Wine yeasts for the future

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Abstract
International competition within the wine market, consumer demands for newer styles of wines and increasing concerns about the environmental sustainability of wine production are providing new challenges for innovation in wine fermentation. Within the total production chain, the alcoholic fermentation of grape juice by yeasts is a key process where winemakers can creatively engineer wine character and value through better yeast management and, thereby, strategically tailor wines to a changing market. This review considers the importance of yeast ecology and yeast metabolic reactions in determining wine quality, and then discusses new directions for exploiting yeasts in wine fermentation. It covers criteria for selecting and developing new commercial strains, the possibilities of using yeasts other than those in the genus of Saccharomyces, the prospects for mixed culture fermentations and explores the possibilities for high cell density, continuous fermentations.

Introduction
International competition within the wine market, consumer demands for newer styles of wines and increasing concerns about the environmental consequences of wine production are providing new challenges for innovation in wine fermentation technology (Bisson et al., 2002; Pretorius & Hoj, 2005). Until about the 1980s, the contribution of yeasts to wine production was seen as a relatively simplistic concept. Essentially, grape juice underwent a natural or a spontaneous alcoholic fermentation that, almost invariably, was dominated by strains of the yeast, Saccharomyces cerevisiae. Consequently, pure cultures of this yeast were isolated and developed as starter cultures for conducting wine fermentations. Many other species of yeasts were known to occur in grape juice and contribute to the first stages of fermentation but, generally, they were considered to be of secondary significance or undesirable to the process. By appropriate inoculation of starter cultures and addition of sulphur dioxide to the juice, winemakers aimed to eliminate or minimize the influence of yeasts other than the strain of S. cerevisiae they inoculated. With this technology, winemakers had established reasonably good control of the process (Benda, 1982; Lafon-Lafourcade, 1983; Reed & Nagodawithana, 1988).

During the last 25 years, major advances have occurred in understanding the ecology, biochemistry, physiology and molecular biology of the yeasts involved in wine production, and how these yeasts impact on wine chemistry, wine sensory properties and appeal of the final product (see reviews of Pretorius, 2000; Fleet, 2003, 2007; Swiegers et al., 2005). Now, the yeast ecology of the fermentation has been found to be much more complex than assumed dominance of the inoculated strain of S. cerevisiae, and the metabolic impact of yeasts on wine character is much more diverse than simple fermentation of grape juice sugars. With this greater knowledge and understanding, alcoholic fermentation is now seen as a key process where winemakers can creatively engineer wine character and value through better yeast management and can strategically tailor wines to a changing market.

This article discusses new directions for exploiting yeasts in wine fermentation. It covers criteria for selecting and developing new commercial strains, the possibilities of using yeasts other than those in the genus of Saccharomyces, the prospects for mixed culture fermentations and explores the possibilities for high cell density, continuous fermentations.

The yeast ecology of fermentation
A sound knowledge of the yeast species that conduct the alcoholic fermentation and a sound knowledge of the kinetics of their growth throughout this fermentation are
essential first steps in understanding how yeasts impact on wine quality and in developing new directions. It has been known for a long time that freshly crushed grape juice harbours a diversity of yeast species, principally within the genera *Hanseniaspora* (anamorph *Kloeckera*), *Pichia*, *Candida*, *Metschnikowia*, *Kluyveromyces* and *Saccharomyces*. Occasionally, species in other genera such as *Zygosaccharomyces*, *Saccharomyces* genes, *Torulaspora*, *Dekkera* and *Schizosaccharomyces* may be present (reviewed in Fleet & Heard, 1993; Fleet, 2003). These yeasts originate from the microbial communities of the grape berry and the microbial communities of the winery environment. It is well known that many of these non-*Saccharomyces* species (especially species of *Hanseniaspora*, *Candida*, *Pichia* and *Metschnikowia*) initiate spontaneous alcoholic fermentation of the juice, but are very soon overtaken by the growth of *S. cerevisiae* that dominates the mid to final stages of the process – most often being the only species found in the fermenting juice at these times (Fleet & Heard, 1993; Fleet, 2003). Based on these early ecological studies, *S. cerevisiae* and the related species *Saccharomyces bayanus* were considered to be the yeasts of main relevance to the process and, logically, they became the species around which starter culture technology was developed (Reed & Nagodawithana, 1988; Degre, 1993).

It remained until the 1980s before a sound understanding was obtained about the quantitative growth of individual yeast species throughout juice fermentation (Fleet et al., 1984; Heard & Fleet, 1986). These studies showed that the non-*Saccharomyces* species usually achieved maximum populations of $10^7$ CFU mL$^{-1}$ or more in the early stages of fermentation before they died off. It was concluded that this amount of biomass was sufficient to impact on the chemical composition of the wine and that the contribution of these yeasts to overall wine character was much more significant than thought previously. Under certain circumstances, such as fermentation at lower temperatures, some non-*Saccharomyces* species did not die off and remained at high populations in conjunction with *S. cerevisiae* until the end of fermentation (Heard & Fleet, 1988). Moreover, it was shown that these indigenous non-*Saccharomyces* yeasts also grew in grape juice fermentations to which starter cultures of *S. cerevisiae* had been inoculated (Heard & Fleet, 1985). Consequently, broadly accepted assumptions that starter cultures would overwhelm the growth of non-*Saccharomyces* yeasts and prevent their contribution to wine character were not necessarily valid. Many studies in various wine regions of the world have now confirmed the important contribution that non-*Saccharomyces* species make to the overall kinetics of yeast growth during both spontaneous and *S. cerevisiae*-inoculated wine fermentations (Mora et al., 1988; Schutz & Gafner, 1994; Lema et al., 1996; Egli et al., 1998; Granchi et al., 1999; Pramateftaki et al., 2000; Powhe-Jemec et al., 2001; Jolly et al., 2003a; Combina et al., 2005a, b; Zott et al., 2008). This conclusion is also supported by more recent ecological studies using culture-independent molecular methods for yeast analyses (Cocolin et al., 2000; Mills et al., 2002; Xufre et al., 2006; Nisiotou et al., 2007). Consequently, the impact of non-*Saccharomyces* yeasts on wine fermentations cannot be ignored. They introduce into the process an element of ecological diversity that goes beyond *Saccharomyces* species and they require specific research and understanding to prevent any unwanted consequences they might cause or to exploit their beneficial contributions (Ciani & Picciotti, 1995; Jolly et al., 2003b).

