# 4
# Developments in Brewery Fermentation

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## Introduction

When embarking on a new article the author is aware of the need to avoid needless duplication of the efforts of others. A search through the literature will reveal that the last review of brewery fermentations in which the process, plant and underlying biochemistry were described in considerable detail appeared almost a decade ago (Macdonald *et al.*, 1984). Since that time Stewart and Russell (1985) have reviewed the modern brewing process in its entirety. The production of ethanol by yeast fermentation, both for use as a beverage and for fuel, has been addressed by Margaritis and Merchant (1987). Maule (1986) and Kleber (1987) have reviewed comprehensively the various fermentation systems that may be used in beer production. The relationships between yeast physiology and beer flavour have been explored by Berry and Watson (1987) and more recently by Quain (1988, 1989). Molzahn (1989) and Anderson (1990) have discussed the need for, and approaches to, improving the control of brewery fermentation. The interested reader is recommended to any of the aforementioned.

The intention of the present chapter is to provide a basic description of the brewing process. Inevitably this will involve some reiteration. Against this back-drop the ways in which the traditional process has been adapted to meet the demands of the current marketplace are discussed. Since these changes are ongoing, possible future trends are highlighted.

## Historical note

To appreciate the developments that have resulted in the modern brewing process, it is instructive to reflect on what has gone before.

The predilection of mankind for ethanolic beverages predates recorded history. Originally this early utilization of a biotechnological process was unwitting, but it is certainly true that in ancient historical times brewing was an organized undertaking, even though the scientific principles were cloaked in mystery. This situation existed for at least four millenia, during which time brewing graduated from a domestic or cottage industry into a large-scale

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commercial enterprise. It was not until the early part of the nineteenth century that the biological basis of fermentation was demonstrated (Anderson, 1989). *Ipso facto* the traditional process developed along entirely empirical lines.

In view of this heritage, it may be supposed that the industry would be innately conservative. In fact this is far from true and efforts have always been made to understand the complexities of the process and to devise improvements. The discoveries made by the Victorian brewing scientists, working for example at the Carlsberg Laboratories in Denmark and those based in Burton on Trent, were largely responsible for the establishment of modern industrial biochemistry (Morgan, 1987; Anderson, 1989). Not only was the biochemical basis for ethanol formation established, but the importance of using monocultures was recognized, as was the need to avoid microbial contamination. Furthermore, techniques such as continuous fermentation and the use of immobilized yeast, which may be supposed to be manifestations of modern biotechnology, were the subject of experiments in the last decade of the nineteenth century.

## Overview of modern developments

Throughout the past 100 years the trend in the UK has been towards fewer and larger brewing companies. In 1870, 133 840 licenced breweries were in operation. By 1906 this had fallen to just 1418 and in 1936 only 453 remained (Watney, 1974). This reduction has continued to the present day, but during the 1960s, in particular, a campaign of mergers resulted in the formation of a handful of dominant brewers. At the present time some 75% of the total beer output of the UK is accounted for by just six companies.

The total UK production volume for the year ending 31 March 1990 was 36·5 million barrels ($6 \times 10^7$ hl) at an average gravity of 1·038. How this relates to previous years is shown in *Figure 1*. It may be seen that current production volumes are essentially the same as those recorded at the beginning of the century, although the average sales gravity declined by approximately 30% during this time. It is noteworthy that the fall in production that occurred just before and during the Second World War was followed by a period of increased output. This growth phase was at its height during the time when the major UK producers were consolidated; however, this situation was not sustained and since 1982 production has been more or less static (*Figure 1B*).

These developments, which have been mirrored in many other countries, have had a major influence on the ways in which the traditional process has evolved. To meet production targets brewers have had to devise methods for increasing output. This has been accomplished by the use of larger batch sizes, which are now typically 1000–4000 hl, roughly tenfold greater than traditional fermenter volumes. Vessel productivity has been further improved by the practice of high-gravity brewing, in which concentrated worts are fermented and the resultant beer diluted to the desired strength prior to sale.

Inevitably, larger batch sizes present a greater financial risk in the event of

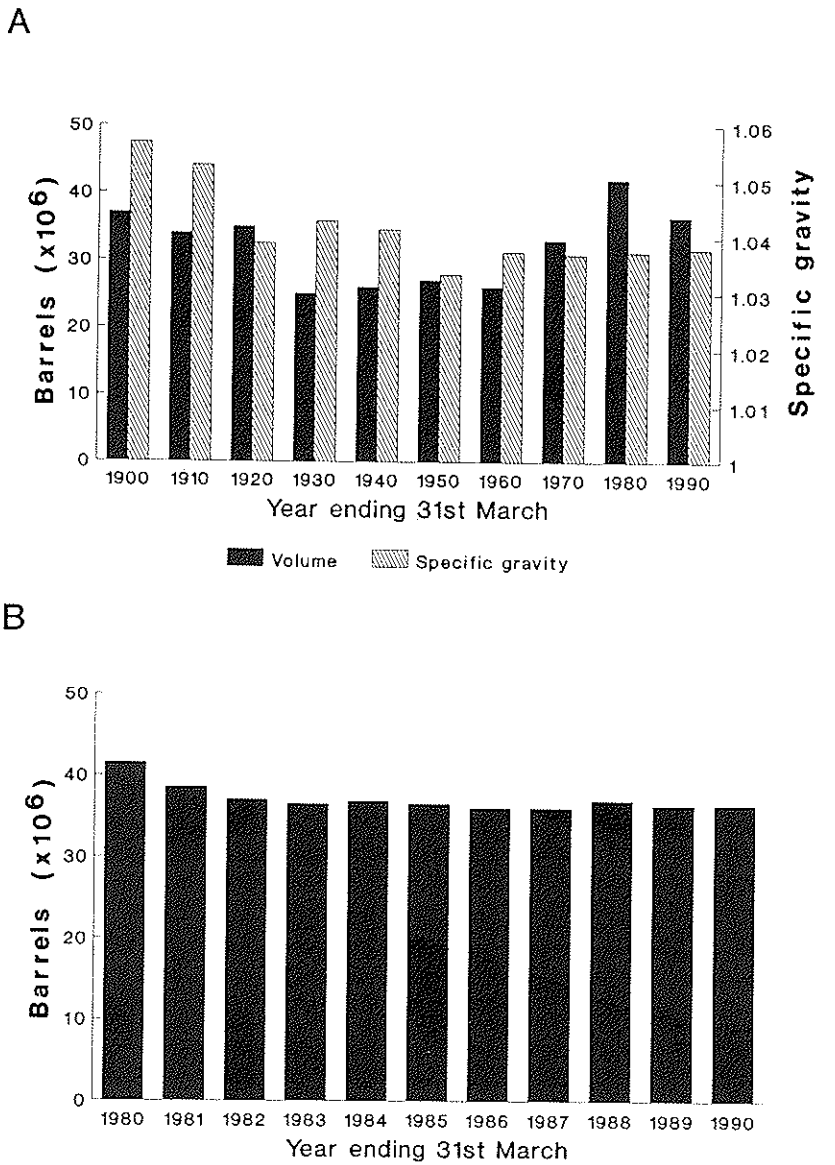


**Figure 1.** UK beer production (Brewer's Society, 1990).

microbial contamination. The chances of this occurring have been much reduced by the adoption of more rigorous hygienic standards than hitherto. Coupled with these measures, the microbiological quality of raw materials and process streams are now subject to strictly defined quality assurance standards. To facilitate this, much effort has been directed towards developing rapid microbiological tests that allow the speedy release of finished product (Hope and Tubb, 1985; Miller and Galston, 1985; Simpson, 1991).

To ensure the quality and consistency of beer derived from large-volume

brewing it has been necessary to seek methods allowing improved control of fermentation. This is particularly important where the same beer type is produced at a number of different locations. The pursuit of this goal has provided the impetus for fundamental studies into elucidating the relationships that exist between fermentation performance and beer quality. Coincidentally, this has required the introduction of novel sensors which allow improved monitoring of fermentation and more precise control over the parameters that are known to be influential in its outcome.

Allied to improved control of fermentation is the need to maintain high process efficiencies. Clearly, it is incumbent on any industry to make the best use of raw materials and maximize product yields. The excise levy to which beer is subject makes this doubly important. In the year 1988–89 Customs and Excise levies on alcoholic beverages, together with value-added tax, accounted for 5·9% of the total UK Government revenues. This amounted to £7·1 billion, of which the duty element from beer alone represented some £2·1 billion (Brewer's Society, 1990).

The Customs and Excise payment to which beer is liable accounts for 60–70% of total production costs. Compared to this, raw material and operating costs are relatively small. In common with Italy, Ireland and the Netherlands, the UK taxation system that is applied to beer is based upon the sugar content and volumes of wort fermented, and not on the alcohol content of the finished product. This has a crucial influence on the way the process is conducted.

From a legal standpoint the brewer has a statutory requirement to maintain meticulous records of the quantities of sugar fermented. This entails the use of approved methods for assessing the sugar content of wort, which is expressed in terms of specific gravity. Similarly, fermenting and other vessels must be gauged such that wort volumes may be accurately measured. In practice, each fermentation has a notional starting point, termed declaration, at which the appropriate measurements are made and the duty liability is assessed. Since at declaration fermentation may have commenced, the duty payment is based upon an analysis that takes into account both the sugar and ethanol content of the partially fermented wort. From these analyses it is possible to calculate the sugar concentration of the uninoculated wort, this being termed the original gravity. The calculation makes allowance for the proportion of wort sugars utilized for the production of new yeast biomass and non-ethanolic metabolites. These relationships were established from practical measurements made in a number of British breweries prior to the First World War (Thorpe and Brown, 1914) and more recently subject to revision (Statutory Instrument, 1979).

Although there are other considerations, as will be discussed, the wort-based excise system provides powerful reasons for retaining the batch system of brewing, since it allows proper auditing procedures to be established. More importantly, because beer production is taxed in terms of potential alcohol there is a clear requirement to maximize fermentation efficiency in order to ensure that this potential is realized.

The need to maximize efficiency has undoubtedly provided the driving

force for seeking methods leading to the improved control of fermentation. It has also had other ramifications, most notably in the area of wort production. Thus, efforts have been directed towards reducing the costs of producing fermentable carbohydrate. This may be accomplished by using sources of sugar less costly than malted barley, the wort may be treated with dextrinases to increase fermentability, or yeast strains may be employed which have been selected on the basis of their ability to utilize an increased spectrum of carbohydrates. Of course, these modifications to the traditional process must not compromise beer quality.

In some respects, a willingness to experiment with wort composition and yeast strain has identified ways for producing novel beers. The present situation of static sales has engendered a spirit of fierce competition. Thus a successful company must fight to maintain, and if possible increase, its share of the market. It is axiomatic that this requires the producer to satisfy the changing tastes of the consumer and, if possible, to predict or create future markets. In the UK the most obvious trend has been the increased production of lager-type beers at the expense of more traditional ales and stouts. In 1960, sales of the latter accounted for 99% and lager 1% of total UK sales. In 1989 the figures were 49.6% and 50.4%, respectively. (Brewer's Society, 1990). Other changes include the production of low-carbohydrate products and low- and zero-alcohol beers. These latter products have arisen within the past 10 years and have necessitated the introduction of technologies entirely novel to the brewing industry.

## The brewing process

Before considering fermentation in detail, it is useful to review briefly the principal steps of the brewing process. These are outlined in *Figure 2*. Although in process terms brewing is complex, the underlying principles are relatively simple. Three main stages may be distinguished.

1. Pre-fermentation steps, in which the wort is produced.
2. Fermentation, in which the wort is converted to green or immature beer.
3. Post-fermentation processing, in which the green beer is matured, clarified and packaged.

Malted barley is still the major source of fermentable carbohydrate. Indeed in Germany the Beer Purity Laws, or *Reinheitsgebot*, preclude the use of other sources of extract (Narziss, 1984). In the UK and other countries, other constituents, termed adjuncts may be used, for example, unmalted cereals, potato starch and sugar syrups from a variety of sources (Lloyd, 1986). During malting the barley grains are allowed to germinate under controlled conditions. This induces various amylases and proteases and results in the partial degradation of the endosperm. This process is halted by kilning and the resultant malt may be stored until required. The stage at which the malt is dried and the temperatures used influence its finished character. Thus, among other factors, the colour and flavour of the beer are decided by the blend of malts used to produce the wort.

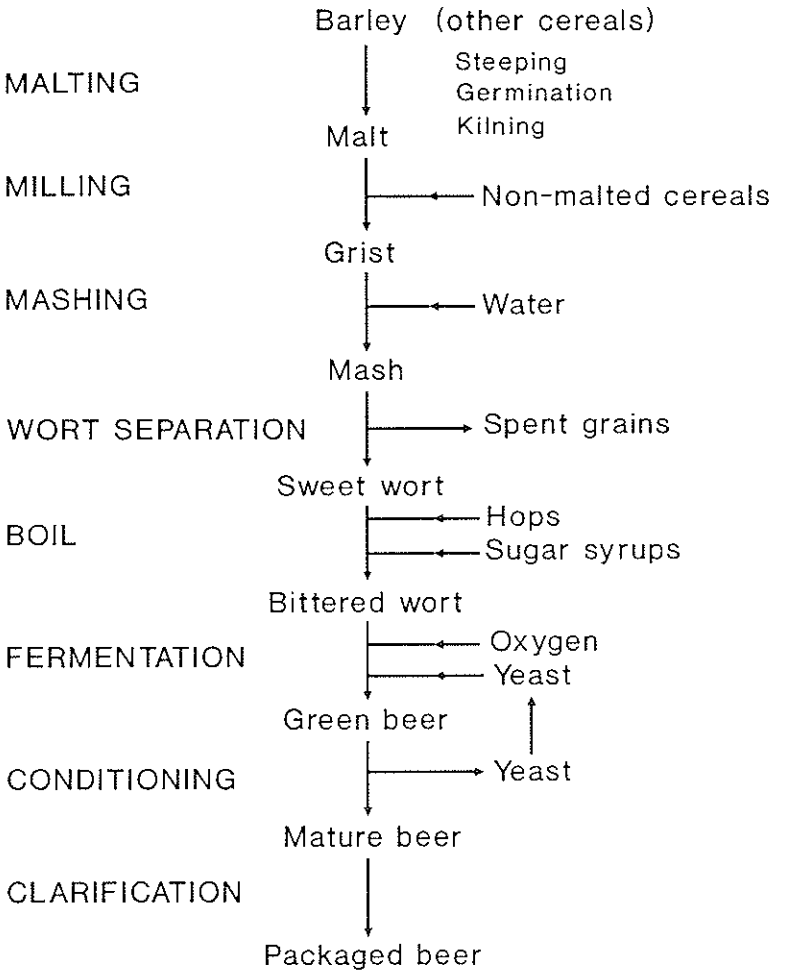


**Figure 2.** Principal steps in the brewing process.

Wort production proper starts with milling of the malt and other ingredients, if used, to produce a coarse powder, termed the grist. This is infused with warm water to form the mash and during this stage the action of the malt endosperm degradative enzymes releases a multitude of soluble components, principally carbohydrates, amino acids and short peptides. This aqueous extract, known as sweet wort, is separated from the unwanted solids and boiled with hops, or more usually, some form of hop extract.

