MINIREVIEW

# The yeast *Saccharomyces cerevisiae* – the main character in beer brewing

Elizabeth J. Lodolo[1], Johan L.F. Kock[2], Barry C. Axcell[3] & Martin Brooks[4]

[1]SAB Ltd Brewing Centre of Excellence, Alrode, South Africa; [2]Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, South Africa; [3]SABMiller, Sandton, South Africa; and [4]SAB Ltd Brewing Centre of Excellence, Alrode, South Africa

**Correspondence:** Elizabeth J. Lodolo, SAB Ltd Brewing Centre of Excellence, PO Box 123902, Alrode 1451, South Africa.
Tel.: +27 82 921 7720;
fax: +27 86 681 4102; e-mail:
bettie.lodolo@za.sabmiller.com

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## Abstract

Historically, mankind and yeast developed a relationship that led to the discovery of fermented beverages. Numerous inventions have led to improved technologies and capabilities to optimize fermentation technology on an industrial scale. The role of brewing yeast in the beer-making process is reviewed and its importance as the main character is highlighted. On considering the various outcomes of functions in a brewery, it has been found that these functions are focused on supporting the supply of yeast requirements for fermentation and ultimately to maintain the integrity of the product. The functions/processes include: nutrient supply to the yeast (raw material supply for brewhouse wort production); utilities (supply of water, heat and cooling); quality assurance practices (hygiene practices, microbiological integrity measures and other specifications); plant automation (vessels, pipes, pumps, valves, sensors, stirrers and centrifuges); filtration and packaging (product preservation until consumption); distribution (consumer supply); and marketing (consumer awareness). Considering this value chain of beer production and the 'bottle neck' during production, the spotlight falls on fermentation, the age-old process where yeast transforms wort into beer.

## Introduction

When considering brewing of beer in its most simplistic form, it probably represents mankind's oldest biotechnology. Whether the discovery of the pleasant beverage can be ascribed to accidental contamination of grains or the natural curiosity of humans, remains a mystery. Archaeological evidence of brewing activity was found on Sumarian tablets dating to about 1800 BC (Katz & Maytag, 1991) although the origins may well go back to around 10 000 years ago (Axcell, 2007). The text found on these tablets sings the praises of the Sumerian goddess of brewing in the 'Hymn to Ninkasi'. Since that time, mankind has discovered that the goddess that caused the 'magic' is in fact the living organism, yeast. Ancient brewers domesticated yeast due to selection of the best fermentation agent, although the full biochemical process was not understood. This intimate association between mankind and *Saccharomyces cerevisiae* was further demonstrated during a study of the genetic diversity among 651 wine yeast strains from 56 geographical origins worldwide (Legras *et al.*, 2007).

The natural curiosity of humans seemed to drive additional discoveries. Antonie van Leeuwenhoek described yeast in 1680 with the aid of handmade wax globules, whereas Charles Cagniard de la Tour reported in 1838 that yeast was responsible for alcoholic fermentation. By the end of the 19th century, improved strains were selected by the use of pure culture technique. Emil Hansen used serial dilutions in 1883 to separate yeast cells based on morphology and showed that different pure cultures of bottom and top fermenters gave unique and reproducible industrial fermentations (Rank *et al.*, 1988).

Since the first records of beer production by mankind, many changes have been made that resulted in the modern beer-brewing process. Many beer styles have developed over time, all with their own unique character and flavour influenced by the milieu of their country of origin (Protz, 1995; Glover, 2001). However, despite all the changes, the one constant factor is the requirement of brewing yeast. Two types of brewing yeast were originally classified based on flocculation behaviour: top fermenting (ale and weiss yeast) (Jentsch, 2007) and bottom fermenting (lager yeast). Their

behaviour is so distinct that the two main classes of beer types (ales and lagers) are based on the two yeast types. Ale yeast is genetically more diverse and, similar to weiss yeasts, ferments at higher temperatures (18–24 °C) whereas lager yeast is more conserved and ferments at lower temperatures (8–14 °C). Weiss yeast produces beer with spicy, clove, vanilla and nutmeg flavour notes due to the presence of the POF gene (*PAD1*) (Meaden & Taylor, 1991). The volatile phenolic compounds such as 4-vinyl guaiacol are formed through the decarboxylation of ferulic acid. The phenotypical characteristics used to distinguish these yeast types include colony morphology, microscopic appearance (chain formation by weiss yeast), fermentation characteristics (flocculation and flavour profiles), growth at 37 °C (ale and weiss yeasts) and utilization of melibiose (lager strains) and the presence of the POF gene (weiss yeasts). Besides the differences in phenotypical behaviour, the yeasts can also be distinguished using electrophoretic karyotyping of their chromosomes (Casey, 1996).

The *Saccharomyces sensu stricto* species complex (Vaughan-Martini & Martini, 1998) contains some of the most important species for the food industry, namely, *S. cerevisiae* (Meyen ex EC Hansen), the agent of wine, bread, ale and weiss beer, and sake fermentations, *Saccharomyces bayanus* (wine and cider fermentations) and *Saccharomyces pastorianus* (EC Hansen), which is responsible for lager beer fermentation (Rainieri *et al.*, 2006). *Saccharomyces pastorianus* (syn *Saccharomyces carlsbergensis*) also contains hybrid strains including lager-brewing strains, which are thought to originate from a natural hybridization event that occurred between a *S. cerevisiae* strain and a non-*S. cerevisiae* strain, probably a *S. bayanus* strain. The existence of two types of genome in lager-brewing strains has been confirmed (Rainieri *et al.*, 2006). The taxonomy of these industrially important strains remains problematic and a generalized term for brewing yeast (*S. cerevisiae*) is used in general in this study.

The simplified graphical depiction of the beer production value chain (Fig. 1) shows the start of the beer-brewing process in the Maltings and the Brewhouse. The Maltings represent the part of the process where barley is converted to malt via the malting process (steeping, germination and kilning). Two types of barley occur naturally (two- and six-rowed) and the malting process activates the natural enzyme systems, which assists in the conversion of starch to fermentable sugars. Malt provides fermentable sugars, flavour and colour (dependent on the level of kilning). The Brewhouse represents the part of the process where the raw materials (malted barley, hops, cereals, adjunct and water) are converted into a liquid medium called wort (Rehberger & Luther, 1995; Kunze, 1996; Goldammer, 2000a; Boulton & Quain, 2006). This part of the process is essential because its primary aim is to produce wort, which will be the nutrient source required for yeast growth and fermentation. It is a rich source of nutrients, containing a mixture of amino acids, carbohydrates, vitamins, inorganic ions and lipids (Hammond, 1993; Bamforth, 2003). Additionally, the use of hops provides bitterness and a 'hoppy' note with the added benefit of resistance to microbial spoilage due to the impact of iso-α-acids on Gram-negative bacteria (Simpson, 1993). However, some Gram-positive bacteria (*Lactobacillus* and *Pediococcus*) have developed resistance to these acids and are of particular concern in breweries (Sakamoto & Konings, 2003).

Wort composition can vary due to the variability in raw materials (Meilgaard, 1976; Gunkel *et al.*, 2002; Van Nierop *et al.*, 2006) and processing factors, with potential negative impacts on yeast performance. Furthermore, the increasing use of raw materials for biofuel production has already impacted negatively on raw material availability, price and quality. The American Renewable Fuel Standard Program (US Environmental Protection Agency, 2007) will increase the volume of renewable fuel required to be blended into gasoline, starting with 4 billion gallons in the calendar year

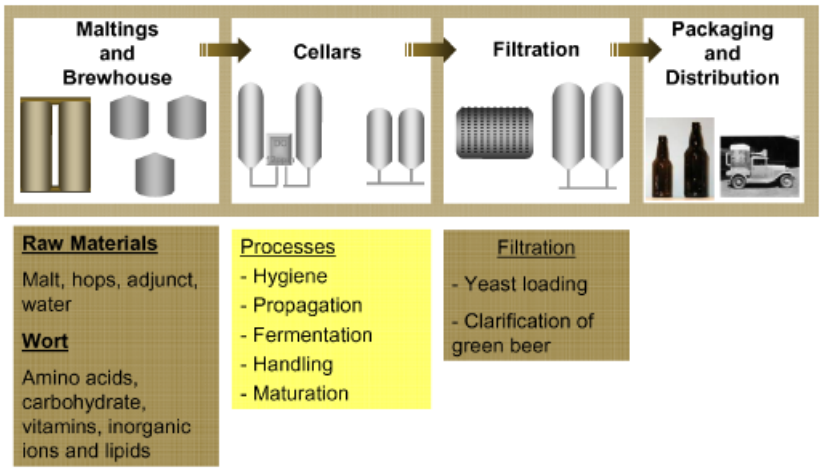


**Fig. 1.** Simplified graphical depiction of the beer production value chain starting at the maltings and finishing at distribution. The cellars area is highlighted as the yeast-related focus area.