Using molecular techniques that enable differentiation of strains within a species, further ecological sophistication of the fermentation has been discovered. Within each species, there is an underlying growth of a succession of different strains. As many as 10 or more genetically distinct strains of *S. cerevisiae* have been found to contribute to the one fermentation (Sabate et al., 1998; Pramateftaki et al., 2000; Cocolin et al., 2004; Ganga & Martinez, 2004; Sipiczki et al., 2004; Santamaria et al., 2005). In some cases, strains of *S. cerevisiae* inoculated as starter cultures were not able to compete successfully with indigenous strains and, therefore, did not dominate the fermentation as expected (Querol et al., 1992; Schutz & Gafner, 1994; Constanti et al., 1997; Egli et al., 1998; Gutiérrez et al., 1999; Ganga & Martinez, 2004; Santamaria et al., 2005). Such failures have important practical consequences, and reasons for their occurrence need to be understood. The evolution of strain diversity throughout fermentation has also been reported within the non-*Saccharomyces* species (Schutz & Gafner, 1994; Powhe-Jemec et al., 2001).

It is now accepted that wine fermentations, whether spontaneous or inoculated, are ecologically complex and not only involve the growth of a succession of non-*Saccharomyces* and *Saccharomyces* species but also involve the successional development of strains within each species. Such complexity presents a challenge to conducting controlled fermentations with particular yeast cultures designed to impose a special character or style on the final product. In such cases, predictable, dominant growth of the inoculated strain or a mixture of strains would be required. Many factors such as grape juice composition, pesticide residues, sulphur dioxide addition, concentration of dissolved oxygen, ethanol accumulation and temperature affect the kinetics of yeast growth during wine fermentations, but little is known regarding how these factors might affect the dominance and succession of individual species and strains within the total population (Fleet & Heard, 1993; Bisson, 1999; Fleet, 2003; Zott et al., 2008). It is generally considered that the successional evolution of strains and species throughout fermentation is largely determined by their different susceptibilities to the increasing concentration of ethanol – the non-*Saccharomyces* species dying off earlier in
the process because they are more sensitive to ethanol than *S. cerevisiae*. However, there are increasing reports of wine isolates of *Hanseniaspora, Candida* and *Kluyveromyces* species with ethanol tolerances similar to those of *S. cerevisiae* (Mills et al., 2002; Pina et al., 2004; Xufre et al., 2006; Nisiotou et al., 2007). In addition to ethanol, other phenomena such as temperature of fermentation, dissolved oxygen content, killer factors, quorum-sensing molecules and spatial density influences are known to affect the competitive interaction between yeast species and strains in wine fermentations (Yap et al., 2000; Fleet, 2003; Nissen et al., 2003; Hogan, 2006; Perez-Nevado et al., 2006), but more research is needed to understand the diversity and mechanisms of such reactions.

**Yeast affect wine character by various mechanisms**

Understanding how yeasts influence the key properties of wine aroma, flavour and colour provides the basic platform for selection of strains for development as starter cultures and management of the alcoholic fermentation. Such mechanisms now extend beyond the simple, glycolytic metabolism of grape juice sugars and, broadly, can be considered as follows:

1. Metabolism of grape juice sugar and nitrogen components.
2. Enzymatic hydrolysis of grape components to affect wine aroma, flavour, colour and clarity.
3. Autolysis.
4. Bioadsorption.

The metabolism of grape juice sugars and amino acids into ethanol and a vast array of other flavour-impacting substances such as organic acids, glycerol, higher alcohols, esters, aldehydes, ketones, amines and sulphur volatiles is well known as the main reaction by which yeasts influence wine character (Lambrchts & Pretorius, 2000; Swiegers et al., 2005). Many studies have reported the qualitative and quantitative profiles of these metabolites for various strains of *Saccharomyces* and non-*Saccharomyces* yeasts (reviewed in Fleet, 1992; Heard, 1999; Lambrchts & Pretorius, 2000; Romano et al., 2003; Swiegers et al., 2005). These profiles vary significantly between yeast species and strains, so that extensive strain screening is necessary to select for those with positive attributes (e.g. enhanced glycerol production, enhanced ester formation) and reject those with distinct negative impacts (e.g. overproduction of acetic acid or hydrogen sulphide). On this basis, species within *Hanseniaspora, Candida, Pichia*, *Zygosaccharomyces*, *Kluyveromyces* and other genera have the potential to contribute additional flavour diversity and complexity to wine production (Ciani & Maccarelli, 1998; Heard, 1999; Romano et al., 2003; Jolly et al., 2003b).

The biochemical transformation of flavour-inactive grape juice constituents into flavour-active components has emerged, in recent years, as an important, additional mechanism whereby yeasts substantially impact on wine aroma and flavour and facilitate greater expression of grape varietal character. Knowledge about the diversity and complexity of these reactions, however, is still in the discovery phase (Swiegers et al., 2005; Hernandez-Orte et al., 2008). Of these reactions, the liberation of terpenes is most studied. Monoterpene alcohols such as citronellol, geraniol, linalool and nerol occur naturally in grapes, especially Muscat, Riesling and other white varieties, giving characteristic fruity, estery, spicy and vegetative aromas. However, a good proportion of these grape terpenes are covalently linked to glucose or disaccharides of glucose and other sugars, where the bound form has no flavour impact. Glycosidases produced by yeasts break down this linkage to release the volatile terpene that significantly impacts on wine character. The production of glycosidases by yeasts varies with species and strain, but there are increasing data suggesting that non-*Saccharomyces* yeasts such as species of *Hanseniaspora, Debaryomyces* and *Dekkera* are stronger producers of such enzymes than *S. cerevisiae* and, therefore, probably have a greater role in the release of terpene aromas (Fia et al., 2005; Maicas & Mateo, 2005; Swiegers et al., 2005; Villena et al., 2007). In a similar way, yeasts can significantly enhance the wine concentration of volatile thiols such as 4-mercapt-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA). These thiols give desirable passionfruit, grapefruit, citrus and other aroma characters that are most important in Sauvignon Blanc wines, but can also be relevant in other varieties such as Riesling, Semillon, Merlot, and Cabernet Sauvignon. These thiols occur in grapes as nonvolatile forms that are conjugated to cysteine. During fermentation, yeasts (*S. cerevisiae, S. bayanus*) produce a cysteine lyase that deconjugates these thiols into their volatile form. The ability to release these volatile thiols varies with the *Saccharomyces* strain (Swiegers et al., 2005; Dubourdieu et al., 2006; Swiegers & Pretorius, 2007). As yet, little is known about their production by other wine yeasts. Thus, yeasts with good properties of terpene and volatile thiol liberation can be particularly significant in the development of varietal flavours for some types of grapes.