The boiling stage serves several purposes, including sterilization, inactivation of degradative enzymes, production of hop-derived flavours and aromas and also promotes the precipitation of proteins, polyphenols and some lipid components. The separation of the latter solids is engineered during the transfer of the wort from the copper to the fermenting vessels. During transfer the wort is cooled by passage through a heat exchanger and oxygen is injected. The cooling stage causes further precipitation of proteins and

polyphenols. The efficient removal of these compounds during the post-fermentation conditioning stage is important in order to avoid haze formation in the packaged beer.

Fermentation commences following inoculation of the yeast, in brewing parlance known as pitching. When the fermentation is judged to be complete the bulk of the yeast is removed and retained for future use and the green beer subject to a maturing process, termed conditioning. In traditional ale breweries conditioning takes place in casks. In this instance the beer is primed with a suitable sugar, flavour and colour adjustments may be made, finings are added to promote clarification and a secondary fermentation occurs, the aim of which is to generate carbon dioxide. More rarely, the secondary fermentation may be carried out in bottles.

In the case of keg and most canned or bottled beers the yeast crop is removed. In some breweries most of the remaining suspended yeast is separated by continuous centrifugation while emptying the fermenter. The conditioning process is performed in brewery vessels and involves a period of storage at lower temperature than that used in primary fermentation. During this time final flavour and carbonation adjustments are made and chilling promotes further precipitation of polyphenols. If high-gravity brewing is practised then, prior to packaging, it is necessary to dilute the beer to the desired strength. This requires a supply of sterile, de-aerated water and the process must be controlled carefully to achieve the desired original gravity and/or alcohol content.

**Wort composition**

Wort composition has a critical impact on fermentation performance and on the nature of the resultant beer. It must provide a complete growth medium, such that fermentable sugars may be converted efficiently to ethanol and carbon dioxide. However, the ratio of sugars to non-carbohydrate components must be such that those products of yeast metabolism which contribute to beer flavour and aroma are formed in appropriate amounts. Furthermore, wort constituents that are not modified by the yeast will persist if not removed during post-fermentation processing, and these will also contribute to the character of the beer.

A consequence of the complex nature of the starting materials and the biochemical intricacies of the malting process means that wort composition remains poorly characterized. In practice, relatively few analytical measurements are made during wort production. It is considered sufficient to examine the brewing qualities of individual batches of malt, on a seasonal basis, to select a suitable blend together with other sources of extract and to adjust the conditions of malting and wort production to generate a feedstock with the desired fermentability. During production, analyses are restricted to those that relate to clarity, total sugar and, possibly, nitrogen content. Thus, a pragmatic view is taken that there is little point in undertaking time-consuming and costly analyses if there is no opportunity to respond to the results. Since there is a legal requirement to declare the quantities of wort

fermented, great care is taken to measure this parameter accurately. In the UK this is measured as the specific gravity at 20°C. Typical worts fall within the range 1·040–1·080, which equates to 10–20% (w/v) dissolved solids.

The chemical composition of wort is predictably complex (MacWilliam, 1968; Hoekstra, 1975). The ensuing discussion does not pretend to provide an exhaustive list of components but is restricted to those presumed to impinge upon fermentation performance. The constituents that may be found are derived from the malt and other sources of extract, those inorganic ions present within the water used to make the wort (in brewing known as liquor) and hop-derived compounds used to impart bitterness and flavour.

Hop chemistry is complex (*see* Hough *et al.*, 1982) and is not considered here, since the compounds that arise within worts take no part in fermentation other than imparting a degree of protection against bacterial contamination (Hough, 1957).

The water used in wort production was originally that available at the particular brewery. Naturally occurring waters of differing ionic balance favour the production of particular beer types and, indeed, this was a major factor in the rise to prominence of major centres of brewing, such as Burton on Trent, Dublin and Pilsen, notable for producing pale ales, stouts and lagers, respectively. In general, the production of lagers requires soft water whereas a degree of hardness is beneficial when brewing ales. The explanations for these differences are several and complex and include the roles of cations and anions in beer flavour, control of pH and promotion of protein precipitation during wort production, activation of amylases in the mash, contribution to the ionic nutrition of the yeast and determination of yeast flocculation (Briggs *et al.*, 1981; Taylor, 1989).

Where a suitable supply of water is not available, it is now usual to either demineralize using ion-exchange resins or to supplement soft water with a salts package, depending on the type of beer produced (Sentfen, 1989). In addition, pollution of water sources, now increasingly prevalent, may be countered by carbon filtration (Sentfen, 1988).

The organic constituents of wort encompass all the major classes of biochemicals (MacWilliam, 1968). Saccharides of various types account for 90–92% of the total dissolved solids. Maltose is the most abundant sugar, representing up to 70% of the total, the remainder consisting mainly of glucose, fructose, sucrose, maltose, maltotriose, maltotetrose and higher dextrins (Enevoldsen, 1974; Hoekstra, 1974; Kieninger and Rottinger, 1974; Taylor, 1974).

Nitrogenous compounds represent 4–5% of the total dissolved solids and are diverse in nature. The bulk of the nitrogen is in the form of free amino acids, small peptides and proteins, which together account for 85–90% of the total (Enari, 1974; Hudson, 1974; Jones, 1974; Lie, Haukeli and Jacobsen, 1974; Moll *et al.*, 1974). Smaller quantities of free ammonia, amines, purines and pyrimidines may also be detected, and, in addition, there are trace quantities of the vitamins biotin, folic acid, inositol, nicotinic acid, pantothenate, pyridoxine, riboflavin and thiamine (MacWilliam, 1968).

The lipids present in wort have been described by Ayrapaa, Holmberg and

Sellmann-Persson (1961), Anness (1984) and Anness and Reed (1985a,b). Several classes are represented, from free fatty acids to esterified forms including acylglycerols, phospholipids, fatty acid esters, glycolipids and sterol esters. Total quantities are small, typically 50–100 $\mu g\ ml^{-1}$, and variable, since a large proportion of the lipid material is removed with other insoluble material during wort production. The efficiency of removal of these solids, which are produced during the copper boil, largely determines the quantities of lipid that persist into the finished wort (Jones, Cope and Rainbow, 1975).

Small molecular weight components of worts include a variety of organic acids, principally TCA-cycle intermediates, as well as pyruvate, oxalate and lactate. Concentrations range between 10 and 100 $mg\ ml^{-1}$ (MacWilliam, 1968).

Sulphur-containing compounds, which contribute to beer flavour and are important as yeast nutrients, are both organic and inorganic in nature. Mandl (1974) reported that the sulphur content of wort amounts to approximately 90 $mg\ l^{-1}$ of which 60% is organic. Inorganic components include $H_2S$ and sulphate, organic sulphur components are principally amino acids, glutathione, coenzyme A and vitamins.

The mineral content of worts is generally sufficient to supply all the anionic and trace metal requirements for yeast growth (Mandl, 1974). However, it is reported that removal of zinc during wort production may result in a deficiency of this metal (Daveloose, 1987). To avoid this possibility, zinc, usually in the form of the sulphate, may be added to wort in the fermenter.

**Brewing yeast**

The characteristic flavour and aroma of any beer is in large part determined by the yeast strain used. In addition, properties such as flocculation, fermentative ability, ethanol tolerance, osmotolerance and oxygen requirement have a crucial impact on fermentation performance. Thus, proprietary strains belonging to individual breweries are usually jealously guarded and conserved.

Brewing yeast strains have been subjected to various taxonomic revisions over the years. In older systems, ale strains were classified as *Saccharomyces cerevisiae* and lager strains as either *S. carlsbergensis* or *S. uvarum* (Lodder and Kreger-van Rij, 1967). Subsequently, all lager strains were placed within the species *S. uvarum* (van der Walt, 1970). The current situation, based on DNA homology studies, is that all strains used for brewing may be assigned to the species, *S. cerevisiae* (Barnett, Payne and Yarrow, 1983).

Irrespective of the current single-species classification, the industry continues to distinguish between individual strains on the basis of their brewing and technological characteristics. With regard to the latter, the flocculation characteristics are of practical importance. Flocculation is the phenomenon by which yeast cells aggregate and form clumps and separate from the suspending medium (Stewart, 1975). This reversible process occurs in late fermentation and assists in separating the yeast crop from the green beer. Good flocculation characteristics favour easier collection of the yeast crop

and the low resultant suspended count in the green beer puts less loading onto the centrifuge. However, premature flocculation may result in fermentation arrest due to an insufficiency of suspended cells within the body of the fermenting wort.

The way in which flocculation occurs during fermentation is strain-specific and may be a determining factor in the type of fermenting vessel employed. Strains used for the production of ales tend to rise to the surface during fermentation. In consequence, shallow open fermenters have traditionally been used for the production of these beers, the yeast being removed from the surface by skimming. Conversely, the flocs produced by lager strains form a sediment. In this case the use of tall, closed cylindrical vessels from which the yeast is recovered from the base, has been widely adopted. In modern practice, the use of the latter type of vessel is predominant for the production of both ales and lagers, bottom-cropping being encouraged by the application of cooling at the end of fermentation.

The physiological basis for these variations in flocculation behaviour is a consequence of differences in cell-surface architecture. Top-fermenting yeast strains form flocs that contain entrapped carbon dioxide bubbles, hence their tendency to rise to the surface. Hinchliffe *et al.* (1985) suggested that such strains have a more hydrophobic cell surface compared to bottom-fermenters and this encourages adherence to carbon dioxide bubbles. Other workers have confirmed these findings and demonstrated that differences in cell-surface hydrophobicity correlated with the N:P surface concentration ratio (Amory and Rouxhet, 1988; Mozes, Leonard and Rouxhet, 1988).

Flocculation in yeast is genetically controlled (Johnston and Reader, 1983) but its manifestation is influenced by environmental conditions. Thus, it is inhibited by the presence of sugars, notably sucrose, maltose, glucose and mannose (Eddy, 1955; Taylor and Orton, 1978), and by low pH (Stratford, Coleman and Keenan, 1988). There is a requirement for $Ca^{2+}$ for flocculation (Taylor and Orton, 1973, 1975; Stratford, 1989b) although $Mg^{2+}$ and $Mn^{2+}$ may also be effective (Stewart and Goring, 1976; Nishihara and Toraya, 1987). Stratford and Keenan (1987) have proposed that for flocculation to occur mechanical energy is required, in order that individual cells may overcome repulsive forces due to surface charge. Flocs may then form either by calcium-ion bridging between cell wall protein components (Stewart, Russell and Garrison, 1975) or by lectin, mannan and calcium-ion interactions (Miki *et al.*, 1982a,b). In a recent paper, Stratford (1989a) concluded that there were two distinct mechanisms of flocculation which occur in different strains. The first group (FLO1 type) is constitutively flocculent and the expression of this is partially inhibited by mannose only. In the second group (NewFLO type), which includes most ale strains, flocculation is completely but reversibly abolished by mannose, maltose, glucose and sucrose. In addition, in NewFLO types flocculence is repressed by ammonium ions. On the basis of these findings the author concluded that a mannose-specific lectin was implicated in the flocculation of FLO1 types, whereas NewFLO strains possessed a broad-specificity lectin.

## YEAST DIFFERENTIATION

The importance of using pure cultures for brewery fermentations was originally recognized by Hansen in 1883 (von Wettstein, 1983). The majority of modern brewers now use pure strains, or occasionally mixtures containing two or three strains in defined proportions. This requires that pure stock cultures are maintained by the brewery and that appropriate identification methods are available. This latter need is of particular pertinence in those breweries that use several individual strains for the production of differing beer qualities.

Traditionally, identification methods have used approaches that reflect the brewing properties of individual strains. Thus, differentiation on the basis of flocculation characteristics (Gilliland, 1951; Hough, 1957) and assessment of fermentation performance and patterns of flavour volatile formation on a laboratory scale (Walkey and Kirsop, 1969; Thorne, 1975) are still in widespread use. Similarly, conventional methods of microbial differentiation such as colonial morphology (Hall, 1954; Richards, 1967) and patterns of carbon assimilation (Lodder and Kreger-van Rij, 1967) are routinely applied to identification.

These established methods are valuable, otherwise their use would have been long discontinued. However, they are expensive in terms of time, a luxury that may not be available in the production environment. Several more rapid and precise methods have been proposed which draw upon the advances that have been made in modern biotechnology. Dowhanick *et al.* (1990) describe a protein fingerprinting method, involving an extraction step followed by polyacrylamide gel electrophoresis, which may be completed within 8 h. Alternatively, restriction endonuclease analysis of mitochondrial DNA (Lee, Knudsen and Poynten, 1985) and chromosomal DNA fingerprinting (Panchal *et al.*, 1987) have been suggested. Undoubtedly all of these methods have merits, although the consensus of opinion supports the view that analysis of chromosomal DNA is likely to represent the most precise method for differentiating yeast strains (Casey, Pringle and Erdmann, 1990; Meadon, 1990).

## STRAIN IMPROVEMENT

The isolation of new strains with altered brewing characteristics affords a route by which perceived deficiencies may be remedied, or existing properties augmented. Using such strains the existing process may be refined or novel products may arise.

Brewing strains are not amenable to classical genetic improvement techniques since they are invariably polyploid or aneuploid, typically possessing between two and four gene copies. In consequence they lack mating ability and exhibit low sporulation frequency (Gilliland, 1981). The polyploid nature of brewing strains would suggest a stable phenotype, but variants with properties different to the parent strain can usually be isolated from any production culture (Tubb and Hammond, 1987). The frequency at which

natural variants occur can, of course, be increased by mutagenesis (Molzahn, 1977).