2006 and nearly doubling to 7.5 billion gallons by 2012. The exact ramification of increasing biofuel demands on beer brewing into the future is unknown and will require innovative approaches to address potential shortages.

This review will not address in detail the brewhouse process or the final stages of beer production such as filtration, packaging and pasteurization. Rather, the spotlight falls on Cellars, where yeast is the main character, set against the backdrop of fermentation systems; quality assurance (QA) management; propagation; fermentation; yeast handling (cropping, storage and pitching); and maturation.

## Cellars

### Fermentation systems

Fermentation systems have evolved over time. The original designs were based on yeast properties (top vs. bottom-fermenting yeast), beer style, tradition as well as availability of construction material (Boulton & Quain, 2006). The types of fermenters range from open and closed squares to horizontal and vertical cylindrical vessels. The most popular fermenter style for batch fermentations is the cylindroconical fermenter where fermentations are conducted in enclosed vertical cylindrical vessels with cone shape bases (cylindroconical vessels-CCVs). CCVs have become the preferred technology in modern breweries due to their lower costs (capital and operating), efficiency (decreased beer losses, increased vessel utilization, $CO_2$ collection and CIP cleaning) and footprint (less area). The most favoured material for CCV construction is stainless steel. Its ideal properties include strength and rigidity, it is inert and corrosion resistant, cleanable, malleable and has good thermal conductivity.

Continuous fermentation systems found limited application in the brewing industry mainly due to its lack of flexibility and the serious consequences of break-down or microbial contamination (Boulton & Quain, 2006; Inoue & Mizuno, 2008). Pilot-scale studies on a 20-L reactor system showed that it was feasible to produce lager beer with a continuous, immobilized system. The main fermentation was stable for more than 14 months both in fermentation efficiency and in flavour compound formation (Virkajärvi, 2001). The development of commercial immobilized reactors has been of particular benefit when applied to rapid beer maturation (Pajunen, 1995). However, independent investigations to optimize different continuous fermentations systems met with mixed success, especially in achieving the required flavour profiles (Dragone *et al.*, 2007; Inoue & Mizuno, 2008). Much more research and optimization of continuous systems will be required before they can replace the current batch fermentation solutions in the brewing industry.

### QA management – the checks and balances towards the final beverage

QA equates with 'customer satisfaction and loyalty' (Gryna, 2001). Systems such as Hazard Analysis and Critical Control Points (HACCP) and ISO 9000 exist to certify the compliance of the manufacturers to good manufacturing practices (Kennedy & Hargreaves, 1997). HACCP is a preventative strategy based on the analysis of existing conditions by identifying potential hazards to food safety.

Ultimately, the brewer aims to produce a beer that will satisfy the consumer time and again. To achieve this promise to the consumer, the product needs to comply with its specification (content, taste and appearance). Control of the microbiological status of raw materials (water, cereals and priming sugars), yeast, wort and plant sterility (fermentation vessels, process gases, postfermentation plant and containers) (Campbell, 2003) is essential to achieve this quest. The challenges the brewer face are numerous. Different microbial contaminants can be present in different parts of the process due to changing environments (pH changes, aerobic vs. anaerobic nature, nutrient concentrations, ethanol and $CO_2$ concentrations). For a review of beer spoilage organisms and the stage of the brewing process at which they occur, refer to Vaughan *et al.* (2005). For a comprehensive discussion on beer spoilage organisms, in general, refer to Rainbow (1981) and Back (2005).

Undoubtedly, the essential foundation for quality is sound hygiene practices. Hygiene refers to practices associated with ensuring good health and cleanliness and can be broadly divided into cleaning (environmental and *in situ*) and sanitization practices. The brewer's task is to ensure that the equipment is as free from unwanted microorganisms as is practically possible by applying hygiene practices. Thus, the aim of the brewer is to sanitize equipment rather than sterilize. Sterilization refers to the total removal of unwanted microorganisms, including spores, whereas sanitizing refers to reduction down to an acceptable level using readily available products and technology (Goldammer, 2000b). Cleaning technology has evolved into an automated practice due to the automated nature of the modern brewing process and the scale of the operations. Enclosed processing plant equipment (vessels, piping, heat exchangers and fittings) is cleaned using an automated cleaning in-place (CIP) process (Curiel *et al.*, 1993; Cluett, 2001). The CIP of vessels are generally conducted through spray balls or rotary jets fitted to the top of the vessel. The solutions are pumped from a central CIP plant that holds water, caustic, acid and sterilant solutions (Cluett, 2001). Effective cleaning is achieved through the synergistic relationship among the four parameters time, temperature, chemical action (concentration) and mechanical action (Boulton & Quain, 2006).

QA measures are in place to verify the effectiveness of the CIP process. Traditional agar plating techniques using selective cultivation media, such as WL actidione agar (for aerobic bacteria), Raka Ray for anaerobic bacteria and lysine (as a sole nitrogen source) agar for selecting non-*Saccharomyces* wild yeast (Walker, 1998; Campbell, 2003), are still in use. The use of swabs to verify cleanliness in selected areas such as packaging halls can be combined with a beer microbial detection medium such as NBB® (Back, 2005). Modern technology supported the developments of rapid microbiological methods (Russell & Stewart, 2003) such as real-time PCR (Kiehne *et al*., 2005), multiplex PCR (Asano *et al*., 2008; Haakensen *et al*., 2008) and ATP bioluminescence (Simpson & Hammond, 1989). The use of direct ATP analysis for the assessment of test surface cleanability and the effectiveness of CIP processes have been reported (Storgårds *et al*., 1999; Stanley, 2005).

The importance of hygiene practices cannot be overstated especially upon considering the 'main character' of brewing. Fermentation is a microbiological process and as such the aim is to exclude unwanted microorganisms (bacterial, fungal or wild yeast) (Rainbow, 1981; Campbell, 2003). It is essential for the brewer to maintain the integrity of the specific culture yeast used to produce the brands. Wild-yeast contamination ('any yeast not deliberately used and under full control' – Gilliland, 1971) will lead to inefficient fermentations and flavour profile changes. The contaminant *Obesumbacterium proteus* (Calderbank & Hammond, 1989) grows in wort and early fermentations and can be found in yeast slurries with the consequence of easy recycling. This organism not only produces the unpleasant off-flavour dimethyl sulphide (DMS) but also reduces nitrate to nitrite, leading to the formation of nitrosamines. Contaminants should thus be avoided to limit health risks (nonvolatile nitrosamines), unpleasant off-flavours, pH changes and filtration problems.

In general, the QA verification of the culture yeast can be divided into microbiological status (free from contaminants), culture integrity (the absence of petites and variants), culture identity verification (fingerprinting) and quality (viability and vitality). Petites (mitochondrial respiratory-deficient mutants/RD mutants) are the most commonly encountered brewing yeast variants and have been reported to impact on fermentation (poor attenuation), flocculation and beer flavour components (Ernandes *et al*., 1993). For this reason, stringent specifications are set to keep the % RD variants low, thus limiting their presence. Petites are generally detected using the tetrazolium overlay technique (Ogur *et al*., 1957).

Culture variant detection can be conducted using agar-plating techniques [Wallerstein Laboratories Nutrient (WLN) agar, Casey & Ingledew, 1981] as well as molecular techniques (Smart, 2007). The relative ease with which the broader yeast groups could be differentiated did not prove to be the case for lager strains. Differentiation of lager strains has been limited because the yeasts within the *Saccharomyces sensu stricto* group consist of closely related yeast that are genetically conserved (Laidlaw *et al*., 1996; Montrocher *et al*., 1998). The capability to differentiate lager strains or variants of a strain differs dependent on the test. Some of these genetic tests include restriction fragment length polymorphism (RFLP) gene mapping (Meaden, 1996), karyotyping, which identifies differences in chromosomal length (Casey, 1996), amplified fragment length polymorphism (AFLP) (de Barros Lopes *et al*., 1999) and PCR-based methods on detecting the Ty transposon (Cameron *et al*., 1979; Coakley *et al*., 1996). More powerful discriminating tools to characterize nine industrial yeast strains, by examining their metabolic footprints (exometabolomes) have been reported (Pope *et al*., 2007). They found that for some strains discrimination not achieved genomically was observed metabolomically.