Yeast produce many other enzymes such as esterases, decarboxylases, sulphite reductases, proteases and pectinases that, in various ways, could impact on wine flavour and other properties (Charoenchai et al., 1997; Fernandez et al., 2000; Strauss et al., 2001), but further studies of these possibilities are needed. The decarboxylation of hydroxycinnamic acids in grape juice to form undesirable amounts of volatile phenols such as 4-ethyl phenol and 4-ethylguaiacol is well known for *Dekkera* species but may...
also occur in other wine yeasts (Dias et al., 2003; Suarez et al., 2007).

The roles of yeast autolysis and bioadsorption in affecting wine flavour have not been given detailed consideration in the past and, possibly, this reflects their minor impact. Nevertheless, such influences may be underestimated and could be of value in future developments. It is well documented, however, that yeast autolysis contributes a unique character and value to champagne and other sparkling wines (Alexandre & Guilloux-Benatier, 2006), and autolytic potential is considered to be a significant property in selecting strains of S. cerevisiae for the secondary fermentation of these wines (Martinez-Rodriguez et al., 2001). During alcoholic fermentation, yeasts grow to produce significant levels of cellular biomass (10⁸–10⁹ CFU mL⁻¹) that consists of a mixture of dead and living cells of different species and strains. These cells, which comprise Saccharomyces and non-Saccharomyces yeasts, sediment towards the end of fermentation, to become a major part of the lees. Such biomass is not inert. Dead yeast cells autolyse, which is characterized by the enzymatic degradation of cell proteins, nucleic acids and lipids to end products such as amino acids, peptides, fatty acids and nucleotides, and the release of soluble mannoproteins from the cell wall (Hernawan & Fleet, 1995; Zhao & Fleet, 2005; Alexandre & Guilloux-Benatier, 2006). Most of these products have flavour impact or flavour-enhancing potential (Charpentier et al., 2004, 2005; Comuzzo et al., 2006), but their specific contributions to wine character require more focused research, and will depend on the extent to which the wine is exposed to the lees (Perez-Serradilla & Luque de Castro, 2008). Moreover, there is evidence that peptides released during yeast autolysis could have antioxidant and other bioactive properties (Alcaide-Hidalgo et al., 2007). Yeast mannoproteins may modulate the perception of flavour metabolites and tannin character (Feuillat, 2003; Caridi, 2007; Chalier et al., 2007). While significant information is known about the autolysis of S. cerevisiae, little is understood about autolysis in other yeast species such as the non-Saccharomyces species associated with wine fermentation. The autolytic behaviour of Kloekera apiculata is somewhat different from that of S. cerevisiae and Candida stellata (Hernawan & Fleet, 1995).

Yeast cells are enveloped by a cell wall that can represent up to 30% of the cell dry weight. The wall is composed mostly of glucan polysaccharides and mannoproteins (Klis et al., 2006) that have the potential to modulate wine flavours by bioadsorption of grape components, including mycotoxins, and microbial metabolites by mechanisms that, as yet, are relatively unknown (Feuillat, 2003; Moruno et al., 2005; Chalier et al., 2007). The bioadsorption properties of mannoproteins appear to be particularly significant and contribute to the colloidal stability of wines by interaction with grape tartrates and proteins (Caridi, 2007; Perez-Serradilla & Luque de Castro, 2008). The fine structure of mannoproteins varies with the yeast species (Klis et al., 2006) and could introduce diversity into their bioadsorption properties.

Yeasts impact on the colour and pigment profiles of wines, especially red wines. Such influences vary with the strain of S. cerevisiae and need to be considered in the portfolio of properties when selecting yeasts for starter culture development (Medina et al., 2005; Hayasaka et al., 2007; Caridi et al., 2007). The impact of non-Saccharomyces yeasts on wine colour is relatively unexplored. Yeasts affect the colour of red wines by several mechanisms. They produce ethanol and other metabolites that extract the anthocyanin pigments from the grape skins. Some yeasts produce extracellular pectinases, and these enzymes could facilitate breakdown of grape tissue and extraction of pigments. After extraction, the anthocyanin–phenolic constituents responsible for colour must remain stable, but there is increasing evidence that yeasts can affect this property. Some of the anthocyanins are glycosylated, and glycosidase production by yeasts may break this linkage and decrease colour (Manzanares et al., 2000). Yeast cell wall polysaccharides can adsorb anthocyanins and, in this way, remove colour from the wine (Caridi, 2007; Caridi et al., 2007). Finally, yeasts may indirectly contribute to the stabilization of wine colour during the maturation stage by production of acetaldehyde and pyruvic acid that facilitate the chemical formation of pyranthocyanins (Morata et al., 2006; Monagas et al., 2007).

Propensity to undergo autolysis and parietal absorption activity are relatively new properties to consider in the selection and development of new wine yeasts (Caridi, 2007; Giovani & Rosi, 2007).

Fermentation options

Microbial fermentations can be conducted as either batch processes or continuous processes. Almost all wines are produced by batch fermentation, which means that the juice is placed in a vessel and the entire batch is kept there until fermentation is completed, usually after 5–10 days (Divies, 1993). Continuous fermentations enable much faster, more efficient processing, and present new directions for the wine industry that will be discussed in a later section.

With batch fermentations, there are two options in wine production: spontaneous or natural fermentation or starter culture fermentation (Pretorius, 2000). The ecological concepts of these two options have already been mentioned. Spontaneous fermentations can give high-quality wines with a unique regional character that provides differentiation and added commercial value in a very competitive market. Unfortunately, reliance on ‘nature’ brings diminished predictability of the process, such as stuck or slow fermentations, and inconsistencies in wine quality.
Nevertheless, a good proportion of wine is commercially produced by this process, especially in European countries (Rainieri & Pretorius, 2000; Mannazzu et al., 2002). Generally, a combination of artisanal and technological expertise is required for success with these fermentations. Starter culture fermentations, in contrast, offer the advantages of a more predictable and rapid process, giving wines with greater consistency in quality. They are well suited for producing mass market wines, and their acceptance by the wine industry has been enhanced by the commercial availability of dried concentrates of selected yeast strains that can be conveniently reconstituted for inoculation into grape juice (Degre, 1993; Manzano et al., 2006). Numerous S. cerevisiae and S. bayanus strains are available as commercial preparations, and these yeasts have been selected on the basis of various criteria (see later), including their ability to tailor wine character (Pretorius, 2000; Bisson, 2004). Nevertheless, there has been increasing recognition that starter culture wines may be lacking in flavour complexity and are too standardized and ordinary in character (Rainieri & Pretorius, 2000; Mannazzu et al., 2002). However, such criticisms are providing new challenges to enhance the appeal and value of wine produced by this fermentation technology. This is being achieved by selecting and developing novel strains of starter culture yeasts and conducting fermentations with controlled mixtures of yeast species and strains. Such initiatives now have a greater chance of a successful outcome because there is a clearer understanding of the yeast ecology of wine fermentations, and how this ecology can be managed under the practical conditions of the winery. Greater understanding of yeast biochemistry and physiology is enabling the selection and development of yeast strains that have defined specific influences on process efficiency and wine quality.