The use of these non-specific methods obviously necessitates much strain-screening. Consequently, more targeted approaches have been proposed. For example, dextrin-utilizing hybrids have been produced using the rare mating technique in which a petite mutant of the parental brewing strain is crossed with an auxotrophic haploid laboratory strain (Tubb *et al.*, 1981; Goodey and Tubb, 1982). An alternative approach, spheroplast fusion, has been used to produce a dextrin-utilizing hybrid which could be used in the manufacture of a low-carbohydrate beer (Hansen, Rocken and Emeis, 1990). Similarly, Crumplen *et al.* (1990) used the same technique to produce a strain with improved osmotolerance.

Undoubtedly, the greatest impetus to strain improvement has been the development of recombinant DNA technology. These techniques offer a direct route to influencing a specific character in a desired fashion and, theoretically, the least risk of perturbing other properties. Aspects of yeast behaviour relevant to brewing, which are being pursued with a view to improving performance using recombinant DNA technology, include accelerated fermentation rate, altered spectrum of carbohydrate utilization and manipulation of specific enzymes involved in the formation of various flavour metabolites (Vakeria, 1991). As discussed by this author, no brewing strain resulting from recombinant DNA technology is as yet in commercial use, even though the baking industry has received permission from the relevant authorities to use a construct with improved dough leavening ability. This reluctance is because the industry perceives possible adverse consumer reaction. It is likely that this situation will be temporary.

## The biochemistry of brewery fermentations

Exposure of yeast to the diversity of nutrients present in wort implies that the results of subsequent metabolism will be equally complex. Undoubtedly this is true, and in fact many of the reactions that constitute the conversion of wort into beer remain to be elucidated. However, much has been achieved, the premise being that a proper understanding of the response of yeast to the conditions encountered in a brewery fermentation is a necessary prerequisite to devising possible improvements.

Fundamental research has pursued three broad avenues of investigation. First, to identify the factors that affect the efficiency of conversion of wort sugars into ethanol. Secondly, to elucidate the pathways that lead to the formation of flavour-active beer components. Thirdly, to establish the relationships between the formation of ethanol and organoleptic metabolites. Practically, this has required a detailed analysis of the metabolism of brewing yeast and, in particular, how it may be modulated by the conditions under which fermentation is conducted. In this regard the following factors may be considered to be relevant:

1. wort composition;

2. temperature;
3. inoculation (pitching) rate;
4. physiological condition of the inoculum;
5. wort oxygen concentration;
6. pH;
7. carbon dioxide concentration;
8. hydrostatic pressure;
9. osmotic pressure (water activity);
10. ethanol concentration.

The gross changes that may be observed to occur in a typical high-gravity fermentation are shown in *Figure 3*. There is an initial lag phase lasting some 12–24 h in which there is little observable increase in yeast biomass or decrease in wort specific gravity. In this period oxygen is rapidly taken up by the yeast and anaerobic conditions pertain for the remainder of the fermentation. Following the lag phase, the yeast biomass (measured in dry weight terms) increases exponentially and active fermentation, as evidenced by the concomitant decline in the specific gravity of the wort, ensues. During this period there is a rapid increase in the ethanol concentration. After some 80–100 h, fermentation rates decline and maximum levels of ethanol and yeast biomass and the minimum wort specific gravity are attained. In numerical terms, a wort of initial specific gravity of 1·060 is reduced to a final value of 1·008–1·012. This conversion produces an ethanol concentration of 40–50 g $l^{-1}$. Yeast biomass, which is inoculated at a comparatively high rate of 0·75–1·0 g $l^{-1}$ dry weight (equivalent to 8–12 × $10^6$ cells $ml^{-1}$) increases four- to fivefold during the course of fermentation.

The transition from wort to beer is associated with a decline in pH, typically by approximately 1 unit from an initial value of pH 5·0–5·3. Wort nitrogen levels, usually expressed as free amino nitrogen decline during fermentation by 50–60% from an initial value of approximately 200–300 mg $l^{-1}$.

In a lager fermentation of this type the temperature is maintained, by the application of cooling, within the range 8–14°C. Ale fermentations are performed at higher temperatures, typically 18–22°C. The contents of the fermenter are not usually physically agitated, and mixing is dependent upon natural convection currents and the generation of carbon dioxide. One consequence of this is that the concentration of suspended yeast cells declines during late fermentation. This is due to flocculation and sedimentation, a process which is encouraged by chilling the green beer to 4–6°C.

## UTILIZATION OF WORT NUTRIENTS

The action of yeast on wort involves the assimilation of a multitude of nutrients, a full discussion of which is beyond the scope of this article. However, the utilization of carbon and nitrogenous components deserves mention since they have a critical impact on fermentation performance and the quality of the resultant beer. This is particularly relevant in that the use of non-malt wort adjuncts can result in an altered C:N ratio with concomitant

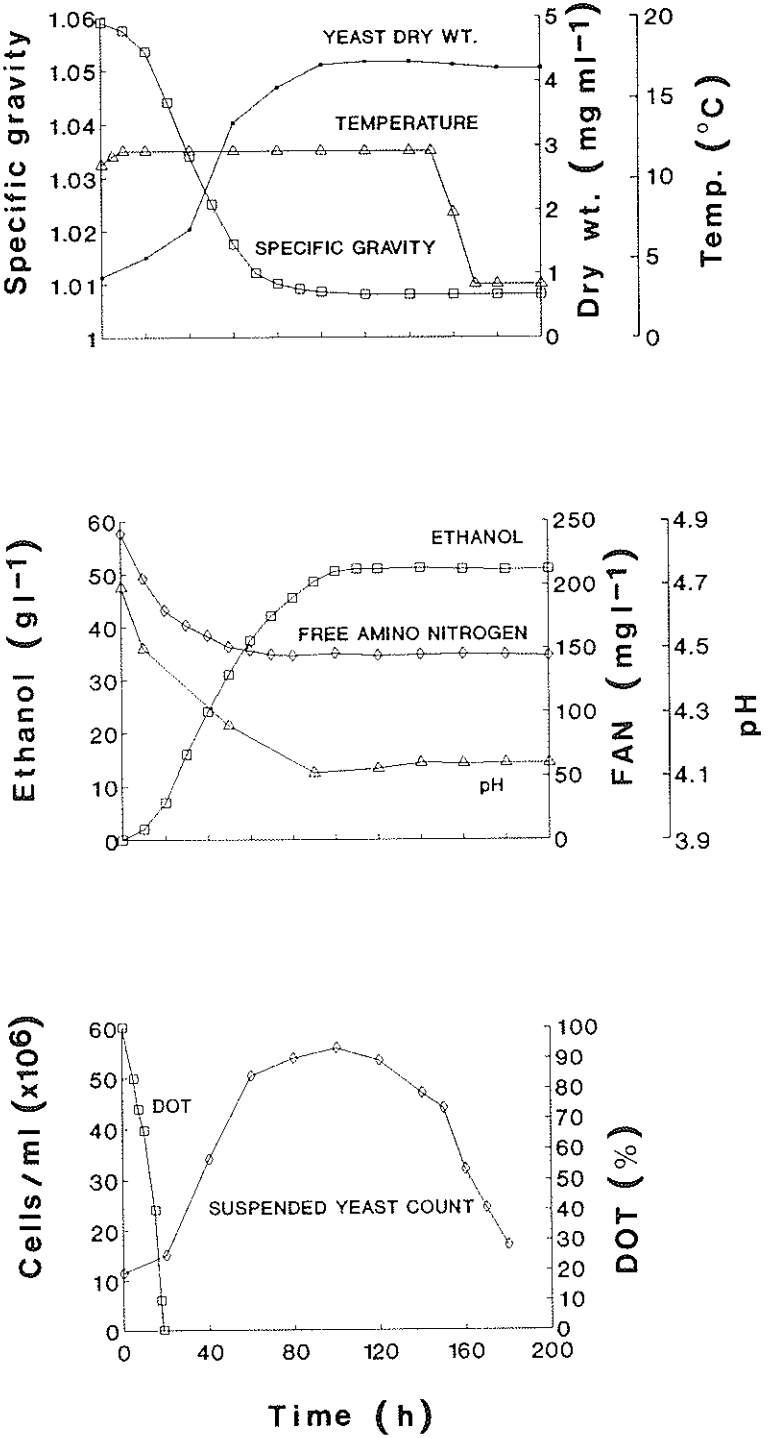


**Figure 3.** Progress of a typical high-gravity lager fermentation (C.A. Boulton, unpublished data).

perturbations in beer flavour. In those instances where adjuncts are used, care is required in the control of other fermentation parameters in order to avoid the development of adverse flavours. Conversely, changing the wort sugar spectrum or the C:N ratio by the use of adjuncts can be deliberate to produce desired changes in fermentation performance (Wilson, 1990).

*Uptake of wort sugars*

Wort sugars are assimilated in ordered fashion, as indicated in *Figure 4*. Sucrose is hydrolysed, resulting in a transient increase in the concentration of fructose. *Saccharomyces cerevisiae* possesses two kinds of invertase, one

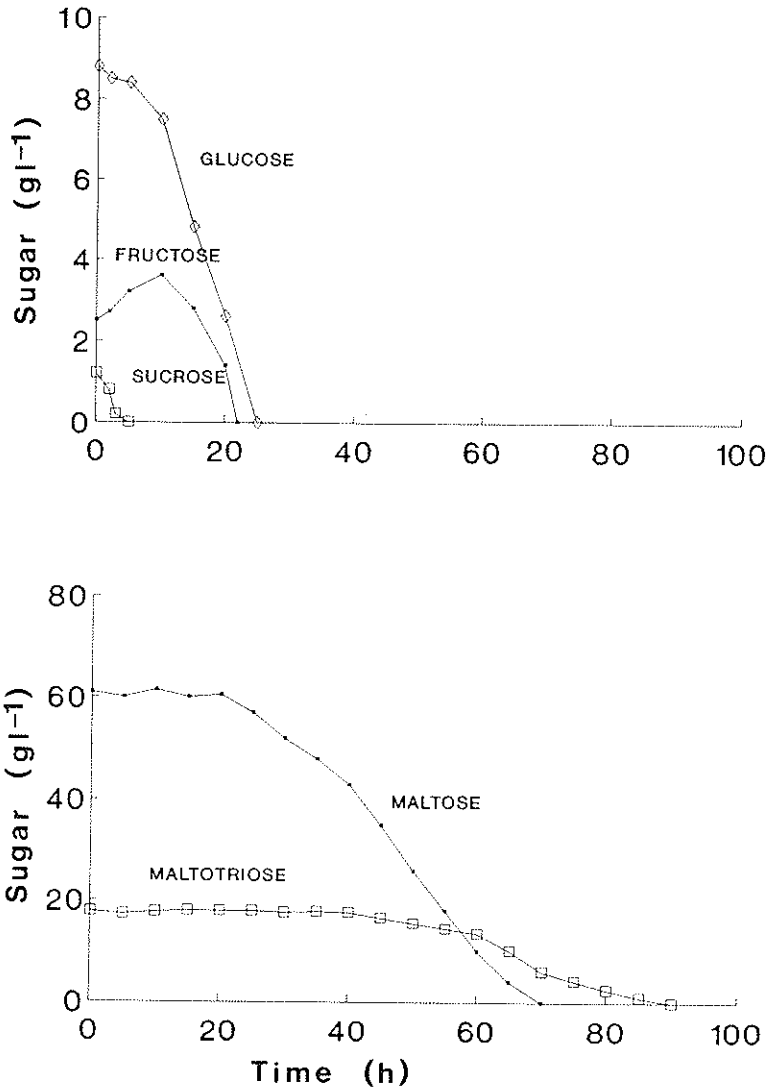


**Figure 4.** Utilization of sugars during fermentation of an ale wort of original gravity 1·040 (Clutterbuck and Boulton, unpublished data).

intracellular and a second which is secreted (Gascon, Newman and Lampen, 1968; Rodriguez *et al.*, 1978). In some strains the secreted form is apparently subject to glucose repression (Mormeneo and Sentandreu, 1986). Fructose and glucose are assimilated more or less simultaneously. The disappearance of glucose from the wort is followed by the utilization of maltose, the major wort sugar. Maltotriose is not taken up until relatively late in fermentation. Higher polysaccharides, the dextrinous component of worts, are not utilized by standard brewing strains.

The sequential uptake pattern of wort sugars is a reflection of the genotype of the particular yeast strain and the ways in which the expression of the genotype is modulated by repression and induction and by carbon catabolite inactivation mechanisms (Gancedo and Serrano, 1989). All strains of *S. cerevisiae* appear to contain two glucose uptake systems, termed high and low affinity. Both are reported to be of the facilitated diffusion type and may also transport fructose. The high-affinity type is repressed by high exogenous sugar concentration and is presumably only active when the wort glucose concentration is low (Bisson, 1988; McClellan and Bisson, 1988; Ramos, Szkutnicka and Cirillo, 1988; Does and Bisson, 1989). Glucose, fructose and maltose are all repressing substrates which have global effects upon yeast metabolism. Thus, when present at high concentration as in wort, respiratory function is absent, even during the aerobic phase of fermentation, and mitochondria are not fully developed (Chapman and Bartley, 1968; Neal, Hoffmann and Price, 1971; Fiechter, Fuhrmann and Kappeli, 1981). Reflecting the lack of mitochondrial competency, the TCA cycle becomes a branched pathway owing to the absence of 2-oxoglutarate decarboxylase (Wales, Cartledge and Lloyd, 1980).

Maltose uptake and utilization is accomplished via a permease and α-glucosidase. Genetic regulation of these enzymes is complex and involves the presence of one of several unlinked loci (Barnett, 1976; Vanoni *et al.*, 1989). Each locus contains a multigene family, elements of which are induced by maltose and repressed by glucose (Federoff, Eccleshall and Marmur, 1983). In addition, Vanoni *et al.* (1989) report that the maltose permease and the α-glucosidase are subject to glucose catabolite inactivation. Maltotriose is reported to be taken up by a constitutive facilitated diffusion system (Michaljanicova, Hodan and Kotyk, 1982).