Determination of yeast quality related to fermentation performance has been elusive. Viability (number of living cells) was defined as a cells' ability to bud and grow, however slowly (Bendiak, 2000). The commonly used viability tests are based on the bright-field stains methylene blue (MB) (Chilver *et al*., 1978) and methylene violet (MV) (Smart *et al*., 1999) and the fluorescent dye 1-anilino-8-naphthalenesulphonic acid (MgANS) (McCaig, 1990). In the MB test, viable cells remain colourless whereas dead cells are stained blue. The colourless 'leuco' form is thought to be the result of the slow uptake of the dye into viable cells to be oxidized whereas the dead cells cannot exclude the dye or perform this reaction. MB is the most commonly used stain in the brewing industry although its reliability has been questioned for viabilities below 90%. An alternative to MB was suggested with the use of MV, which contains fewer impurities and should result in less subjective errors by the operator (Smart *et al*., 1999). MgANS differentiates dead cells clearly by entering nonviable cells and binding to cytoplamic proteins to generate yellow/green fluorescence (McCaig, 1990). Unfortunately, the requirement for fluorescent microscopy has precluded it as a practical application in breweries.

Yeast vitality determination has historically been hampered by the complexity of the test and the brewers' requirements for a test that is affordable, rapid, simple and reproducible. Numerous reviews discussing the various proposed methods have been published (Imai, 1999; Bendiak, 2000; Heggart *et al*., 2000). Vitality has been defined as the capacity of yeasts to initiate metabolism rapidly after transfer from a nutrient-poor to a nutrient-rich environment (Kaprelyants & Kell, 1992) the ability to endure stress and still perform (Axcell & O'Connor-Cox, 1996) and the physiological state of the viable cell population (Smart,

1996). Advances in technology afford science the opportunity to now study yeast population characteristics at a cellular level. Flow cytometric measurement allows for the analysis of single cells as the suspended yeast cells pass the detector in a single stream. A laser beam is directed on the flow channel and detects cellular components using fluorescent probes. This technology has been applied to brewing yeast by numerous workers and has also been used to determine the physiological state during propagation (Novak *et al.*, 2007) or vitality determination before fermentation (Lodolo & Cantrell, 2007). In this holistic vitality approach, the capacity of the yeast population to perform once it is introduced into production wort is measured (Lodolo & Cantrell, 2007). Yeast response to the environment is determined using flow cytometry to measure DNA concentration changes relative to nutrient limitation or natural antimicrobial inhibitors in the wort (Van Nierop *et al.*, 2006).

The advent of consumer demand for novel products requires additional rigour in hygiene practices due to compositional changes in these new brands. The composition on balance can result in an increased microbiological risk due to pH changes, low alcohol or low-hopped beers or products with higher residual sugar concentrations at the end of fermentation. Appropriate QA methodologies need to be in place to enable verification of these novel beverages.

## Propagation

Propagation is a stepwise, aerobic process where the brewing strain of choice is removed from storage to be grown such that sufficient cell numbers, free of contaminants and of the desired physiological condition, are obtained to adequately inoculate (pitch) the zero-generation fermentations (Voigt & Walla, 1995). Culture maintenance methodologies have also evolved over time considering the initial exclusive use of agar slants that developed into alternative methods of storage in liquid nitrogen at $-196\,^{\circ}C$ (Wellman & Stewart, 1973) and cryovials at $-70\,^{\circ}C$ (Hulse *et al.*, 2000). The use of storage methodology depends on sample integrity preservation, ease of use and transport implications.

Investment in the propagation process is required despite the availability of excess yeast, because serial repitching and cropping result in the deterioration of the yeast. This deterioration could be hygiene related (cross-contamination with other brewing cultures, wild yeast or bacteria), selection of crops with specific characteristics (trub enriched, increased flocculance, age and cell size) and yeast quality related (genetic changes, petite mutants and physiological changes due to stress). Stresses imposed on the yeast during fermentation and yeast handling can lead to cell death (Powell *et al.*, 2000). Two pathways have been identified: necrosis and senescence. Necrosis was defined as the accumulation of irreparable damage to intracellular components compromising cell integrity, leading to death and autolysis. Senescence is the predetermined cessation of life as a result of the genetically controlled progression from youth to old age (Powell *et al.*, 2000). Replicative senescence has been demonstrated in brewing strains. The mean lifespan is strain specific within the range of 10–30 divisions (Powell *et al.*, 2000). The experience of the brewers in the past dictated the need for fresh propagations. The awareness of replicative senescence and strain-specific lifespan supports the rationale for this practice.

Traditionally, propagation is a stepwise aerobic process with incremental changes in volume and temperature (Andersen, 1994) and can be divided into laboratory propagation and brewery/plant propagation. The main purpose of plant-scale propagation is to provide favourable conditions for yeast growth. Thus, the requirements of propagators are hygienic design, sufficient oxygen supply to maintain an aerobic environment (including mixing) and sufficient nutrients (amino acids, carbohydrates, vitamins and inorganic ions). The laboratory stage is initiated from the stored culture (cryovial or slope), which is typically inoculated into 15 mL and then transferred to 200 mL, with the final stage in the Carlsberg flask (Andersen, 1994). Various plant propagation systems are in use today. The design considerations include the yield required (terminal yeast count in propagator) and the target inoculation count required for the first fermentation. Based on these factors, scale-up ratios can be designed, resulting in propagation vessels of incremental sizes.

One disadvantage of propagation plants is the relatively high cost, and alternative approaches are therefore of interest. Of these alternative approaches, the use of dried yeast or fed-batch (assimilation technology) is advocated.

Initial investigations into the application of dried yeast showed that under comparable conditions, the same strain of yeast performed similarly when it was dried or fresh (Muller *et al.*, 1997). However, separate investigations found that ale yeast strains, which exhibited similar results both in dried and in fresh states, did not appear to be affected to the same extent as the lager strains (Finn & Stewart, 2002). Furthermore, dried lager yeast samples showed characteristics different from those of the identical fresh samples, in terms of flocculation and haze formation. The average viability of dried yeast was 20–30% lower than that of freshly propagated yeast, impacting on haze and foam stability (Finn & Stewart, 2002).

Another alternative advocated is the use of assimilation technology/fed-batch yeast propagations (Thiele & Back, 2007). The process is based on maintaining cells in the logarithmic phase by leaving a certain quantity of yeast suspension in the tank after removal of assimilated yeast, followed by topping up with fresh wort. The implementation

of assimilation technology/fed-batch yeast propagations in many commercial plants demonstrated certain advantages. Because of the uniform high yeast vitality, fermentation times are shorter, pH drop proceeds faster and concentrations of diacetyl and other unwanted fermentation by-products are lower. The risk of contamination also decreases due to high yeast vitality. The resulting beers are characterized by uniform, good beer quality and less foam-negative compounds (Thiele & Back, 2007).

## Fermentation

Fermentation is the cumulative effect of the growth of yeast on wort, ultimately resulting in the spent growth medium, beer. Fermentation is the 'magic' early mankind saw. A typical lager fermentation requires around 12 days to complete and therefore causes a 'bottle neck' in the process. The design of breweries compensate for this 'bottle neck' by increasing fermenter size as well as the number of fermenters. CCVs provide the solution due to their small footprint and ideal construction material (stainless steel).

Although science has provided a solid understanding of the biochemical processes (Florkin, 1972), fermentation management remains an art of balancing yeast growth and metabolism such that the desired flavour compounds are formed within the required process time. A successful outcome is dependent on the use of a highly viable/vital, pure yeast strain of choice. The process control parameters such as sufficient nutrient supply, correct inoculation (pitching) rate, optimized dissolved oxygen (DO) addition and temperature control assist in controlling yeast growth rate and extent. Typically, oxygen is the growth-limiting nutrient and serves as a fermentation control point. Overall balance in growth (nutrient uptake and by-product release) is required for consistent, brand-specific beer quality.