Criteria for selecting and developing new strains of wine yeasts

Criteria for the selection and development of yeasts for wine fermentation have evolved over many years and are discussed in Degre (1993), Rainieri & Pretorius (2000), Mannazzu et al. (2002), Pretorius & Bauer (2002), Bisson (2004) and Schuller & Casal (2005). Basically, these criteria can be considered under three categories: (1) properties that affect the performance of the fermentation process, (2) properties that determine wine quality and character and (3) properties associated with the commercial production of wine yeasts. Within each category, there are properties of varying degrees of significance and importance, some being essential and some being desirable.

Fast, vigorous and complete fermentation of grape juice sugars to high ethanol concentrations (>8% v/v) are essential requirements of wine yeasts. The yeast should be tolerant of the concentrations of sulphur dioxide added to the juice as an antioxidant and antimicrobial, exhibit uniform dispersion and mixing throughout the fermenting juice, produce minimal foam and sediment quickly from the wine at the end of fermentation. These processing properties should be well expressed at low temperatures (e.g. 15 °C) for white wine fermentations and at higher temperatures (e.g. 25 °C) for red wine fermentations. It is most important that the yeast does not give slow, sluggish or stuck fermentations (Bisson, 1999).

With respect to wine quality and character, it is essential that any yeast produces a balanced array of flavour metabolites, without undesirable excesses of volatiles such as acetic acid, ethyl acetate, hydrogen sulphide and sulphur dioxide. It should not give undesirable autolytic flavours after fermentation. It should not adversely affect wine colour or its tannic characteristics. In essence, the yeast must give a wine with a good clean flavour, free of sensory faults, and allow the grape varietal character to be perceived by the consumer (Lambrechts & Pretorius, 2000; Bisson, 2004; Swiegers et al., 2005).

Companies that produce yeasts for the wine industry also have basic needs that must be built into the selection and development process (Degre, 1993). The cost of production needs to be contained so that the final product is affordable to the wine industry. Consequently, the yeast must be amenable to large-scale cultivation on relatively inexpensive substrates such as molasses. Subsequently, it needs to be tolerant of the stresses of drying, packaging, storage and, finally, rehydration and reactivation by the winemaker (Soubeyrand et al., 2006). These requirements need to be achieved without loss of the essential and desirable wine-making properties.

Although technologies are well established for the selection, development and production of wine yeasts with good basic oenological criteria, there are increasing environmental pressures for a wine industry that is more efficient and more sustainable, and there are increasing demands from consumers for wines with more distinctive and specific styles, including those with a healthier appeal (e.g. less ethanol, increased antioxidant levels; Bisson et al., 2002; Bisson, 2004). These directions require a more strategic approach to the development of wine yeasts than in the past. For this purpose, a desired wine attribute is identified, and the property to give that quality is designed into the yeast selection and development process, without compromising the essential oenological criteria already mentioned. Pretorius and colleagues (Pretorius & Bauer, 2002; Pretorius & Hoj, 2005; Verstrepen et al., 2006) and Bisson (2004) broadly describe these developmental targets under five categories. These are, with some examples, as follows: (1) Improved fermentation performance (e.g. yeasts with greater efficiency in sugar and nitrogen utilization, increased ethanol tolerance, decreased foam production).
(2) Improved process efficiency (e.g. yeasts with greater production of extracellular enzymes such as proteases, glucanases and pectinases to facilitate wine clarification; yeasts with altered surface properties to enhance cell sedimentation, floatation and flor formation, as needed; and yeasts that conduct combined alcoholic-malolactic fermentations).

(3) Improved control of wine spoilage microorganisms (e.g. yeasts producing lysozyme, bacteriocins and sulphur dioxide that restrict spoilage bacteria).

(4) Improved wine wholesomeness (e.g. yeasts that give less ethanol, decreased formation of ethyl carbamate and biogenic amines, increased production of resveratrol and antioxidants).

(5) Improved wine sensory quality (e.g. yeasts that give increased release of grape terpenoids and volatile thiols, increased glycerol and desirable esters, increased or decreased acidity and optimized impact on grape phenolics).

Sources of new wine yeasts

During the past 50–75 years, wine production has been transformed into a modern, industrialized process, largely based around the activities of only two yeast species: S. cerevisiae and S. bayanus. Future developments will continue to be based on innovation with these species, but opportunities for innovation using other species of yeasts cannot be overlooked. As mentioned already, various species of Hanseniaspora, Candida, Kluyveromyces and Pichia play significant roles in the early stages of most wine fermentations, and there is increasing interest in more strategic exploitation of these species as novel starter cultures (Ciani & Maccarelli, 1998; Heard, 1999; Ciani et al., 2002; Jolly et al., 2003b). Their limitations with regard to ethanol tolerance may not be a hurdle in the production of wines with lower, final ethanol contents. Various species of Zygosaccharomyces, Saccharomyces and Schizosaccharomyces are strong fermenters and are ethanol tolerant. Although they are generally considered as spoilage yeasts, there is no reason to doubt that a good programme of selection and evaluation within these yeasts would not discover strains with desirable winemaking properties (Romano & Suzuki, 1993; Fleet, 2000a). It needs to be recalled that not all strains of S. cerevisiae produce acceptable wines, and that a systematic process of selection and evaluation was needed to obtain desirable strains (Degre, 1993). Consequently, in searching for and developing new yeasts, the wine industry of the future must look beyond Saccharomyces species. In addition, it must look beyond grapes and give broader consideration to other fruits as the starting raw material. With such vision, many new yeasts and wine products await discovery.

Essentially, there are two strategies for obtaining new strains of wine yeasts for development as commercial starter cultures: (1) isolation from natural sources and (2) genetic improvement of natural isolates. Once a prospective isolate has been obtained, it is screened in laboratory trials for essential oenological criteria as mentioned already. Isolates meeting acceptable criteria are then used in micro-scale wine fermentations and the resulting wines are then subjected to sensory evaluation. Strains giving good fermentation criteria and acceptable-quality wines under these conditions are then selected for further development as starter culture preparations. Mannazzu et al. (2002) have outlined an experimental strategy for the selection and evaluation of yeast strains for starter culture development, and some applications of this approach can be found in Regodon et al. (1997), Perez-Coello et al. (1999), Esteve-Zarzoso et al. (2000), Cappello et al. (2004), Cocolin et al. (2004) and Nikolaou et al. (2006).