### *Uptake of wort nitrogen*

The bulk of utilizable wort nitrogen is in the form of amino acids. As with wort sugars the assimilation of amino acids is ordered (Pierce, 1987). Four groups of amino acids have been identified on the basis of patterns of assimilation (*Table 1*). Those in group A are utilized immediately following yeast inoculation, whereas those in group B are assimilated more slowly. Utilization of group C amino acids commences when group A types are fully assimilated. Proline, the sole group D amino acid, is utilized poorly or not at all.

The regulation of amino acid uptake by *S. cerevisiae* is complex, involving

**Table 1.** Classification of wort amino acids based on order of assimilation (from Pierce, 1987)

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

carriers specific to certain amino acids and a general amino acid permease of broad substrate specificity (Hinnebusch, 1987). O'Connor-Cox and Ingledew (1989) have discussed the utilization of wort nitrogen in brewing and concluded that the uptake pattern for amino acids is due to a combination of the range of permeases present, their specificity, and feedback inhibition effects resulting from the composition of the yeast intracellular amino acid pool. The usually observed non-utilization of proline is a consequence of anaerobiosis, since its dissimilation requires the presence of a mitochondrial oxidase (Wang and Brandriss, 1987).

The metabolism of assimilated amino nitrogen is dependent on the phase of the fermentation and on the total quantity provided in the wort. Pierce (1987) reported that the majority of amino nitrogen is ultimately utilized in protein synthesis and, as such, is vital for yeast growth. However, it would appear that amino acids are not usually incorporated directly into proteins but are involved in transamination reactions, a significant proportion of the amino acid skeletons of yeast protein being derived via the catabolism of wort sugars. This explains why the total amino content of wort is important in determining the extent of yeast growth, the amino acid spectrum being somewhat secondary.

The amino acid spectrum of wort does influence beer flavour (Pierce, 1987). In this respect this author further subdivides wort amino acids on the basis of their 'essential' nature (*Table 2*). The initial concentration of class 1 amino acids was considered relatively unimportant since they may be incorporated directly from the wort when available, or synthesized from sugar metabolism and transamination in later fermentation. Deficiencies in class 2 and 3 amino acids can have considerable effects on beer quality. Thus, in the later stages of fermentation when the supply of exogenous amino acids is exhausted, the keto-acid moiety of class 2 amino acids must be synthesized

**Table 2.** Classification of wort amino acids on the basis of effect on fermentation performance and beer quality (from Pierce, 1987)

| Class 1 | Class 2 | Class 3 |
|---|---|---|
| Aspartate | Isoleucine | Lysine |
| Asparagine | Valine | Histidine |
| Glutamate | Phenylalanine | Arginine |
| Threonine | Glycine | Leucine |
| Serine | Tyrosine | |
| Methionine | | |
| Proline | | |

solely from sugars. Carbonyl by-products of the syntheses of certain of these keto-acids impart deleterious flavours to beers if present in excess. A major aim of the management of fermentation is to ensure that these carbonyls, the vicinal diketones, are present at an appropriate concentration in the finished beer (pp. 152–156). Clearly, this will be facilitated if the wort contains a suitable proportion of class 2 amino acids. In the case of class 3 amino acids, the contribution made by the sugar synthetic route is small and the yeast is dependent on an adequate exogenous supply. Therefore, a deficiency in class 3 amino acids results in major perturbations in nitrogen metabolism, yeast growth and, by inference, beer flavour.

It is apparent that the amino nitrogen composition of wort has far-reaching effects upon fermentation performance and on beer flavour. However, where malt is used as the principal source of extract, the quantity and composition of amino acids are such that these problems are not encountered. However, care must be exercised when using adjuncts, many of which are relatively deficient in amino nitrogen.

## FERMENTATION EFFICIENCY

A crude mass balance showing the principal reactants and products of a typical high-gravity fermentation are given in the following equation:

| | | | | |
|---|---|---|---|---|
| Sugars + | Amino-N + | Yeast + | Oxygen | → |
| (150 g $l^{-1}$) | (150 mg $l^{-1}$) | (1 g $l^{-1}$) | (25 mg $l^{-1}$) | |
| Ethanol + | Carbon dioxide + | Yeast + | Glycerol | |
| (45 g $l^{-1}$) | (42 g $l^{-1}$) | (5 g $l^{-1}$) | (2 g $l^{-1}$) | |

On a molar basis some 80–85% of fermentable sugars are metabolized to ethanol and carbon dioxide. The yield of carbon dioxide is less than theoretical since a proportion is re-utilized in anabolic carboxylation reactions (Oura, Haarasitta and Londesborough, 1980). The proportion of fermentable sugar not converted to ethanol and carbon dioxide is utilized in the formation of new yeast biomass and, to a lesser extent, in the production of extracellular metabolites associated with yeast growth, which may contribute to beer flavour. The regulation of carbon flux between ethanologenesis and that utilized to fuel the production of new biomass is crucial to fermentation efficiency and to beer quality.

A key determinant of fermentation efficiency is the quantity of oxygen supplied to the wort. Failure to administer oxygen results in sluggish fermentations, yeast growth is reduced and the utilization of sugars is poor. This is a consequence of pitching with yeast derived from a previous fermentation. Such yeast is of anaerobic physiology and is deficient in essential lipids, sterols and unsaturated fatty acids (Andreason and Stier, 1953, 1954; David and Kirsop, 1973). These lipids are required for proper membrane function and their synthesis involves molecular oxygen (Quinn, Joo and Vigh, 1989; Weete, 1989). Although wort does contain some sterol

and unsaturated fatty acid (Anness and Reed, 1985a) the proportion synthesized during the aerobic phase of fermentation is crucial in determining the extent of subsequent yeast growth. Thus, as growth proceeds during the anaerobic phase of fermentation further synthesis of sterol and unsaturated fatty acids is precluded and the pre-formed pool is therefore diluted amongst daughter cells.

The quantity of oxygen required for satisfactory fermentation performance is strain-dependent. Quantitatively, the requirement ranges from approximately 4 to over 40 mg $l^{-1}$ (Kirsop, 1974; Jacobsen and Thorne, 1980). The biochemical basis of the differing oxygen requirements is unresolved, although variations in the spectrum of sterols synthesized have been implicated (Kirsop, 1974). From a practical standpoint there is a tendency to over-oxygenate worts to avoid the possibility of a sticking fermentation. In strains of low to medium oxygen requirement this can result in excessive yeast growth and a concomitant reduction in ethanol yield (Boulton and Quain, 1987).

There is much evidence to suggest that the lack of membrane function in pitching yeast requires that the carbon and energy for sterol synthesis is provided by the dissimilation of endogenous glycogen reserves (Quain, 1981; Quain and Tubb, 1982). As these authors point out, glycogen may account for up to 40% of the dry weight of yeast at the end of fermentation. This suggests that some 4% of fermentable carbohydrate is utilized in the synthesis of this reserve polymer. During the aerobic phase of fermentation, glycogen is rapidly mobilized, and a stoichiometric relationship between the quantities of sterol synthesized and glycogen dissimilated may be demonstrated. The onset of anaerobiosis is accompanied by a gradual acceleration in the rate of glycogen accumulation. In the later stages of fermentation glycogen reserves are again utilized, albeit more slowly, for the maintenance of viability.

The patterns of glycogen accumulation and dissimilation evident during fermentation imply the presence of mechanisms that regulate carbon flux between glycolysis and gluconeogenesis. In this regard the ratio of fermentable sugar to other yeast nutrients would appear to be the determining factor. Thus, during active fermentation growth gradually becomes limited due to the disappearance of nutrients other than carbon. Under these conditions glycogen accumulation and ethanologenesis is favoured. This phase is terminated by the exhaustion of assimilable carbon and the utilization of glycogen for cellular maintenance is initiated (Quain, 1988).

The degree of metabolic control exerted over glycolysis during fermentation is controversial. Unfortunately, the majority of published work applies to aerobic systems. The amenability of phosphofructokinase activity to regulation, *in vitro*, by a variety of effectors is well attested (Sols, 1981). However, Schaaf, Heinisch and Zimmerman (1989) have demonstrated that overproduction of glycolytic enzymes in mutant strains did not result in an increase in rates of ethanol production. Furthermore, the necessity for phosphofructokinase activity for ethanol production from glucose has been questioned (Breitenback-Schmitt *et al.*, 1984; Heinisch and Zimmermann, 1985). It is argued that ethanol accumulation is explainable in terms of the limited

respiratory capacity of *S. cerevisiae* and the kinetic properties of the principal enzymes responsible for the regulation of carbon flow through the major metabolic branch-point, pyruvate. Thus, it is suggested that under conditions of high glycolytic flux only a limited proportion of the carbon flow may be chanelled through pyruvate dehydrogenase and thence into the respiratory pathways of energy transduction. The remainder of the carbon flow is therefore diverted into ethanologenesis via pyruvate decarboxylase, in what may be regarded as an overspill phenomenon (Barford and Hall, 1981; Rieger, Kappeli and Fiechter, 1983; Auberson *et al.*, 1989).

In the case of brewery fermentation the transient exposure to oxygen and repressing concentrations of sugars ensure that respiratory capacity does not develop. In this situation the proportion of carbon utilized for growth, via pyruvate dehydrogenase, is comparatively small and that utilized for ethanol formation is concomitantly high. However, the provision of exogenous carbon in excess of that required for energy maintenance results in increased rates of gluconeogenesis as fermentation proceeds. The external trigger for the accumulation of storage carbohydrates is then a nutrient limitation, usually nitrogen (Ingledew, 1975; Lillie and Pringle, 1980; Francois, Villanueva and Hers, 1988).

Ethanol productivity during fermentation progressively declines, the diminution being most marked as growth ceases. The metabolic basis for this effect has been studied extensively by Dombek and Ingram (1986a, b, c, 1987, 1988; Alterthum, Dombek and Ingram, 1989). These authors concluded that contributory factors included the effects of nutrient limitation, which influence adenylate energy charge; progressive loss of membrane function because of sterol and unsaturated fatty acid depletion; and the toxic effects of accumulated ethanol.

The effects of ethanol on yeast physiology have been much studied, since lack of tolerance to this metabolite may be a limiting factor in the fermentation of ultra-high-gravity worts. Ethanol inhibition is manifested as a reduction in the specific growth rate and specific fermentation rate. In extreme cases loss of viability may occur and this is exacerbated by elevated temperature (D'Amore and Stewart, 1987; Jones, 1989). The metabolic basis for ethanol inhibition is complex and several possible sites of action have been reported. These include non-specific osmotic effects (Jones and Greenfield, 1987a; D'Amore, Panchal and Stewart, 1988) and specific effects in which membrane function is disrupted (Jones and Greenfield, 1987b; Littleton, 1979; Petrov and Okorokov, 1990). In addition, other intermediates of ethanologenesis have been implicated as inhibitors of fermentation. Evidence for this is provided by the observation that endogenously generated ethanol is apparently more toxic to yeast cells than that supplied exogeneously (Nagodawithana and Steinkraus, 1976; Novak *et al.*, 1981; Dasari *et al.*, 1985). This is not a consequence of intracellular accumulation of ethanol (Jones, 1988) but is due to the presence of intermediates of ethanol formation. Metabolites cited as being implicated in these effects include acetaldehyde (Jones, 1989) higher alcohols (Viegas, Sa-Correira and Novais,

1985; Okolo, Johnston and Berry, 1987) and acetic acid (Pampulha and Loureiro, 1989; Pampulha and Laureiro-Dias, 1990).

The toxic effects of ethanol, or other intermediates, can be ameliorated by manipulating the composition of the wort. This is of obvious importance in ensuring that high-gravity worts contain an appropriate balance of nutrients. Casey, Magnus and Ingledew (1984) reported that supplementation of worts with additional nitrogen, ergosterol and oleic acid allowed the production of beers containing 16·3% v/v ethanol. These authors suggested that this was simply a consequence of increased growth and not altered ethanol tolerance. In other reports, the requirement for magnesium and calcium in addition to that normally found in typical worts as a practical method for increasing ethanol yields and fermentation rates has been highlighted (Dombek and Ingram, 1986b; Nabais *et al.*, 1988; Stewart *et al.*, 1988; Dasari *et al.*, 1990).

## PRODUCTION OF FLAVOUR-ACTIVE METABOLITES

Wort composition, the yeast strain employed and the conditions established at the beginning of fermentation are all influential in determining beer flavour and aroma. It is essential to ensure that the finished beer contains a desired balance of flavour-active components. In this regard the proper management of fermentation is crucial and it is important to appreciate that changes in wort composition or in process conditions that affect yeast growth will also be reflected in changes in the production of metabolic by-products which may contribute to beer flavour. These relationships impose constraints on the ways in which the conditions of fermentation may be manipulated with a view to effecting improvements in beer yield or vessel turn-round time.

Flavour-active by-products of yeast metabolism produced during fermentation include organic and fatty acids, higher alcohols, esters, carbonyls and sulphur compounds.

### *Organic and fatty acids*

Some 110 acids, both organic and short- to medium-chain-length fatty acids occur in beer (Meilgaard, 1975). In part these are derived from malt or other wort constituents, but a proportion arise during fermentation as a result of yeast metabolism. Organic acids contribute to the decrease in pH observed during fermentation and many are flavour-active. They derive from carbohydrate metabolism and include pyruvate, succinate, citrate, malate and acetate (Coote and Kirsop, 1974; Klopper *et al.*, 1986). It is presumed that most of these arise in consequence of the incomplete TCA cycle which occurs under conditions of anaerobiosis (Wales, Cartledge and Lloyd, 1980). In an early study Coote and Kirsop (1973) reported that pyruvate was secreted into the wort during the phase of active fermentation and that in the later stages, when yeast growth had ceased, it was re-utilized and the accumulation of acetate occurred. Presumably this observation may be regarded as further evidence for the overspill model of ethanol formation. Thus, for pyruvate secretion to

occur it would suggest that under conditions of high glycolytic flux the pathways devolving from pyruvate are rate-determining.

Medium-chain-length fatty acids ($C_6$–$C_{10}$) arise via the activity of fatty acid synthetase as intermediates in the formation of longer-chain-length fatty acids, which are incorporated into the various classes of yeast lipids (Taylor and Kirsop, 1977). In addition, a proportion derive from the assimilation and further metabolism of wort lipids (Chen, 1980). The release of medium- and long-chain-length fatty acids during fermentation is probably associated with some loss of yeast viability and subsequent cell lysis. This may also occur during the conditioning phase (Masschelein, 1986).