Yeast flocculation characteristics dictate the fermenter design. Ale yeast (top yeast) exhibit flotation and have the ability to trap $CO_2$ bubbles to form a yeast 'head' at the top of fermentation vessels where the yeast can be scooped off. CCVs are ideally suited to lager strains (bottom yeasts) because the cells clump together, resulting in flocs that sediment from the medium to settle in the bottom of CCV cones. This strain-dependent phenomenon is termed flocculation (Speers *et al.*, 1992; Verstrepen *et al.*, 2003) and evidence exists that a number of *FLO* genes play a role (Teunissen & Steensma, 1995). Various factors affect flocculation. They are factors that affect *FLO* gene activity (nutrition, temperature, ethanol and other); the genetic background of the specific strain (sensory mechanisms, transcription and translation of *FLO* genes, incorporation of proteins in cell walls) and factors affecting cell–cell interactions in floc formation (agitation, calcium concentration, carbohydrate concentrations, cell shape and size, ionic strength, osmolality, pH, rheology of suspending medium, temperature and zymolectins) (Speers & Ritcey, 1995; Verstrepen *et al.*, 2003; Strauss, 2005).

The changes in flocculation patterns can negatively impact the brewing process in two ways. Poor flocculation results in elevated cell numbers which impacts on filtration of beer. Conversely, 'premature' yeast flocculation (PYF) is the early flocculation of cells during fermentation in the presence of high sugar concentrations. This results in high residual extract, lower ethanol and other quality concerns (Van Nierop *et al.*, 2006).

The lectin type cell–cell interaction (Miki *et al.*, 1982) was proposed to explain flocculation although cell surface hydrophobicity (Straver *et al.*, 1993) has been identified as the second major factor responsible for flocculation onset. This observation was further supported by the accumulation of hydrophobic carboxylic acids i.e. 3-hydroxy (OH) oxylipins on cell surfaces during flocculation onset (Strauss *et al.*, 2006).

During fermentation the propagated yeast or cropped yeast from a previous fermentation is inoculated into the wort. The CCVs generally feature entry/exit mains, sampling points, thermometer pockets, automatic temperature control (cooling jackets with a coolant), antivacuum relief valve, $CO_2$ exhaust, CIP inlet and a man way door. Some constraints of CCVs are mixing efficiency, maximum height to diameter ratios and homogeneous temperature control (Knudsen, 1978).

The fermentation process initiates with the utilization of glucose in the glycolytic pathway to pyruvate, the major branch point between the fermentation process and the citric acid cycle (Krebs cycle). During fermentation, a net of two ATP molecules are formed as pyruvate is converted via acetaldehyde to ethanol and carbon dioxide. The carbon dioxide formed during fermentation can potentially have a negative or a positive impact on overall brewery performance. The potential negative impact has been observed when yeast exhibits poor fermentation performance. Carbon dioxide is toxic to the yeast cell and efficient nucleation from the fermenting wort is required to minimize impacts on yeast (Kruger *et al.*, 1992). Supersaturation of carbon dioxide due to poor pressure release in fermenters exacerbates the toxic effects. One practice advocated is the use of 'cloudy' wort rather than 'clear' wort with the potential proposed benefits of lipid material and carbon dioxide nucleation sites (O'Connor-Cox *et al.*, 1996b; Stewart & Martin, 2004). However, a negative impact of cloudy wort on fermentation performance and product quality was demonstrated. It was argued that better process control can be achieved by rather supplying the DO requirements for optimal lipid formation and to provide nucleation sites with the addition of yeast stimulants (O'Connor-Cox *et al.*, 1996b). The recovery of carbon dioxide from fermenters

has been weighed against the capital expenditure of fitting a recovery plant. However, the global concern regarding the control of gas emissions into the atmosphere and the availability of carbon dioxide for beverage carbonation makes carbon dioxide recovery a more viable option with positive ramifications for a brewery.

The performance of yeast during fermentation will be considered in more detail under the topics: carbohydrate utilization, nitrogen uptake, mineral requirements, oxygen effects on yeast metabolism, mitochondrial roles and petites, flavour formation and beer sulphur components.

## Carbohydrate utilization

The two major nutrient classes impacting brewing yeast performance are carbohydrates and nitrogenous compounds. Assimilation of the individual nutrients is dependent on the yeast response to the various components. Brewing strains can utilize various carbohydrates (glucose, sucrose, fructose, maltose, galactose, raffinose and maltotriose), with the major distinguishing difference between ale and lager strains being the capability of lager yeasts to ferment melibiose. The generalized sugar uptake pattern initiates with sucrose, which is hydrolysed, causing an increase in glucose and fructose concentrations. The uptake then follows the route of simplest sugars (the monosaccharides glucose and fructose) first, followed in increasing order of complexity by disaccharides (maltose) and trisaccharides (maltotriose) excluding maltotetraose and other dextrins (Boulton & Quain, 2006; Stewart, 2006).

Fructose and glucose are transported across the cellular membrane by common membrane transporters in a facilitated diffusion process (Zimmermann & Entian, 1997). Two glucose uptake systems are recognized: low-affinity (constitutively expressed) and high-affinity transporter, which is repressed in the presence of high glucose concentrations. Catabolite repression (inactivation of the high-affinity transport system) has been shown to occur only in fermentative yeast strains. The affinity of the glucose carrier towards its substrate may not only depend on the availability of glucose but also the presence of oxygen and the growth rate and energy status of the cell (Walker, 1998).

Maltose is the major sugar of wort, accounting for 50–55% of the total carbohydrate content of wort compared with 10–14% for maltotriose. The maltose uptake mechanism involves two systems: an energy-dependent maltose permease (ATP converted to ADP) which transports the maltose intact across the cell membrane, and maltase (α-glucosidase), which hydrolyses maltose internally to yield two units of glucose. Maltotriose has an independent energy-dependent permease for intact transport but shares the α-glucosidase, which hydrolyses maltotriose to yield three units of glucose. The high-affinity maltose transporter has been characterized and three maltose utilization genes (*MAL* genes) are involved (Walker, 1998). The expression is regulated by maltose induction and repressed by high glucose concentrations (>1% w/v) (Stewart, 2006).

Different carbohydrate ratios have been shown to affect fermentation performance negatively, particularly in worts where glucose is the predominant carbohydrate. Separate investigations provide some explanation of the underlying mechanisms impacting negatively on yeast. Exponentially growing cultures under fermenting conditions with glucose as the carbon source (YPD, 1% yeast extract, 2% peptone, 2% glucose) or under respiring conditions with glycerol (YPG, 1% yeast extract, 2% peptone, 3% glycerol) were challenged with hydrogen peroxide ($H_2O_2$) for 45 min. There was a marked increase in protein damage in cells grown in YPD with a moderate increase in protein carbonyls for cells growing in YPG (Cabiscol *et al.*, 2000). The work on calorie restriction (CR) is of particular interest because CR is known to extend lifespan. Imposing moderate CR in *S. cerevisiae* by reducing the glucose concentration from 2% to 0.5% in rich media has extended the replicative lifespan (Easlon *et al.*, 2007). These findings were further supported when cultures growing in maltose and glucose media were compared and yeast exhibited higher viabilities in maltose than in glucose media (Stewart, 2006). The impact of carbohydrate ratios on yeast fermentation performance is thus a matter that requires careful consideration by the brewer.

One option a brewer has to address plant capacity constraints is to ferment at higher gravities. However, high-gravity worts (>16°P) exert a negative effect on fermentation performance. Although elevated osmotic pressure was identified as the major negative influence, the dilution of other essential nutritional factors such as amino acids also contributes towards poor fermentation performance. Global gene analysis studying the osmotic stress response during anaerobiosis showed that the osmotic stress response encompasses the presumed oxidative stress response. Anaerobic cells adapted faster due to the high intrinsic glycerol production (Krantz *et al.*, 2004). This highlighted the role of the high-osmolarity glycerol (HOG)-signalling pathway (Hohmann, 2002) in cellular adaptation to hyperosmotic shock. Tolerance to stress, including osmotic pressure, is associated with accumulation of trehalose. Evidence of trehalose accumulation under osmotic stress conditions was demonstrated in lager yeast fermentations using very high gravity wort (up to 25°P) (Majara *et al.*, 1996).