Natural sources

Generally, wine yeasts for starter culture development have been sourced from two ecological habitats, namely, the vineyard (primarily the grapes) and spontaneous or natural fermentations that have given wines of acceptable or unique quality.

As mentioned already, yeasts are part of the natural microbial communities of grapes (Fleet et al., 2002). Understandably, therefore, grapes are always considered a potential source of new wine yeasts. Moreover, there is an attraction that unique strains of yeasts will be associated with particular grape varieties in specific geographical locations and, through this association, they could introduce significant diversity and regional character or ‘terroir’ into the wine-making process (Veizinh et al., 1992; Pretorius et al., 1999; Jolly et al., 2003a; Martinez et al., 2007; Raspor et al., 2006; Valero et al., 2007). Thus, in the interests of preserving biodiversity and regional influence on wine character, grapes of the region would represent an important source of yeasts for starter culture development.

Yeasts associated with grape berries have been studied for over 100 years (reviewed in Fleet et al., 2002), although more detailed studies are needed to obtain a clearer understanding of their ecology and factors that affect this ecology. The yeast species and populations evolve as the grape berry matures on the vine and are influenced by climatic conditions such as temperature and rainfall, application of agrochemicals and physical damage by wind, hail and attack by insects, birds and animals. The predominant semi-fermentative and fermentative yeasts isolated from grapes at the time of maturity for wine-making are mostly species of Hanseniaspora (Kloekera), Candida, Metschnikowia, Pichia and Kluyveromycetes, although the data are not always consistent (Martini et al., 1996; Jolly et al., 2003a; Prakashaiwattana et al., 2004; Combina et al., 2005a, b; Renouf et al., 2005, 2007; Raspor et al., 2006; Barata
et al., 2008). If the berries are over-ripe, become damaged or are infected with filamentous fungi (mould), the yeast populations tend to be higher and include a greater incidence of fermentative species such as those of Saccharomyces, Zygosaccharomyces, Saccharomycesceae and Zygoascus (Barata et al., 2008; Nisiotou et al., 2007). Consequently, grapes will be a very good source of non-Saccharomyces yeasts, should there be a future direction for using such species as novel starters in wine fermentations.

It is difficult to isolate Saccharomyces species from mature, undamaged grapes by direct culture on agar media, but they are frequently found by enrichment culture methods, suggesting their presence in very low numbers. Grape berries that are aseptically harvested from vines and crushed will eventually ferment and strains of S. cerevisiae and S. bayanus are easily isolated from the fully fermented must (Vezinhet et al., 1992; Khan et al., 2000; van der Westhuizen et al., 2000; Demuyter et al., 2004; Schuller et al., 2005; Mercado et al., 2007; Valero et al., 2007). Strains of Saccharomyces paradoxus, capable of producing wine, have also been isolated from grapes (Redzepovic et al., 2002). However, recovery of Saccharomyces species from such fermentations is not always consistent and can be determined by many factors that are likely to affect the occurrence and survival of yeasts on the grape surface, such as amount of rainfall, temperature and applications of agrichemicals. We have observed that the frequency of isolation of Saccharomyces species from aseptically harvested and crushed grapes can be significantly increased by removing the skins from the macerate and allowing the juice without skins to ferment (S.S. Bae & G.H. Fleet, unpublished data). Possibly, such modification gives low initial numbers of Saccharomyces a better chance to compete with the higher populations of other species. As mentioned already, damaged grape berries are more likely to yield Saccharomyces species than nondamaged grapes. Based on molecular analyses using pulsed field gel electrophoresis and restriction fragment length polymorphism of mtDNA, grape isolates of S. cerevisiae exhibit substantial genomic diversity, because many different strains have been obtained from grapes within the one vineyard or geographical region. In some cases, particular strains have been unique to one location, leading to the notion of a yeast ‘terroir’ (Vezinhet et al., 1992; Khan et al., 2000; van der Westhuizen et al., 2000; Raspor et al., 2006). In other cases, strain diversity within the one location and over consecutive years has been too extensive and inconsistent to support a ‘terroir’ concept (Versavaud et al., 1995; Schuller et al., 2005; Valero et al., 2007). However, further research at the biochemical, physiological and oenological level is required to determine whether S. cerevisiae strains have adapted to grapes in certain geographical locations.

Wines that have undergone a successful natural fermentation have been a main source of yeasts for development as starter cultures. In the past, the focus has been to isolate suitable strains of S. cerevisiae and S. bayanus, but these wines will also be a good reservoir of non-Saccharomyces species. The precise origin of the indigenous strains of Saccharomyces in these wine fermentations has been a question of much debate and controversy (Martini et al., 1996; Mortimer & Polsinelli, 1999). Clearly, the grape itself is a primary source of the yeasts that occur in the juice and it is logical to conclude that any Saccharomyces strains from this source would be prominent in the final fermentation. However, processing of the juice and its transfer to fermentation tanks contributes added microbial communities. These microbial communities originate as contamination from the surfaces of winery equipment and are widely considered to be ‘residential’ flora that have built up in the winery over time, through a process of adaptation and selection, despite cleaning and sanitation operations. These flora are dominated by fermenting ethanol-tolerant yeast species such as S. cerevisiae and S. bayanus because of the selective conditions presented by the properties of fermenting grape juice. Many researchers consider winery flora to be the main source of the diversity of strains of Saccharomyces involved in grape juice fermentations and, in recent years, this has been demonstrated using molecular techniques to track strain origin and development. Strains isolated from winery equipment, in addition to any inoculated strain, are found in the fermenting wine, and similar strains can be found in the one winery in consecutive years, suggesting a carry-over from 1 year to the next (Constanti et al., 1997; Gutierrez et al., 1999; Cocolin et al., 2004; Santamaria et al., 2005; Mercado et al., 2007). Although data are still very little, it seems that S. cerevisiae strains on the grape may have only minor contributions to the overall fermentation (Mercado et al., 2007). However, this could depend on their initial populations and how these are influenced by grape-crushing procedures, cold-maceration processes and grape maturity (Hierro et al., 2006; Sturm et al., 2006). Presumably, the Saccharomyces flora in the winery originally came from grapes and evolved with time. The source of Saccharomyces yeasts on the grapes is still a mystery, but contamination from insects in the vineyard is thought to be a likely possibility (Mortimer & Polsinelli, 1999; Fleet et al., 2002).