The concentration of fatty acids formed as a result of yeast metabolism is inversely related to fermentation rate. Thus, those parameters that increase fermentation rate, such as elevated temperature and pitching rate, result in decreased accumulation of fatty acids (Engan, 1981). However, the provision of oxygen would appear to be of overriding importance. High concentrations of wort oxygen favour yeast growth, with a concomitant requirement for increased synthesis of membrane lipids. This depletes the acetyl-CoA pool such that less is available for the formation of medium-chain-length fatty acids (Berry and Watson, 1987). Wort composition is also influential in determining the extent of fatty acid accumulation. This may be due to a general effect on fermentation rate, as discussed. In addition, the nitrogen content is important since acids such as isobutyric and isovaleric may be excreted as intermediates in the formation of the corresponding amino acids.

### *Higher alcohols*

In flavour terms the higher alcohols of major importance that occur in beer are *n*-propanol, isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol. However, more than 40 other alcohols have been identified (Engan, 1981). Regulation of the biosynthesis of higher alcohols is complex since they may be produced as by-products of amino acid catabolism or via pyruvate derived from carbohydrate metabolism (Ayrapaa, 1965, 1967a, b, 1968).

The catabolic route involves a pathway in which the keto-acid produced from an amino acid transamination is decarboxylated to the corresponding aldehyde, then reduced to the alcohol via an NAD-linked dehydrogenase. In this way, for example, isobutanol may be produced from valine, 3-methyl-1-butanol from leucine and 2-methyl-1-butanol from isoleucine.

The anabolic route utilizes the same pathways as those involved in the biosynthesis of amino acids. As in the catabolic route, the keto-acid intermediate is decarboxylated and the resultant aldehyde reduced to the alcohol.

The relative contribution made by the two routes varies with individual higher alochols. Since there is no corresponding amino acid, the anabolic route would seem to be the sole mechanism for the formation of *n*-propanol. Chen (1978) concluded that the biosynthetic pathway was of progressively reduced importance with increase in the carbon number of the higher alcohol. Inoue (1975) investigated the production of higher alcohols during the course of fermentation and reported that the catabolic route predominated during

the early phase when exogenous amino nitrogen was plentiful. In the later stages when the wort became deficient in assimilable nitrogen, the anabolic route was the major source of higher alcohols.

The total concentration of higher alcohols produced during fermentation is linearly related to the extent of yeast growth (Quain and Duffield, 1985). Thus, conditions that promote growth, such as an increased provision of oxygen, will result in increased production of higher alcohols. Similarly, supplementation of worts with additional amino nitrogen also results in a stimulation of the synthesis of higher alcohols. In this case the nature of the amino acids present is reflected in the spectrum of higher alcohols produced (Berry and Watson, 1987). Application of pressure during fermentation, which may be accomplished by restricting the release of evolved carbon dioxide, results in reduced yeast growth and this is accompanied by a similar reduction in the extent of higher alcohol formation (Arcay-Ledzema and Slaughter, 1984; Nielsen *et al.*, 1987).

### *Esters*

Esters are important flavour components which impart flowery and fruit-like flavours and aromas to beers. Their presence is desirable at appropriate concentrations but failure to properly control fermentation can result in unacceptable perturbations in beer ester levels. Organoleptically important esters include ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl caproate and 2-phenylethyl acetate. In total, some 89 distinct esters have been detected in beer (Engan, 1981).

The mechanisms underlying ester formation have been reviewed recently by Peddie (1990). Esters arise from a reaction between an alcohol, which may be ethanol or of longer chain-length and a fatty acyl-CoA ester. The reaction is catalysed by an alcohol acetyl transferase (Howard and Anderson, 1976), which is probably located within subcellular structures termed vacuomes (Malcorps and Dufour, 1987). The acyl component of the activated fatty acid may be acetate, produced by the action of pyruvate dehydrogenase. Alternatively, acetate and longer-chain-length acids may be activated directly by an acyl-CoA synthetase (Peddie, 1990).

It is accepted brewing dogma that pathways that compete for the available pool of CoA influence the extent of ester synthesis. In this regard, factors that affect lipid synthesis would appear to be critical. Thurston, Quain and Tubb (1982) investigated the specific rates of ester synthesis during wort fermentation and noted that there was a marked increase at the mid-point when rates of synthesis of saturated fatty acids and squalene were restricted. Conditions that prolonged the period of active growth, and consequently lipid synthesis, such as continued low levels of aeration, reduced ester synthesis. Conversely, supplementation of wort with the unsaturated fatty acid, linoleic acid, suppressed the synthesis of ethyl and isoamyl acetates by 80%, compared to an unsupplemented control. The authors speculated that this could be due to the specific inhibition of the alcohol acetyltransferase. In a more recent paper (Malcorps *et al.*, 1991) this proposition has been refuted. These authors

suggest that linoleic acid represses the synthesis of alcohol acetyltransferase. The provision of oxygen was reported to exert a similar effect.

The spectrum of esters produced is largely strain-specific (Engan, 1981). This may reflect the presence of a family of alcohol acetyltransferases with different substrate specificities (Peddie, 1990). The relative activities of these enzymes will be dependent to some extent on the availability of their respective substrates. In this regard, as Berry and Watson (1987) point out, the rate of formation and type of ethyl ester produced are influenced by the availability of the respective fatty acids. Presumably these latter may be synthesized *de novo* or assimilated from the wort. In the case of the synthesis of acetate esters the availability of the corresponding higher alcohol is important.

The total quantities of esters produced during fermentation are influenced by the wort gravity, the availability of oxygen and the temperature. Since temperature should not be a process variable, the first two of these are of practical importance.

An increase in the concentration of oxygen supplied to wort at the start of fermentation is associated with a progressive decline in the ester content of the resultant beer. It is assumed that since increased oxygen availability promotes greater yeast growth more of the acetyl-CoA pool is utilized in biosynthetic reactions, thereby restricting that available for ester synthesis. Alternatively, the possibility that oxygen represses the activity of alcohol acetyltransferase has been described (Malcorps *et al.*, 1991).

The effect of wort gravity is particularly relevant to modern pratice since an increase in this parameter is associated with elevated ester levels. Although other factors are pertinent, this phenomenon defines an upper limit to the concentration of wort that can be used in high-gravity brewing. Thus, Whitworth (1978) reported that a lager of sales gravity of 1·040 could be fermented up to a gravity of 1·060 with no perturbation in beer flavour after dilution. However, fermentation at gravities greater than 1·060 resulted in an exponential increase in ester synthesis such that the flavour profile of the diluted beer was considered unacceptable.

The explanation for the relationship between wort gravity and ester levels would appear to reside in the use of sugar adjuncts in concentrated worts. This increases the C:N ratio of the wort such that growth becomes limited by nitrogen depletion, thereby allowing the excess carbon to be metabolized to acetyl-CoA and hence provide a supply of substrate for ester synthesis. In addition, the concentration of unsaturated fatty acid may be diluted, which would tend to promote ester synthesis by relieving repression of the alcohol acetyltransferase. Casey, Chen and Ingledew (1985) supported the contention that high-gravity worts must be of a suitably balanced composition by demonstrating that supplementation with nitrogen and lipid suppressed the formation of excessive ester concentrations.

Practical measures which may be taken to control ester levels are self-evident from the foregoing discussion. Provision of wort with a suitably low C:N ratio and adequate supply of oxygen, both of which promote yeast growth, will minimize ester synthesis. However, since this will also reduce

fermentation efficiency, it is usual to settle on a compromise. It has been suggested that the application of pressure during fermentation reduces both yeast growth and ester synthesis (Nielsen *et al.*, 1986, 1987). An explanation for this observation is still required, though the effect of intracellular carbon dioxide accumulation caused by pressure increases, resulting in perturbations in cellular pH control with concomitant disruption of enzyme function, seems a likely mechanism. Recently, Hodgson and Moir (1990) have reported that the ionic composition of wort may be influential in determining levels of ester synthesis. These authors reported that zinc, which is routinely added to wort to ensure adequate yeast growth, may also encourage the formation of the acetate esters of higher alcohols. The effect was reported to be a consequence of zinc stimulating the production of the higher alcohol from the corresponding oxo-acid, thereby increasing the supply of precursors for subsequent ester synthesis.

### *Carbonyls*

Some 200 carbonyl compounds are reported to contribute to the flavour of beer and other alcoholic beverages (Berry and Watson, 1987). Those influencing beer flavour, produced as a result of yeast metabolism during fermentation, are various aldehydes and vicinal diketones, notably diacetyl.

Quantitatively, acetaldehyde is the most important aldehyde. This is produced via the decarboxylation of pyruvate and is an intermediate in the formation of ethanol. It may be present in beer at concentrations above its flavour threshold, at which it imparts an undesirable 'grassy' character. Acetaldehyde accumulates during the period of active growth. Levels usually decline in the stationary phase of growth in late fermentation (Pessa, 1971; Geiger and Piendl, 1976).

The extent of formation of acetaldehyde is governed by the kinetic properties of the enzymes that catalyse pyruvate catabolism, principally pyruvate dehydrogenase and decarboxylase, and those responsible for acetaldehyde dissimilation, aldehyde and alcohol dehydrogenase. Jones (1989) has discussed the relevant biochemistry and its metabolic significance in comprehensive detail. He reports that under conditions of high glycolytic flux, and where fermentative conditions apply, redox considerations favour acetaldehyde accumulation. This effect is enhanced since the aldehyde and alcohol dehydrogenases are susceptible to inhibition by acetaldehyde whereas the producing enzyme, pyruvate decarboxylase, is less sensitive.

Although in brewing terms the presence of high acetaldehyde concentrations in beer is viewed with concern because of its undesirable flavour, Jones (1989) argues persuasively that its true significance resides in its cytotoxic properties. Thus, the ability of the aldehyde group to form Schiff bases with amino residues can inhibit specific enzymes, and disrupt the synthesis of nucleic acids and proteins. The global effects of acetaldehyde toxicity may be manifested as inhibition of cellular growth and mutagenesis. It is the author's view that these effects have been wrongly ascribed to ethanol. This may explain the observation that endogenously generated ethanol exerts more

toxic effects than exogenously supplied ethanol at the same concentration (Jones and Greenfield, 1985). It is assumed that the excretion of acetaldehyde into the fermenting wort represents a strategy used by the yeast to reduce intracellular accumulation and thereby minimize the toxic effects. A similar role may be ascribed to the extracellular accumulation of acetate reported by Coote and Kirsop (1974).

As with the higher alcohols and esters, the extent of acetaldehyde accumulation is determined by the yeast strain and the conditions of the fermentation. Geiger and Piendl (1976) reported that the yeast strain was of primary importance. However, elevated wort oxygen concentration, pitching rate and temperature all favoured acetaldehyde accumulation. In addition, MacDonald *et al.* (1984) considered that premature separation of yeast from fermented wort did not allow the re-utilization of excreted acetaldehyde associated with the latter stages of fermentation.

Other important flavour-active carbonyls, whose presence in beer is determined by the fermentation stage of brewing, are the vicinal diketones, diacetyl (2,3-butanedione) and 2,3-pentanedione. Both compounds impart a 'butterscotch' flavour and aroma to beers. Quantitatively, diacetyl is the most important since its flavour threshold, 0·1–0·2 mg $l^{-1}$ in lagers and 0·4 mg $l^{-1}$ in ales, is approximately tenfold lower than that of 2,3-pentanedione (Berry and Watson, 1987). The organoleptic properties of vicinal diketones contribute to the overall palate and aroma of ales but in lagers they impart an undesirable character. An important aspect of the management of lager fermentations and subsequent processing is to ensure that the mature beer contains concentrations of vicinal diketones lower than their flavour threshold.

Diacetyl and 2,3-pentanedione arise in beer as by-products of the pathways leading to the formation of valine and isoleucine (*Figure 5*). The α-acetohydroxy acids, which are intermediates in these biosyntheses, are in part excreted into the fermenting wort. Here they undergo spontaneous oxidative decarboxylation, giving rise to vicinal diketones. Further metabolism is dependent on yeast NAD-dependent dehydrogenases, which may be alcohol dehydrogenase (Brenner and Kamimura, 1969). Thus, diacetyl is reduced to acetoin and hence 2,3-butanediol. Similarly, 2,3-pentanedione is reduced to the corresponding diol (Wainwright, 1973). The flavour threshold concentrations of the latter groups of compounds are relatively high and therefore the final reductive stages of vicinal diketone metabolism are critical to obtaining a beer with acceptable organoleptic properties.