## Nitrogen uptake

One of the major functions of malt is to supply assimilable nitrogen sources to the yeast. The main sources of nitrogen in wort are amino acids, ammonium ion and some di- and tripeptides. The majority of wort-free amino nitrogen

(FAN) is utilized by yeast for protein formation (structural and enzymic) required for yeast growth (Pierce, 1987) and other functions such as osmoregulation (Hohmann, 2002). The level and composition of wort FAN also has a huge impact on higher alcohol, ester, vicinal diketone (VDK) and $H_2S$ formation due to the role of amino acid metabolism in the formation of these flavour compounds (Pierce, 1987; O'Connor-Cox & Ingledew, 1989). Conditions that stimulate fast yeast growth (high temperature and high DO concentrations) will lead to high FAN utilization, which can lead to flavour imbalances. Amino acids are utilized by yeast in a sequence that appears to be independent of the fermentation conditions. Based on this observation, the wort amino acids were catagorized into groups (Pierce, 1987). However, this uptake pattern may be strain dependent (Kruger *et al*., 1991). Group A amino acids are utilized first (arginine, asparagine, aspartate, glutamate, glutamine, lysine, serine and threonine), followed by the slower disappearance from wort of Group B amino acids (histidine, isoleucine, leucine, methionine and valine). Group C amino acids (alanine, glycine, phenylalanine, tyrosine, tryptophan as well as ammonia) are only absorbed after the complete removal of group A amino acids.

Group D contains only proline, which is poorly utilized. It has no free amino group and therefore cannot be transaminated. The uptake of the amino acids requires a number of permeases, some specific for individual amino acids and a general amino acid permease (GAP). Uptake is subjected to nitrogen catabolite repression (Grenson, 1992) and is active, requiring energy (Hinnebusch, 1987).

## Mineral requirements

Minerals are required in the micromolar or the nanomolar range as trace elements necessary for growth (Jones & Greenfield, 1984). In nature, the trace minerals can be found in wort in levels adequate for fermentation performance. However, the advent of higher gravity fermentations and poor barley crops can result in wort deficient in these minerals. For the purpose of this review, only the trace elements calcium, copper, iron, magnesium and zinc will be discussed. The roles of these trace elements are diverse as is demonstrated by the selected examples below. The significance of calcium uptake in yeast lies in the multifunction roles as a second messenger in the modulation of growth and metabolic responses of cells to external stimuli. Calcium also plays a crucial role in flocculation although the exact function is still debateable. Copper and iron act as cofactors in several enzymes including the redox pigments of the respiratory chain. Iron is required as a building block for haeme formation (Abbas & Labbe-Bois, 1993). Iron was further implicated in cellular redox homeostasis, oxidative stress resistance and lifespan through the modulation of iron levels by Inositolphosphosphingolipid phospholipase C (Isc1p) (Almeida *et al*., 2008). Isc1p require magnesium for optimal activity and is posttranslationally activated by translocation into the mitochondria (Almeida *et al*., 2008). Magnesium is the most abundant intracellular divalent cation in yeast where it acts primarily as an enzyme cofactor and a correlation has been demonstrated between cellular $Mg^{2+}$ uptake and alcoholic fermentation in industrial strains of *S. cerevisiae* (Walker, 1998). Trace levels of zinc are essential for the structure and function of $>300$ enzymes such as alcohol dehydrogenase (the terminal step in alcoholic fermentation) and the critical component of structural motifs such as zinc fingers. In *S. cerevisiae*, zinc homeostasis is regulated by the controlled activity of zinc uptake transporters in the plasma membrane and transporters responsible for intracellular zinc compartmentalization. The activity of these transporters is regulated at both transcriptional and posttranscriptional levels in response to zinc (Eide, 2003).

Analysis of fermentations using defined media experimental fermentations showed that yeast fermentation performance depended on complex interactions between potassium, magnesium and calcium (Chandrasena *et al*., 1997). Statistical modelling based on wort composition could prove to be a useful tool to predict yeast performance; however, the malt wort fermentations failed to match the model predictions, indicating the influences of other components in brewer's wort. The true complexity of wort composition, and all the factors that contribute towards its unique properties to support brewing yeast fermentations, is still not fully understood.

## Oxygen effects on yeast metabolism

DO supply to brewing yeast fermentations is necessary to achieve balanced fermentations resulting in the desired end product (Kirsop, 1982; Lodolo, 1999). Molecular oxygen has multifaceted roles in yeast physiology (Fig. 2) and various genes are differentially expressed in response to different oxygen environments to regulate cellular metabolism (Zitomer & Lowry, 1992). In addition to differences in gene expression under aerobic and anaerobic growth conditions, cells respond to decreases in oxygen tension. These genes (hypoxic genes) are induced at low oxygen tension (DOT) and are repressed by haeme, which serves as an intermediate in the signalling mechanism for oxygen levels in yeast cells (Zitomer & Lowry, 1992). The biosynthesis of haeme requires oxygen, and the supply of oxygen to yeast cells at the start of fermentation has a primary role in its biosynthesis.

Secondly, oxygen is an essential nutritional element for the biosynthesis of ergosterol and unsaturated fatty acids (UFA) (Rattray *et al*., 1975). The sterols and UFA are

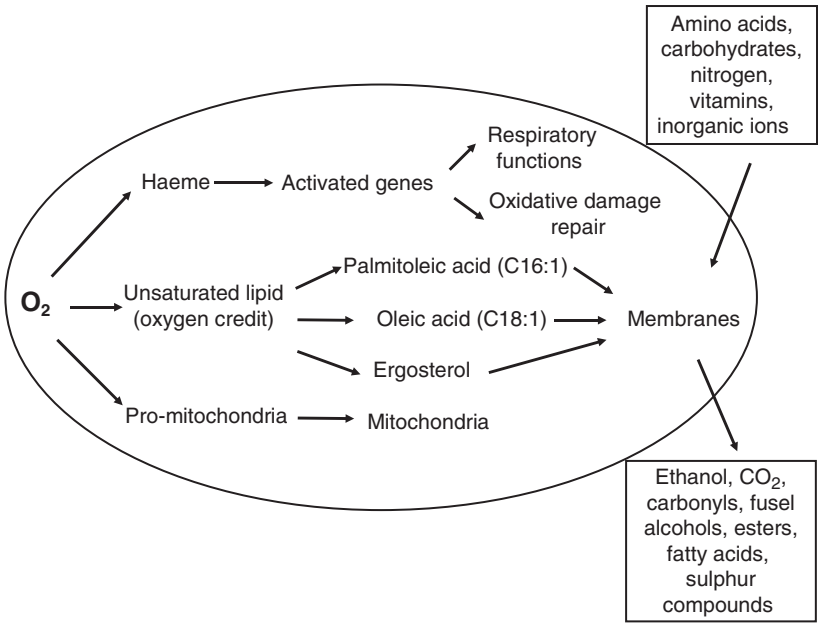


**Fig. 2.** Graphical summary of the multifaceted anabolic roles of oxygen in yeast metabolism.

incorporated into the membrane bilayer structure (Prasad & Rose, 1986) of growing cells. Cell membranes support cellular function because the uptake of nutrients occurs across this natural barrier (Walker, 1998). Yeast that is not provided with an adequate oxygen supply will possess inferior membranes with a reduced transport capability and a reduced ability to withstand osmotic stresses and high exterior ethanol levels (Piper *et al.*, 1984).

The potential negative impact of oxygen is founded on the knowledge of the numerous metabolic processes that can lead to reactive oxygen species (ROS) such as $H_2O_2$ and hydroxyl radicals ($OH^-$) (Halliwell & Gutteridge, 1999). The ROS are able to oxidize nucleic acids, proteins, lipids and carbohydrates affecting membrane activity and cellular functions important for viability (Manon, 2004; Belinha *et al.*, 2007). Oxidative damage is prevented by antioxidant defences, such as superoxide dismutase (SOD), catalase, peroxidases and glutathione. When, on balance, the ROS levels exceed the antioxidant capacity, cells undergo an oxidative stress that has been linked with ageing (Santoro & Thiele, 1997). The importance of optimized oxygen supply is supported by the scientific evidence of the impact of ROS on chromosomal rearrangements and cell death (Ragu *et al.*, 2007). Furthermore, evidence exists around the central role of the hydroperoxyl ($HO_2^{\bullet}$) radical and its role in mediating toxic side-effects during aerobic respiration (De Grey, 2002).