Genetic improvement of isolates

Through genetic improvement and metabolic engineering technologies, it is now possible to develop wine yeasts with a vast array of specific functionalities as mentioned already in the section on essential and desirable criteria for selecting wine yeasts. Basically, the desired property is defined (e.g. strain with enhanced glycerol production; strain with bacteriocin production), and then the appropriate genetic tools are applied to construct the strain with that property.
Usually, it is necessary to start with a proven wine yeast at the outset, and to ensure that any genetic manipulation does not adversely affect its basic winemaking properties. The principles of these technologies and their application to wine yeasts have been reviewed by Barre et al. (1993), Pretorius (2000), Pretorius & Bauer (2002), Bisson (2004), Giudici et al. (2005), Schuller & Casal (2005) and Verstrepen et al. (2006) and include:

1. Mutagenesis.
2. Spheroplast fusion.
3. Intraspecific and interspecific hybridization.
4. Transformation and recombinant DNA techniques.
5. Adaptive evolution.

Although early studies (reviewed in Barre et al., 1993) showed that yeast mating and hybridization methods could be used to develop strains of *S. cerevisiae* with improved properties (e.g. flocculation, less hydrogen sulphide production), these classical approaches were overtaken by more precise and convenient recombinant DNA techniques that have now yielded a plethora of strains with well-defined oenological traits as listed in criteria already described (see also, tables in Rainieri & Pretorius, 2000; Pretorius & Bauer, 2002; Bisson, 2004; Schuller & Casal, 2005). Recent reports on the metabolic engineering of wine strains of *S. cerevisiae* that give enhanced release of volatile thiols (Swiegers et al., 2007) and decreased ethyl carbamate production (Coulon et al., 2006) are further examples that illustrate the ‘targeted’ application of this technology. However, consumer and government concerns about the public health and environmental safety of microbial strains engineered by recombinant DNA technologies remain a hurdle to the commercial use of these yeasts (Akada, 2002; Schuller & Casal, 2005; Verstrepen et al., 2006). So far, only one recombinant strain of wine yeast has received approval for commercial use (in North America), and this is a strain of *S. cerevisiae* constructed to contain a malate-permease gene from the yeast, *Schizosaccharomyces pombe*, and the malolactic gene from the bacterium, *Oenococcus oeni*. This strain offers the advantage of improved process efficiency by eliminating the need for bacterial malolactic fermentation that is usually conducted after alcoholic fermentation (Husnik et al., 2007; Main et al., 2007).

Hybridization (Giudici et al., 2005; Belloch et al., 2008), adaptive evolution (Barrio et al., 2006; McBryde et al., 2006) and systems biology (Bisson et al., 2007; Borneman et al., 2007; Pizarro et al., 2007) are strategies that do not compromise consumer and government sensitivities with respect to safety and environmental risks, and now attract significant focus for the development of a new generation of wine yeasts. Inter- and intraspecies hybrids within strains of *Saccharomyces* (e.g. *S. cerevisiae* × *S. bayanus* and *S. cerevisiae* × *S. kudriavzevii*) have been isolated from spontaneous fermentations and similar hybrids, now commercially available, have been produced by mating yeasts under laboratory conditions (Gonzalez et al., 2006, 2007; Belloch et al., 2008). Hybrids between *S. cerevisiae* and other species within *Saccharomyces* are also available (e.g. *S. cerevisiae* × *S. cariocanus*, *S. cerevisiae* × *S. paradoxus* and *S. cerevisiae* × *S. mikatae*). A list of commercially available strains of wine yeasts, including newly developed hybrid strains, has been compiled by Henschke (2007). Hybridization expands the tolerance of some strains to the stresses of winemaking such as temperature of fermentation and ethanol concentration and increases the pool of strains available to enhance diversity in wine flavour (Belloch et al., 2008; Bellon et al., 2008).

Adaptive evolution is another concept for selecting strains with oenological performance and flavour profiles matched to a particular winemaking need. In this case, yeasts are continuously and repeatedly cultured under a defined combination of conditions from which strains that have specifically adapted to these conditions can be isolated (McBryde et al., 2006). In essence, it is a laboratory approach for speeding up what occurs naturally, over time, in winery habitats (Barrio et al., 2006). Systems biology exploits knowledge of the total genome and bioinformatic methods to select and develop new strains of wine yeasts with very specific functionalities and criteria, as determined by production, consumer and environmental demands (Borneman et al., 2007; Pizarro et al., 2007). Because genomic information about wine yeasts is still very limited, this approach is at a conceptual stage of development and practical outcomes are yet to be realized.

**Controlled fermentations with mixed strains or species of yeasts**

The realization that yeasts other than *Saccharomyces* species are ecologically and metabolically significant in wine fermentation has laid the platform for more creative and controlled exploitation of their use in wine production. The potential of using them as stand-alone, single starter cultures in the development of new styles of fermented beverages has already been mentioned, but not seriously explored to date. However, some of these species are limited in their ability to fully ferment the grape juice sugars and in their ability to produce sufficient concentrations of ethanol. Some may grow too slowly in comparison with other indigenous yeasts, and not establish themselves. Nevertheless, they have other properties of oenological relevance that would be worth exploiting. For example, some *Hanseniaspora/Kloeckera* species may produce more appealing mixtures of flavour volatiles, and higher amounts of glycosidases and proteases than *Saccharomyces* species (Zironi et al., 1993; Capece et al., 2005; Mendoza et al., 2007; Moreira...
et al., 2008), C. stellata gives increased levels of glycerol (Ciani & Comitini, 2006), Kluyveromyces thermodtolerans gives increased levels of lactic acid (Mora et al., 1990), Torulaspora delbrueckii produces less acetic acid (Lafon-Lafourcade et al., 1981; Ciani et al., 2006; Bely et al., 2008) and Schizosaccharomyces species decrease wine acidity through malic acid metabolism (Gao & Fleet, 1995; Taillandier et al., 1995). Some wine strains of C. stellata have recently been reclassified as Candida zemplinina (Csoma & Sipiczki, 2008). Conducting wine fermentations by controlled inoculation of mixtures of different yeast starter cultures is one strategy to harness the unique activity of such yeasts. This concept is not new and early studies have been reviewed by Heard (1999) and Ciani et al. (2002). However, it now attracts greater interest because of its potential to introduce specific characteristics into wine and also because winemakers have a more thorough knowledge of the ecology and biochemistry of wine fermentation and how to manage this process (Jolly et al., 2003b). This area of wine technology is very likely to grow in future applications; consequently, it is useful to collate significant studies to date (Table 1).