The pattern of diacetyl formation and subsequent breakdown in relation to yeast growth and gravity loss in a typical high-gravity lager fermentation is shown in *Figure 6*. It is evident that the peak diacetyl concentration occurs towards the end of the period of active growth. The reduction of diacetyl takes place in the latter stages of fermentation when active growth has ceased. In terms of practical fermentation management the requirement to achieve a desired diacetyl specification may be the factor which determines when the beer may be moved on to the conditioning phase. Thus, vicinal diketone

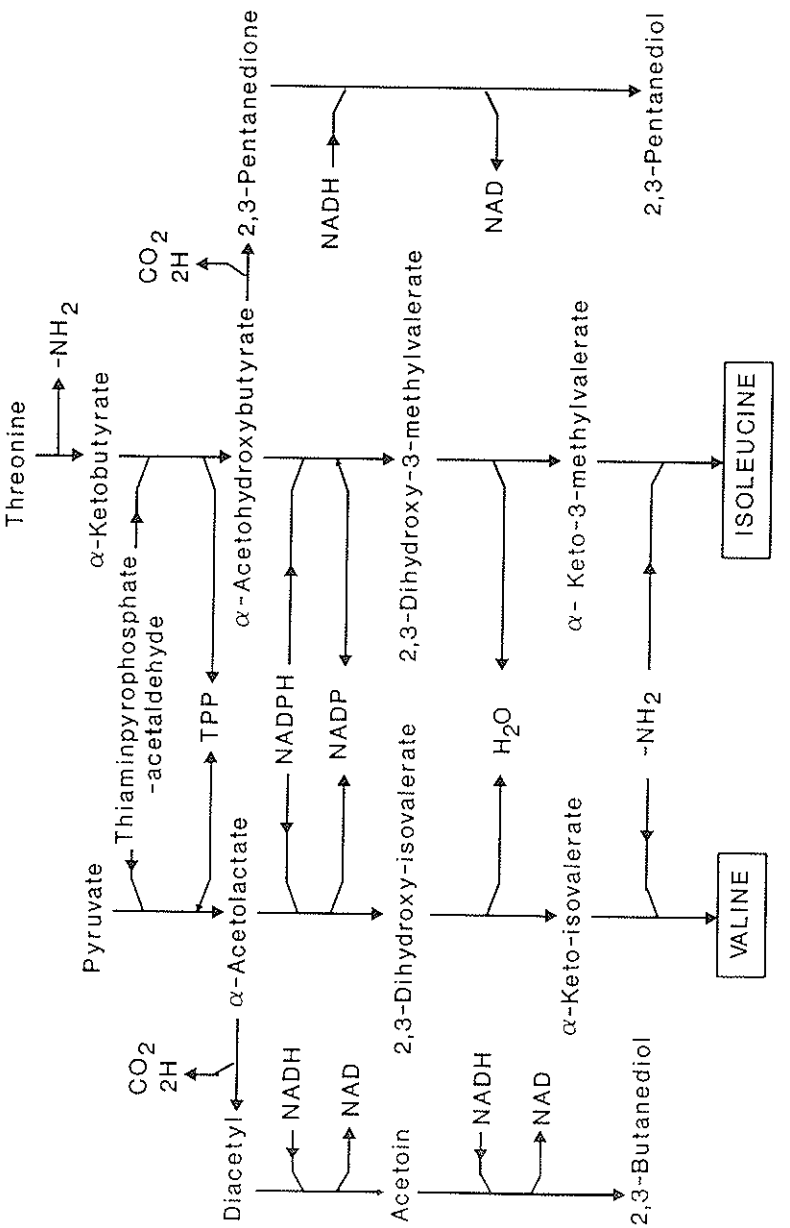


**Figure 5.** Pathways leading to the formation of vicinal diketones in beer.

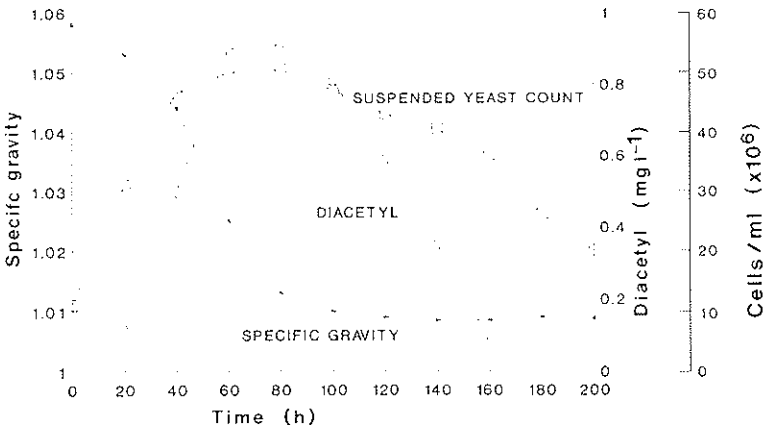


**Figure 6.** Pattern of diacetyl formation and reduction in relation to gravity loss and suspended yeast count in a high-gravity lager fermentation (C.A. Boulton, unpublished data).

metabolism can be an important determinant of overall vessel residence time, which clearly affects the efficiency of plant utilization.

The concentration of diacetyl present in fermenting wort is a function of the rate of formation of α-acetolactate precursor, oxidative decarboxylation of the precursor to form diacetyl and reduction of diacetyl to acetoin (Wainwright, 1973). These reactions are influenced by the yeast strain, both in terms of its biochemistry and technological behaviour and how these are, in turn, affected by wort composition, the type of fermenting vessel used and the fermentation conditions. These complex interactions have been studied extensively, frequently producing contradictory results. In this regard, it is crucial to consider how the fermentation conditions affect the entire diacetyl cycle and not just its individual steps.

Conditions that favour yeast growth rate, and consequently an increased requirement for amino acid biosynthesis from pyruvate, would be expected to lead to elevated levels of α-acetolactate. The conditions would include high temperatures and pitching rates and an increased provision of oxygen, but may be modulated by the wort composition. Thus, where the assimilable amino-nitrogen level is high, there will be a reduced requirement for amino acid synthesis. In addition, the presence of valine and isoleucine specifically inhibits the formation of α-acetohydroxy acids (Nakatani *et al.*, 1984a, b).

It does not follow that elevated α-acetolactate levels will result in high diacetyl concentrations in the beer at the end of fermentation. Indeed, the α-acetolactate may persist in the finished beer if the conditions do not favour its oxidation. However, this is undesirable since diacetyl formation may occur during subsequent processing when no yeast is present to catalyse its reduction.

The non-enzymic oxidative decarboxylation of α-acetolactate is considered to be the rate-determining step in the diacetyl cycle (Wainwright, 1973). The process does not require oxygen since metal ions such as $Cu^{2+}$, $Fe^{3+}$ and $Al^{3+}$ may serve as alternative electron donors (Inoue *et al.*, 1968a, b). It follows that if these metal ions are present in low concentration, or are otherwise

sequestered, then diacetyl formation will only occur to an appreciable degree if oxygen is available. The rate of formation of diacetyl from α-acetolactate is also influenced by pH. Within the range encountered in fermenting worts, a low pH promotes diacetyl formation. A rapid reduction in wort pH is associated with high rates of yeast growth, which again serves to illustrate how the conditions established at the start of fermentation will influence subsequent diacetyl metabolism.

The reduction of vicinal diketones in the later stages of fermentation requires the presence of adequate yeast within the body of the fermented wort. Thus, where the yeast is particularly flocculent, premature separation will be reflected by low rates of diacetyl reduction. This effect is most pronounced where fermenting vessels of high aspect ratio are employed. In addition, wort ionic composition may play a role, since flocculation is influenced by the balance of metal ions present, particularly $Ca^{2+}$ (Stratford, 1989b).

Diacetyl removal is also affected by the physiological condition of the yeast. It is perhaps to be expected that when the pitching yeast is in poor condition, such that the primary fermentation performance is suboptimal, then the yeast present during the latter stages will also be stressed and the period of diacetyl reduction will be prolonged. Since the reduction of diacetyl requires a supply of reduced nicotinamide co-factor, it is likely that the redox state of the yeast will be influential.

In conventional brewing operations, two strategies may be adopted for ensuring that beer diacetyl specifications are attained. In the first, which is not considered in detail here, diacetyl removal is undertaken post-fermenter in the conditioning stage of brewing. Since this may be a slow process, expensive in terms of time and conditioning capacity, it is desirable to ensure that minimum diacetyl concentrations are achieved before the beer is removed from the fermenter. In this case, it is incumbent on the brewer to select fermentation conditions, viz. pitching rate, wort oxygen concentration and attemperation regime, which provide an optimum profile. In practice, the aim is to promote the formation of maximum α-acetolactate levels as early as possible, such that the resultant diacetyl may be rapidly reduced due to presence of a high suspended yeast count. The latter reductive phase may be encouraged by increasing the fermentation temperature at the mid-point of fermentation.

The measures that are taken to control diacetyl levels must not adversely affect other flavour parameters or fermentation efficiency. The inevitable compromise usually results in the diacetyl-rest determining total vessel residence time.

To overcome this problem, it has been suggested that brewing yeast strains may be genetically modified such that the propensity for diacetyl formation is reduced. Two strategies have been proposed (Gjermansen *et al.*, 1988). These are that the ILV2 gene, coding for the α-acetohydroxy acid synthase, may be deleted and thereby reduce the supply of diacetyl precursor. Alternatively, the ILV5 gene, whose product is α-acetohydroxy acid isomerase, which catalyses the reductive step in the synthesis of valine and

isoleucine, could be amplified. It is suggested that this would also reduce the pool size of diacetyl precursor, in this instance by promoting the synthesis of valine and isoleucine. A lager brewing strain with increased levels of the ILV5 gene has been constructed (Villanueba, Gossens and Masschelein, 1990) which, in laboratory-scale fermentations, produced 70–80% less diacetyl than the wild type. Other fermentation properties were reportedly unaltered.

Several bacterial species possess α-acetolactate decarboxylase activity, which catalyses the direct formation of acetoin from α-acetolactate (Godtfredsen, Larck and Sisgaard, 1983; Godtfredsen *et al.*, 1983). This enzyme has been inserted into brewing yeast and it has been demonstrated that during fermentation, again at laboratory scale, diacetyl formation was reduced (Sone *et al.*, 1988; Shimizu, Sone and Inoue, 1989). Although the use of genetically modified yeast is an attractive proposition, since it allows precise manipulation of specific brewing characteristics, none are or have been used in commercial brewing. This is principally because of a fear of adverse public reaction. No doubt when the benefits of the new technology become more widely appreciated by the public these concerns will disappear.

### *Flavour metabolites and cellular redox*

The formation of flavour metabolites during fermentation has received considerable attention from the point of view of identifying the means whereby their concentrations can be controlled. However, their metabolic significance has been less well studied. As Quain (1989) points out, the yeast cell is entirely selfish in respect to the strategies it adopts to enable it to grow under the conditions of a brewery fermentation. Thus, by-products, excreted into the fermenting wort, which are coincidently flavour-active are produced for sound metabolic reasons.

Under anaerobic conditions the cell requires mechanisms for maintaining redox balance. NADH generated during glycolysis is re-oxidized in the terminal step of ethanologenesis. However, a proportion of the carbon flow devolving from glycolysis is used in biosynthetic reactions via pyruvate dehydrogenase. This depletes the NAD pool and alternative redox balancing reactions are required. The formation of glycerol, which is present in concentrations of up to 2 g $l^{-1}$ in beer, may fulfil this role (Oura, 1977). In addition, it is notable that the pathways leading to the formation of higher alcohols, and the terminal reductive steps in vicinal diketone metabolism, also result in the re-oxidation of NADH. Quain (1989) convincingly argues that these reactions could be manifestations of 'fine tuning' of cellular redox control.

### *Sulphur compounds*

The majority of sulphur compounds present in beer are not associated with fermentation but are derived from the raw materials used (Soltoft, 1988). However, the concentrations of hydrogen sulphide and sulphur dioxide are dependent on yeast activity. Failure to manage fermentation properly can

result in unacceptably high levels of these compounds persisting into the finished beers.

The concentrations of hydrogen sulphide and sulphur dioxide formed during fermentation are primarily determined by the yeast strain used, although the composition of the wort and fermentation conditions are major factors, particularly where levels are abnormally high. Both compounds arise as by-products of the synthesis of cysteine and methionine from sulphate or sulphite (Stewart and Russell, 1981). These syntheses are influenced by wort composition in that the yeast will preferentially assimilate sulphur-containing amino acids. It is only when the wort is depleted in these that the biosynthetic route comes into operation. (MacDonald *et al.*, 1984).

Nagami *et al.* (1980) have demonstrated that the peak of hydrogen sulphide and sulphur dioxide production occurs in the second or third day of fermentation. Presumably, at this time the sulphur-containing amino acids present within the wort will have been utilized. These same authors produced evidence that the time courses for hydrogen sulphide production and active bud formation were negatively correlated. Yeast growth during fermentation is roughly synchronous and hydrogen sulphide evolution was shown to occur in a number of peaks which corresponded to the phase of the yeast cell cycle just prior to the onset of budding.

The formation of excessive levels of hydrogen sulphide during fermentation is therefore associated with conditions that restrict yeast growth. In this regard the provision of adequate oxygen at the time of pitching is a critical factor. Since hydrogen sulphide is volatile, it follows that a vigorous fermentation will promote its removal via carbon dioxide stripping. The type of fermenting vessel is also influential. Conversely, sluggish fermentations promote hydrogen sulphide formation and more is retained in the vessel. This is exacerbated in that such fermentations may result in considerable yeast autolysis, and additional hydrogen sulphide may arise from the desulphydration of cysteine and methionine. This process may also occur in the post-fermentation stages, particularly if measures are not taken to prevent oxygen ingress (MacDonald *et al.*, 1984).

Assuming that a suitable yeast strain is used and that the wort has a balanced composition, it follows that hydrogen sulphide levels can be controlled by ensuring that yeast growth is sufficient to ensure a vigorous fermentation. Since efficiency and other flavour considerations require that yeast growth is to some extent restricted, other more direct measures may have to be taken. This may take the form of ensuring that the beer is subjected to a treatment of carbon dioxide purging during conditioning.

### Fermenter design

Modern brewing is still predominantly a batch process. Undoubtedly, this situation is likely to pertain for the foreseeable future unless the economic climate dictates that non-batch methods can be used to commercial advantage. It is true that during the 1960s and 1970s continuous processes were introduced by some major brewers (Bishop, 1970; Seddon, 1975); however,

these have not generally persisted. The comments of Portno (1978) are still pertinent, i.e. continuous systems are inflexible and therefore not suitable where several beer qualities are produced within a single brewery and where volume requirements vary throughout the year, as is of course the norm. Start-up times are long, as is down-time in the event of plant failure or microbial contamination. To avoid these possibilities there is a need for skilled technical personnel available at all times with consequent increased labour costs.

Although continuous systems have not found favour in the brewing industry, their use, particularly with immobilized yeast, has attracted considerable attention for the production of fuel alcohol (Margaritis and Merchant, 1987). It is suggested (Ryder and Masschelein, 1985; Masschelein, 1987) that similar technology, particularly the use of fluidized-bed reactors, could be profitably applied to brewing. Whether or not this proves to be the case, remains to be seen. In its favour, it is true that there is now a tendency towards larger breweries producing fewer product lines which would be more suited to continuous processes.

With regard to batch fermenters, the need for increased brewing capacity has been responded to by the introduction of larger vessels. Several vessel designs are in current usage (Maule, 1986). Of these, the cylindro-conical fermenter, originally introduced by Nathan (1930a, b) is the most popular (*Figure 7*). Modern versions are constructed from stainless steel and are cylindrical with a base consisting of a cone with an included angle of approximately 70°. Aspect ratios are usually about 3:1 and fermenter heights are between 10 and 20 metres. Operating volumes are chosen to suit the requirements of the brewery, but are typically 1500–2000 hl.

Filling and emptying of vessels occurs at the base of the cone, using a complex valving system. If required, the fermenter may be pressurized up to $2–3 \times 10^5$ Pa by restricting the venting of evolved carbon dioxide. This latter may be voided to the atmosphere or collected for use elsewhere in the brewing process. Vessels are lagged to facilitate attemperation. Temperature control is achieved by probes mounted at suitable points within the fermenter and the application of chilling using a number of cooling jackets fitted to the vessel walls. Facilities are provided for automatic cleaning and sterilization.