Clarkson *et al.* (2004) studied the effects of a sudden transition from aerobiosis to anaerobiosis (including the reverse) and found the most significant change in the specific activity of copper, zinc-SOD (SOD1), which showed a rapid increase in activity on transition from anaerobiosis to aerobiosis. Increases in citrate synthase and manganese-SOD were also noted after a significant lag period. Furthermore, anaerobically grown cells showed a rapid loss in viability on exposure to oxygen while aerobically grown cells were unaffected. They concluded that the toxic effect of oxygen was due to superoxide (or species derived from it) and that SOD1 played a role in protecting the cells. Other workers (Corson *et al.*, 1999) also demonstrated that the absence of the predominantly cytosolic SOD1 caused vacuolar fragmentation in *S. cerevisiae*. The vacuole is reported to be a site of iron storage and iron reacts with ROS to generate toxic side products such as the hydroxyl radical. Their findings supported the hypothesis that oxidative stress alters cellular iron homeostasis, which in turn increases oxidative damage.

The potential negative impacts of oxygen on brewing yeast necessitate optimization of the DO requirements. These include the amount of oxygen to supply at the correct time (O'Connor-Cox & Ingledew, 1990; Lodolo, 1999). Owing to capacity requirements, fermenter tank volumes exceed brewhouse capacity, thereby necessitating fermenter filling with multiple brews. Various strategies have been followed to achieve the optimized DO concentration at the right time by applying different DO concentrations/brew (Yokoyama & Ingledew, 1997; Jones *et al.*, 2007).

Alternative means to supply yeast with oxygen includes the aeration of yeast slurries before pitching (Fujiwara & Tamai, 2003). The underlying molecular mechanisms triggered by aeration under nutrient-lacking conditions were analysed. Higher hypoxic repressor gene (*ROX1*) transcription levels resulted with increased aeration. The expressions of the Δ-9 fatty acid desaturase gene (*OLE1*) transcription levels were initially depressed but then increased with

increased aeration. The ratio of unsaturated fatty acids to total fatty acid composition increased with increased aeration. The findings suggested that aeration of the yeast-containing medium, even under nutrient-lacking conditions, stimulated oxygen-signalling pathways (Fujiwara & Tamai, 2003).

## Mitochondrial roles and petites

Oxygen is also required for the differentiation of promitochondria to mitochondria (Plattner *et al.*, 1971; O'Connor-Cox *et al.*, 1996a). Many key metabolic functions (such as the activity of manganese-SOD, the synthesis and oxidation of fatty acids and the synthesis of some amino acids) can only take place in partially or fully developed mitochondria (Guerin, 1991). Mitochondria play a role in ethanol tolerance (Aguilera & Benitez, 1985; Costa *et al.*, 1997) and functional mitochondria give rise to increased cell viability under extreme conditions of stress (Jiminez & Benitez, 1988). It is also known that alterations in mitochondrial membranes lead not only to respiratory deficiencies but also to changes of the cell surface (Zaamoun *et al.*, 1995). Additional links between cell surface changes and mitochondrial functionality were reported where 3-hydroxy oxylipin formation was inhibited by the addition of acetylsalicylic acid (Strauss *et al.*, 2007). The link between mitochondria and oxygen metabolism becomes more interesting if one considers ferrochelatase. This mitochondrial inner membrane-bound enzyme catalyses the incorporation of ferrous iron into protoporphyrin, the last step in protohaeme biosynthesis (Abbas & Labbe-Bois, 1993).

The role of mitochondria in ageing is becoming more apparent. The mitochondrial transmembrane proteins ensure longevity and mitochondrial DNA are required for oxidative stress. Analysis of proteins that are oxidatively damaged showed that, besides some cytosolic proteins, mitochondrial proteins such as E2 subunits of both pyruvate dehydrogenase and α-keto-glutarate dehydrogenase, aconitase and heat-shock protein 60 were the major targets (Cabiscol *et al.*, 2000). Furthermore, Spanish researchers demonstrated that glutaredoxin *GRX5* localized in mitochondria protected *S. cerevisiae* cells against oxidative damage by enzymes containing Fe–S clusters (Rodríguez-Manzaneque *et al.*, 2002).

Instability of the mitochondrial genome (mtDNA) leads to the formation of petite mutants. Mutations in nuclear genes involved in mtDNA metabolism (replication, repair and recombination) can cause a compete loss of mtDNA ($rho^0$ petites) and/or lead to truncated forms ($rho^-$) (Contamine & Picard, 2000). Mutations that increase mtDNA instability act indirectly and they lie in genes controlling diverse functions such as mitochondrial translation, ATP synthase, iron homeostasis, fatty acid metabolism and mitochondrial morphology (Contamine & Picard, 2000). Brewers have historically ignored mitochondrial functions due to the Crabtree-positive nature of brewers yeast (O'Connor-Cox *et al.*, 1996a; Walker, 1998). The Crabtree effect refers to the repression of respiratory activity by glucose (respiratory bottleneck) under aerobic conditions and the focus was thus on the fermentation pathway. However, the additional evidence related to mitochondrial function and process impacts due to the presence of petite mutants requires the brewer's attention. QA efforts thus aim to minimize the presence of petite mutants to avoid potential negative performance effects such as sluggish fermentations, reduced yeast growth, changed flocculation and altered flavour profiles (Ernandes *et al.*, 1993).

## Flavour formation

The unique flavour profiles of beer can largely be attributed to the biochemical activities within the yeast cell during fermentation. The flavour compounds are intermediates in pathways leading from the catabolism of wort components (sugars, nitrogenous compounds and sulphur compounds) to the synthesis of components required for yeast growth (amino acids, proteins, nucleic acids, lipids, etc.) (Fig. 3). The yeast-derived flavour-active compounds can broadly be listed as ethanol, $CO_2$, carbonyls (aldehydes/ketones), higher/fusel alcohols, esters, vicinal diketones (VDK) (diacetyl and pentanedione), fatty and organic acids and sulphur compounds. Ethanol and carbon dioxide are the primary by-products formed during fermentation as indicated in Fig. 3.

Carbonyls (aldehydes and ketones) contain a functional group composed of a carbon atom double bonded to an oxygen atom. Aldehydes are formed during wort preparation (such as Maillard reactions and lipid oxidation) and as part of the anabolic and catabolic pathways for higher alchohol formation during fermentation. Acetaldehyde is the major aldehyde to consider due to its importance as an intermediate in the formation of ethanol and acetate. Acetaldehyde has a flavour threshold of 10–20 mg $L^{-1}$ and its presence in beer above the threshold value results in 'grassy' off-flavours (Meilgaard, 1975). However, many tasters can detect this compound at much lower levels.

Numerous VDK can be present in beer but the most important when considering beer flavour is diacetyl (2,3-butanedione) and 2,3-pentanedione. They both impart a butterscotch aroma but the flavour threshold of diacetyl (0.15 mg $L^{-1}$) is sixfold lower than that of 2,3-pentanedione (0.9 mg $L^{-1}$) (Meilgaard, 1975). VDK formation is linked to amino acid metabolism (Boulton & Quain, 2006). Wort deficient in valine results in elevated diacetyl levels and similarly wort deficient in leucine result in increased 2,3-pentanedione. Contaminants such as *Lactobacillus* and

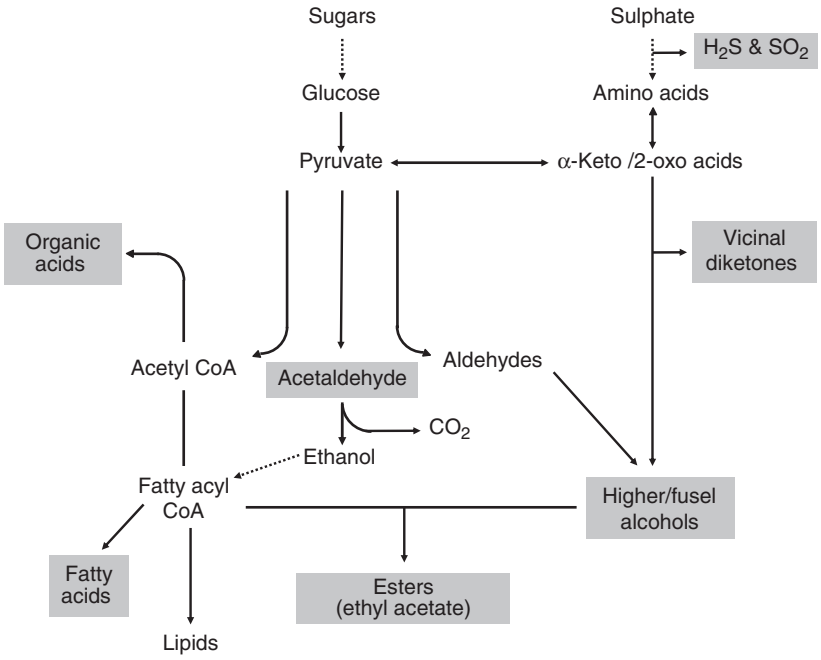


**Fig. 3.** Interrelationships between the main metabolic pathways contributing towards flavour-active compounds in beer (adapted from Hammond, 1993).