Most studies (Table 1) have aimed to understand how non-Saccharomyces species grow interactively with S. cerevisiae in comparison with monocultures of the respective yeasts. Growth profiles are generally reported, along with glucose and fructose utilization, and the production of key metabolites such as ethanol, acetic acid, glycerol, ethyl acetate and, in some cases, various higher alcohols, higher acids and other esters (e.g. see Jolly et al., 2003b). Essentially, these studies confirm that non-Saccharomyces yeasts grow in sequential patterns similar to those observed for spontaneous wine fermentations, but conditions such as temperature, sulphur dioxide addition, inoculum levels and time of inoculation can be manipulated to enhance the extent of their survival and contribution to the overall fermentation. Inoculating ethanol-sensitive or slower-growing non-Saccharomyces yeasts into the grape juice several days before inoculating S. cerevisiae (sequential inoculation) is one strategy for enhancing their contribution to the fermentation. Data are not always consistent on how interactive growth affects the production of flavour metabolites, but several studies show that the unacceptably high volatile acid and ester production by some Hanseniaspora/Kloeckera species does not occur when these yeasts are grown in mixed culture with S. cerevisiae (Herraiz et al., 1990; Zironi et al., 1993; Zohre & Erten, 2002; Mendoza et al., 2007; Moreira et al., 2008). In some cases, overall ethanol production is decreased in mixed culture fermentations, but in other cases this is not observed. Using different Saccharomyces species and strains, Howell et al. (2006) concluded that mixed culture impacts on the metabolic performance of individual strains within the mixture. Wines made with mixed cultures of the yeasts gave a combination of volatile aroma

### Table 1. Studies of wine fermentation inoculated with defined mixtures of yeast species

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<th>Yeast species inoculated</th>
<th>Objective of study</th>
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<td>Nissen et al. (2003)</td>
<td>S. cerevisiae, T. delbrueckii, K. thermodtolerans</td>
<td>Ecological interactions</td>
</tr>
<tr>
<td>Ciani et al. (2006)</td>
<td>S. cerevisiae, H. uvarum, K. thermodtolerans, T. delbrueckii</td>
<td>Ecological interactions</td>
</tr>
<tr>
<td>Perez-Nevado et al. (2006)</td>
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<td>Ecological interactions</td>
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<td>Mendoza et al. (2007)</td>
<td>S. cerevisiae, K. apiculata</td>
<td>Ecological interactions</td>
</tr>
<tr>
<td>Herraiz et al. (1990)</td>
<td>S. cerevisiae, K. apiculata, T. delbrueckii</td>
<td>Flavour modulation</td>
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<tr>
<td>Moreno et al. (1993)</td>
<td>S. cerevisiae, T. delbrueckii</td>
<td>Flavour modulation</td>
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<td>Zironi et al. (1993)</td>
<td>S. cerevisiae, H. guilliermondii, K. apiculata</td>
<td>Flavour modulation</td>
</tr>
<tr>
<td>Gil et al. (1996)</td>
<td>S. cerevisiae, H. uvarum, K. apiculata</td>
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<td>Soden et al. (2000)</td>
<td>S. cerevisiae, C. stellata</td>
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<td>Garcia et al. (2002)</td>
<td>S. cerevisiae, D. vanrij</td>
<td>Flavour modulation</td>
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<td>Clemente-Jimenez et al. (2005)</td>
<td>S. cerevisiae, P. fermentans</td>
<td>Flavour modulation</td>
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<td>Kurita (2008)</td>
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<td>Moriera et al. (2008)</td>
<td>S. cerevisiae, Hanseniaspora spp.</td>
<td>Flavour modulation</td>
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<td>Mora et al. (1990)</td>
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<td>Silva et al. (2003)</td>
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<td>Ciani &amp; Comitini (2006)</td>
<td>S. cerevisiae, C. stellata</td>
<td>Glycerol enhancement</td>
</tr>
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<td>Bely et al. (2008)</td>
<td>S. cerevisiae, T. delbrueckii</td>
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<td>S. cerevisiae, S. bayanus (three strain mixtures)</td>
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<td>Jolly et al. (2003b)</td>
<td>S. cerevisiae, C. colliculosa</td>
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<td>S. cerevisiae, C. stellata</td>
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<td>S. cerevisiae, K. apiculata</td>
<td>Flavour modulation</td>
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<td>S. cerevisiae, C. pulcherrima</td>
<td>Flavour modulation</td>
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metabolites different from that obtained by blending together monoculture wines made with the same component yeast strains. Thus, with respect to production of flavour volatiles in wine, the metabolic interactions of yeasts during mixed culture could be quite complex and difficult to predict. Consequently, the ultimate evaluation of such fermentations should be based on sensory testing but, unfortunately, few studies in Table 1 report such data. In this context, the study of Soden et al. (2000) is significant and reports the chemo-sensory outcome in a Chardonnay wine produced by fermentation with a mixed culture of C. stellata and S. cerevisiae. The sensory impact of C. stellata, as well as its production of increased levels of glycerol, were only evident when the yeast was used in a sequential inoculation protocol (i.e. inoculated 15 days before S. cerevisiae), as compared with coinoculation of the two species at day zero. Jolly et al. (2003b) conducted small-scale wine fermentations with S. cerevisiae mixed with either Candida colliculosa, C. stellata, Kloeckera apiculata or Candida pulcherrima. Wines with enhanced sensory appeal were obtained with many of the mixed yeast fermentations, but this depended on the grape variety and the length of time the wine had been aged.

The impact of non-Saccharomyces yeasts in mixed culture with S. cerevisiae can be more definitive when specific wine properties are targeted, such as decreasing malic acid concentrations using Schizosaccharomyces species or using Torulaspora delbrueckii to prevent volatile acidity production in sweet wine fermentations. Sequential inoculation of S. pombe before S. cerevisiae appears to be necessary for a successful deacidification but, unfortunately, this yeast can give off-flavours to the wine (Snow & Gallander, 1979; Silva et al., 2003). Possibly, a programme of selection for this yeast could avoid these problems. In the case of T. delbrueckii, coinoculation with S. cerevisiae was necessary because it causes stuck fermentations if inoculated before S. cerevisiae (Bely et al., 2008).

The studies listed in Table 1 provide an excellent platform for future development of wine fermentation technology based on the controlled use of mixed cultures. Further research is required to understand the biological mechanisms of how yeasts interact ecologically and metabolically when grown in coculture and sequential culture under winemaking conditions. These fundamental studies must be linked with well-controlled sensory evaluations of wine flavour and colour so that useful practical outcomes are achieved.

More efficient wine fermentation

Alcoholic fermentation is conducted as a batch process, where the grape juice is placed in tanks or barrels, and kept there until the fermentation is completed. In most modern wineries, large stainless-steel, cylindro-conical vessels are used. Good understanding of the growth and biochemical activities of yeasts in these batch systems, as well as ability to control variables such as temperature, agitation and aeration, have ensured the widespread successful use of this technology (Moresi, 1989; Divies, 1993). Nevertheless, batch fermentations have inherent inefficiencies. The process is relatively slow because the duration of fermentation depends on the growth of yeast cells to final populations of at least $10^8$ cells mL$^{-1}$. Most wine fermentations, therefore, require 4–7 days or longer. The process is discontinuous because a new batch of wine cannot be fermented until the previous batch is finished and removed from the vessel. Consequently, large fermenter capacity and associated capital investment are required. At the end of the process, microbial cells need to be separated from the product, thereby adding to inefficiency and product losses. From time to time, winemakers experience frustration with these limitations, especially during vintage when there are peak demands on volume capacity, or when there are unforeseen delays due to stuck or sluggish fermentations. Consequently, the question arises – can wine fermentations be conducted more efficiently, and what are the alternatives?