Vessels are not usually mechanically agitated unless a particularly flocculent yeast strain is employed. However, small impellers may be provided to ensure homogeneity during fill. Alternatively, this same end may be achieved by gas rousing or use of a recirculation loop. During fermentation, adequate mixing is achieved by the high rates of carbon dioxide evolution. This may be promoted by the application of differential cooling using the multiple cooling jackets (Maule, 1976).

In operation, hot wort is pumped to the empty and pre-sterilized vessel via a heat exchanger. This cools the wort to a desired temperature, after which oxygen and the yeast may be injected in-line. With large vessels, filling takes several hours, possibly needing several individual batches of wort. When the fermentation is judged to be complete, the yeast is encouraged to sediment by cooling the beer to 4–6°C. Yeast settling is facilitated by the inclined angle of

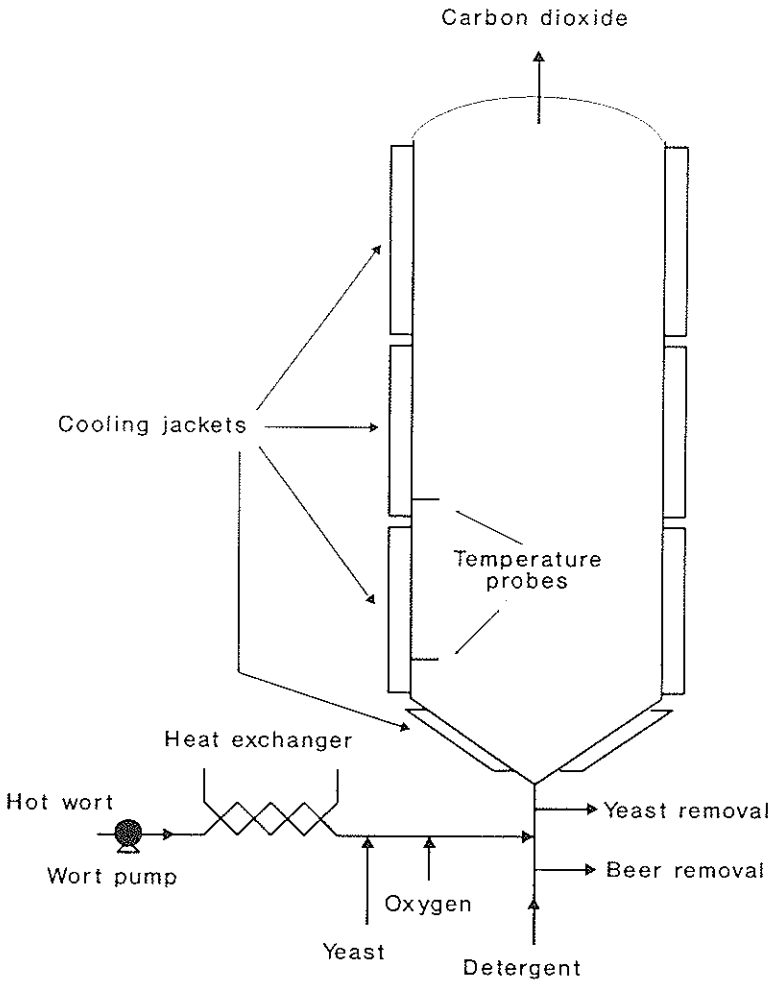


**Figure 7.** Diagrammatic representation of a cylindro-conical fermenting vessel.

the cone. Once chilled the bulk of the yeast may be removed and stored for future use. In a 'uni-tank' operation the beer may then be further chilled and conditioned *in situ*. Alternatively, the green beer may be decanted, the bulk or the remaining yeast removed by continuous centrifugation and conditioned in separate purpose-built vessels.

Cylindro-conical vessels, particularly when used for both fermentation and conditioning, have several advantages. Their high aspect ratio means that several can be conveniently located together in the form of a tank farm which uses the minimum of floor area. They can be used for all beer qualities and in operation wetting losses are reduced compared to more traditional vessels since the yeast crop is more cleanly collected. Their enclosed nature and stainless steel construction facilitates good hygienic practice and their operation is more suited to automation, thereby providing opportunities for reducing labour costs.

Some disadvantages have been recognized (Maule, 1986). The hydrostatic pressure in vessels of high aspect ratio results in over-carbonation, which may require adjustment post-fermenter. The high rates of agitation due to gas-lift action can influence fermentation efficiency and beer flavour. This may be via gas-purging of volatiles or by a direct effect on the extent of yeast growth. Thus, Masschelein (1987) demonstrated that agitated fermentations were associated with enhanced growth. The relationships between yeast growth and the production of flavour-active metabolites have been discussed. It follows that care is required to ensure proper flavour matching when the same beer quality is produced in fermenters of disparate capacity and aspect ratio.

## Monitoring of fermentations

In traditional brewing the eye and experience of the brewer were the most valuable tools available for monitoring the progress of fermentations. In practice this subjective procedure was supplemented by intermittent off-line measurement of temperature and specific gravity, the latter being performed using a hydrometer. In many modern breweries this relative lack of technical sophistication is still the norm. However, in the industry in general there is considerable interest in introducing novel sensors for the automatic monitoring of critical fermentation parameters.

The impetus for these developments has been provided by the scale and competitive nature of modern brewing. Apart from the need to reduce labour costs, the ability to accurately and rapidly monitor the progress of fermentation is an obvious prerequisite to introducing regimes which allow improved control. Continuous monitoring permits early identification of suboptimal performance such that corrective measures can be taken.

The requirements of a brewery sensor are robustness, accuracy, good replication and possession of a long, preferably maintenance-free, life-span. Of course not all of these ideals are likely to be met and, in any case, they have to be tempered by economic considerations. Since beer is a high-volume, relatively low-value product, the introduction of novel sensors must be justifiable in terms of real commerical gain. Undoubtedly, this explains why many elegant monitoring and control systems have been proposed which have failed to progress beyond the laboratory or pilot plant scale.

Fermentation parameters that are already measured and are suitable for automation are temperature and specific gravity. Related parameters that provide information on fermentation rates are pH, exothermy and carbon dioxide evolution. Those indicative of fermentation efficiency are ethanol concentration and yeast count. In terms of final beer quality, analysis of fermenter exhaust gases for volatile compounds such as esters, higher alcohols and diacetyl could be profitably performed.

Several methods have been devised for the automatic monitoring of specific gravity. In one system the gravity is determined using the output from pressure-sensitive cells mounted within the fermenting vessel and separated by a predetermined distance (Cumberland, MacDonald and Skinner, 1984). A similar device, termed the platometer, has been described by Moller

(1975). Another method detects the upthrust exerted on a displacer of known volume submerged in the fermenting wort (Gravibeam, PCA Associates, Congleton, Cheshire, UK). Specific gravity may be determined by passing wort through an oscillating U-tube and measuring the degree of damping (Armstrong and Forrest, 1986). In this instance, a recirculation loop is required and care must be taken to avoid gas break-out. A totally non-invasive method has been described which uses ultrasonic detection (Forrest and Cuthbertson, 1986). This has the added advantage of simultaneously determining ethanol concentration and, by calculation, original gravity.

In-line measurement of fermentation temperature is not problematic since suitable thermometers are readily available. However, in large vessels care is required in locating the sensors to ensure that cooling is properly applied (Maule, 1976). Advances in regard to both monitoring and control of fermentation temperature have been achieved by the introduction of computer-controlled systems which service many vessels, simultaneously (Lloyd, 1979; Pridden, 1986).

The evolution of carbon dioxide during fermentation is stochiometrically related to the rate of sugar utilization and, by inference, decrease in specific gravity and rate of ethanol production. It may be measured by diverting part of the exhaust gas from the fermenting vessel via a thermal mass flow meter (Stassi *et al.*, 1987; Eyben, 1989; Daoud and Searle, 1990). This is an attractive method for monitoring fermentation since it uses tried and tested technology which is totally non-invasive. However, it does suffer the drawback that in early fermentation no carbon dioxide is evolved until the wort has become saturated. As discussed in the next section, it is vital for any practical interactive control regime that the chosen parameter for monitoring provides early indication of non-ideal performance.

Monitoring of fermentation by measuring the heat produced as a by-product of yeast metabolism has been discussed by Ruocco, Coe and Hahn (1980). In this case it was suggested that a computer could be used to compare measured exothermy with a predetermined standard. As with carbon dioxide evolution, this approach also fails to provide usable data in the crucial early stages of fermentation.

Ethanol formation may be determined by non-invasive ultrasonic measurement (Forrest and Cuthbertson, 1986) or by gas chromatography of liquid samples taken from an in-line loop system (Knol *et al.*, 1988). Alternatively, gas chromatography analysis may be applied to the head-space of the fermenter (Pfisterer *et al.*, 1988). Undoubtedly, the ability to determine the ethanol concentration present within fermenting wort would provide a useful measure of fermentation efficiency. However, whether or not the use of sophisticated systems, such as on-line gas chromatography, can be cost-justified is a moot point.

The ability to measure yeast growth would also be a useful monitor of fermentation progress and efficiency. At present there is no practical method for achieving this on a production scale. Leonel and Moll (1986) have demonstrated that, using a pilot-scale fermenter fitted with an external recirculation loop, the yeast count may be determined using an electronic

counting device. However, to ensure homogeneity, the fermenter was stirred. In production-scale vessels it is likely that yeast cells are not evenly distributed during all stages of fermentation, and therefore such an approach is probably not feasible. As an alternative, Nielsen, Anderson and Jakobsen (1990) have demonstrated that the quantity of assimilable nitrogen utilized during fermentation is proportional to yeast growth. At present this analysis must be performed off-line.

On-line monitoring of flavour-volatile formation by gas chromatography on a laboratory scale has been investigated by Mathis *et al.* (1989). This would be of particular value if applied to diacetyl since it would enable a rapid and accurate identification of the end-point of fermentation. However, cost and robustness considerations are likely to prevent introduction to the production environment, at least in the short term.

## Fermentation control

In the majority of modern breweries the control of fermentation is still relatively unsophisticated. This is surprising since the crucial role of fermentation in determining total process efficiency and product quality is self-evident.

Two strategies for improving fermentation control have been advocated. The first of these is perhaps the more pragmatic, merely seeking to improve current practice. In essence this is a passive regime, the aim of which is to regulate pertinent process variables at the start of fermentation and then assume that the desired profile may be achieved by precise control of temperature. The second is to use a true interactive strategy in which a chosen parameter is monitored during the course of fermentation, deviation from a pre-determined ideal being countered by appropriate manipulation of other process variables.

The passive control strategy requires that at the start of fermentation wort of known volume and specific gravity, which contains a predetermined dissolved oxygen concentration and suspended yeast count, is delivered to the fermenting vessel. Hitherto the control of these parameters has been less than ideal and, in consequence, efforts are now being made to remedy these deficiencies.

In many breweries it is usual to pump concentrated wort into the fermenter and then dilute with water to achieve the desired specific gravity. Since the specific gravity of the concentrated wort may be variable, the final volume of diluted wort will also be variable. This can result in pitching rate errors, since this parameter may be calculated using a notional rather than measured wort volume. To overcome this problem methods have been devised for in-line wort dilution using high-precision flow-meters (Collins, 1989). If used in conjunction with in-line specific gravity measurement, wort of known concentration and volume may be automatically delivered to the fermenter.

The addition of oxygen to wort is usually achieved by injection into the cooled wort as it passes from the brewhouse to the fermenter. In older installations this process was relatively uncontrolled, it being considered

sufficient to use either oxygen or air and a simple flow-meter. In modern systems the quantity of oxygen supplied is regulated precisely using a thermal mass flow meter. This may be further refined by measuring the dissolved oxygen concentration using a suitable in-line probe mounted downstream of the injection point (Hale, Teasca and Mitchell, 1985).

Undoubtedly, proper control of the yeast pitching rate is a critical process parameter. Failure to properly regulate the pitching rate will compromise both fermentation efficiency and beer quality, since subsequent yeast growth will also be variable. Clearly, the aim of pitching rate control is to achieve a desired suspended viable yeast count in the wort at the start of fermentation. In fact, traditional systems of pitching rate control use indirect methods of assessing the achieved suspended yeast count. Thus, the yeast may be stored as either pressed cake or a slurry, in which the suspending fluid is beer or water. In each case, the pitching rate is determined by adding a known wet weight of yeast cake, or weight or volume of slurry, to a given volume of wort. This makes the assumptions that a constant relationship exists between yeast wet weight and cell number, that all the yeast is viable and that all the solid material is actually yeast. The first of those assumptions is perhaps reasonable, errors due to the second may be corrected for by determining the viability of the yeast and adjusting the pitching rate accordingly. However, the suspended solids component of yeast slurries that have been cropped from the cone of conical fermenters are likely to contain a large and variable proportion of non-yeast material. This will result in large errors in pitching rate control if not corrected.

Two recent developments seek to address these deficiencies and to allow the automatic control of pitching rate. The first of these automatically doses yeast into wort using in-line near-infra-red turbidometry. A dual beam arrangement corrects for non-yeast suspended solids in the unpitched wort (Riess, 1986). This method affords considerable improvements compared to traditional systems of pitching rate control, although it still requires a separate viability collection and does not account for non-yeast material present within the yeast slurry.

These problems may be overcome using a novel biomass sensor. This measures the capacitance resulting when intact yeast cells are subjected to a signal of the frequency of radio waves (Harris *et al.*, 1987). The biomass probe produces an output that provides an instantaneous measure of the viable fraction of a yeast slurry. It is unaffected by the presence of non-yeast solids, the nature of the suspending medium and gas bubbles. It would appear to have utility in the accurate and automatic control of pitching rate (Boulton *et al.*, 1989).

Although advances have been made in ensuring that the pertinent fermentation parameters are subject to rigorous control, this begs the question as to what values should be ascribed to the critical variables. The influence of wort composition, yeast strain and fermenter geometry has been discussed. Temperature cannot be regarded as a true process variable since, although it is used to regulate fermentation rate, in practice only a narrow range is usable for any beer quality. Thus, lagers are typically produced using temperatures

of 8–13°C and ales at 18–22°C. Although vessel productivity for lager fermentations could be improved by the use of higher temperatures, this is not possible because of the resultant unacceptable perturbations in flavour.