*Pediococcus* can produce diacetyl. Brewers use the ratio of diacetyl : pentanedione to gain an indication of whether elevated diacetyl concentrations are due to contaminants or fermentation by-products.

Higher/fusel alcohols contribute to the overall beer flavour character and can be synthesized by two routes via α-keto/2-oxo-acids (Fig. 3). The first anabolic pathway is synthesis from wort carbohydrates via pyruvate, whereas the second catabolic process is as by-products of amino acid metabolism (Ehrlich pathway) (Äyräpää, 1968). The 'Ehrlich pathway' describes the conversion of branched aminoacids to fusel alcohols by three enzymatic steps (transamination, decarboxylation and reduction). However, the molecular mechanisms of the genes encoding the enzymes involved in the pathway remain unclear. Genome-wide gene analysis of yeast expression profiles during flavour formation when cultivated on L-leucine and ammonia revealed a group of 117 genes that were more than twofold up- or downregulated (Schoondermark-Stolk *et al.*, 2006). The gene expression groups consisted of genes encoding proteins involved in amino acid metabolism. It was concluded that amino acid metabolism pathways, other than the branched chain amino acids (BCAA) pathway, play significant roles in the formation of volatile compounds. Regarding the second step of the Ehrlich pathway, *ARO10* (phenylpyruvate decarboxylase gene) with a broad 2-oxo acid decarboxylase activity, was strongly induced and *PDX1* (pyruvate dehydrogenase complex protein X) showed a significant increase in gene expression during flavour formation (Schoondermark-Stolk *et al.*, 2006). These and other genes are now under investigation to fill in the gaps in the understanding of the molecular mechanisms. The metabolic purpose for higher alcohol formation is not clear and may appear wasteful. However, Quain & Duffield (1985) proposed that similar to glycerol, these metabolites form part of the overall cellular redox balance. Interestingly, the yeast plasma membrane is well adapted to the use of glycerol as an osmolyte and cellular glycerol content is controlled at the level of export (Hohmann, 2002).

Ester formation is closely linked to lipid metabolism and growth and is a product of fermentation. Over 100 different esters resulting in the floral/fruity aromas in beer have been identified (Meilgaard, 1975). Two potential routes for ester formation have been recognized. These are the reaction between an alcohol (such as ethanol) or higher alcohols with a fatty acyl-CoA ester (Nordström, 1963) and by esterases working in a reverse direction (Soumalainen, 1981). Different alcohol acetyl transferases (*ATF* genes) have been identified and the expression of the gene is required for ester formation (Lyness *et al.*, 1997). Evidence from gene disruption and expression analysis of members of the *ATF* gene family indicated that different ester synthases are involved in the synthesis of esters during alcoholic fermentation. Control mechanisms that underpin the oxygen-mediated regulation of *ATF1* gene transcription appear to be closely linked to those involved in the regulation of fatty acid metabolism (Mason & Dufour, 2000). Some common esters that impact on beer flavour are ethyl acetate (fruity/solvent), isoamyl acetate (banana) and ethyl caproate (apple/aniseed).

The presence of over 110 organic or short-chain fatty acids has been reported (Meilgaard, 1975). They are derived

from wort as well as during the course of fermentation, particularly from pyruvate or from the repressed tricarboxylic acid cycle. Organic acids (such as pyruvate, citrate, malate, acetate and succinate) impart sour flavours and contribute towards the lowering of pH during fermentation. The two critical oxo-acids (α-acetolactate and α-acetohydroxy acids) are of particular interest due to their roles as precursors of diacetyl and 2,3-pentanedione, respectively (Fig. 3).

Chen (1980) investigated the presence of free fatty acids in wort and beer and concluded that long-chain fatty acids in wort (palmitic, linoleic, stearic and oleic) were assimilated by growing yeast whereas the short-chain fatty acids in beers were released by-products from lipid synthesis. The short-chain fatty acids are generally undesirable due to their impact on taste and foam.

## Beer sulphur components

The main sulphur components impacting on beer flavour are sulphur dioxide ($SO_2$), hydrogen sulphide ($H_2S$), dimethyl sulphide (DMS) and mercaptans (Van Haecht & Dufour, 1995). The two main compounds that are influenced by yeast metabolism are $H_2S$ and $SO_2$ (Fig. 3). Reduced sulphur is an essential constituent of proteins (sulphur-containing amino acids such as cysteine and methionine), coenzymes (CoA, biotin, thiamine and pyrophosphate) and other cellular metabolites (glutathione, sulphides and thiols). Radiochemical tracer experiments showed that all of the $SO_2$ formed during fermentation is derived from the inorganic sulphur source, sulphate, in wort. $SO_2$ production during fermentation should be controlled due to possible negative flavour impacts. However, sulphite can function as an antioxidant and can react with stale-tasting carbonyls (acetaldehyde and *trans*-2-nonenal) to form flavour-inactive carbonyl-sulphite adducts.

Regulation of sulphur metabolism involves feedback inhibition and gene repression. The sulphur amino acid biosynthetic pathway was investigated by Thomas & Surdin-Kerjan (1997) and reviewed in wine yeast (Swiegers & Pretorius, 2007). *S*-Adenosyl-methionine (AdoMet/SAM) has a central role as a cofactor in numerous reactions as well as repressing transcription of the enzymes involved in sulphate uptake.

Tracking sulphur production during fermentations has been problematic at an industrial level. Positive progress was made with a high-throughput method that was developed to screen the formation of $H_2S$ and $SO_2$ in the industrial setting (Duan *et al.*, 2004). Both compounds were produced in greater quantities by yeast when grown in the presence of increasing concentrations of cysteine. Methionine repressed the cysteine-induced increase in the $H_2S$ production but had no effect on the formation of $SO_2$. Differences were also seen in $H_2S$ compared with $SO_2$ production in response to nitrogen levels in wort. It was found that, although $H_2S$ and $SO_2$ production are closely linked biochemically, environmental conditions can have different effects on their rate of formation (Duan *et al.*, 2004).

DMS can arise via two routes: the first from the thermal degradation of *S*-methylmethionine (SMM) during wort boiling and the second via the reduction of dimethyl sulphoxide (DMSO) by yeast during fermentation (Anness & Bamforth, 1982; Dickenson, 1983). The final DMS concentration in beer is the result of DMS present in wort at pitching, DMS formed from reduction of DMSO during fermentation and DMS lost by $CO_2$ stripping during fermentation. Thus, the aim of the brewer is to manage the process such that these unpleasant sulphury flavours are minimized in the final product.

## Yeast handling (cropping, storage and pitching)

The various yeast management processes that include mechanical or physical treatment of yeast are collectively called 'Yeast Handling' (O'Connor-Cox, 1997, 1998a, b; Kennedy, 2000). The modern yeast handling circuits are designed for the movement of yeast from one vessel (fermenter) to be pitched in the next. During the yeast handling cycle (Fig. 4), the brewing yeast (slurry) is recovered (cropped) with the use of cropping pumps (O'Connor-Cox, 1997) from the cone of the cylindroconical fermentation vessel (FV) after the fermentation process. During fermentation the nutrients were utilized and the major metabolites (ethanol and $CO_2$) were formed. The yeast crop is thus exposed to various stresses that include fluctuations in DO concentration, carbon dioxide, pH, pressure, ethanol concentration, nutrient limitation and temperature (Heggart *et al.*, 1999; Gibson *et al.*, 2007). A certain percentage of the crop is scrapped due to the presence of older or dead cells sedimenting in the cone

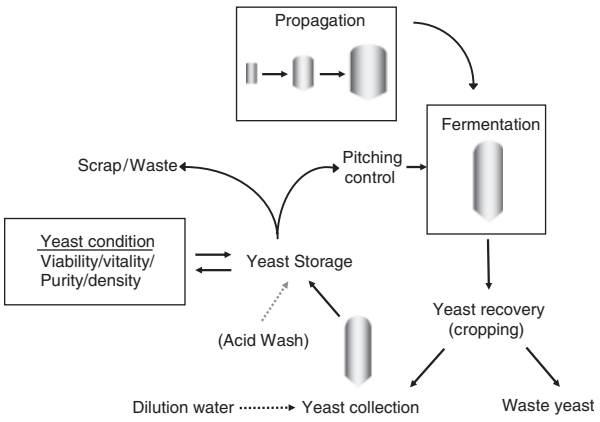


**Fig. 4.** The main activities during lager yeast handling (cropping, storage and pitching) in CCVs.

(Powell *et al.*, 2003, 2004). Seeding a fermentation with yeast consisting mainly of aged yeast would result in an extended lag phase whereas if the crop consisted mainly of newly budded virgin cells the time taken to reach the critical size required for the onset of division would result in a delay in the onset of growth (Powell *et al.*, 2000). It is, therefore, essential that yeast handling practices in a brewery do not select for yeast populations enriched in very young or aged cells.