During the last 20–30 years, technologies have been developed to immobilize microbial cells and apply them to commercial fermentations. The basic concept is that very high concentrations of microbial cells can be entrapped within or attached to a solid matrix without destroying their viability or biochemical activity. Immobilized or entrapped populations of $10^6$–$10^{10}$ cells mL$^{-1}$ can be achieved, compared with the levels of $10^7$–$10^8$ cells mL$^{-1}$ usually obtained in batch cultures. These high cell densities are very reactive, and can conduct biochemical reactions, such as sugar-alcohol fermentations, at rates 10–100 times faster than those that occur during batch culture (reviewed in Pilkington et al., 1998; Verbel et al., 2006; Bai et al., 2008). Several strategies have been used for the capture and immobilization of high densities of microbial cells and these include:

1. Physical entrapment within a porous matrix (e.g. gels of calcium alginate or k-carrageenan, usually in the form of beads about 2 mm in diameter).
2. Attachment or adsorption to the external surface of an inert solid matrix such as cellulose particles, porous glass, volcanic rock, diatomaceous earth, particles of gluten and fruits.
3. Confinement within a defined space by membrane filters (e.g. membrane bioreactors).
4. Self-aggregation of highly flocculent cells.

Immobilized cell reactors enable faster, more efficient fermentations, which can be conducted on a batch or a continuous basis. Usually, the immobilized cell matrix is packed into columns, and the material for fermentation is then passed through the column, giving the transformed
product on exit. Their application to wine production has been discussed in several reviews (Divies, 1993, 1994; Fleet, 2000b; Ciani et al., 2002; Kourkoutas et al., 2004; Strehaiano et al., 2006). Most focus has been on the use of bacterial reactors for malo-lactic fermentation, and the use of yeasts entrapped in alginate gels for the secondary fermentation in sparkling wines. Schizosaccharomyces pombe metabolizes malic acid, and several studies have described the experimental use of alginate-immobilized cells of this yeast to deacidify wines (reviewed in Strehaiano et al., 2006). Silva et al. (2003) successfully used S. pombe entrapped within beads of alginate in a batch process to deacidify grape juice before fermentation with S. cerevisiae, and suggest that this process is at a stage for commercialization.

Application of new bioreactor technologies to the alcoholic fermentation grape juice is still at beginning stages of development. Some early laboratory studies showed that the length of batch fermentations could be decreased using concentrates of yeast cells, freely suspended or immobilized on alginate, recycled from previous fermentations (Rosini, 1986; Suzzi et al., 1996). Wine strains of S. cerevisiae immobilized on a wide range of supports such as volcanic rock, alumina, calcium alginate, cellulose, gluten pellets and particles of apple, quince, grape skin, watermelon and raisin berries have been used to conduct batch fermentations. The immobilized yeasts could be easily recovered from each fermentation and reused many times, reportedly giving fermentations two to three times faster than control fermentations (Kourkoutas et al., 2004). Even faster fermentations could be achieved when the grape juice was continuously passed through columns packed with yeast cells immobilized on these matrices (Fleet, 2000b; Kourkoutas et al., 2004; Tsakris et al., 2004; Reddy et al., 2008). Candida stellata, immobilized on calcium alginate, has been used in conjunction with S. cerevisiae to enhance glycerol production in both batch and continuous wine fermentations (Ciani & Ferraro, 1996; Ferraro et al., 2000; Ciani et al., 2002).

Membrane bioreactors operated on a continuous, cell-recycle basis offer greater efficiencies and faster reactions compared with other reactor technologies because much higher cell densities can be achieved, and the yeast cells are in direct contact with the grape juice. Their application to wine production has been briefly mentioned by Strehaiano et al. (2006) and Divies (1993, 1994). We have shown that a laboratory-scale, 1-L membrane bioreactor charged with $10^8$–$10^{10}$ cells mL$^{-1}$ of S. cerevisiae could produce 1 L of fully fermented wine per hour. The reactor performed continuously for 10 days without loss of fermentative activity (Charoenchai, 1995). Similar experiences with these reactors have been described by Takaya et al. (2002). Crapisi et al. (1996) reported an interesting use of a hollow-fibre membrane reactor, charged with cells of Kloeckera apiculata/Hanseniaspora uvarum to produce a low-alcohol wine.

There is little doubt that new bioreactor technologies have the potential to significantly enhance the efficiency of wine fermentation and facilitate innovations with yeasts other than Saccharomyces species. However, their scale-up to commercially successful operations presents a number of challenges that require further research. Some of these issues are discussed in Fleet (2000b), Kourkoutas et al. (2004) and Bai et al. (2008). Any particulate material in the juice or wine will, in due course, physically clogs column reactors or fouls membranes in membrane reactors, eventually stopping their performance. Similarly, indigenous bacteria and yeasts in the juice or wine are likely to accumulate in the reactor and compromise its performance. Prior clarification and pasteurization of the juice or wine may be necessary, with consequences on processing economy and product quality. Long-term stability and reactivity of yeast cells in the reactors are essential to maintaining processing costs. Yeast cells are exposed to many new physical, chemical and biological stresses in reactors that need to be understood and optimized to enhance their long-term performance. Large amounts of carbon dioxide are produced, whose consequences need to be managed. Finally, wines produced by new reactor technologies must have acceptable sensory qualities. The metabolic behaviour of yeast cells operating under reactor conditions may be significantly different from that for cells growing in batch culture, thereby giving a different profile of flavour volatiles in the final product. A programme of strain selection and development will be required to obtain wine yeasts that perform under continuous, high cell density conditions.

**Conclusion**

The wine industry is faced with an exciting but challenging future. Continuing advances in yeast biology and fermentation technology provide many opportunities for innovation and adaptation to a changing market. These will enable further refinements of existing technologies and products but also the development of new products based on the exploitation of new strains of Saccharomyces yeasts, non-Saccharomyces yeasts, novel bioreactor technologies and the use of fruits other than grapes. Environmental issues present new challenges. Current agrichemical use in the vineyard will not be sustainable, and climate change is likely to have a cascading effect on grape cultivation, grape juice composition, the yeast ecology of wine production, fermentation kinetics and wine character.

**References**