It follows that the provision of oxygen and the yeast pitching rate are the only user-accessible process variables that may be modulated to achieve the desired fermentation profile. Clearly, these are intimately related. The role of oxygen in determining the extent of sterol synthesis has been described. Depending on the yeast strain, an increased provision of oxygen is associated with increased yeast growth (Boulton and Quain, 1987). However, the determining factor is the quantity of sterol formed per yeast cell and this is influenced by the pitching rate. Typical pitching rates for ale fermentations are $5–10 \times 10^6$ cells $ml^{-1}$, whereas those for lagers are usually higher at $10–30 \times 10^6$ cells $ml^{-1}$. These differences reflect the lower temperatures used for the latter. Irrespective of the beer type, a low pitching rate and low oxygen concentration results in a slow fermentation rate and low yeast growth, since sterol is limiting. With low pitching rate but high oxygen concentration yeast growth is promoted, since high rates of sterol synthesis per yeast cell are permitted. Conversely, high pitching rate and low oxygen concentrations result in low fermentation rates and extents of yeast growth, since the sterol synthesis per cell is again limited. As the pitching rate is progressively increased fermentation rates also increase until a maximum value is attained. This is associated with a concomitant progressive decline in the extent of new yeast growth due to either restriction in the quantity of sterol formed by each cell or by the availability of other wort micronutrients (Anderson, 1990).

In order to ensure a satisfactory fermentation rate, with the minimum of yeast growth and the formation of a suitable balance of flavour compounds, it is clearly necessary to control both pitching rate and wort oxygen concentration. However, a satisfactory outcome is also dependent on the physiological condition of the pitching yeast. Since the pitching yeast is usually derived from a previous fermentation, its physiological state will be influenced by the conditions to which it was exposed in that fermentation and the procedures used to manage the yeast in the interval between cropping and re-pitching.

The widespread adoption of cylindro-conical fermenters in modern brewing has done little to improve the condition of the cropped yeast. Yeast which has settled in the cone is subject to environmental stresses, including starvation, high ethanol concentration, low water activity, high carbon dioxide concentration and elevated pressure. The effects of these may be exacerbated by high local temperatures since proper attempteration of yeast plugs is difficult (Leonel *et al.*, 1987). Under these conditions, if residence time is lengthy, yeast death and autolysis are inevitable, which will compromise the quality of the yeast crop and the beer. Even if reductions in yeast viability are not evident, the lack of external nutrients requires that glycogen reserves are mobilized to provide maintenance energy. Quain and Tubb (1982) have suggested that such yeast performs poorly when re-pitched. Leonel *et al.* (1987) have demonstrated that the stresses to which yeast are subjected in the fermenter cone may be ameliorated by ensuring that it is

rapidly cooled to 1°C more or less immediately after removal to the storage vessel.

In large-scale brewing the storage of yeast as a slurry suspended in beer has been almost universally adopted. However, there seems little consensus as to how this should be best performed. Normal production demands require that pitching yeast is held for a variable period of 1–3 days prior to re-use. Clearly, during this time the aims are to ensure microbiological purity and to minimize metabolic activity.

Good microbiological practice and proper design of the plant should ensure that the former is not a problem. In any event, bacterial loading of pitching yeast may be reduced by pre-pitching treatments. Thus, contaminating bacteria may be killed by reducing the pH of slurries with either food-grade phosphate or sulphuric acid. The lethal effects towards bacteria of acidification may be increased by supplementation with the oxidizing agent, ammonium persulphate (Simpson, 1987). Providing the treatment is performed at 2–4°C, the pH is controlled at 2·1–2·3 and the process is terminated by either immediate pitching or neutralization, no deleterious effects on the yeast are observed (Simpson and Hammond, 1989). In fact, Jackson (1988) reported that acid washing of pitching yeast improved subsequent fermentation performance, possibly due to its deflocculating effect.

The metabolic activity of pitching yeast is minimized during storage by ensuring that the temperature is maintained at 2–4°C using closed jacketed vessels. Good attemperation is assisted by gentle stirring of the yeast slurry. McCaig and Bendiak (1985) considered that static or intermittent stirring of slurries was the most appropriate storage condition, since continuous stirring resulted in high rates of glycogen breakdown and this was associated with loss of viability. However, since in this case an agitation rate of 300 r.p.m. was used, then perhaps shear rates were influential.

It seems that a convincing body of information supports the view that prolonged storage at an inappropriate temperature promotes glycogen dissimilation and such yeast performs poorly on pitching (Pickerell, Hwang and Axcell, 1991). Although temperature is a critical parameter with regard to yeast storage, exposure to oxygen will also have far-reaching effects on physiology. Quain and Tubb (1982) demonstrated that such exposure resulted in the dissimilation of glycogen being linked to limited sterol synthesis. Such yeast when pitched produces a rapid fermentation but one that is associated with excessive yeast growth and therefore losses in efficiency (Boulton and Quain, 1987). Conversely, in other reports (Murray, Barich and Taylor, 1984; Sall, Seipp and Pringle, 1988) exposure of yeast to oxygen during storage had debilitating effects such that subsequent fermentation performance was impaired.

It is difficult to reconcile these apparently contradictory results and form a coherent hypothesis as to the significance of the physiological changes that occur during yeast storage. Possibly the observations are explicable in terms of differing strain-specific responses to oxygen. In addition, variability in the condition of the yeast at the time of cropping could be a contributory factor. Whatever the true explanation, it seems certain that the physiological

condition of the stored yeast is likely to be more consistent if exposure to oxygen is avoided, residence times are held to a minimum and proper regard is paid to the slurry temperature.

Even with optimum storage conditions some variability in yeast physiological state is inevitable. In the majority of breweries this variability is disregarded. It is usual to assess the viability of the pitching yeast using the redox dye, methylene blue. The industry-recommended method for this involves direct examination using a haemocytometer. It has been suggested that the procedure may be more easily quantified by adopting a spectrophotometric approach (Bonora and Mares, 1982). Other workers report that methylene blue overestimates viability in severely stressed yeast slurries compared to slide-culture methods (Jones, 1987). In consequence, the use of other dyes, such as the fluorochrome, 1-anilino-8-naphthalene sulphonic acid (McCaig, 1990) or eosine, rhodamine B and crystal violet (King, Schisler and Ruocco, 1981) have been advocated.

Viability assessment tests are of limited value. They may be used as the basis of a fitness to pitch decision. In addition, if the particular batch of yeast is considered satisfactory, they may be used to determine the appropriate pitching rate. It has been suggested that other rapid assessment tests are required which provide information on the physiological condition of the viable fraction of the pitching yeast and which are, therefore, predictive of subsequent fermentation performance. Several have been proposed. These include a measure of the ATP content of yeast using a luminometric technique (Manson and Slaughter, 1986), determination of glycogen content by iodine staining (Quain, 1981), estimation of the rate at which a defined quantity of yeast consumes oxygen (Kara, David and Searle, 1987) and a measure of the ability of the yeast to acidify an unbuffered medium, both spontaneously and in the presence of exogenous glucose (Opekarova and Sigler, 1982; Kara, Simpson and Hammond, 1988; Fernandez, Gonzalez and Sierra, 1991).

Whether or not these tests will ever see widespread adoption is debatable. To be of value, they must provide information in addition to the usual viability measurement. Thus, the results must indicate the appropriate pitching rate and level of wort oxygen to produce a desired fermentation profile. In this regard the crucial impact of the glycogen:sterol ratio has already been discussed.

This would tend to rule out ATP measurement. It seems likely that low cellular ATP concentrations merely reflect extensive nutrient starvation, and such yeast would be judged unfit to pitch. Similarly, low glycogen content would indicate extensive starvation, which should be responded to by either rejection or increasing pitching rates and/or wort oxygen concentration. However, if yeast is exposed to oxygen during handling and storage, glycogen dissimilation may be coupled to limited sterol synthesis (Quain and Tubb, 1982). In this instance, pitching rates and wort oxygen levels should be reduced to avoid excessive yeast growth during fermentation due to increased sterol synthesis. Unfortunately, at present there is no method that allows the rapid assessment of yeast sterol content.

The rate at which pitching yeast consumes oxygen is related to membrane function and, by inference, sterol content (Boulton and Quain, 1987; Kara, David and Searle, 1987). These authors have demonstrated that this parameter is correlated with subsequent fermentation performance and it can be used to compute the optimum oxygen concentration to provide controlled yeast growth. However, this approach has, as yet, failed to progress beyond the laboratory scale.

The acidification power test reflects the ability of the yeast to maintain a constant internal pH in response to changes in the external environment (Kara, Simpson and Hammond, 1988). Thus, a measure of endogenous proton efflux is considered to reflect the turnover of glycogen reserves, whereas that measured in response to added glucose is indicative of membrane function. As with the oxygen uptake method, the results of this test show a good correlation with subsequent fermentation rates. However, it has also been demonstrated that the correlations are no better than those obtained using the methylene blue viability assessment (Boulton, Jones and Hinchliffe, 1991).

Several genuine interactive control regimes have been proposed. In each case a suitable parameter is monitored immediately after pitching, and suboptimal performance is corrected by the provision of additional pitching yeast and/or oxygen.

Suggested parameters for monitoring are the rate of carbon dioxide evolution (Stassi *et al.*, 1987; Daoud and Searle, 1990), decline in specific gravity (Armstrong, 1986), decline in pH (Leedham, 1983) and by ultrasonic detection (Forrest, 1986).

Such approaches are attractive since they should ensure that every fermentation is controlled precisely and therefore performs within specified limits. However, in the context of brewing they are subject to two practical shortcomings. The first of these is technical. For any successful control strategy, it is essential that deviation from non-ideal behaviour is detected and responded to as early as possible. With the exception of oxygen uptake rate, the parameters described show little change in the critical first hours of fermentation. Thus, the application of corrective measures may be too late to be of benefit. The second is economic. There is no doubt that a workable interactive control strategy can be devised. However, it would clearly be of little use if the costs of installation and operation outweighed the benefits accruing from improved control. In a modern brewery the cost of fitting suitable in-line sensors to every fermenter, coupled with the associated microprocessor control, may well be considered prohibitive.

The need to balance process improvements against costs and profitability will probably ensure that, in the short term, most efforts will continue to be directed towards better passive control regimes. One obvious approach is to reduce the number of process variables. The proclivity of cropped yeast for synthesizing sterol at the expense of the glycogen reserve when exposed to oxygen has been described. This may be encouraged in a controlled process whereby pitching yeast is subject to vigorous oxygenation. Such yeast has no requirement for wort oxygenation, and good fermentation control may be

achieved by careful regulation of the pitching rate (Quain and Boulton, 1987; Molzahn, 1989). This technique has been successfully tested at production scale (Boulton, Jones and Hinchliffe, 1991).

It is, of course, true that many of the present causes of fermentation inconsistency are a direct consequence of pitching with yeast derived from a previous fermentation. It is this practice that produces the requirement for wort oxygenation. Furthermore, the need to store cropped yeast until required allows the possibility for variations in physiological condition to occur.

It is usual to allow the yeast to be used for 8–10 serial fermentations, after which time a new culture is introduced from laboratory stocks. This ensures that there is no strain drift due to selection of a variant and it guards against contamination by wild yeast. Propagation of the new yeast line is performed using a series of cultures of gradually increasing volume until sufficient is available to pitch the first production-scale fermentation (Ahlquist, 1986; Ashurst, 1990). Propagation stages performed within the brewery use wort with intermittent or continuous low rates of oxygenation to promote growth. It is a common observation that newly propagated yeast does not perform well in the first fermentation, for example, poor utilization of wort sugars (Hammond and Wenn, 1985). Probably some of these effects are due to the first production fermentation being underpitched, although poor physiological condition of the propagated yeast would also appear to be implicated.

This would imply that current propagation procedures require some development. Avenainen and Makinen (1981) indicated that fully aerobically propagated yeast could be used to ferment anaerobic wort with no changes in the composition of the resultant beer.

At present in countries such as the UK, which have wort-based excise laws, yeast propagation is an expensive undertaking, since the sugar used attracts the same levy as that used for producing beer. However, in the near future the UK seems certain to move towards an alcohol-based tax. In this case the economics of more frequent propagation of yeast become more favourable. It could perhaps be envisaged that the fed-batch techniques already used within the bakers' yeast industry to produce sterol-rich catabolite-depressed yeast might be profitably employed. Using such yeast it could, perhaps, be possible to employ a non-conservative strategy in which the crop from a purely anaerobic fermentation is discarded, thereby negating the problems associated with serial re-pitching.

## The future

Undoubtedly, there will always be a place for the small-scale brewer using a traditional process. However, the world trend towards fewer larger companies operating under conditions of fierce competition seems destined to continue. In this climate the latter will be driven towards utilizing all the resources that modern biotechnology has to offer in order to generate a consistent high quality product while minimizing operating costs.

A prerequisite will be a fuller understanding of the biochemistry of

fermentation. Considerable advances have been made during the past decade or so but much remains to be done. Further elucidation of the complex interactions of yeast and wort will underpin improved control strategies and will identify specific ways in which the properties of brewing strains may be manipulated to improve their performance.

It is anticipated that the cautious approach towards the application of genetically engineered strains will eventually change and steps will be taken to capitalize on the exciting opportunities offered by recombinant DNA technology. Areas where genetically modified strains may be of value (which have not been discussed) would include the ability to utilize carbon sources other than those encountered in conventional worts. In addition, there will be an increasing need to produce novel beverages.

In this regard, low- or zero-alcohol beers are likely to be an important and growing market sector. Since these are commonly produced using post-fermentation de-alcoholization procedures, they have not been dealt with here. However, some are manufactured using limited fermentation in a one-step process (Muller, 1990). Such techniques still require considerable development in order to achieve proper flavour balancing. Clearly, the use of a suitably genetically modified strain could be of value both in terms of influencing ethanol yield and the production of flavoursome metabolites.

A deeper understanding of the fundamental biochemistry of fermentation will pave the way for introducing further improvements to process control. This will require relatively low cost, robust and specific sensors, which may be used to effect in-line measurements. In this regard the ability to monitor and consequently control the formation of major beer flavour components would be of obvious benefit.

The predominance of the batch method of fermentation has been described. No doubt this will continue unless significant product quality, process efficiency or economic reasons for changing to other systems can be demonstrated. Since the majority of brewers are already investing substantial sums in order to further improve the performance of batch fermenters, a change to other systems seems unlikely.

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