The remainder of the crop is collected into a collection vessel, where it can be treated with dilution liquor in order to decrease potential negative impacts of ethanol toxicity (Van Uden *et al.*, 1983; Lentini *et al.*, 2003; Thiele & Back, 2007). This recovered yeast is then stored until it is required for fermentation. Should the yeast crop be older than the required generation (numbers of cycles used) or fail QA tests, then the crop will go to waste (scrapped) and freshly propagated culture will be required.

Cropping, storage and pitching of yeast need to support QA targets of correct strain integrity, phenotypical homogeneity (flocculence, metabolism and age), freedom from contamination and high viability or vitality. Nonadherence to good practice (cold storage at 4 °C, effective agitation for homogeneity, atmospheric pressure, effective cleaning and sterilization) will lead to deterioration in crop condition with a concomitant impact on fermentation performance (Pickerell *et al.*, 1991).

Acid washing of pitching yeast to eliminate bacterial contaminants with bactericidal substances such as phosphoric acid is a common practice in many breweries (Cunningham & Stewart, 1998). The typical procedure requires a reduction in pH to a value of pH 2.2–2.5 for a few hours at a temperature below 4 °C. However, the disadvantages of this process are that it may not kill all bacteria and it may negatively impact the yeast condition. Reports related to an approximate sixfold decrease in cell viability (Van Bergen & Sheppard, 2004) and changes in cell surface charge and hydrophobicity (Wilcocks & Smart, 1995) indicated the potential dangers of this practice even when applied correctly. Incorrect use (acid 'hot spots', elevated temperatures and extended exposure periods) will lead to increased yeast damage impacting on fermentation performance.

### Maturation (conditioning, lagering, ageing and diacetyl stand)

Maturation of green beer (immature beer) is required to refine the finished aroma, flavour and clarity of lager beer. The main objectives of green beer maturation are flavour adjustment (diacetyl, $SO_2$ and DMS), yeast sedimentation, carbonation and colloidal stability. Maturation can be divided into two parts, the first being the warm (ruhr) maturation and the second a cold (lagering) process.

The aim of the warm maturation process is to reduce flavour-active components such as diacetyl and, to a lesser extent, 2,3-pentanedione. The presence of the diketones at levels higher than the flavour threshold is generally not acceptable in lager beer styles due to the butterscotch aroma. High levels of diacetyl at the completion of fermentation are not limited to imbalances during fermentation performance. Other factors such as *Pediococcus* contaminations (*Sarcina* sickness) and the presence of respiratory-deficient 'petite mutants' can contribute to increased diacetyl concentrations (Ernandes *et al.*, 1993). Additionally, the initial valine concentration of wort (between 130 and 140 mg $L^{-1}$) was found to contribute to the total diacetyl profile with longer transition phases and the second maxima that occurred later (Petersen *et al.*, 2004). This resulted in extended diacetyl reduction times.

The removal of diacetyl is the rate-limiting step in the maturation of beer. Brewers use various strategies to manage diacetyl profiles in beer. The general practice is to hold the fermenter at higher temperatures (relative to the fermentation temperature) to allow for the decarboxylation of α-acetolactate to diacetyl. The yeast then reduces diacetyl to the less flavour-active products acetoin and butanediol.

Other strategies include fermentation control to limit the final diacetyl concentrations, the use of immobilization technology (Yamauchi *et al.*, 1995; Moll & Duteurtre, 1996) and the use of enzymes such as α-acetolactate decarboxylase, which transforms α-acetolactate directly to acetoin without the formation of diacetyl (Yamano *et al.*, 1995).

The cold part of the maturation process ( − 2 °C) aims at improving the colloidal stability of beer such that no haze will develop over time. Factors that play a role are glucans excreted by yeast during stressful fermentations as well as protein and polyphenol (tannin) complexes that are formed. These can result in visible haze that may not be attractive from a consumer's perspective. At − 2 °C, insoluble protein–tannin complexes precipitate and are then removed by the subsequent filtration process before packaging. Typical bright beer haze requirements after filtration are 0.5–1 EBC as measured at 90° scatter. The final product is now ready to proceed to packaging, the final step to ensure delivery of this intricate beverage to the consumer.

## Future yeast research

Arguably, one of the biggest breakthroughs in yeast research was the sequencing of the complete genome in 1996, the first complete sequence of a eukaryote (Goffeau *et al.*, 1996). This knowledge supported the development of microarray technology. Transcript-level analysis with DNA microarrays has become a powerful tool to study gene expression and metabolic changes and has been applied to the brewing yeast transcriptome during production-scale lager beer

fermentation (Olesen *et al.*, 2002). However, the application in lager yeast was hampered by the availability of lager yeast-specific arrays, which have a more complex genetic background than *S. cerevisiae* (Kobayashi *et al.*, 2007). Future developments in this field can provide clarity regarding the best brewing practices to optimize yeast performance during propagation, yeast handling and fermentation. The impact of wort composition fluctuations on yeast performance remains a complex area. The identification of specific gene activities linked to wort compositional changes will allow for more directed research. The recent developments in metabolomics have shown that future developments have the potential to not only differentiate but also effectively select brewing strains for new brands.

Progressive developments in yeast genetics and molecular biology of laboratory cultures provided the scientific basis for genetic manipulation of industrial strains. Examples of genetic transformation of brewing yeast include introduction of the maltose-permease gene, glucoamylase from *S. cerevisiae var. diastaticus*, yeast flocculation genes (*FLO1*), bacterial acetolactate decarboxylase (ALDC) to bypass diacetyl formation, elimination of *MET10* to increase $SO_2$ production and β-glucanase ex *Bacillus subtilis* (Boulton & Quain, 2006). However, the initial upbeat predictions of the future application of genetic engineering in the brewing industry (Stewart & Russell, 1986) were not realized. Consumer reticence about genetically modified food and the media interest make it a nonviable option in the highly competitive global market. Fortunately, the opportunity to exploit *S. cerevisiae* as a model eukaryote is not lost (Goffeau *et al.*, 1996). The intrinsic advantages of this eukaryotic experimental system are used to understand the underlying molecular mechanisms of ageing (Belinha *et al.*, 2007) as well as diseases that plague mankind (Czabany *et al.*, 2007). Thus, besides providing mankind with pleasurable beverages to consume, yeast also serves as a model system to find cures for human diseases.

## Conclusion

Not only is *S. cerevisiae* the main character in the beer-brewing process but yeast also has the centre stage role in other biotechnological industries benefiting mankind (alcoholic beverage production, bread baking, fine chemicals and bioethanol). The earlier predictions that yeast fermentation for the production of bioethanol as a replenishabe energy source is being realized because industry is now embracing this technology. The main difference between bioethanol fermentation and beer fermentation is the final product requirements. Bioethanol fermentations require ethanol as the main product at the highest yield for the process. In contrast, beer fermentation aims for well-balanced flavourful products containing ethanol. The script of the process is based on years of investigations to understand the requirements of yeast and how best to treat the yeast for enhanced performance and balanced flavour production. The various aspects discussed under cellars (hygiene requirements, propagation, fermentation and yeast handling) showed that the multifaceted process has various interlinked factors that need to be considered in the design of economic and effective brewing processes. The reward for careful planning and optimization, based on the requirement of the main character *S. cerevisiae*, ultimately results in an enjoyable and refreshing beverage.

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