | 23.9 |
| 5 | 13.1 | 15.1 | 16.5 | 19.2 | 24.8 |
| 5.5 | 13.2 | 15.2 | 17.1 | 20.3 | 25.2 |
| 6 | 13.2 | 15.2 | 17.2 | 20.9 | 25.4 |
| 6.5 | 13.2 | 15.2 | 17.2 | 20.9 | 25.4 |
| 7 | 13.2 | 15.2 | 17.2 | 20.9 | 25.4 |
| 7.5 | 13.2 | 15.2 | 17.2 | 20.9 | 25.4 |
| 7.5 | 13.2 | 15.2 | 17.2 | 20.9 | 25.4 |

**TABLE A3: Ethyl Caproate concentrations at various temperatures in ppm**

| Time(Days) | $14^0$C | $16^0$C | $18^0$C | $20^0$C | $23^0$C |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.2 | 0.2 | 0.3 | 0.7 | 1.1 |
| 1 | 0.3 | 0.6 | 0.8 | 1.2 | 1.8 |
| 1.5 | 0.5 | 0.9 | 1.4 | 1.9 | 2.4 |
| 2 | 0.7 | 1.4 | 1.6 | 2.6 | 3.2 |
| 2.5 | 0.9 | 1.8 | 2.0 | 3.0 | 3.9 |
| 3 | 1.0 | 2.1 | 2.6 | 3.5 | 4.4 |
| 3.5 | 1.3 | 2.5 | 3.0 | 3.9 | 5.1 |
| 4 | 1.6 | 3.0 | 3.5 | 4.7 | 5.9 |
| 4.5 | 1.9 | 3.6 | 4.2 | 5.1 | 6.9 |
| 5 | 2.3 | 4.0 | 5.0 | 6.0 | 7.3 |
| 5.5 | 2.7 | 5.2 | 5.8 | 6.7 | 8.2 |
| 6 | 3.2 | 5.8 | 6.3 | 7.2 | 9.3 |
| 6.5 | 3.8 | 5.8 | 6.3 | 7.2 | 9.3 |
| 7 | 3.8 | 5.8 | 6.3 | 7.2 | 9.3 |
| 7.5 | 3.8 | 5.8 | 6.3 | 7.2 | 9.3 |

**TABLE A4: Isoamyl acetate concentration at various temperatures in ppm**

| Time(days) | 14$^{o}$C | 16$^{o}$C | 18$^{o}$C | 20$^{o}$C | 23$^{o}$C |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.1 | 0.2 | 0.1 | 0.3 | 0.9 |
| 1 | 0.4 | 0.3 | 0.4 | 0.5 | 1.2 |
| 1.5 | 0.7 | 0.6 | 0.8 | 1.0 | 1.8 |
| 2 | 1.0 | 0.9 | 1.1 | 1.3 | 2.3 |
| 2.5 | 1.2 | 1.2 | 1.3 | 1.7 | 2.9 |
| 3 | 1.4 | 1.4 | 1.5 | 2.1 | 3.4 |
| 3.5 | 1.5 | 1.7 | 1.9 | 2.5 | 4.1 |
| 4 | 1.6 | 2.0 | 2.3 | 2.9 | 5.4 |
| 4.5 | 1.8 | 2.3 | 2.5 | 3.3 | 5.8 |
| 5 | 1.9 | 2.6 | 2.9 | 3.7 | 6.1 |
| 5.5 | 2.0 | 2.9 | 3.3 | 4.1 | 6.3 |
| 6 | 2.2 | 3.1 | 3.7 | 4.5 | 6.4 |
| 6.5 | 2.3 | 3.3 | 3.9 | 4.9 | 6.4 |
| 7 | 2.3 | 3.3 | 3.9 | 4.9 | 6.4 |
| 7.5 | 2.3 | 3.3 | 3.9 | 4.9 | 6.4 |

**TABLE A5: Vicinal diketones (VDK) concentrations at various temperatures in ppb**

| Time(days) | $14^0$C | $16^0$C | $18^0$C | $20^0$C | $23^0$C |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.17 | 21 | 39 | 63 | 82 |
| 1 | 44 | 40 | 52 | 94 | 107 |
| 1.5 | 59 | 63 | 68 | 128 | 163 |
| 2 | 69 | 79 | 84 | 140 | 144 |
| 2.5 | 80 | 94 | 112 | 126 | 121 |
| 3 | 94 | 112 | 128 | 98 | 101 |
| 3.5 | 109 | 105 | 116 | 70 | 87 |
| 4 | 98 | 92 | 102 | 50 | 71 |
| 4.5 | 85 | 81 | 87 | 43 | 52 |
| 5 | 63 | 60 | 74 | 34 | 34 |
| 5.5 | 40 | 54 | 59 | 25 | 25 |
| 6 | 32 | 30 | 45 | 22 | 19 |
| 6.5 | 29 | 23 | 32 | 20 | 16 |
| 7 | 25 | 16 | 22 | 17 | 12 |
| 7.5 | 18 | 16 | 13 | 14 | 11 |
| 8 | 14 | 15 | 12 | 11 | 8 |

**TABLE A6: Beer colour changes with time in EBD**

| Time(days) | $14^0$C | $16^0$C | $18^0$C | $20^0$C | $23^0$C |
|---|---|---|---|---|---|
| 0 | 11.5 | 11 | 11.3 | 11 | 11.2 |
| 0.5 | 11 | 10.7 | 10.6 | 10.2 | 10.2 |
| 1 | 10.5 | 10.2 | 10.1 | 9.9 | 9.9 |
| 1.5 | 10.2 | 9.9 | 9.8 | 9.7 | 9.7 |
| 2 | 9.9 | 9.8 | 9.8 | 9.5 | 9.5 |
| 2.5 | 9.8 | 9.8 | 9.7 | 9.4 | 9.4 |
| 3 | 9.8 | 9.7 | 9.6 | 9.3 | 9.3 |
| 3.5 | 9.8 | 9.7 | 9.6 | 9.2 | 9.2 |
| 4 | 9.8 | 9.7 | 9.5 | 9.2 | 9.2 |
| 4.5 | 9.8 | 9.7 | 9.5 | 9.2 | 9.2 |
| 5 | 9.8 | 9.7 | 9.5 | 9.2 | 9.2 |
| 5.5 | 9.8 | 9.7 | 9.5 | 9.2 | 9.2 |
| 6 | 9.8 | 9.7 | 9.5 | 9.2 | 9.2 |
| 6.5 | 9.8 | 9.7 | 9.5 | 9.2 | 9.2 |
| 7 | 9.8 | 9.7 | 9.5 | 9.2 | 9.2 |
| 7.5 | 9.8 | 9.7 | 9.5 | 9.2 | 9.2 |